Journal of Ornamental Plants
Available online on: www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Modification of Flower Color Pigments and Color Composition with Hormonal Treatments and Sucrose in *Tulipa gesneriana* 'Kingsblood'

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Received: 26 June 2017 Accepted: 09 January 2018
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Abstract

Three separate experiments were conducted to investigate the interplay between three phytohormones and sucrose for the change of flower color composition and the plant secondary metabolites in *Tulipa*. The variations of the physiological and morphological characteristics, with ABA at 5 and 10 mg/L (as experiment one), GA_3 at 300 and 500 mg/L (as experiment two), 50 and 100 µM of JA (as experiment three) and their interactions with sucrose at 1 and 2 g/L were analyzed. By reviewing the HPLC charts and UV-Vis spectra, it was found that the production of plant secondary metabolites, total flavonoids, and anthocyanins composition pigments was influenced by the foliar application of the plant hormones. The sucrose alone had no significant effect on the quantification of different phytochemicals, but the interaction with JA and ABA showed a considerable variation in the anthocyanin accumulation and total flavonoids. Both JA and ABA hormones, in spite of enhanced anthocyanin accumulation and increased cyanidin and pelargonidin pigment percentage, were associated with reduced vegetative growth parameters as well as vase life, compared to the control plants. However, GA_3 at 500 mg/L without sucrose played a key role in the accumulation of anthocyanin, postharvest performance, and the increase in the three major anthocyanin pigments. Moreover, the data provided evidence of interference between the sucrose and GA_3 in the regulation of the anthocyanin accumulation.

Keywords: Abscisic acid, Anthocyanins pigments, Gibberellic acid, Jasmonic acid, Total flavonoids.

INTRODUCTION

Anthocyanin pigments play a major role in the color composition of tulip flowers. Therefore, enhancement of flower color by altering anthocyanin production and the composition of pigments for the purpose of improving the quality of the flowers can be of great importance in the flower industry. The variation of flower color is influenced by many endogenous and exogenous factors, but the plant growth regulators and sucrose have the greatest impact (Zhao and Tao, 2015).

Recent studies have shown that sucrose as the main form of transport of carbon, in addition to its contribution to the carbon skeleton of the plant cells and as a source of energy, acts as a molecular signal. The role of sucrose as an inducer molecule or molecular signal is certainly proven in many plants (Bolouri Moghaddam *et al.*, 2010). Moreover, sucrose can activate the expression of the genes involved in the biosynthesis of anthocyanins and flavonoids in different *in vivo* and *in vitro* cultures (Teng *et al.*, 2005). The ability of sucrose to induce the biosynthesis of anthocyanin pigments in various species of plants such as Arabidopsis (Solfanelli *et al*., 2006), grapevine (Pirie and Mullins, 1976), and radish (Hara *et al.*, 2003) has been proven.

The activation process of anthocyanin biosynthesis pathway in the petals and other organs is the result of a complex interaction between plant growth regulators and sucrose (Weiss, 2000). In previous studies, it has been proven that the three main groups of phytohormones include gibberellic acid (GA_3), jasmonic acid (JA) and abscisic acid, which are involved in the biosynthesis of anthocyanin pigments (Medina-Puche *et al.*, 2014). Each of these hormones has a different situation process to change and regulate anthocyanin biosynthetic pathway and the synthesis of secondary metabolites. However, all of these hormones, whether directly or indirectly, act by affecting the expression or suppression of related genes involved in the biosynthesis of anthocyanin and flavonoids (Loreti *et al.*, 2008).

The effect of GA_3 on anthocyanin synthesis has been reported in many different plants. Ohlsson and Berglund (2001) studied the influence of GA_3 on anthocyanin metabolism in cell culture of *Catharanthus roseus* and observed increased anthocyanin accumulation. The influence of GA_3 on *in vivo* and *in vitro* cultures of periwinkle (Piovan and Filippini, 2007), gerbera (Danaee *et al.*, 2011) and *Hyacinthus* (Hosokawa, 1999) has been observed. The involvement of JA in anthocyanin synthesis is demonstrated by some experimental evidence in various plant species and tissues (Steyn *et al.*, 2002). The effects of JA in *Arabidopsis thaliana* (Qi *et al.*, 2011), *Tulipa* (Saniewski *et al.*, 1998a), grapevine cell cultures (Belhadj *et al.*, 2008), and in apple fruits (Kondo *et al.*, 2001) were also reported. The results obtained from the exogenous applications of ABA showed that depending on the concentration and plant species, ABA stimulated the synthesis of anthocyanin (Jaakola, 2013). Furthermore, some studies show that ABA can induce biosynthesis pigment in *Fragaria ananassa* (Medina-Puche *et al.*, 2014), *Litchi chinensis* (Lai *et al*., 2014), and sweet cherry (Shen *et al.*, 2014). The aim of this research was to study the interaction between sucrose and plant hormones on the variation of flavonoids, anthocyanin, and color composition pigments, as well as to evaluate the physiological and morphological changes of tulip plants.

MATERIALS AND METHODS

Plant materials and treatments

This experiment was carried out in November 2015 at the Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The tulip plants (*T. gesneriana* 'Kingsblood') used for this study were grown in the fields of the Netherlands with a cover of 11-12 cm in circumference and were purchased from a commercial grower. The bulbs were grown in individual pots filled with three growth beds: peat, cocopeat, and perlite in equal ratio. After the planting, these pots were stored at 5°C for 14 weeks, and then they were transferred to a greenhouse according to a randomized complete block design in three separate tests to be grown at 20°C/15°C

(day/night). During the vegetative phase, the plants were irrigated with the normal Hoagland nutrient solution. The plants were separately sprayed at three stages of inflorescence development with ABA at the rate of 5 or 10 mg/L and sucrose at the rate of 1 or 2 g/L (as experiment one), GA_3 at the rates of 300 or 500 mg/L and sucrose at the rates of 1 or 2 g/L (as experiment two), and 50 or 100 µM of JA and sucrose at the rates of 1 or 2 g/L (as experiment three). In all the treatments, the spray solutions contained 0.2 % Tween-20 as a surfactant. At flowering, the characteristics measured in this experiment included days to bud appearance, shoot weight, plant length, flower stalk length, flower fresh weight, vase life, leaf water content, flower water content (Fig. 1).

Fig. 1. Pots arrangement based on a completely randomized design in the greenhouse.

In April 2016, the petal samples for total anthocyanin, flavonoid, and HPLC detection were collected from the tulip plants in a fully developed stage when the petals were fully pigmented (Fig. 2). The samples were collected, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until use.

Fig. 2. The tulip plants in a fully developed stage and the petals were fully pigmented ready for laboratory measurements.

The estimation of proline content

The free proline content was determined according to the method followed by Bates *et al*. (1973) by measuring the quantity of the colored reaction product of proline with ninhydrin acid. The absorbance was read at 520 nm. The amount of proline was calculated from the previously plotted standard curve and expressed in µmol/g FW.

Total flavonoid content

Aluminum chloride colorimetric method of Woisky *et al*. (1998) was used to determine total flavonoids. The plant extract (0.5 mL of 1:10 g/mL) was mixed with 1.5 ml of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 ml of distilled water. It was placed at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm in triplicate. The calibration curve was prepared with quercetin solutions at the concentrations of 10 to 100 µg/mL in methanol.

Isolation of anthocyanin and estimation of total anthocyanin content

Petal slices (0.5 g of each treatment) were macerated in 1 ml acidic ethanol solution (1% HCL in MeOH) in a test tube and allowed to equilibrate overnight at 4°C. These samples were centrifuged at 12000 g for 10 min at 4°C, leaving the anthocyanin in the supernatant. Then, the total anthocyanin of the petals extracted was measured using the pH differential method described by Lee *et al.* (2005). Quantities of anthocyanin were determined in cyanidin-3-glucoside (MW=449.2, molar absorbance, ε=26.900) for tulip materials.

Identification and quantification of anthocyanin pigments in perianthes by HPLC

The flower colors of perianthes were evaluated by comparing the data from UV–Vis spectra and co-chromatographed analysis. The preparation of anthocyanin samples for HPLC analysis on each of the plant material was made by the procedure described by Nakayama *et al.* (2004). The HPLC analysis was conducted using a model Shimadzu performance liquid chromatography. An Inertsil ODS-2 column (φ10 × 250 mm), from GL Sciences, was used. Two solvents were used for elution: solvent A with 1.5 % phosphoric acid and solvent B with 1.5% phosphoric acid, 25% CH_3CN, and 20% HCO_2H. The anthocyanin samples were separated by gradient elution: in 20 min linear 0 to 40% B in A + B and in 20 min 40% B in A + B (A, 10% acetic acid and 0 .1% phosphoric acid in water; B, 50% acetonitrile in water) and the flow rate was 0.6 ml/min. Anthocyanins were detected at 520±20 nm with a Shimadzu spectrophotometer. Retention times and quantitative calculations of the anthocyanin peaks were obtained with a Shimadzu Chromatopac. The identification procedures of anthocyanins were carried out by comparing the known and unknown samples by using the visible l max and co-chromatography. The standards of cyanidin, delphinidin, and pelargonidin were obtained from Chromadex (Santa Ana, CA). For quantification, peak areas were correlated with concentrations in accordance with the calibration plot. The quantification of the amounts of anthocyanin in the HPLC chromatograms was calculated from peak areas with reference to respective standard and expressed in mg/g fresh weight sample (Torskangerpoll *et al.*, 2005).

Statistical analysis

Three separate experiments were conducted based on complete randomized block design. The data obtained from the measurements of the pots, and the laboratory observations were subjected to the analysis of variance using SPSS software and Duncan's mean separation test procedure was applied. All treatments had six pots in three replications.

RESULTS AND DISCUSSION

Vegetative growth parameters

The results indicated dramatic differences between the different concentrations of plant growth regulator and sucrose ($P < 0.05$) (Table 1). The use of any of these hormones had significant effects on the characteristics of the tulip plants. According to the results represented in Table 1, almost all of the traits, except for flower stalk length, leaf water content and flower water content,

were affected by the hormonal treatments.

Effect of abscisic acid on vegetative growth parameters (Experiment one)

Data obtained from ABA treatments showed delayed flowering by about three days and reduced vegetative growth parameters like plant length (45.21 cm) that was almost 10 cm smaller than the control plants (Table 2). The results showed the inhibitory effect of ABA on shoot growth and flowering induced by endogenous GA_3 in tulips. Moreover, our observations showed that ABA (10 mg/L) could lead to unwanted early senescence of tepals of cut tulips. The lowest vase life of cut flowers was related to 10 mg/L ABA with 1 g/L sucrose in which it was 5.08 days. Furthermore, ABA may play an important role in the regulation of flower senescence and exogenous application of ABA accelerates the symptoms of flower senescence in carnation, rose and daylily cut flowers (Panavas *et al*., 1998). In ethylene-sensitive flowers like carnation, exogenous ABA triggered endogenous ABA synthesis. Therefore, the effects of ABA might be mediated through an increase in ethylene synthesis resulted from ABA application or through activation of ethylene action (Onoue *et al.*, 2000). Although ethylene seems not to be directly involved in the senescence of tulip tepals, we observed that exogenously applied high concentrations of ABA may trigger a higher rate of ethylene production, resulting in unusual senescence and abscission of the tepals (van Doorn, 2002). Endogenous content of ABA has been reported to increase during senescence in several flowers (Panavas *et al*., 1998; Hunter *et al*., 2004) and this may be due to water soaking or conversion of carotenoids to ABA (Milborrow, 2001).

Besides, the least amount of flower water content (5.86 g) and leaf water content (8.56 g) was related to 10 mg/L ABA treatments and the highest amount of flower water content (7.17 g) and leaf water content (15.53 g) was recorded in 1 g/L sucrose treatments and control plants, respectively (Table 2). So, the relative water content of tepals and leaves were reduced by ABA as compared to control plant, since the reduction of the water content of leaves and flowers is directly related to the early aging of flowers. Thus, we conclude that ABA plays a pivotal role in reducing the vase life of flowers and flower quality in 'Kingsblood' tulips.

Effect of gibberellic acid on vegetative growth parameters (Experiment two)

The GA_3 treatment caused the tulip plants to be heavier, longer and earlier in flowering with effective prolonging of vase life without abscission. As can be seen in Table 3, the highest shoot weight of 65.42 g and the tallest plants (63.21 cm) were obtained from the application of 500 mg/L GA_3. Furthermore, we detect significantly faster growth in the GA_3-treated plants so that the effect of the treatment was to increase the growth period resulting in longer flower stalk length (45.17 cm) (Table 3). GA_3 treatment of various plants has accelerated growth and cell elongation (Smith *et al.*, 1996) and stem elongation (Yim *et al.*, 1997). Although sucrose alone, in most of the cases, had no significant effect on morphological characteristics, the interaction between sources and exogenous GA_3 at low concentrations led to the improvement of some of the measured traits like delayed senescence in cut flowers and increased vegetative growth parameters. According to the results of Table 3, GA_3 treatments extended vase life by about two days (11.58 days) compared to control plants and improved the quality of flowers. Moreover, the interactive effects between GA_3 and sucrose showed that GA_3 (500 mg/L) without sucrose increased the vase life of cut flowers compared to control plants and improved the quantitative and qualitative characteristics of the tulips. These results suggest that the increase in vase life with GA_3 was greater than that in plants sprayed with GA_3 combined with sucrose. These results correspond to the observations by Imsabai *et al.* (2013). Furthermore, there was a positive correlation between vase life and water content and dry weight of tepals after treatment with GA_3. The water content of tepals and leaf were significantly affected by GA_3 treatments (Table 3). The application of GA_3 in combination

Table1. ANOVA of the effect of different concentrations of plant hormones and sucrose on physiological parameters in *Tulipa gesneriana* 'Kingsbloods'

	SoV	df	Budap-pearance	Shoot weight	Plant length	Flower stalk length	Flower fresh weight	Vase life	Leaf water content	Flower water content	Flavonoid-content	Anthocyanin content	Proline content	Dry weightof flower
Expr-One	ABA	8	5.27 **	43.45 **	63.22 **	14.97 ns	0 .45 *	13.53 **	27.17 **	0.42 *	7.98**	0.83 **	1.08 **	3443.48 **
	Error	18	0.77	6.24	3.40	6.19	0.15	0.73	2.67	0.14	0.28	0.07	0.08	147.88
	CV (%)		3.98	4.82	3.58	7.47	5.54	11.59	14.24	5.69	2.76	7.53	4.83	4.15
Expr-Two	GA_3	8	4.68 **	83.08**	44.92**	81.08 *	0.67 *	2.86 **	14.59 **	0.5 ns	4.65 **	0.46**	1.20 **	1262.79 **
	Error	18	0.47	15.53	8.02	12.41	0.23	0.35	2.44	0.26	0.18	0.06	0.26	141.62
	CV (%)		3.50	6.80	4.98	9.53	6.61	5.67	11.26	7.39	2.48	4.2	8.876	3.81
Expr-Three	JA	8	7.77 **	72.73 **	82.62 **	47.39 **	3.98 **	5.44 **	5.28 ns	3.80 **	3.89**	1.17 *	1.18 **	2219.52 **
	Error	18	0.55	11.54	4.97	2.63	0.28	0.16	2.41	0.28	0.35	0.15	0.15	100.62
	CV (%)		3.23	6.98	4.63	5.25	9.76	4.25	11.67	10.29	3.21	7.76	7.77	3.48

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

Table 2. Effect of different concentrations of abscisic acid and sucrose on vegetative growth parameters in *Tulipa gesneriana* 'Kingsblood'.

Treatments		Vegetative growth parameters																								
		Bud appearance (day)			Shoot weight (g)			Plant length (cm)			Flower stalk length (cm)			Flower fresh weight (g)			Vase life (day)			Leaf water content (g)			Flower water content (g)		
		Sucrose (mg/L)																								
		0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-One	ABA (mg/L) 0	20.67 cd	20.25 d	20.42 d	54.83 abc	56.33 ab	58.33 a	54.88 bc	56.63 ab	58.42 a	ns	ns	ns	7.03 ab	7.52 a	6.99 abc	9.83 a	10.42 a	9.75 a	15.53 a	15.45 a	15.05 a	6.69 ab	7.17 a	6.67 ab
	5	22.67 ab	22.08 bc	21.75 bcd	53.92 abc	53 bcd	49.92 de	51.54 cd	52.08 cd	50.88 d	ns	ns	ns	7.11 ab	6.98 ab	6.61 ab	6.75 bc	7.08 b	6.75 bc	10.52 b	10.6 b	10.27 b	6.81 ab	6.69 ab	6.32 bc
	10	23.17 ab	23.33 ab	23.75 a	50.33 cde	50.08 cde	46 e	46.88 e	46.88 e	45.21 e	ns	ns	ns	7.17 ab	6.8 abc	6.13 c	5.25 cd	5.08 d	5.25 cd	8.57 b	8.77 b	8.56 b	6.93 ab	6.53 abc	5.86 c

* In each column, means with the similar letter (s) are not significantly different at 5% level of probability using Duncan's multiple range tests.

with sucrose not only improved leaf water content but also increased the dry weight of flowers by more than 25% in the treated tulips (Table 3). It has been scientifically proven that exogenous application of GA_3 serves to improve petal water relations by increasing the level of osmotic solutes, and it delays cell death. All these effects of GA_3 treatment manifest its great potential for improving the quality and yield of 'Kingsblood' tulips grown for the cut flower. Generally, GA_3 may be synergistically improving post-production quality of 'Kingsblood' tulips. Therefore, we conclude that sprays containing GA_3 might be of commercial value in enhancing the post-production quality of tulip flowers.

Effect of jasmonic acid on vegetative growth parameters (Experiment 3)

The results of JA treatments showed a contradictory effect compared to GA_3 treatment. According to Table 4, most vegetative characteristics of the treated plants showed a considerably significant decrease, i.e. in height, weight, and vase life. Moreover, our results revealed that the treated flowers exhibited post-harvest tepal abscission with slight wilting of the leaves. JA strongly inhibits IAA-stimulated elongation possibly by blocking incorporation of glucose into cell wall polysaccharides (Ueda *et al.*, 1995) and for this reason, it reduced plant weight and length. On the other hand, the comparison of data obtained from JA treatments showed delayed flowering by about four days and reduced vegetative growth parameters like plant length (41.75 cm), which was about 16 cm smaller than that in the control plants (Table 4). Our results indicate that JA (100 µM) could lead to unwanted early senescence of mature tepals of cut tulips and the shortest vase life of cut flowers was related to this treatment (7.25 days). So, based on our observations, JA plays a pivotal role in tepal senescence, leaf chlorosis, and gummosis in 'Kingsblood' tulips during the post-production evaluation. Furthermore, the ability of JA to inhibit the expression of genes involved in photosynthesis suggests that jasmonate could help reduce the plant's capacity for carbon assimilation. JA inhibits genes encoding the photosynthetic apparatus and would eventually balance energy absorbing and using capacities (Creelman and Mullet, 1997). Therefore, the senescence of tulip flowers seems to be coordinately mediated by the interactions of plant hormones in a way that ethylene plays a minor role (Kim and Miller, 2008). Unfortunately, a complete analysis of JA levels in senescing leaves has not been carried out. Thus, although JA can induce senescence-like symptoms, the role of this hormone in mediating senescence is presently unclear. In addition, the relative tepal water content was reduced by JA as compared to control plants (Table 4). The lowest amount of flower water content (3.59 g) was related to 100 µM JA treatments. Since there is a close relationship between the flower water content and the quality of flowers, JA treatments reduced post-harvest performance. Totally, we conclude that sprays containing JA treatments not only reduced the qualitative characteristics like vase life, bud appearance, and post-production quality but also reduced the quantitative characteristics i.e. flower water content, plant weight and plant length of the 'Kingsblood' tulips.

Proline content

Due to protein degradation in plant tissue, the accumulation of free amino acids, especially proline content was increased, and they could not be mobilized to other plant organs. Though expected, protease activity is a sign of senescence (Silveira *et al.*, 2003). Alongside the increase in proline levels, a widely-distributed multi-functional osmoprotectant (Szabados and Savoure, 2010) is also often associated with the increased sucrose levels, demonstrating that proline synthesis also depends on sucrose-specific signaling events. The increased levels of endogenous sucrose increase the biosynthesis of pigments (van den Ende and El-Esawe, 2014). The increase in the levels of proline, on one hand, signifies active genes involved in anthocyanin biosynthesis, and on the other hand, it is a sign of senescence. A previous study showed that the application of some plant growth

Table 3. Effect of different concentrations of gibberellic acid and sucrose on vegetative growth parameters in *Tulipa gesneriana* 'Kingsblood'.

Treatments		Vegetative growth parameters																							
		Bud appearance (day)			Shoot weight (g)			Plant length (cm)			Flower stalk length (cm)			Flower fresh weight (g)			Vase life (day)			Leaf water content (g)			Flower water content (g)		
		Sucrose (mg/L)																							
		0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Two	GA3 (mg/L)																								
	0	21.08 ^{a}	21.33 ^{a}	20.58 ^{ab}	53.08 ^{cd}	53.75 ^{bcd}	50.25 ^{d}	53.33 ^{cd}	52.5 ^{cd}	51.08 ^{d}	31.5 ^{cd}	31.04 ^{d}	29.83 ^{d}	7.2 ^{abc}	6.53 ^{c}	7.09 ^{bc}	9.5 ^{bc}	8.67 ^{c}	9.83 ^{b}	11.42 ^{de}	10.56 ^{e}	12.62 ^{cde}	ns	ns	ns
	300	19.25 ^{cd}	19.75 ^{bc}	19.83 ^{bc}	59.42 ^{abc}	56.58 ^{bcd}	56.67 ^{bcd}	58.75 ^{ab}	56.96 ^{bc}	56.92 ^{bc}	37.63 ^{bc}	37.54 ^{bc}	41.54 ^{ab}	7.14 ^{bc}	6.98 ^{bc}	6.98 ^{bc}	10.5 ^{ab}	11.08 ^{a}	10 ^{b}	13.99 ^{bcd}	13.46 ^{bcde}	14.03 ^{bcd}	ns	ns	ns
	500	18.17 ^{de}	18.5 ^{de}	17.92 ^{e}	65 ^{a}	61.08 ^{ab}	65.42 ^{a}	63.21 ^{a}	59.42 ^{ab}	59 ^{ab}	38.27 ^{b}	40.08 ^{ab}	45.17 ^{a}	8.08 ^{a}	7.41 ^{abc}	7.85 ^{ab}	11.58 ^{a}	11.17 ^{a}	11.33 ^{a}	15.93 ^{ab}	15.35 ^{abc}	17.58 ^{a}	ns	ns	ns

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using Duncan's multiple range tests.

Table 4. Effect of different concentrations of jasmonic acid and sucrose on vegetative growth parameters in *Tulipa gesneriana* 'Kingsblood'.

Treatments		Vegetative growth parameters																							
		Bud appearance (day)			Shoot weight (g)			Plant length (cm)			Flower stalk length (cm)			Flower fresh weight (g)			Vase life (day)			Leaf water content (g)			Flower water content (g)		
		Sucrose (mg/L)																							
		0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Three	JA (µM)																								
	0	21.67 ^{cd}	21.5 ^{cd}	21 ^{d}	56.17 ^{a}	55/92 ^{a}	51.67 ^{ab}	57.54 ^{a}	54.75 ^{a}	50.67 ^{b}	37.17 ^{a}	34.54 ^{a}	35.46 ^{a}	6.97 ^{a}	6.55 ^{ab}	6.25 ^{ab}	10.42 ^{ab}	10.92 ^{a}	10.83 ^{a}	ns	ns	ns	6.65 ^{a}	6.23 ^{ab}	5.93 ^{ab}
	5	22.5 ^{bc}	23.08 ^{b}	22.42 ^{bc}	46.33 ^{bc}	47.08 ^{bc}	45.25 ^{bc}	46.21 ^{c}	47.75 ^{bc}	46.21 ^{c}	29.88 ^{bc}	30.64 ^{b}	29.29 ^{bc}	5.85 ^{b}	5.78 ^{b}	5.6 ^{b}	9.08 ^{d}	9.83 ^{bc}	9.25 ^{cd}	ns	ns	ns	5.57 ^{b}	5.49 ^{b}	5.31 ^{b}
	10	24.67 ^{a}	24.75 ^{a}	25.5 ^{a}	43.25 ^{c}	43.92 ^{c}	46.42 ^{bc}	43.58 ^{cd}	41.75 ^{d}	44.58 ^{cd}	25.5 ^{d}	28.67 ^{bc}	27 ^{cd}	3.96 ^{c}	4.3 ^{c}	3.83 ^{c}	8.58 ^{d}	7.5 ^{e}	7.25 ^{e}	ns	ns	ns	3.71 ^{c}	4.02 ^{c}	3.59 ^{c}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using Duncan's multiple range tests.

regulators can promote the biosynthesis of proline in water-limited environments (Bhaskara *et al.*, 2015). Based on our results, JA treatments increased the proline content and showed the shortest display life and poor vegetative growth parameters (Table 7). The application of JA to leaves decreases the expression of nuclear and chloroplast genes involved in photosynthesis, so JA causes the loss of chlorophyll from leaves and cell cultures (Weidhase *et al.*, 1987). Furthermore, the ability of Jasmonate to cause chlorosis led to the suggestion that this compound plays a role in plant senescence (Ueda *et al.*, 1995). In our study, the amounts of proline were found to be significantly increased in JA treatments, comparable to the control leaves (Table 7). Although different values were determined for proline content with the use of GA_3, there was no significant difference from the control leaves (Table 6). The lowest amount of proline was found in 300 mg/L GA_3 (0.69 µmol/g FW) applied to leaves and the highest free proline accumulation resulted from 100 µM of JA treatment (8.10 µmol/g FW) while the exogenous foliar application of ABA led to a slight increase in the proline content (1.24 µmol/g FW) as can be seen in Table 5. Therefore, it is evident that JA did accelerate protein degradation and increased the free level of proline content, resulting from proteolysis, which indicates typical processes of senescence. A role has been suggested for jasmonic acid in protein storage in plants in part because jasmonate levels are high in vegetative sinks. In the short term, JA-mediated induction of vegetative storage protein synthesis under conditions of high sugar accumulation creates a sink for carbon and nitrogen and releases phosphate from sugar phosphate pools for further carbon fixation. Carbon and nitrogen may also accumulate in cells located in meristematic regions for the use during rapid cell growth. For osmotic reasons, cells are only able to accumulate a limited amount of sucrose and amino acids. Therefore, large amounts of carbon and nitrogen accumulate as polymers in the form of starch, fructan, and protein. In general terms, tulips that are sprayed with JA demonstrate this phenomenon best. The results prove that senescence in tulip tepals is closely associated with free proline accumulation, similar to what is found in rice (Yang *et al.*, 2000).

Table 5. Effect of different concentrations of abscisic acid and sucrose on physiological parameters in *Tulipa gesneriana* 'Kingsbloods'.

Treatments			Physiological parameters											
			Flavonoid content (mg/g FW)			Anthocyanin content (mg/L)			Proline content (µmol/g FW)			Dry weight of flower (mg)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-One	ABA (mg/L)	0	2.244 ^{c}	2.306 ^{c}	2.32 ^{c}	1.588 ^{c}	1.476 ^{c}	1.662 ^{c}	0.77 ^{c}	0.70 ^{d}	1.24 ^{a}	335.75^{a}	341.83^{a}	321.58 ^{a}
		5	2.616 ^{b}	2.624 ^{b}	1.098 ^{a}	1.352 ^{cd}	2.256 ^{b}	2.764 ^{a}	0.87 ^{b}	0.73 ^{dc}	1.24 ^{a}	294^{b}	311.5^{bc}	288.42^{bc}
		10	2.488 ^{bc}	2.382 ^{c}	1.666 ^{d}	1.588 ^{c}	1.176 ^{d}	1.214 ^{d}	0.70 ^{d}	0.87 ^{b}	0.73^{d}	239.67 ^{d}	280.92 ^{c}	270.17 ^{c}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using Duncan's multiple range tests.

Table 6. Effect of different concentrations gibberellic acid and sucrose on physiological parameters in *Tulipa gesneriana* 'Kingsbloods'.

Treatments			Physiological parameters											
			Flavonoid content (mg/g FW)			Anthocyanin content (mg/L)			Proline content (µmol/g FW)			Dry weight of flower (mg)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Two	GA_3 (mg/L)	0	2.124 ^{d}	2.22 ^{d}	2.314 ^{cd}	1.582 ^{d}	1.552 ^{d}	1.678 ^{d}	0.93 ^{b}	1.04 ^{a}	0.72 ^{d}	311.75^{bc}	287.42^{d}	281.75 ^{d}
		300	2.536 ^{c}	2.732 ^{bc}	2.644 ^{bc}	2.102 ^{b}	1.944 ^{c}	2.104 ^{b}	0.81 ^{c}	1.00 ^{a}	0.69 ^{d}	309.17 ^{abc}	311.5^{bc}	302.75 ^{cd}
		500	2.912 ^{a}	2.34 ^{cd}	2.252 ^{d}	2.406 ^{a}	2.112 ^{b}	2.124 ^{b}	0.87 ^{b}	0.89 ^{b}	0.81 ^{c}	346.42 ^{a}	331.08 ^{ab}	326.33 ^{ab}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using Duncan's multiple range tests.

Table 7. Effect of different concentrations of jasmonic acid and sucrose on physiological parameters in *Tulipa gesneriana* 'Kingsbloods'.

Treatments			Physiological parameters											
			Flavonoid content (mg/g FW)			Anthocyanin content (mg/L)			Proline content (µmol/g FW)			Dry weight of flower (mg)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Three	JA (µM)	0	2.33 ^{c}	2.334 ^{c}	1.984 ^{c}	1.586 ^{c}	1.596 ^{c}	1.712 ^{bc}	0.70 ^{f}	0.72 ^{f}	1.17 ^{e}	316.67 ^{a}	315.67 ^{a}	326.33 ^{a}
		50	2.346 ^{b}	2.856 ^{a}	1.68 ^{d}	1.52 ^{c}	2.256 ^{a}	1.838 ^{b}	3.24^{d}	5.24 ^{c}	7.22 ^{b}	283.67 ^{b}	292.33 ^{b}	270.17 ^{b}
		100	1.276 ^{e}	1.464 ^{d}	1.986 ^{c}	1.792 ^{b}	1.052 ^{d}	1.152 ^{d}	3.54^{d}	7.35 ^{b}	8.10 ^{a}	248.75 ^{c}	280.75 ^{b}	245.83^{c}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using Duncan's multiple range tests.

The effect of the treatments on total flavonoids content

The total flavonoid content in PGRs treatments was found to have a significantly wide variation according to the results from three experiments.

In experiments 1, the results obtained after the use of ABA showed a significant impact on the total flavonoid content so that the hormones increased flavonoid content, but at higher concentrations, they had negative effects (Table 5). ABA at 5 mg/L with sucrose at 2 g/L had a decreasing effect on the flavonoid content and reduced to 1.09 mg/g FW, while the interaction of this hormones with sucrose at low concentrations exhibited a synergetic effect, and the highest range of the total flavonoid content was obtained from ABA at 5 mg/L and sucrose at 1 g/L (2.62 mg/g FW). In the case of *Orthosiphon aristatus*, the use of ABA could enhance the production of primary and secondary metabolites, which our results confirm it (Ibrahim and Jaafar, 2013). Our study showed that the increased application of ABA improved the production of total phenolics and flavonoids. It has long been proven that ABA naturally accumulates in grape skin at the onset of ripening, a time when anthocyanin and other phenolic compounds also increase (Koyama *et al*., 2010).

In experiment two, the highest total flavonoid content was found in the perianthes of the tulips sprayed with 500 mg/L GA_3 (2.91 mg/g FW) and the lowest concentration was recorded in the control plants (2.12 mg/g FW) and those treated with 1 g/L sucrose (2.22 mg/g FW), as can be seen in Table 6. The results indicated that both sucrose and GA_3 increased the flavonoid content

at lower concentrations, but at higher concentration, they reduced the total flavonoid content to 2.25 mg/g FW. The two compounds act to suppress each other's effect at higher concentrations. These results support the findings of Sarrou *et al.* (2015) who reported that flavonoid levels of plants were significantly affected by GA_3, and GA_3 also significantly promoted secondary metabolites.

Furthermore, in experiment three, flavonoids production is enhanced by 50 mM JA to up to 2.85 mg/g FW, but increasing the level of sucrose to 2 g/L led to a decreased range of flavonoid content to 1.08 mg/g FW. It is already known that low concentrations of jasmonate induce the expression of the genes encoding enzymes of flavonoid biosynthesis (Wasternack and Parthier, 1997). Our data showed that the increase in the levels of JA and sucrose led to a decrease in the total flavonoid content. As represented in Table 7, we had the minimum range in 100 mM of JA and 0 g/L of sucrose (1.27 mg/g FW). The low concentrations of jasmonate have been shown to induce gene expression of enzymes involved in flavonoid biosynthesis (Walker *et al.*, 2002).

Moreover, this conclusion is in accordance with the results we got for the total flavonoid content. There were similar effects on the flavonoid content with the use of ABA and JA. However, at low concentrations both ABA and JA had an increasing effect on flavonoid content, but at higher concentrations, they led to a decreased range of the total flavonoid content.

Perianthes coloration and accumulation of anthocyanins

Endogenous anthocyanins of perianthes in dark red tulip were analyzed by HPLC and UV-Vis spectra. Six anthocyanins including pelargonidin 3-rutinoside (Peak 3), pelargonidin 3-(2'''-acetylrutinoside) (Peak 6), cyanidin 3-rutinoside (Peak 2), cyanidin 3-(2'''-acetylrutinoside) (Peak 4), delphinidin 3-rutinoside (Peak 1), and delphinidin 3-(2'''-acetylrutinoside) (Peak 5) were identified by comparing the known samples using the visible l max (Fig. 3).

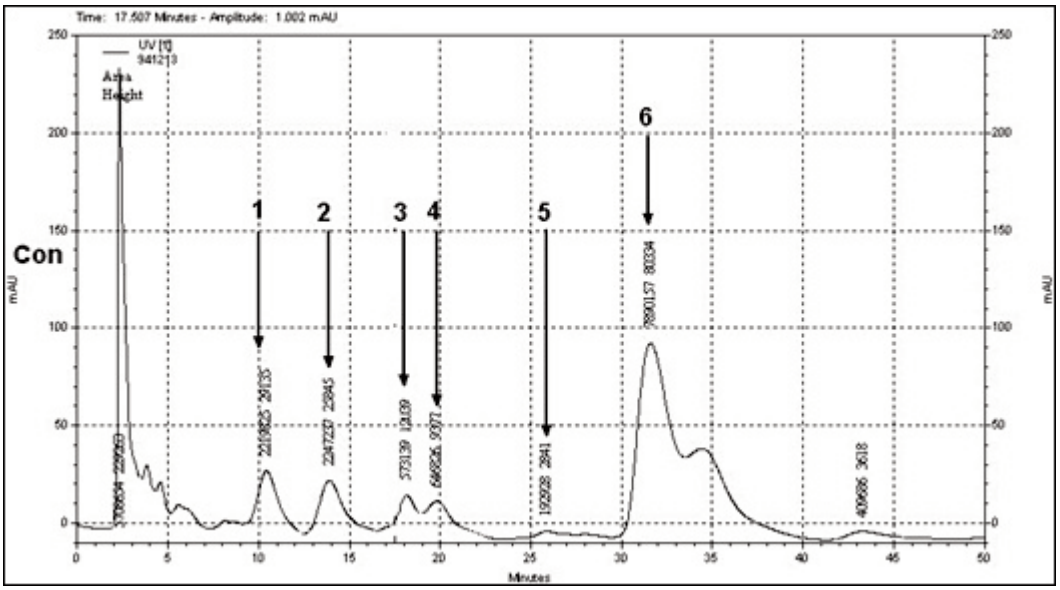

Fig. 3. HPLC separation of anthocyanins in *Tulipa gesneriana* 'Kingsblood'.

The distribution and amounts of individual anthocyanins are presented in Table 8. The retention times (Rt) and absorption spectra (l max) of anthocyanins 5 and 3 coincided with those of pelargonidin 3-rutinoside and cyanidin 3-rutinoside, respectively. Anthocyanins 2 and 4 were extracted from tulip perianthes and purified by using chromatography methods. The molecular weights (M^+) were 621 and 637, suggesting that these anthocyanins are the acetyl ester of pelargonidin 3-rutinoside and cyanidin 3-rutinoside (Table 8).

Table 8. Retention times (Rt) and **l** max of the individual anthocyanins in *Tulipa gesneriana* 'Kingsblood'.

Anthocyanins	**Analytical data of anthocyanin**			
	[x] M^+	**[Y] Rt (min) ODS**	**[z] l max (nm)**	**Peak no.**
Delphinidin 3-rutinoside	611	12.27	529, 269	1
Delphinidin 3-(2'''-acetylrutinoside)	653	22.55	534, 269	5
Cyanidin 3-rutinoside	595	16.40	559, 254	2
Cyanidin 3-(2'''-acetylrutinoside)	637	25.45	559, 254	4
Pelargonidin 3-rutinoside	579	24.00	516, 276	3
Pelargonidin 3-(2'''-acetylrutinoside)	621	28.35	516, 276	6

[Y] **Rt**: Retention time is a measure of the time taken for a salute to pass through a chromatography ODS column. It is calculated as the time from injection to detection.
[x] **M^+**: Molecular weight.
[z] **l max**: Maximum absorbance.

The effect of abscisic acid on perianthes coloration and anthocyanins accumulation

The plant hormone ABA has been suggested to play an efficient role in the development of flower color. It is observed that the anthocyanin content correlates with the exogenous application of ABA, and high amounts of anthocyanins accumulation were recorded in the plants treated with 5 mg/L ABA and 2 g/L sucrose (2.76 mg/L) as can be seen in table 5. Although, sucrose alone showed a slight but insignificant increase in the accumulation of anthocyanins, its combination with ABA had an impressive effect on their accumulation (Table 5). There was an enhancement stimulant between ABA and sucrose in the induction of anthocyanin biosynthesis. These results demonstrate a synergistic effect between ABA and sucrose on the induction of anthocyanins. Several lines of evidence illustrate that there is a synergistic effect on ABA, and sugar signaling pathways provide a physiological basis for ABA activity. The hormone stimulated the accumulation of mRNA of several genes involved in anthocyanin biosynthesis, and the anthocyanin concentration was increased after the application of ABA (Jaakola, 2013). It was also demonstrated that ABA provoked the expression of anthocyanin biosynthesis-related genes such as *CHS, CHI, DFR*, and *UFGT*, as well as the regulatory factor *VvmybA1* in grape skin (Jeong *et al.*, 2004). A similar effect was observed in *Arabidopsis* by Solfanelli *et al.* (2006). With low concentrations of ABA, we had an increase in total anthocyanin content, but with increased concentrations, there was a decrease in anthocyanin content. It is probable that a lower dosage of ABA could have a sufficient effect on color development. The representative HPLC chromatograms for ABA treatment have been presented in table 8. Among these types, analysis of data indicates that sucrose alone increased delphinidin and cyanidin pigments, but the interaction of sucrose with ABA reduced both pigments. The application of ABA showed a substantial increase in the amount of pelargonidin pigments, and the highest levels of the total peak areas in the HPLC-chromatogram were detected in combination of 5 mg/L ABA and 2 g/L sucrose. As can be seen in table 9, increasing the level of ABA concentration had a positive effect on pelargonidin pigment percentage and we recorded the highest percentage of pelargonidin in 10 mg/L ABA with 1g/L sucrose (73.49 %) as is evident in table 9.

But this treatment led to a reduced amount of total anthocyanin content and two other pigments. As already mentioned, anthocyanin synthesis is under the control of a sucrose-specific pathway in *Arabidopsis*, but extensive cross-interaction with hormonal signaling pathways can be expected, especially with ABA. ABA caused a hypersensitive response leading to anthocyanin accumulation in rice (Zhou *et al.*, 2009). In other experiments, it was found that the exogenous application of ABA promoted anthocyanin accumulation, like in grapes (Koyama *et al*., 2010). In this study, we found that ABA promoted anthocyanin accumulation in tulip flowers.

Table 9. Effect of different concentrations of abscisic acid and sucrose on anthocyanin composition pigments in *Tulipa gesneriana* 'Kingsbloods'.

Treatment			Distribution and amounts of individual anthocyanins											
			[x] Pelargonidin (%)			[x] Cyanidin (%)			[x] Delphinidin (%)			[y] TRA(mg/g FW)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-One	ABA (mg/L)	0	66.150	61.714	65.844	30.285	34.478	30.30	3.5634	3.8069	3.8496	0.35240	0.3683	0.400
		5	71.498	70.063	69.117	25.485	28.690	29.944	3.0167	1.2467	0.9376	0.32582	0.386	0.467
		10	68.769	73.498	63.922	28.968	24.725	34.174	2.2690	1.7764	1.903	0.39227	0.282	0.212
		100	74.480	81.621	77.908	24.085	16.519	19.283	1.4336	1.8587	2.8077	040299	0.2409	0.203

[x] The area of each pigment in the HPLC-chromatogram relative to the total area of three pigments for this sample detected at 520±20 nm.
[y] TRA: (Total relative amount of anthocyanin); Data represent relative amount of anthocyanin composition from total absorbed peaks areas that were detected at 520±20 nm by HPLC-chromatogram.
Each value is a mean of three replicates. Assignments are supported by published data (Nakayama *et al*., 2004; Torskangerpoll *et al*., 2005).

The effect of gibberellic acid on perianthes coloration and anthocyanins accumulation

The results showed that GA_3 had a significant effect on anthocyanin content and increased three major anthocyanin pigments. The highest range of anthocyanin content (2.4 mg/L) was observed at 500 mg/L GA_3 without sucrose (Table 6). It was found that the interaction between sucrose and GA_3 treatments had no significant effect on anthocyanin content. Moreover, data provided evidence about a conflict between the sucrose and GA_3 in the regulation of the anthocyanin accumulation. When sucrose is used alone, the amount of the accumulated pigments is more than when sucrose is used in combination with GA_3. The results were in accordance with those of Loreti *et al.* (2008) indicating that GA_3 inhibited the sucrose-induction of dihydroflavonol 4-reductase expression the anthocyanin content. The main role of sugar in the enhancement of GA_3 responses is as a source of carbohydrates for carbon metabolism, probably to be used for energy. It should be noted that GA_3 induces not only the expression of anthocyanin biosynthetic genes but also that of several other genes (Ben-Nissan and Weiss, 1995). In the review of the HPLC charts, we observed an increasing effect of GA_3 application on delphinidin pigment and the highest amount of delphinidin pigments was obtained from 300 mg/L without sucrose (4.92 %). Similar results were observed in cyanidin pigment percentage in 500 mg/L without sucrose (36.10%). Generally, sucrose reduces the effect of GA_3 as can be seen in Table 10. The highest range of anthocyanin was achieved without sucrose, and this concurred with the findings of Roussos *et al*. (2009). Finally, the higher rate of the accumulation of anthocyanins was the plants' response to 500 mg/L GA_3 without sucrose (2.4 mg/L) as can be seen in Table 10, and a similar effect was observed in Arabidopsis by Solfanelli *et al*. (2006). Khandaker *et al.* (2013) observed that GA_3 significantly promoted the biosynthesis of secondary metabolites in fruits with the highest antioxidant activity. The GA_3 treatment increased phenylalanine ammonia lyase (PAL) enzyme activity, which is correlated with anthocyanin accumulation (Jeong *et al*., 2004).

Table 10. Effect of different concentrations of gibberellic acid and sucrose on anthocyanin composition pigments in *Tulipa gesneriana* 'Kingsbloods'.

Treatment			Distribution and amounts of individual anthocyanins											
			[x] Pelargonidin (%)			[x] Cyanidin (%)			[x] Delphinidin (%)			[y] TRA(mg/g FW)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Two	GA_3 (mg/L)	0	67.930	61.207	65.754	28.783	34.907	30.967	3.2853	3.8845	3.2785	0.34701	0.624	0.326
		300	59.371	63.266	64.803	35.705	33.160	30.776	4.9233	3.5731	4.4198	0.55279	0.3348	0.482
		500	59.854	62.982	63.718	36.106	33.348	32.742	4.0392	3.6695	3.5393	0.68768	0.438	0.384

[x] The area of each pigment in the HPLC-chromatogram relative to the total area of three pigments for this sample detected at 520±20 nm.
[y] TRA: (Total relative amount of anthocyanin); Data represent relative amount of anthocyanin composition from total absorbed peaks areas that were detected at 520±20 nm by HPLC-chromatogram.
Each value is a mean of three replicates. Assignments are supported by published data (Nakayama *et al*., 2004; Torskangerpoll *et al*., 2005).

The effect of jasmonic acid on perianthes coloration and accumulation of anthocyanins

The amount of anthocyanin content in perianthes was enhanced by the use of JA and sucrose (Table 7). The highest range of total anthocyanin was quantified in flowers that received 50 µM JA and 2 g/L sucrose (1.83 mg/L), and the HPLC chart shows that higher amounts of anthocyanin accumulated in the petals that received this treatment. On the other hand, the highest amount of absorbed peak was recorded for 50 µM JA treatment and 2 g/L sucrose (0.60 mg/L) as is evident in Table 11. There are many research studies proving that jasmonates induce the formation or the accumulation of anthocyanin, for example in peach shoots (Saniewski *et al.*, 1998b), tulip stems (Saniewski *et al.*, 1998a), and *Kalanchoe blossfeldiana* (Saniewski *et al.*, 2003). Generally, in the review of HPLC charts, it was found that the application of JA increased the percentage of pelargonidin and cyanidin pigments (Table 11). It was clearly observed that the treatment with JA reduced the percentage of delphinidin pigments and increased the percentage of pelargonidin pigments. Interestingly, there was a linear effect of the use of JA on the percentage of pelargonidin pigments. It was reported in previous publications that in tulip flowers, an antagonism existed between delphinidin and pelargonidin synthesis and that the synthesis of both anthocyanidins strongly depended on the presence of cyanidin combinations. Although the positive effect of JA on anthocyanin accumulation is well known (Loreti *et al.*, 2008), the only exception has been buckwheat (*Fagopyrum esculentum*), where JA was found to decrease anthocyanin content (Horbowicz *et al.*, 2009). Nevertheless, in the tulip plants which have received the higher concentration of JA (100µM), there was a negative effect on the content of anthocyanins content. The increased levels of JA decreased the percentage of pelargonidin and cyanidin pigments percentage extremely. Similar results were observed by Horbowicz *et al.* (2011) who showed that higher concentrations of methyl jasmonate resulted in a decreased accumulation of anthocyanin. Furthermore, the research by Horbowicz *et al.* (2008) showed that the increased concentration of methyl jasmonate inhibited the synthesis and accumulation of anthocyanins in buckwheat hypocotyls. Sucrose individually showed a positive effect on the percentage of cyanidin pigments, but did not bring about a significant change in the percentage. There is a strong correlation between sucrose inductions of anthocyanin biosynthesis-related genes (Shan *et al.*, 2009). Although JA and a number of transcription factors are potential regulators of the anthocyanin pathway in Arabidopsis (Wasternack and Song, 2017), it has been shown that JA and sucrose in a complex signaling network can modulate anthocyanin accumulation and, notably, sucrose signaling seems to be a primary and essential component in this network (Loreti *et al.*, 2008). Moreover, the amount of anthocyanin of perianthes was enhanced by increasing sucrose. Sucrose exhibited a synergetic effect with JA on anthocyanin

content and changing the percentage of pigments. According to this research, JA might affect sugar metabolism in tulip bulbs and, as a result, the released sugar molecules could contribute to promoting anthocyanin formation in flower.

Table 11. Effect of different concentrations of jasmonic acid and sucrose on anthocyanin composition pigments in *Tulipa gesneriana* 'Kingsbloods'.

Treatment			Distribution and amounts of individual anthocyanins											
			[x] Pelargonidin (%)			[x] Cyanidin (%)			[x] Delphinidin (%)			[y] TRA(mg/g FW)		
			Sucrose (mg/L)											
			0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000
Expr-Three	JA (µM)	0	67.770	52.366	54.602	28.706	43.205	41.008	3.5224	4.4279	4.3891	0.34913	0.394	0.525
		50	61.697	64.490	71.496	35.159	32.680	26.264	3.1427	2.8288	2.2393	0.38218	0.4371	0.602
		100	74.480	81.621	77.908	24.085	16.519	19.283	1.4336	1.8587	2.8077	040299	0.2409	0.203

[x] The area of each pigment in the HPLC-chromatogram relative to the total area of three pigments for this sample detected at 520±20 nm.

[y] TRA: (Total relative amount of anthocyanin); Data represent relative amount of anthocyanin composition from total absorbed peaks areas that were detected at 520±20 nm by HPLC-chromatogram.

Each value is a mean of three replicates. Assignments are supported by published data (Nakayama *et al*., 2004; Torskangerpoll *et al*., 2005).

CONCLUSION

In conclusion, the hormones of all three plants improve flower color pigmentation in *Tulipa gesneriana* 'Kingsblood', but each had a different effect on the physiological and morphological characteristics of tulip plants. This study found that the exogenous application of foliar ABA and JA can be a useful tool to enhance the properties of secondary metabolites in this plant and improve the production of phytochemicals (total anthocyanins content and flavonoids). Furthermore, the application of both these hormones reduced vegetative growth parameters, acted as senescence enhancer in vase life, and delayed flowering, but improved flower color and color composition pigments coefficient, compared to control plants. More importantly, ABA and JA at higher concentrations had a negative effect on the stimulation of anthocyanins biosynthetic pathway. Additionally, we showed that exogenous sucrose can significantly modulate anthocyanin accumulation and enhance the stimulating effect of ABA and JA on anthocyanin accumulation. It is conclusively proven that the application of GA_3 results in improving growth and flowering, prolonging flower life, and improving bulb attributes of tulip. It can be concluded that GA_3, particularly, at the rate of 500 mg/L, could stimulate tulip growth and pigmentation, as well as the vase life of tulips. The treatment with GA_3 accelerated bud appearance, flavonoids, and anthocyanin accumulation. Interestingly, it has been shown that the interaction between GA_3 and sucrose in a complex signaling network can modulate anthocyanin accumulation. Finally, the data provide evidence that GA_3 treatments may be used for extending the flowering stage and improve the quality of flower. The results obtained in the present study may be useful in providing important information for the cut-flower industry.

ACKNOWLEDGMENTS

We would like to gratefully thank all the members of the Ferdowsi University of Mashhad, Faculty of Agriculture, and the Department of Horticulture. We extend special thanks to Mahdelaleha Company for providing the facilities to carry out this work, and supporting tulips materials and standards.

Literature Cited

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39 (1): 205-207.

Belhadj, A., Telef, N., Saigne, C., Cluzet, S., Barrieu, F., Hamdi, S. and Mérillon, J.M. 2008. Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. Plant Physiology and Biochemistry, 46: 493-499.

Ben-Nissan, G. and Weiss, D. 1995. Developmental and hormonal regulation of a triosephosphate isomerase gene in *Petunia corollas*. Journal of Plant Physiology, 147: 58-62.

Bhaskara, G.B., Yang, T.H. and Verslues, P.E. 2015. Dynamic proline metabolism: Importance and regulation in water-limited environments. Frontiers in Plant Science, 6: 484. doi: 10.3389/fpls.2015.00484.

Bolouri Moghaddam, M.R., Le Roy, K., Xiang, L., Rolland, F. and van den Ende, W. 2010. Sugar signalling and antioxidant network connections in plant cells. The FEBS Journal, 277: 2022-2037.

Creelman, R.A. and Mullet, J.E. 1997. Biosynthesis and action of jasmonates in plants. Annuals Review of Plant Biology, 48: 355-381.

Danaee, E., Mostofi, Y. and Moradi, P. 2011. Effect of GA_3 and BA on postharvest quality and vase life of gerbera (*Gerbera jamesonii.* cv. Good Timing) cut flowers. Horticulture, Environment, and Biotechnology, 52: 140-144.

Hara, M., Oki, K., Hoshino, K. and Kuboi, T. 2003. Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyl. Plant Science, 164: 259-265.

Horbowicz, M., Grzesiuk, A., Dębski, H., Koczkodaj, D. and Saniewski, M. 2008. Methyl jasmonate inhibits anthocyanins synthesis in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). Acta Biologica Cracoviensia Series Botanica, 50 (2): 71–78.

Horbowicz, M., Kosson, R., Wiczkowski, W., Koczkodaj, D. and Mitrus, J. 2011. The effect of methyl jasmonate on accumulation of 2-phenylethylamine and putrescine in seedlings of common buckwheat (*Fagopyrum esculentum*). Acta Physiologiae Plantarum, 33: 897–903.

Horbowicz, M., Mioduszewska, H., Koczkodaj, D. and Saniewski, M. 2009. The effect of cis-jasmone, jasmonic acid and methyl jasmonate on accumulation of anthocyanins and procyanidins in seedlings of common buckwheat (*Fagopyrum esculentum* Moench). Acta Societatis Botanicorum Poloniae, 78: 271–277.

Hosokawa, K. 1999. Cell layer-specific accumulation of anthocyanins in responss to gibberellic acid in tepals of *Hyacinthus orientalis*. Bioscience, Biotechnology, and Biochemistry, 63: 930-931.

Hunter, D.A., Ferrante, A., Vernieri, P. and Reid, M.S. 2004. Role of abscisic acid in perianth senescence of daffodil (*Narcissus pseudonarcissus* "Dutch Master"). Physiologia Plantarum, 121: 313-321.

Ibrahim, M.H. and Jaafar, H.Z. 2013. Abscisic acid induced changes in production of primary and secondary metabolites, photosynthetic capacity, antioxidant capability, antioxidant enzymes and lipoxygenase inhibitory activity of *Orthosiphon stamineus* Benth. Molecules, 18: 7957-7976.

Imsabai, W., Leethiti, P., Netlak, P. and van Doorn, W.G. 2013. Petal blackening and lack of bud opening in cut lotus flowers (*Nelumbo nucifera*): Role of adverse water relations. Postharvest Biology and Technology, 79: 32-38.

Jaakola, L. 2013. New insights into the regulation of anthocyanin biosynthesis in fruits. Trends in Plant Science, 18: 477–483.

Jeong, S.T., Goto-Yamamoto, N., Kobayashi, S. and Esaka, M. 2004. Effects of plant hormones

and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. Plant Science, 167: 247-252.

Khandaker, M.M., Boyce, A.N., Osman, N., Golam, F., Rahman, M.M. and Sofian-Azirun, M. 2013. Fruit development, pigmentation and biochemical properties of wax apple as affected by localized application of GA_3 under field conditions. Brazilian Archives of Biology and Technology, 56: 11-20.

Kim, H.J. and Miller, W.B. 2008. Effects of GA_{4+7} and benzyladenine application on postproduction quality of 'Seadov' pot tulip flowers. Postharvest Biology and Technology, 47: 416-421.

Kondo, S., Tsukada, N., Niimi, Y. and Seto, H. 2001. Interactions between jasmonates and abscisic acid in apple fruit, and stimulative effect of jasmonates on anthocyanin accumulation. Journal of the Japanese Society for Horticultural Science, 70: 546-552.

Koyama, K., Sadamatsu, K. and Goto-Yamamoto, N. 2010. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. Functional and Integrative Genomics, 10: 367-381.

Lai, B., Li, X.J., Hu, B., Qin, Y.H., Huang, X.M., Wang, H.C. and Hu, G.B. 2014. *LcMYB1* is a key determinant of differential anthocyanin accumulation among genotypes, tissues, developmental phases and ABA and light stimuli in *Litchi chinensis*. PLoS One, 9: 86293. https://doi.org/10.1371/journal.pone.0086293.

Lee, J., Durst, R.W. and Wrolstad, R.E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International, 88(5):1269-1278.

Loreti, E., Povero, G., Novi, G., Solfanelli, C., Alpi, A. and Perata, P. 2008. Gibberellins, jasmonate and abscisic acid modulate the sucrose induced expression of anthocyanin biosynthetic genes in *Arabidopsis*. New Phytologist, 179: 1004-1016.

Medina-Puche, L., Cumplido-Laso, G., Amil-Ruiz, F., Hoffmann, T., Ring, L., Rodríguez-Franco, A., Caballero, J.L., Schwab, W. and Muñoz-Blanco, J. 2014. *MYB10* plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of *Fragaria× ananassa* fruits. Journal of Experimental Botany, 65: 401-417.

Milborrow, B.V. 2001. The pathway of biosynthesis of abscisic acid in vascular plants: A review of the present state of knowledge of ABA biosynthesis. Journal of Experimental Botany, 52: 1145-1164.

Nakayama, M., Okada, M., Taya-kizu, M., Urashima, O., Kan, Y., Fukui, Y. and Koshioka, M. 2004. Coloration and anthocyanin profile in tulip flowers. Japan Agricultural Research Quarterly, 38: 185–190.

Ohlsson, A.B. and Berglund, T. 2001. Gibberellic acid-induced changes in glutathione metabolism and anthocyanin content in plant tissue. Plant Cell, Tissue and Organ Culture, 64: 77-80.

Onoue, T., Mikami, M., Yoshioka, T., Hashiba, T. and Satoh, S. 2000. Characteristics of the inhibitory action of 1, 1-dimethyl-4-(phenylsulfonyl) semicarbazide (DPSS) on ethylene production in carnation (*Dianthus caryophyllus* L.) flowers. Plant Growth Regulation, 30: 201-207.

Panavas, T., Walker, E.L. and Rubinstein, B. 1998. The role of ABA in programmed cell death of daylily petals. Journal of Experimental Botany, 49: 1987-1997.

Piovan, A. and Filippini, R. 2007. Anthocyanins in *Catharanthus roseus in vivo* and *in vitro*: A review. Phytochemistry Reviews, 6: 235-242.

Pirie, A. and Mullins, M.G. 1976. Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. Plant physiology, 58: 468-472.

Qi, T., Song, S., Ren, Q., Wu, D., Huang, H., Chen, Y., Fan, M., Peng, W. and Ren, C. 2011. The jasmonate-ZIM-domain proteins interact with the *WD-Repeat/bHLH/MYB* complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. The Plant Cell, 23: 1795-1814.

Roussos, P., Denaxa, N. and Damvakaris, T. 2009. Strawberry fruit quality attributes after application of plant growth stimulating compounds. Scientia Horticulturae, 119: 138-146.

Saniewski, M., Horbowicz, M., Puchalski, J. and Ueda, J. 2003. Methyl jasmonate stimulates the formation and the accumulation of anthocyanin in *Kalanchoe blossfeldiana*. Acta Physiologiae Plantarum, 25: 143-149.

Saniewski, M., Miszczak, A., Kawa-Miszczak, L., Wegrzynowicz-Lesiak, E., Miyamoto, K. and Ueda, J. 1998a. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO2 evolution in uncooled and cooled tulip bulbs. Journal of Plant Growth Regulation, 17: 33-37.

Saniewski, M., Miyamoto, K. and Ueda, J. 1998b. Methyl jasmonate induces gums and stimulates anthocyanin accumulation in peach shoots. Journal of Plant Growth Regulation, 17: 121-124.

Sarrou, E., Chatzopoulou, P., Dimassi-Theriou, K., Therios, I. and Koularmani, A. 2015. Effect of melatonin, salicylic acid and gibberellic acid on leaf essential oil and other secondary metabolites of bitter orange young seedlings. Journal of Essential Oil Research, 27: 487–496.

Shan, X., Zhang, Y., Peng, W., Wang, Z. and Xie, D. 2009. Molecular mechanism for jasmonate-induction of anthocyanin accumulation in *Arabidopsis*. Journal of Experimental Botany, 60: 3849-3860.

Shen, X., Zhao, K., Liu, L., Zhang, K., Yuan, H., Liao, X., Wang, Q., Guo, X. and Li, F. 2014. A role for *PacMYBA* in ABA-regulated anthocyanin biosynthesis in red-colored sweet cherry cv. Hong Deng (*Prunus avium* L.). Plant and Cell Physiology, 55: 862-880.

Silveira, J.A.G., de Almeida Viégas, R., da Rocha, I.M.A., Moreira, A.C.D.O.M., de Azevedo Moreira, R. and Oliveira, J.T.A. 2003. Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. Journal of Plant Physiology, 160: 115-123.

Smith, R.C., Matthews, P.R., Schünmnn, P.H. and Chandler, P.M. 1996. The regulation of leaf elongation and xyloglucan endotransglycosylase by gibberellin in 'Himalaya' barley (*Hordeum vulgare* L.). Journal of Experimental Botany, 47: 1395-1404.

Solfanelli, C., Poggi, A., Loreti, E., Alpi, A. and Perata, P. 2006. Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. Plant Physiology, 140: 637-646.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M. and Jacobs, G. 2002. Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytologist, 155: 349-361.

Szabados, L. and Savoure, A. 2010. Proline: A multifunctional amino acid. Trends in Plant Science, 15: 89-97.

Teng, S., Keurentjes, J., Bentsink, L., Koornneef, M. and Smeekens, S. 2005. Sucrose-specific induction of anthocyanin biosynthesis in *Arabidopsis* requires the MYB75/PAP1 gene. Plant Physiology, 139: 1840-1852.

Torskangerpoll, K., Norbaek, R., Nodland, R., Ovstedal, E. and Andersen, O.M. 2005. Anthocyanin content of *Tulipa* species and cultivars and its impact on tepal colours. Biochemical Systematics and Ecology, 33: 499-510.

Ueda, J., Miyamoto, K. and Kamisaka, S. 1995. Inhibition of the synthesis of cell wall polysaccharides in oat coleoptile segments by jasmonic acid: Relevance to its growth inhibition. Journal of Plant Growth Regulation, 14: 69-76.

van den Ende, W. and El-Esawe, S.K. 2014. Sucrose signaling pathways leading to fructan and anthocyanin accumulation: A dual function in abiotic and biotic stress responses. Environmental and Experimental Botany, 108: 4-13.

van Doorn, W.G. 2002. Effect of ethylene on flower abscission: A survey. Annals of Botany, 89: 689-693.

Walker Travis, S., Harsh, P.B. and Vivanco, M.J. 2002. Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's Wort). Phytochemistry, 60: 289-293.

Wasternack, C. and Parthier, B. 1997. Jasmonate-signalled plant gene expression. Trends in Plant Science, 2: 302-307.

Wasternack, C. and Song, S. 2017. Jasmonates: Biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. Journal of Experimental Botany, 68: 1303-1321.

Weidhase, R.A., Kramell, H.M., Lehmann, J., Liebisch, H.W., Lerbs, W. and Parthier, B. 1987. Methyljasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Science, 51: 177-186.

Weiss, D. 2000. Regulation of flower pigmentation and growth: Multiple signaling pathways control anthocyanin synthesis in expanding petals. Physiologia Plantarum, 110: 152-157.

Woisky Ricardo, G. and Antonio, S. 1998. Analysis of propolis: Some parameters and procedures for chemical quality control. Journal of Apicultural Research, 37: 99-105.

Yang, C.W., Lin, C.C. and Kao, C.H. 2000. Proline, ornithine, arginine and glutamic acid contents in detached rice leaves. Biologia Plantarum, 43: 305-307.

Yim, K.O., Kwon, Y.W. and Bayer, D.E. 1997. Growth responses and allocation of assimilates of rice seedlings by paclobutrazol and gibberellin treatment. Journal of Plant Growth Regulation, 16: 35-41.

Zhao, D. and Tao, J. 2015. Recent advances on the development and regulation of flower color in ornamental plants. Frontiers in Plant Science, 6: 261. doi: 10.3389/fpls.2015.00261.

Zhou, X., Hua, D., Chen, Z., Zhou, Z. and Gong, Z. 2009. Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in *Arabidopsis*. The Plant Journal, 60: 79-90.

How to cite this article:

Hojjati, Y., Shoor, M., Tehranifar, A. and Abedi, B. 2019. Modification of flower color pigments and color composition with hormonal treatments and sucrose in *Tulipa gesneriana* 'Kingsblood'. *Journal of Ornamental Plants,* 9(2), 73-91.

URL: http://jornamental.iaurasht.ac.ir/article_665134_e01142e179e87038d5033f4dbe4e5f10.pdf