



Morphology and responses of *Tulipa gesneriana* L. to light quality in combination with GA and cold storage time

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ABSTRACT

Tulips are one of the important bulbous flowers and valuable as cut flowers. Hence, the present experiment was conducted in order to study effect of different concentrations of gibberellic acid (GA), light sources, and cold storage time on tulip flowers. The results of the present study revealed that day to sprouting and flowering of tulip decreased by T1×G2B, and T1×G2B and T2×G2B conditions, respectively. Plant height as well as the first internode length was also increased at T1×CD and T2×CD conditions. Furthermore, all light treatments, except of the dark conditions, enhanced the amount of anthocyanin and chlorophyll content of tulips plants. Similarly, the same trend recorded for dry weight of leaf and bulb of tulip. In conclusion, application of different light qualities in combination with GA and cold storage treatments resulted in an improvement in the growth parameters as well as the morphological and the flowering characteristics of tulip.

Key words: Cold storage; Flowering; Gibberellic acid; Light sources; Tulip.

INTRODUCTION

Almost all aspects of plant's life is regulated by environmental signals (Pashkovskiy *et al.*, 2016). Several environmental factors, such as temperature, light, watering, and fertilization etc., have been identified to modify plant behaviors. Among these factors, light is an important factor for plant growth, flowering, and photosynthesis (Pashkovskiy *et al.*, 2016). Light quality (spectrum or wavelength) also plays a key role in plants (Sumitomo *et al.*, 2012). The environments with blue light-deficient promote stem elongation in many long-day plants (Runkle and Heins, 2001). For instance, in chrysanthemum, the effect of blue light on plant height depended on the presence or absence of FR light (Reddy *et al.*, 1996).

Another important factor is gibberellic acid (GA₃) as a plant growth regulation. GA performs significant role in the plant flowering which reduce forcing period of tulip bulbs caused saving energy as well as reducing physiological disorders (Shakarami *et al.*, 2013). Several studies have been evaluated effect of GA on tulip in reducing duration of cold treatment. Shakarami *et al.*, (2013) found that GA treatment reduced stem length, internodes length, and precocity period of tulip cv. Lacourtine. Ramzan *et al.* (2014) also reported that pre-planting application of GA₃ had beneficial effect on quality tulip production, plant height, stalk length, and diameter and flower size. GA₃ can even be effective in the performance of fruit (Kumar and Sharma, 2016). For

example, in *Abelmoschus esculentus*, (Linn) GA at 30 ppm gave maximum fruit length, fruit thickness, green fruits yield and dry weight of fruits (Verma *et al.*, 2016).

Tulip (*Tulipa gesneriana* L.) is an important ornamental bulbous plant and one of the most famous as cut, pot, and garden flowers cultivated in the world (Benschop *et al.*, 2010). This bulbous plant has a dormancy period to occur most physiological change (Xu and Niimi, 2008). Tulip bulbs often require a cold period time in order to develop sufficiently long stem and promote rapid growth and flowering (Xu and Niimi, 2008). Therefore, the main goal of most researchers is increasing stalk, flower quality, and stimulate early flowering. Thus, they generally used different temperature, light, and plant growth regulation (Kurtar and Ayan, 2005). Hence, the objective of the present study was to evaluate the flowering behavior and morphological characteristics of tulip plants under various light quality, GA concentrations, and cold storage time.

MATERIALS AND METHODS

Plant materials and treatments: This study was conducted during 2015-2016 at Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran (Iran). Healthy and uniform bulbs of tulip cv. 'Princess Catharina-Amalia' with circumferences 12 cm were collected from Mahde Laleha, Institute of Gachsar (Iran). Treatments were included cold storage time (0 as control, 10, and 15 weeks), GA₃ (0, 300, and 600 ppm), and light treatment (white, dark,

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blue, and red light). Treatments were performed when the pistil of bulbs are reached to G stage (pistil has 3 lobes and anthers are specified). Bulbs were cooled at 4 °C for 0, 10, and 15 weeks. At the end of each storage treatment, the bulbs were soaked for 24 hours in GA₃ at concentrations of 0, 300, and 600 ppm. To prevent fungal diseases, the bulbs were disinfected with benomyl 3/1000 for 25 min and then (remove) exposed to air for two hours. Afterwards, bulbs were planted in 10 cm plastic pots contain 70% peat and 30% perlite and were surfaced by sandy soil. Plants were placed in laboratory under three fluorescent lamps for each color (Philips TLD) with 36 W power at 60 cm above soil level; white color (370-700 nm), blue color (400-580 nm), red color (600-700 nm) for 16/8 h light/dark conditions, and dark treatment (without any light application during experiment). The photon flux density (PFD) at the surface of the pot was (was) 25.0 μmol m⁻² s⁻¹. Optimum temperature during experiment was in the range of 15-17 °C and relative air humidity was 60-70%. Irrigation was done every three days and no fertilization was done. it is worth mentioning that artificial lights were the only means of light supply, and they were well isolated to prevent potential contamination with other light sources.

Measurements: Days to sprouting of bulbs, days to flowering, percentage of sprouting and flowering plants, plant height, length of upper and lowest internodes, length and width of the lowest leaf, flower diameter at the time of flower opening, length of flower bud, fresh and dry weight of bulb and shoot were determined when the flower buds were fully colored. The chlorophyll content index (SPAD) was also measured using a chlorophyll meter (SPAD-502). Anthocyanin contents was quantified by measuring the amount of anthocyanins in fresh ray florets as described by Meng and Wang (2004).

Statistical analysis: The research was designed as a completely randomized design with factorial concept and 3 replications. Each replication was consisting (consisting) of 6 experimental units. Data were analyzed by SAS software (Version 9.1, SAS Institute Inc., Cary, NC, USA) by using Duncan’s methods at 5%.

RESULTS AND DISCUSSION

As shown in results of Table 1, all of treatments even dark conditions caused bulbs sprouting.(in table use sprouting) The highest day to sprouting was observed at T0×CD, T0×CW, and T0×CR and the lowest day was observed at T1×G2B treatment. Blue light application decreased day to sprouting in T0×G1 treatment. Similarly, day to flowering significantly decreased at T1×G2B and T2×G2B treatments. All light conditions significantly decreased day to flowering at T1 and T2, especially in GA applications. In non-application GA conditions, excessive bulbs storage time reduced the quality of the bulbs, so that sprouting and flowering percentage at 10 weeks of cold

Table 1: Integrated effects of light quality (D, B, W, and R as dark, blue, white, and red, respectively), cold storage time (T0, T1 and T2 as 0, 10 and 15 weeks, respectively), and GA (C, G1 and G2 as control, 300 and 600 ppm, respectively) treatments on sprouting and flowering traits of tulip

Treatments	Sprouting(%)			Flowering(%)			Days to sprouting(day)			Days to flowering(day)		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
CD	77.77	88.88	88.88	14.28	43.75	37.5	23.6a	20.2c-f	20.5b-f	44.6a	43.4abc	42.8a-d
CB	77.77	100	94.44	21.42	83.33	75	22.0abc	18.4fg	19.3ef	44.6a	38.8hij	39.8e-h
CW	72.22	100	88.88	23.07	88.88	76.47	23.0a	19.7def	20.0c-f	44.0ab	38.7ghi	39.4fgh
CR	72.22	100	94.94	23.07	88.88	76.47	23.3a	20.0c-f	20.2c-f	44.3ab	38.7hij	40.2c-f
G1D	100	100	100	16.66	77.77	66.66	22.4ab	15.2hij	16.8gh	44.6a	37.8hij	38.1g-j
G1B	88.88	100	100	26.66	94.44	88.88	15.3hij	14.6ij	15.3hij	42.4a-e	33.5lm	33.8klm
G1W	83.33	100	100	33.33	100	94.44	21.6a-d	16.4hi	16.2hi	44.0ab	36.2i-l	36.5jkl
G1R	83.33	100	100	31.25	94.44	94.44	21.0b-e	16.0hi	16.4hi	43.0a-d	36.5jkl	34.5klm
G2D	83.88	100	100	20	77.77	72.22	20.8b-e	15.2hij	16.2hi	44.6a	37.8hij	37.8hij
G2B	88.88	100	100	31.25	94.44	94.44	20.5b-f	13.3j	14.8hij	42.8a-d	33.1m	33.0m
G2W	88.88	100	100	25	100	100	20.2c-f	15.3hij	15.7hi	20.2c-f	34.3klm	34.1klm
G2R	88.88	100	100	31.25	100	94.44	21.0b-e	14.6ij	16.2hi	41.6b-f	34.6klm	34.1klm

Means followed by the same letter are not significantly different at 5% level, using Duncan.

treatment were more than 15 weeks and bulbs sprouted earlier (Table 1).

Our findings revealed that GA application and cold storage time increased the percentage of bulbs sprouting, flowering as well as early sprouting of bulbs and rapid flowering. In agreement with the present study, the results obtained by Ramzan *et al.* (2014) showed that tulip bulbs treated with 100 mg/L GA₃ sprouted in less number of days, higher sprouting percentage, and stalk diameter. They explained that flowering in plant related with hormonal and sugar changes. GA elevated starch hydrolysis into reducing sugars in the stem and flower, due to improved quality of cut flowers by enhanced water balance (Emongor, 2004). Kumar *et al.* (2013) and Navale *et al.* (2010) found that GA supplementation caused an early flowering in tulip and chrysanthemum. The results of pervious study showed that GA₃ reduced the sprouting time and also increased sprouting percentage in bulbs of *Iris nigricans* and *Callistephus chinensis* L. Nees (Al-Khassawneh *et al.*, 2006; Parvan Kumar *et al.*, 2015). Geng *et al.* (2007) explained that GA had suppressing effect on ABA which caused early sprouting in bulbs.

The highest plant height and first internode was observed at T1×CD and T2×CD conditions, respectively (Table 2). All light conditions, except dark condition, increased length of last internode in T0 in GA₃ and non-GA₃ application. In addition, blue light increased last internode at T2 in combination with GA (Table 2).

Flowers quality and plant height were increased by increasing the cold period. Under the cold treatment conditions cause the balance accumulation of cytokinin and gibberellic acid in bulb of plant (Okubo and Uemoto, 1985). Therefore, it increase (remove) growth and flowering of tulip bulbs. The results indicated that GA application had beneficial effect in non-cold treatment in increasing flower quality and decreasing sprouting and flowering time. Since, GA did not produce an acceptable percentage of flowering in non-cold treatment bulbs that completely cannot act as an alternative for the low temperature. However, GA caused a decrease in day to spourting and flowering, and an increase in percentage of flowering and flowering uniformity of tulips.

In our study, almost all morphological parameters in tulip plants promoted at both cold storage time and GA application. The reason of this phenomenon explained by Khan *et al.* (2007) who expressed that GA application stimulate and accelerate cell division, elongation, and enlargement. Furthermore, in this study, dark conditions increased plant height through increasing first internodes while last internodes increased at blue light. Similarly, Okubo and Uemoto (1985) reported that dark conditions resulted in an increasing in the length of the first internode of tulips, whereas the last internode was the longest one when grown under natural light conditions. Stem elongation in response

Table2: Integrated effects of light quality (D, B, W, and R as dark, blue, white, and red, respectively), cold storage time (T0, T1 and T2 as 0, 10 and 15 weeks, respectively), and GA (C, G1 and G2 as control, 300 and 600 ppm, respectively) treatments on some morphological traits of tulip

Treatments	Plant height(cm)			Length of first internode(cm)			Length of last internode(cm)			Length of flower bud(cm)			Flower diameter(cm)		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
CD	38.1a-d	42.1a	41.5ab	3.3klm	3.2lm	3.3j-m	2.6j	2.8hij	2.7ij	3.3klm	3.2lm	3.3j-m	2.6j	2.8hij	2.7ij
CB	31.3fg-j	39.5abc	10.0d-h	3.7g-m	4.6a-f	4.4a-g	3.7c-g	3.7c-h	3.8c-g	3.7g-m	4.6a-f	4.4a-g	3.7c-g	3.7c-h	3.8c-g
CW	29.1ghi	32.1f-j	7.1ghi	3.4j-m	4.8abc	5.2ab	3.3e-i	3.8e-f	4.1b-e	3.4j-m	4.8abc	5.2ab	3.3e-i	3.8e-f	4.1b-e
CR	28.1h-k	33.0d-i	6.6hi	4.0c-j	3.9e-m	4.8a-d	4.1b-e	5.4a	5.5a	4.0c-j	3.9e-m	4.8a-d	4.1b-e	5.4a	5.5a
G1D	38.0a-e	39.8ab	40.0ab	3.4j-l	3.6i-m	3.4j-m	2.6j	2.8hij	3.4e-j	3.4j-l	3.6i-m	3.4j-m	2.6j	2.8hij	3.4e-j
G1B	30.3g-k	38.2a-d	40.0ab	3.7g-m	4.4b-f	4.5a-g	3.6c-i	4.0c-f	4.0c-f	3.7g-m	4.4b-f	4.5a-g	3.6c-i	4.0c-f	4.0c-f
G1W	25.5kl	33.6d-h	34.5c-f	4.3c-i	3.9d-k	5.2a	3.0g-j	3.9c-g	4.9ab	4.3c-i	3.9d-k	5.2a	3.0g-j	3.9c-g	4.9ab
G1R	22.1l	33.6d-h	33.6d-h	3.8g-m	3.2i-m	4.9abc	3.6c-i	3.9c-g	4.5bc	3.8g-m	3.2i-m	4.9abc	3.6c-i	3.9c-g	4.5bc
G2D	36.3b-f	41ab	41.0ab	3.1m	3.3j-m	3.6h-m	2.7ij	3.5d-j	3.7d-i	3.1m	3.3j-m	3.6h-m	2.7ij	3.5d-j	3.7d-i
G2B	28.0jkl	32.8e-i	40.8ab	3.4j-m	4.1c-i	4.5a-g	3.5d-j	3.8c-g	3.9c-g	3.4j-m	4.1c-i	4.5a-g	3.5d-j	3.8c-g	3.9c-g
G2W	27.3kj	33.6d-h	30.9f-j	4.0c-j	4.1c-i	4.7a-e	3.1f-j	3.7e-i	4.4bcd	4.0c-j	4.1c-i	4.7a-e	3.1f-j	3.7e-i	4.4bcd
G2R	25.3kl	31.6f-j	31.0f-j	3.6h-m	3.6h-m	4.8a-d	4.4b-i	4.1b-e	4.9ab	3.6h-m	3.6h-m	4.8a-d	4.4b-i	4.1b-e	4.9ab

Means followed by the same letter are not significantly different at 5% level, using Duncan.

to changes in light quality may be mediated by changes in GA level (Morgan, 1994) or sensitivity to GA (Ross *et al.*, 1990). On the other hands, blue light increased last internodes in tulips. In agreement with our results, Gautam *et al.* (2015) missing in reference founded that blue light increased stem elongation and plant height and promote flowering compared to control and red light conditions. The results of Smigielska *et al.* (2014) (italic) showed that blue light forced the length of first internode in tulip, while red light stimulated last internode elongation.

The results, presented in Table 3, showed that all light conditions, with the exception of dark conditions, significantly improved the amount of anthocyanin and chlorophyll content of tulips plants. Furthermore, there was no observed significant difference in applications of GA and cold storage treatments. However, the highest anthocyanin and chlorophyll contents were at T1×G1B and T2×G2B, and T1×G2B conditions, respectively. Dry leaf weight increased by G2R at T1 and dry weight of bulb increased at G2W, G2R at T2. Dry weight of leaf and bulb increased by all light applications at all time and GA treatment in comparison with dark conditions (Table 3). In addition, applications of GA and cold storage time treatments clearly enhanced the amount of dry weight of leaf and bulb of tulips. In line with our results, Vieira *et al.* (2010) (italic) showed the beneficial effect of GA on increasing characteristics of several flowers such as Azalea, Impatiens, Iris, Aglaonema, Hyacinthus, Lilium, Liatris, Rhododendron, and Muscari as an alternative for the low temperature. Khan *et al.* (2007) also reported a similar trend, indicating that growth, physiological behavior and flower quality of tulip increased (remove)by plant growth regulators. Additionally, in agreement with our results, sufficient cold storage treatment enhanced characteristics of tulip plants (Moe and Wickestrom, 1979).

Chlorophylls as macromolecules are one of the important light absorbent pigments in chlorophylls Tilakoid membrane, and also are very vulnerable to stress conditions (Hopkins, 1999). Majidian *et al.* (2012) (italic) showed that pre-treatment of calla lily corms with GA leads to the increase of chlorophyll in leaves. In recent years, several researchers stated some concepts of anthocyanin function in plants that may be related to balance of quality and quantity light receptions, protection of light stay, and protection of UV-B destroying effects (Mizuno *et al.*, 2011). Therefore, there is evidence that mentions, both growth and anthocyanin expression could be efficiently controlled by shifting of light quality between mixed light sources (Lee *et al.*, 2010). Moreover, accumulation of anthocyanins in plants by different environmental motivations such as UV (Reddy *et al.*, 1994), low temperature (Christie *et al.*, 1994), and plant growth regulators [cytokinin (Deikman and Hammer, 1995), ethylene (Woltering and Somhorst, 1990), and gibberellic acid (Akinwunmi, 2001)] is possible. Nevertheless, there is

Table 3: Integrated effects of light quality (D, B, W, and R as dark, blue, white, and red, respectively), cold storage time (T0, T1 and T2 as 0, 10 and 15 weeks, respectively), and GA (C, G1 and G2 as control, 300 and 600 ppm, respectively) treatments on some parameters of tulip

Treatments	Anthocyanin(mg/g FW)			Chlorophyll(SPAD)			Leaf dry weight(g)			Bulb dry weight(g)		
	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
CD	0.27j	0.24j	0.25j	7.9m	8.4m	8.7m	0.57ij	0.80hij	0.90f-i	3.68h	5.29e	5.31e
CB	0.57abc	0.52a-e	0.55a-e	31.8f-j	34.7b-f	34.0c-g	0.81hij	1.17fg	1.49de	4.40g	6.27c	6.20c
CW	0.43e-h	0.47c-f	0.46c-f	27.2i	28.4kl	29.2jkl	0.81hij	1.21ef	1.70bcd	4.5g	6.29c	6.24c
CR	0.46c-f	0.44d-g	0.43e-h	27.7kl	28.6kl	29.3i-l	0.84ghi	1.22ef	1.93abc	4.3lg	6.24c	6.15c
G1D	0.33g-j	0.36f-i	0.37f-i	7.9m	8.4m	8.5m	0.49j	0.98fgh	0.99fgh	4.97f	5.66d	5.52de
G1B	0.60ab	0.64a	0.60ab	36.6a-e	37.1a-d	37.7abc	0.87ghi	1.81bcd	1.74bcd	5.75d	7.48b	7.55b
G1W	0.52b-e	0.53a-e	0.54a-e	29.6h-l	31.5fg-j	33.4d-i	0.85ghi	1.83bc	1.83bc	5.81d	7.64ab	7.63ab
G1R	0.57abc	0.51b-e	0.56a-d	31.1f-l	28.6kl	32.9f-j	0.93fgh	1.92abc	1.94abc	5.73d	7.67ab	7.67ab
G2D	0.32hij	0.33g-j	0.34g-j	8.0m	8.3m	8.6m	0.69hij	0.99fgh	0.99fgh	4.93f	5.62d	5.58de
G2B	0.59ab	0.56a-d	0.64a	38.4ab	38.8a	38.3ab	1.02fgh	1.79bcd	1.68cd	5.72d	7.76ab	7.23ab
G2W	0.52b-e	0.51b-e	0.53a-e	30.2g-l	33.2d-j	33.7e-h	0.92fgh	1.86bc	1.91abc	5.73d	7.50b	7.96a
G2R	0.52a-e	0.56a-d	0.54a-e	30.4g-l	32.8e-j	32.8e-j	0.90f-i	2.22a	2.03ab	5.73d	7.71ab	7.49a

Means followed by the same letter are not significantly different at 5% level, using Duncan.

no yet any publication about integrated effects of light sources, GA, and cold storage time on tulip cv. 'Princess Catharina-Amalia'.

CONCLUSION

The interaction of GA and cold storage showed that cold treatment needed for vegetative growth and flowering. Moreover, cold requirement cannot entirely replace with light and GA treatments, although these had beneficial effect on flower quality and flowering time. In spite of the fact that the application of light quality in combination with GA and

cold storage can increase the morphological and biological parameters of tulip plants but light treatments, with the exception of dark conditions in some case, performed higher effects on characteristics of tulip plants than the other treatments applied in this study. Therefore, by changing the light quality can be produced plants with varied heights for different purposes such as the production of potted plants or may be effectively applied to tulip cut-flower production as well as saving energy by reducing growing period in greenhouse.

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