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The optimum conditions for quantitative and qualitative flowering in chrysanthemum and the endogenous hormones associated with flowering

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Abstract

The work aimed to determine the optimum conditions for the production of chrysanthemum flowers all the year, and identify the endogenous hormones associated with flowering. Three factors, including plant age, photoperiod and cultivar, were studied on potted chrysanthemum under fiberglass house conditions. Plants were used 1.5 months after the pruning of mother plants (old plants) or after propagation via terminal cuttings (new plants). Plants were exposed to five photoperiods including 4, 6, 8, 10 and 12 hours. The study was conducted on four commercial cultivars of different flower characteristics; yellow with small (YS) or big (YB) inflorescence, and red with small (RS) or big (RB) inflorescence. Old plants were better than new plants where their flowers production was 4 times higher. No flowers were obtained under 4 hours photoperiod. Flowers quantity increased with increasing photoperiod till 10 hours, the maximum flowering, then decreased under 12 hours. Flowering of chrysanthemum was cultivar dependent since cultivars of small flowers produced higher flowers quantity compared to those of big flowers, and the yellow cultivar gave the highest flowering. The optimum conditions allowed the production of 20-28 flowers per plant, depending on cultivar, with the highest quality expressed as flower stem length and flower diameter. The analysis of endogenous hormones showed that Indole Acetic Acid (IAA) and Gibberellic Acid (GA_3) had an important role in flowering, on the contrary of Abscisic Acid (ABA). Both hormones correlated positively with flowering however, ABA correlated negatively with it. The concentration of IAA and GA_3 during flowering was 3-4 times higher than ABA, and both were in high levels under the optimum conditions. The reported results could be useful on the applied level for the commercial production of chrysanthemum all the year, and the fundamental level for understanding the physiology of flowering process leading to plant improvement.

Keywords: Chrysanthemum, Flowering, Photoperiod, Age, Cultivar, Hormones.

Introduction

Chrysanthemum, family Asteraceae, is one of the leading flowering plant presenting the third most economically important cut flowers in the international market after rose and carnation. It includes a huge number of horticultural cultivars of different colors, flower size and petal shape. Chrysanthemums are in high demand for their multiple uses as cut flowers, potted flowering plants or bedding plants for interior and exterior decoration. The nature of chrysanthemum as a short-day plant presents a barrier preventing its commercial production all the year to face its increasing demand. Understanding the physiology of its flowering could also lead to improve its production.

Flowering is a complex phenomenon controlled by many environmental and agricultural factors. Many efforts were made to improve flowering of chrysanthemum as a highly commercial and economic plant. Many studies were carried out on the effect of

temperature on the growth and flowering of chrysanthemum **(Bonaminio and Larson 1980; Whealy *et al*., 1987; Carvalho *et al*., 2005)**. Photoperiod duration was also studied by some researchers. The submission of *C. morifolium* cv. Reagan Sunny to three photoperiods including 8, 10 and 12 hours at flower bud initiation and flower bud development stages showed a positive effect for 8h in promoting flower initiation, and buds did not develop into flowers under 12h (**Kahar, 2008**). The application time of short day was also studied where covering from 5h00 to 9h00 AM was found to be the best treatment for flowering **(Nxumalo and Wahome, 2010)**. Response of chrysanthemum changed according to planting time showing an important effect for seasonal changes, and the best ones were from March-May to September- November (**Jerzy and Bres, 2011**). The response of chrysanthemum seemed to be cultivar dependent where varietal difference in the response to light and the ability of floral differentiation was found among twelve cultivars of chrysanthemum **(Ochiai *et al*., 2015)**.

Plant hormones are the main internal factors controlling growth and development of plants at different stages. They play a vital role in most physiological processes including flowering **(Koshita *et al*., 1999)**. Endogenous plant hormones include five groups comprising auxins, cytokinins, gibberellins, abscisic acid and ethylene **(Muller and Munne-Bosch, 2011)**. Foliar application of the exogenous plant hormones, including GA3, IAA, IBA, NAA or 2,4D at blooming stage were investigated on cashew, and GA_3 was found to be the best hormone improving flowering **(Aliyu *et al*., 2011)**. In chrysanthemum, the effect of hormonal levels on inflorescence differentiation was investigated. GA_3 was required for the flowering of chrysanthemum (**Sumitomo *et al*., 2009**), and IAA was found to be necessary for inflorescence differentiation whereas, GA_3 and ABA were stable during crown formation (**Jiang *et al*., 2010**). It was also reported that levels of IAA and ABA change in apical and lateral buds according to growth stage and cultivar **(Jiang *et al*., 2012)**.

From the previous review, it appears clearly that photoperiod and cultivar are important factors affecting flowering of chrysanthemum, and the endogenous hormones played an important role in this process. However, No report is available on the effect of some important factors like plant age which is a well known factor affecting flowering of many plant species including strawberry **(Verheul *et al*., 2005)**. No report is also available on the effect of multiple factors allowing the determination of the optimum conditions for chrysanthemum flowering all the year for commercial purposes. Furthermore, no enough information is available on the role of endogenous hormones associated with flowering of different cultivars which is essential to understand the physiology of flowering leading to plant improvement. We previously reported on the improvement of rose flowering as the first cut flowers plant **(Hassanein, 2010)**. Concerning chrysanthemum, we optimized its propagation via stem cuttings **(Hassanein, 2013)**, and reported on the optimum irrigation and fertilization conditions for its growth and flowering **(Hassanein, 2015)**. The actual work aimed to determine the optimum conditions for its continuous flowering with high quantity and quality all the year, and define the endogenous hormones associated with its flowering.

Materials and Methods

Establishment of work and studied factors

This work aimed to optimize flowering of the most commercially cultivars of *Chrysanthemum morifolium* to be produced throughout the year, and identify the endogenous hormones associated with flowering. Three factors including plant age, photoperiod and cultivar were investigated to achieve this aim. Experiments were carried out under fiberglass house conditions at the floriculture experimental farm, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia during two successive seasons at April and October 2014. Four different chrysanthemum cultivars, highly diffused in markets, were selected to perform this study. They included two cultivars of yellow flowers with small (YS) or big (YB) inflorescence, and two cultivars of red flowers with small (RS) or big (RB) inflorescence **(Figure 1)**. Two plant ages were studied where mother plants were used after 1.5 month of pruning at 10 cm height (old plants) or new plants were used after 1.5 month of propagation via terminal cuttings of 10 cm (new plants). Both plant types were exposed to long-day conditions during this 1.5 month for vegetative growth development before the study. Five photoperiods were tested in small houses of 2 × 2 × 2 m dimensions. Photoperiods durations were 4, 6, 8, 10 or 12 hours using black covering for dark and sunlight or lamps for light. Both plant types of all cultivars were exposed to the studied photoperiods for 16 weeks. All agricultural practices were carried out similarly as recommended for all the studied treatments.

Figure 1. Chrysanthemum cultivars including yellow with small (YS) or big (YB) flowers, and red with small (RS) or big (RB) flowers.

Experimental design and measured parameters

Experiment was arranged in a split-split plot design containing three factors with five replicates. The first factor was the plant age including two levels (old and new plants) in the main plot, the second one was the photoperiod durations including five levels (4, 6, 8, 10 and 12h) in the sub-plot and the third factor was the cultivar comprising four cultivars (YS, YB, RS and RB) in the sub-sub-plot. Experiment was repeated twice at two different periods as above mentioned. Flowers number and the total flowering (flowers + flowering buds) per plant were counted for all treatments throughout the experiment starting from the beginning of flowering till the end of experiment. To assess the quality of flowering, average of flower stem and flower diameter were determined for five randomizely selected flowers per replicate for each treatment at the end of experiment.

Analysis of Endogenous hormones

To identify the endogenous hormones associated with flowering, the concentration of Indole-Acetic Acid (IAA), Abscisic Acid (ABA) and Gibberellic Acid (GA_3) in apical buds and the newest fresh leaves were determined for all treatments at the end of experiment. Levels of hormones were quantified by liquid chromatography (HPLC, LC-20, Shimadzu) according to **Kelen *et al*. (2004)** and **Muller and Munne-Bosch (2011)**. Frozen materials (500 mg) were ground in liquid nitrogen then extracted in 10 ml of extraction solvent (Methanol : Isopropanol : Acetic acid, 20 : 79 :1) using ultra sonication (10,000 rpm for 20 minutes at 4 ^{o}C). The supernatant was collected and re-extracted in 1 ml solvent three times. The supernatants were combined and dried under a nitrogen stream then re-dissolved in 300 µl of methanol with centrifugation at 10,000 rpm for 5 min. HPLC was adjusted following to the conditions explained by **Kelen *et al*. (2004)**. The mobile phases used were acetonitrile-water (26:74; v/v). The Luna C18 column (250 / 4.6 ml) was equilibrated for each mobile phase condition with a time limit of 30 min. The flow rate was 0.8 ml / min and the signals were monitored at 208 nm wave length. Hormones were determined in three independent samples of 10 µl then the average was calculated for each treatment. Quantification was done by the creation of calibration curves for each hormone followed by the calculation of concentration in µg/g.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) to determine significant differences followed by the comparison of means at significant level of 5% using SAS 9.1.3. For curves, means were shown with confidence interval at α= 0.05.

Results

Flowering of chrysanthemum

Production of whole flowers and the total flowering, including flowers and flowering buds, per plant as affected by plant age, photoperiod and cultivar factors are shown in **Table (1a)**. The effect of all factors and their interactions were significant **(Table 1b)**. Generally, old plants produced significantly higher flowers and total flowering compared to new plants where their production were about four times higher. Photoperiod was also an effective factor affecting flowers production and total flowering per plant. No flowers were produced under 4 hours photoperiod where very little number of small flowering buds were observed and they did not develop into whole flowers. Flowering increased with increasing photoperiod durations where it reached the maximum under 10 hours photoperiod then decreased under 12 hours photoperiod. Cultivars of small flowers, whatever their color, gave significantly higher total flowering per plant compared to cultivars of big flowers.

The yellow cultivar with small flowers also showed the highest flowers production compared to other studied cultivars. Concerning interaction effect, significant differences were found among different interactions and the best flowering was recorded with old plants exposed to 10 hours photoperiod, old plants of small-flower cultivars and these cultivars under 10 hours photoperiod. The maximum total flowering (26-28) and the highest flowers number (22) per plant were obtained from old plants of small-flower cultivars exposed to 10 hours photoperiod, however, no flowers were obtained from any cultivar, whatever plant age, under 4 hours photoperiod **(Figure 2)**..

Table 1a. Flowering of chrysanthemum as affected by plant age, photoperiod and cultivar factors after 16 weeks of culture

Plant age	Photo-period	Flowers number per plant				Mean (photo-period)	Total flowering per plant				Mean (photo-period)
		YS	YB	RS	RB		YS	YB	RS	RB	
New	4 hours	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.3	0.1
	6 hours	0.3	0.8	0.0	1.5	0.7	1.3	2.0	0.3	5.2	2.2
	8 hours	1.7	0.3	1.8	3.0	1.7	3.0	1.3	5.0	5.3	3.7
	10 hours	1.0	4.7	3.2	3.2	3.0	2.7	5.3	5.9	4.0	4.5
	12 hours	3.5	1.7	1.7	2.5	2.3	5.5	2.2	2.0	5.0	3.7
Mean (new)		1.3	1.5	1.3	2.0	*1.5*	2.5	2.2	2.6	4.0	*2.8*
Old	4 hours	0.0	0.0	0.0	0.3	0.1	0.3	1.8	0.0	1.3	0.9
	6 hours	9.2	6.2	1.0	4.2	5.1	17.0	10.8	2.5	5.5	9.0
	8 hours	8.7	6.0	6.0	3.0	6.0	18.0	14.5	20.0	10.0	15.6
	10 hours	22.0	6.0	7.5	3.8	9.8	25.7	20.0	27.3	13.7	21.7
	12 hours	5.3	9.0	11.0	15.2	10.1	14.0	13.5	25.0	19.3	18.0
Mean (old)		9.0	5.4	5.1	5.3	*6.2*	15.0	12.1	15.0	10.0	*13.0*
New × Old	4 hours	0.0	0.0	0.0	0.2	0.05	0.3	0.9	0.0	0.8	0.5
	6 hours	4.8	3.5	0.5	2.9	2.9	9.2	6.4	1.4	5.3	5.6
	8 hours	5.2	3.2	3.9	3.0	3.8	10.5	8.0	12.5	7.7	9.6
	10 hours	11.5	5.3	5.3	3.5	6.4	14.2	12.7	16.6	8.9	13.0
	12 hours	4.4	5.3	6.3	8.8	6.2	9.8	7.9	13.5	12.2	10.9
Mean (cultivars)		5.1	3.5	3.2	3.7		8.8	7.2	8.8	7.0	

Total flowering includes whole flowers plus flowering buds

Table 1b. The least significant difference at 0.05 for the principal factors and their interactions

LSD (0.05)	Age	Photoperiod	Cultivar	A × P	A × C	P × C	A× P×C
Flowers / plant	0.80	1.09	0.63	1.78	0.89	1.41	2.00
Total flowering	0.53	1.86	0.93	1.19	1.31	2.08	2.94

Figure 2. Flowering of cultivars under 10 hours photoperiod compared to no flowers under 4 hours

Flowering development

Follow up flowering development during experiment starting from the beginning of flowering till the end of experiment is shown as average for all studied factors and for plants under the best conditions **(Figure 3)**. Old plants initiated flowering buds early where buds were observed 4-6 weeks after treatment compared to 8-10 weeks for new plats. Old plants continued the production of new flowering buds and the development of whole flowers; from 6 to 14 flowers per plant as average in 8 weeks. However, flowering of new plants was slow and did not exceed 3 flowers per plant as average during flowering period. Plants exposed to 4 hours photoperiod did not produced any flowers till the end of experiment and only non-developing buds were observed (0.1 per plant). Plants started producing flowering buds and flowers under all other studied photoperiods but 10 hours was more early, rapid and permanent than other ones **(Figure 3)**. Flowering of plants increased with time but the increments were higher under 10 and 12 hours photoperiods. Furthermore, flowering of plants under 10 hours photoperiod continued increasing till the end of experiment however it decreased under 12 hours photoperiod. Cultivars also showed different responses to studied factors where cultivars with small flowers produced higher flowers than those with big flowers. It should be mentioned that cultivars of the same color gave similar flowering at the beginning but those with small flowers showed higher flowering than cultivars with big flowers for both colors. Under the best conditions, exposing old plants to 10 hours photoperiod, flowering was increased with time for all cultivars and plants continued the production of flowers and flowering buds till the end of experiment. Flowering reached to 28 per plant for small-flower cultivars versus 17 as average for big-flower cultivars. Despite the similarity of production for both small-flower cultivars, yellow cultivar with big-flower gave higher production than the red one.

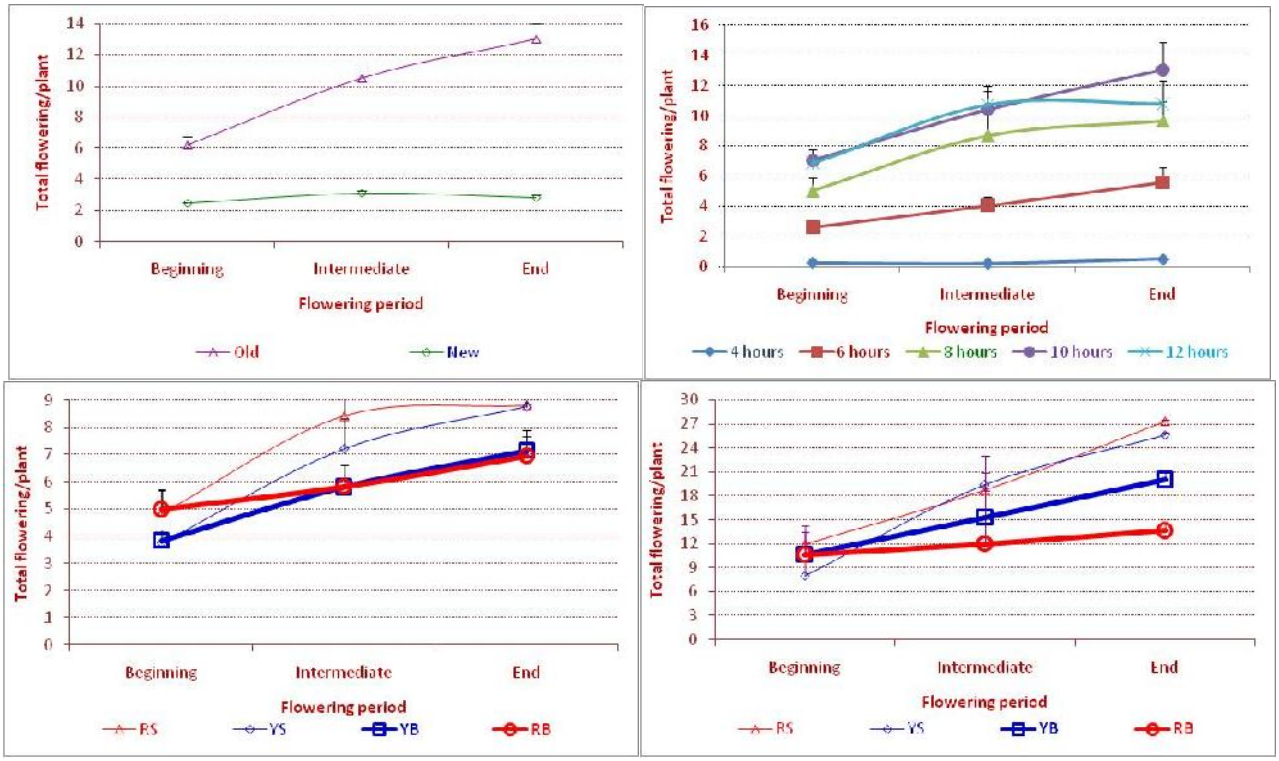

Figure 3. Average of flowers production during flowering period for the three studied factors, and for cultivars using old plants under 10 hours photoperiod as the best conditions (bottom-right fig.).

Flowers quality

Quality of flowers, expressed as flower stem length and flower diameter, as affected by plant age, photoperiod and cultivar is shown in **Table (2a)**. No flowers were produced under 4 hours photoperiod, so, this level was excluded during statistical analysis for more accuracy. Results of variance analysis after the exclusion of 4 hours level showed significant effects for all studied factors and their interactions except plant age **(Table 2b)**. Generally, quality of flowers produced on new and old plants were similar.

Quality of flowers was also the same under most studied photoperiods but flowers produced under 6 hours photoperiod had significantly higher flower stem length and lower flower diameter than those produced under 12 hours photoperiod. Cultivars also gave flowers of similar quality but the yellow cultivar with small flowers had higher flower stem length and flower diameter than that with big flowers. Concerning the interactions, it was recorded that new plants produced flowers with stem length taller than that of old plants under any photoperiod except 6 hours where old plants were better in flower stem and flower diameter. The old plants of the yellow and small-flowers cultivar also gave the best flower characteristics. This cultivar also gave the best flowers under 10 hours photoperiod and thus red and big-flower cultivar under 6 hours. Characteristics of flowers differed intra and inter cultivars according to culture condition **(Figure 4)**. The longest flower stem length (9 cm) was obtained from old plants of red cultivars under 6 hours photoperiod or yellow and small-flower cultivars under 10 hours photoperiod. The largest flower diameter (6 cm) was also obtained from old plants of red cultivars exposed to 6 or 12 hours photoperiod.

Table 2a. Flowers quality of chrysanthemum as affected by plant age, photoperiod and cultivar factors after 16 weeks of culture

Plant age	Photo-period	Flower stem (cm)				Mean (photo-period)	Flower diameter (cm)				Mean (photo-period)
		YS	YB	RS	RB		YS	YB	RS	RB	
New	4 hours	-	-	-	-	-	-	-	-	-	-
	6 hours	3.7	3.8	0	3.2	2.7	1.5	2.1	0	2.8	1.6
	8 hours	4.4	3.0	8.2	7.2	5.7	2.2	2.7	3.2	4.7	3.2
	10 hours	4.6	3.5	8.2	5.7	5.5	3.6	3.3	3.2	3.2	3.3
	12 hours	5.7	5.4	3.7	4.4	4.8	4.4	3.6	2.2	2.0	3.0
Mean (new)		4.6	4.0	5.0	5.1	*4.7*	3.0	3.0	2.1	3.2	*2.8*
Old	4 hours	-	-	-	-	-	-	-	-	-	-
	6 hours	4.3	6.5	9.0	9.2	7.2	2.5	3.1	3.5	5.5	3.6
	8 hours	7.3	3.2	1.6	0.8	3.2	4.2	2.5	1.1	0.6	2.1
	10 hours	8.5	2.0	2.4	1.2	3.5	3.8	3.0	3.0	1.5	2.8
	12 hours	3.6	3.8	2.6	4.6	3.6	3.7	2.0	5.7	2.6	3.5
Mean (old)		6.0	3.9	3.9	4.0	*4.4*	3.5	2.6	3.3	2.5	*3.0*
New × Old	4 hours	-	-	-	-	-	-	-	-	-	-
	6 hours	4.0	5.2	4.5	6.2	5.0	2.0	2.6	1.8	4.2	2.6
	8 hours	5.8	3.1	4.9	4.0	4.4	3.2	2.6	2.1	2.6	2.6
	10 hours	6.6	2.8	5.3	3.4	4.5	3.7	3.1	3.1	2.3	3.0
	12 hours	4.7	4.6	3.1	4.5	4.2	4.0	2.8	4.0	2.3	3.3
Mean (cultivars)		5.3	3.9	4.5	4.5		3.2	2.8	2.7	2.9	

The level 4 hours photoperiod, with zero flowers, was excluded from statistical analysis for accuracy

Table 2b. The least significant difference at 0.05 for the principal factors and their interactions

LSD (0.05)	Age	Photoperiod	Cultivar	A × P	A × C	P × C	A× P×C
Flower stem	NS	1.01	0.75	1.17	1.06	1.51	2.13
Flower diameter	NS	0.70	0.35	0.65	0.50	0.70	0.99

Figure 4. Quality of flowers for yellow with big-flower cultivar (YB) and red with small flower cultivar (RS).

Endogenous plant hormones

Levels of Indole Acetic Acid (IAA), Abscisic Acid (ABA) and Gibberellic Acid (GA_3) in apical buds during flowering at the end of experiment are shown for different conditions in **Table (3)**. Generally, the level of IAA and GA_3 were higher 3-4 times in all plants during flowering period compared to the level of ABA. Old plants contained higher levels of all hormones compared to new plants regardless photoperiod duration or cultivar type, and the greatest difference was in GA_3 level. Among the studied photoperiods, the maximum levels of IAA and GA_3 were found in plants exposed to 10 hours photoperiod. However, ABA level differed slightly under various photoperiods. The yellow cultivars contained higher level of IAA and intermediate level of GA_3 compared to the red cultivars which were similar in IAA level and different in GA_3 level. The level of ABA was approximately stable in the studied cultivars. Old plants exposed to 10 hours photoperiod showed the highest levels of IAA and GA_3 however, the new plants under 4 hours photoperiod showed the least levels. Despite the similar ABA content in new plants under different photoperiods, old plants showed the highest ABA content under 4 hours and the least content under 8 hours. Yellow cultivars showed higher IAA content under both plant ages compared to red ones. The level of GA_3 in old plants was also higher than that in new plants for most cultivars. ABA also showed little variance for the interaction between the plant age and cultivar. Under 10 hours photoperiod, the yellow cultivar with small flowers showed the highest IAA content, and the same cultivar with big flowers showed the highest GA_3 content. The last cultivar also showed the highest ABA content under 6 hour photoperiod. The maximum content of IAA (607 µg/g) was obtained from new plants of yellow cultivar with small flowers under 10 hours photoperiod, and the maximum GA_3 (664 µg/g) content was found in old plants of red cultivar with small flowers under the same photoperiod. However, the maximum content of ABA (202 µg/g) was recorded in new plants of yellow and big flowers under 6 hours photoperiod.

Relationship between Endogenous plant hormones and flowering

Results of flowering were in agreement with those of endogenous hormones specially for IAA and GA_3 **(Table 1 and 3)**. The comparison between the average of total flowering and the endogenous hormones under different factors justified this relationship **(Figure 5)**. Regarding plant age, levels of all hormones were higher in old plants which also produced higher flowers compared to new plants. Among the studied photoperiods, the maximum flowering was obtained with 10 hours photoperiod and the minimum flowering was recorded under 4 and 6 hours. Similarly, the maximum levels of IAA and GA_3 were obtained under 10 hours and the minimum level of GA_3 was found under 4 and 6 hours photoperiods. ABA showed different trend in comparison with flowering and the other two hormones. Levels of IAA and GA_3 were also changed with cultivars in the contrary of ABA **(Figure 5)**. The study of relationships and correlations between flowering and the levels of the three hormones proved that flowering is mostly correlated with IAA and GA_3 levels where they positively correlated with flowering. However, a negative correlation was found between flowering and ABA concentration.

Discussion

The aim of the study was to determine the optimum conditions allowing production of chrysanthemum, short-day plant, at any time throughout the year and identify the endogenous hormones associated with its flowering. Plant age, photoperiod duration and cultivar were found to be very effective factors affecting

Table 3. Endogenous hormones content in the apical buds of chrysanthemum during flowering as affected by plant age, photoperiod and cultivar

Plant age	Photo-period	IAA (µg/g)				Mean (photo-period)	ABA (µg/g)				Mean (photo-period)	GA_3 (µg/g)				Mean (photo-period)
		YS	YB	RS	RB		YS	YB	RS	RB		YS	YB	RS	RB	
New	4 hours	21.3	136.4	0.7	-	52.8	48.6	32.1	34.3	-	38.3	190.6	90.3	260.8	-	180.6
	6 hours	305.6	0.9	74.7	67.3	112.1	17.3	202.0	0.1	41.0	65.1	330.7	221.0	163.5	228.1	235.8
	8 hours	354.3	361.7	24.3	82.3	205.7	60.4	25.6	56.7	19.1	40.5	359.3	144.0	324.5	0.5	207.1
	10hours	607.0	353.0	127.7	1.0	272.2	78.7	36.5	0.0	124.3	59.9	387.0	430.9	122.0	193.8	283.4
	12 hours	216.0	498.7	114.0	-	276.2	43.7	76.6	54.3	-	58.2	266.5	113.5	301.3	-	227.1
Mean (new)		300.8	270.1	68.3	50.2	*172.4*	49.7	74.6	29.1	61.5	*53.7*	306.8	199.9	234.4	140.8	*220.5*
Old	4 hours	-	300.4	36.6	237.7	191.6	-	32.5	177.3	77.2	95.7	-	351.5	508.1	158.4	339.3
	6 hours	195.3	53.7	0.0	14.3	65.8	5.9	104.5	49.0	26.3	46.4	265.9	442.1	470.1	394.7	393.2
	8 hours	297.0	138.0	-	-	217.5	29.0	2.7	-	-	15.9	189.8	392.0	-	-	290.9
	10 hours	423.7	519.7	480.0	308.0	432.9	53.6	35.6	57.9	107.3	63.6	244.0	476.2	663.6	224.0	402.0
	12 hours	0.0	71.0	24.3	-	31.8	58.6	73.5	105.0	-	79.0	187.8	246.5	226.3	-	220.2
Mean (old)		229.0	216.6	135.2	186.7	*191.9*	36.8	49.8	97.3	70.3	*63.5*	221.9	381.7	467.0	259.0	*332.4*
New × Old	4 hours	21.3	218.4	18.7	237.7	122.2	48.6	32.3	105.8	77.2	67.0	190.6	220.9	384.5	158.4	260.0
	6 hours	250.5	27.3	37.4	40.8	89.0	11.6	153.3	24.6	33.7	55.8	298.3	331.6	316.8	311.4	314.5
	8 hours	325.7	249.9	24.3	82.3	211.6	44.7	14.2	56.7	19.1	28.2	274.6	268.0	324.5	0.5	249.0
	10 hours	515.4	436.4	303.9	154.5	352.5	66.2	36.1	29.0	115.8	61.7	315.5	453.6	392.8	208.9	342.7
	12 hours	108.0	284.9	69.2	-	154.0	51.2	75.1	79.7	-	68.6	227.2	180.0	263.8	-	223.7
Mean (cultivars)		244.2	243.4	90.7	128.8		44.4	62.2	59.1	61.4		261.2	290.8	336.5	169.8	

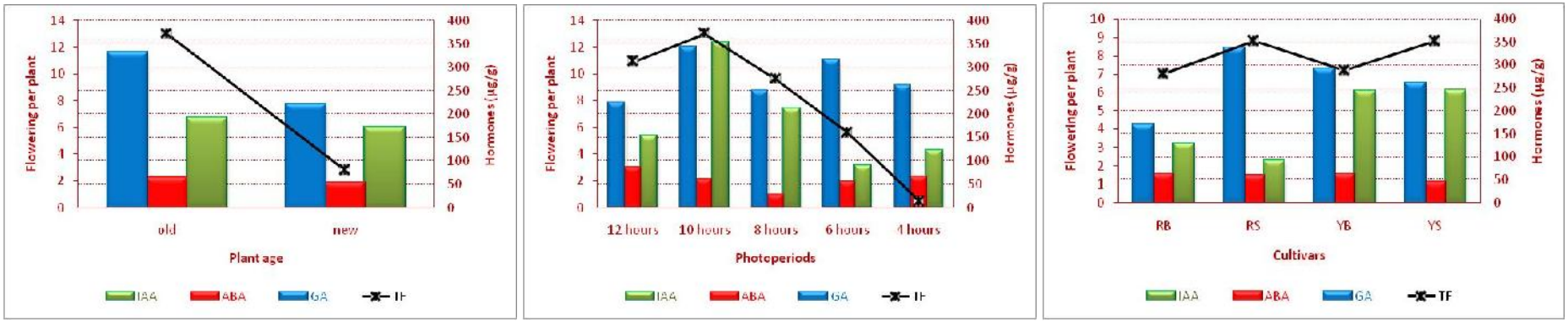

Figure 5. The relationship between the total flowering (TF) and the endogenous hormones content for the three studied factors

quantity and quality of chrysanthemum flowering. Old plants responded better and produced flowers 4-5 times higher than new plants. This result may be related to the early readiness of old plants to flowering and its higher nutrients content absorbed by its developed root system. It is also well known that plants should reach to suitable vegetative growth before flowering stage. The favorable role of plant age was previously reported on other plant species as strawberry **(Verheul *et al.*, 2005)**. Photoperiod duration was vital factor for flowering of chrysanthemum. Despite the nature of chrysanthemum as short-day plant, no flowering was obtained under 4 hours photoperiod. This result could be explained by the inappropriate quantity of light causing deficiency in photosynthesis process and subsequently insufficient carbohydrate content for flowering. Flowering of chrysanthemum increased with increasing photoperiod and reached its maximum at 10 hours then decreased at 12 hours. This result could be related to the critical duration of photoperiod where flowering is affected if it increased or decreased. Our results proved that the optimum photoperiod was 10 hours which is different from that previously reported on chrysanthemum; 8 hours **(Kahar, 2008)**. This difference may be related to the light type or intensity. The reduction of flowering at 12 hours could be also related to the temperature resulted from the long photoperiod causing the increment of respiration and subsequently the consumption of carbohydrate needed for flowering. This result is also in agreement with that previously found by **Kahar (2008**). Our results also proved that flowering of chrysanthemum is cultivar dependent where studied cultivars showed different responses which may be related to the genetic diversity recently proved in chrysanthemum **(Chen *et al.*, 2013)**. Different responses to light and different floral ability has been found among twelve cultivars of chrysanthemum **(Ochiai *et al.*, 2015)**. This could explain the variation among the studied cultivars. It was also observed that cultivars of the same colors gave similar flowering at the beginning but those with small flowers gave higher flowering at the end of experiment. The superiority of small-flower cultivars in flowers production compared to the big-flower cultivars may be related to the lower nutrients requirement needed for the production of small flowers. Our results proved that the best conditions, allowing the greatest flowers quantity, gave similar or better flowers quality compared to the other conditions of low flowers production. The higher quality of flowering with the optimum conditions ensure their efficiency, however, the similar quality of flowering in some cases could be explained by the distribution of nutrients content on many flowers under the optimum conditions compared to few flowers under the other conditions. Good relationship was previously found between flower diameter and light **(Nothnagl and Larsen, 2002)**. The superiority of flower quality in yellow cultivar with small flowers compared to that with big flowers could be also related to its lower nutrient requirements needed for the production of its small flowers. The analysis of endogenous hormones showed high concentrations of IAA and GA_3 during the flowering period. They were also of high concentration in plants giving the best flowering. These two hormones were also correlated positively with flowering. All these indicators proved the role of both hormones in flowering process. The role of GA_3 in the improvement of flowering was previously reported in some plant species **(Sumitomo *et al.*, 2009; Aliyu *et al.*, 2011)**. IAA was also found to be necessary for flowering of other chrysanthemum cultivar and coffee **(Schuch *et al.*, 1994; Jiang *et al.*, 2010**). However, in our study, ABA was almost stable under different conditions or correlated negatively with flowering which exclude its role in flowering. Indeed, this hormone has different roles in the inhibition of vegetative growth and the tolerance of plants to stress. It was also reported that levels of IAA and ABA change in apical and lateral buds according to growth stage and cultivar **(Jiang *et al.*, 2012)**. The studied cultivars differed in their content of hormones which may relate to the genetic variability. The high concentration of IAA and GA_3 in the cultivars of high flower production and the stability in ABA may prove the role of IAA and GA_3 in flowering. Also, the high level of IAA and GA_3 in old plants and under 10 hours photoperiod compared to new plants and 4 hours photoperiod, showing lower level of both hormones and high level of ABA, could justify this hypothesis. The optimum conditions reported in this study could be very beneficial for the commercial production of high quantitative and qualitative flowers of chrysanthemum all the year. The relationships between flowering and endogenous hormones could also help in the understanding of flowering physiology and the improvement of flowering using these hormones.

Conclusion

It can be concluded that plant age, photoperiod and cultivar are effective factors affecting quantity and quality of chrysanthemum flowering. Using old plants after pruning was found to be better than using new plants resulted from cuttings where the first could

produce flowers 4 times higher than the last. Photoperiod duration was vital factor where no flowers produced under 4 hours. Flowers production increased with increasing photoperiod to 10 hours, allowing the maximum flowering, followed by the reduction of flowering under 12 hours. Cultivar was also an important factor affecting flowering where cultivars showed different responses to induction factors. Cultivars of small flowers, whatever their color, produced significantly higher flowers quantity compared to cultivars of big flowers, and the yellow cultivar with small flowers gave the highest flowers production. The optimum conditions allowing the maximum flowering quantity, 20-28 flowers per plant depending on cultivar, were to expose old plants after 1.5 months of pruning to 10 hours photoperiod. These conditions could allow continuous flowering at any time throughout the year. They also allowed the best characteristics of flowering quality expressed as flower stem length and flower diameter. The analysis of endogenous hormones showed that IAA and GA_3 are responsible on flowering while ABA has no role in this process. The concentration of both hormones during flowering was 3-4 times higher compared to that of ABA. Their concentration was also higher under the optimum conditions for flowering, in the contrary to ABA which was stable or lower. The levels of both hormones correlated positively with flowering however, ABA had negative correlation with it. This result could be useful for improving flowering using IAA or GA_3. The whole results could be beneficial on applied level for the commercial production of chrysanthemum and the fundamental level for the understanding flowering process.

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