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Effects of time and duration of short-day treatments under long-day conditions on flowering of a quantitative short-day sunflower (*Helianthus annuus* L.) 'Sunrich Orange'.

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ABSTRACT

The effects of short-day (SD) treatment on differentiation, development and anthesis of flower buds, and on cut flower quality were investigated in a leading cut sunflower
15 (*Helianthus annuus* L.) cultivar, Sunrich Orange, a quantitative SD plant. Plants were cultivated under long-day (LD) conditions with night interruption lighting from 2200 to 200 HR. SD treatment (11.5 h photoperiod) for 1, 2, or 3 weeks was commenced at the cotyledon, two true leaves, or four true leaves stage. The control plants were

grown under LD conditions throughout the experimental period. SD treatment for 1 or 2 weeks promoted flower bud initiation even when delivered at the cotyledon stage (9 days after sowing), indicating that the juvenile phase is very short in this cultivar. Flower differentiation and development were also accelerated by the SD treatment, and 5 resulted in 19-39 days earlier anthesis. The quality of cut flowers was improved by SD treatment to desirable characteristics such as decreased weight, shortened stem and reduced stem diameter. The present study suggests that under LD conditions such as found in summer, SD treatment for 2 weeks from the cotyledon or two true leaves stage can promote LD-delayed flowering of quantitative SD sunflower plants without 10 reducing cut flower quality.

Keywords: Ornamental sunflower, Flowering, Photoperiod

1. Introduction

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Ornamental sunflowers (*Helianthus annuus* L.) are widely cultivated for use as cut flowers, potted plants or in the garden (Blacquiere et al., 2002; Schuster, 1985). As a popular summer flower, the demand for cut sunflowers increases from May to August;

however, most sunflower cultivars are quantitative SD plants in which flowering occurs under any photoperiod, but is accelerated under SD conditions (Pallez et al., 2002). Cut flower production takes a longer period under LD conditions during the summer. As a result, flower quality declines with longer and heavier stems than desirable for cut flowers. The desirable stem length and cut flower weight are approximately 80 cm and 70-100 g, respectively. Although some cultivars are day-neutral and flowering is not affected by photoperiod (Robinson et al., 1967; Schuster, 1985; Vince-Prue, 1975; Yanez et al., 2004; 2005), the number of such cultivars is limited. Therefore, establishing a method to promote flowering of quantitative SD sunflower cultivars under LD conditions is highly desirable.

Tanemura and Kurashima (2004) reported that in 20 of 23 sunflower cultivars used SD treatment beginning at the fourth leaf stage accelerated flowering and reduced stem length; however, these investigators did not examine the effect of the number of SD cycles on flowering and flower quality. Damann and Lyons (1993) studied the effect of the number of inductive LD cycles on flowering with a LD plant *Coreopsis lanceolata*, and demonstrated that limited inductive photoperiod (i.e. LD cycle number) inhibited stem elongation of 'Early Sunrise' plants along with slightly late flowering. In a facultative (or quantitative) SD plant celosia, timing, not but duration , of inductive SD

treatment beginning after the development of 1-5 nodes impacted node number below the terminal inflorescence (Warner, 2009). Delaying the beginning of SD treatment increased node and leaf number below the terminal inflorescence, however, flowering was still accelerated compared with plants grown under constant LD. When photoperiod treatment was started immediately after germination, the duration of SD treatment interacted with timing of SD treatment and cultivar to impact node number below the terminal inflorescence. These results indicate variability in impacts of the number of photoinductive cycles on flowering and reproductive development across photoperiodic plant species, cultivars and developmental stages.

In order to promote earlier flowering and to improve flower quality of qualitative SD sunflower cultivars, we investigated the effects of time and duration of SD treatment on flower bud development and flower quality of sunflower ‘Sunrich Orange’ grown under LD conditions. This cultivar was chosen because it is a quantitative SD plant (Yanez et al., 2005).

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2. Materials and Methods

2.1. Plant material and treatments

All experiments were performed in a greenhouse at Shizuoka University (34°58'N

latitude). Seeds of sunflower ‘Sunrich Orange’ (five seeds per pot) were sown in 15.5 cm-diameter plastic pots (20 cm tall) using Kureha-soil mix (Kureha Corporation, Tokyo, Japan) as a potting medium on October 8, 2003. The medium was supplemented with a controlled release fertilizer, Long 70 (14N-5.2P-11.6K, Asahi Kasei Corporation, Tokyo, Japan) at a rate of 5 g per pot. Two seedlings per pot were selected for uniformity and further cultivated in the greenhouse at 13-30°C (heated at 15°C and ventilated at 25°C) night-day. LD conditions were given as 4-hour night interruption lighting (from 2200 to 200 HR) each day using 75-W incandescent bulbs (K-RD110V75W, Panasonic Corporation, Osaka, Japan). The bulbs were strung overhead (1.8 m above the ground) and provided 2-3 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ photosynthetic photon flux density (PPFD) at the level of 1 m above the pot. Natural light intensity was not recorded. Hyponex liquid fertilizer (20N-17.5P-25K, 200 ppm N, Hyponex Japan Inc. Osaka, Japan) was applied weekly. SD treatment was conducted by transferring plants to SD-conditioned areas in the same greenhouse at the cotyledon, two true leaves, or four true leaves stage. We considered the plants to have reached a leaf stage when the laminae were fully expanded. Blacquiere et al. (2002) reported that the critical photoperiod for sunflower was around 12 h; therefore, the day-length under SD conditions was 11.5 h (530 to 1700 HR) and created by automatically opening and closing photo-protective plastic film. The

duration of SD treatment was 1, 2, or 3 weeks. After the SD treatment, the plants were returned to the LD conditions and were cultivated until flowering. Control plants were grown under the LD conditions described above throughout the experimental period.

5 *2.2. Data collection and statistical analysis*

Shoot tip samples of ten plants per treatment were taken when the second, fourth, sixth, and eighth leaves were fully expanded and were dissected under a binocular microscope to examine flower bud differentiation and development. Based on the method of Marc and Palmer (1981) with slight modification, flower bud development was divided into 9 stages as shown in Table 1. Twenty plants per treatment were used to determine time to visible flower bud (VFB) and days to flower. At flower opening, cut flower quality was evaluated for 10 plants per treatment. Flower opening was defined as when ray florets had expanded perpendicular to the stem attachment. Data taken for quality evaluation involved: stem length from soil surface to calyx attachment; weight of cut flowers harvested at the stem base; stem diameter at 10 cm below calyx attachment; the number of true leaves, node number including cotyledonary one; ray floret number; and capitulum diameter (the longest distance between the tips of opposite ray florets). Data were subjected to an analysis of variance (ANOVA) and

means were compared by Scheffe's multiple-range test.

3. Results

SD treatment promoted flower bud initiation and development (Table 1). In SD
5 treatments for 2 or 3 weeks starting at the cotyledon stage and for 1, 2 or 3 weeks
starting at the 2 true leaves stage, flower bud initiation (dome-shaped apex) was
observed 20 days after sowing in plants with 4 true leaves. In plants exposed to SD for 1
week starting at the cotyledon stage or for 1, 2 or 3 weeks starting at the 4 true leaves
stage, flower bud initiation occurred 24 days after sowing in plants with 6 true leaves.
10 Flower bud initiation of control plants occurred at 28 days after sowing in plants with 8
true leaves. When 8 true leaves had expanded, corolla and anther differentiation (stage
8) was observed in the 2 week SD treatment of plants starting at the cotyledon stage.
The control plants had just started flower bud initiation at the 8 true leaves stage.

In all SD treatments flower bud development and anthesis occurred earlier than
15 in the control plants that were grown under continuous LD conditions (Table 2).
Flower buds became visible 27 days after sowing in the 2-week SD treatment beginning
at the cotyledon stage, or 28 days earlier than for the control plants. Regardless of the
developmental stage at which the SD treatment commenced, flower opening was more

delayed in the 1-week SD treatment than the treatments given for 2 or 3 weeks. The number of days to flower was shortest for 3-week SD-treated plants starting at the cotyledon stage. These plants flowered 39 days earlier than the control. As the length of the SD treatment increased, the number of days to flower decreased.

5 The quality of cut flowers was also affected and improved by SD treatment (Table 3). The stem was shorter with longer durations of SD treatment. The shortest stem length was 73.6 cm in the 3-week SD treatment beginning at the cotyledon stage, followed by 74.8 cm in the 3-week SD treatment beginning at the 2 true leaves stage. In these treatments producing short stems, the days to flower were shortest (Table 2). The weight of cut flowers and stem diameter were reduced to 70 g and 0.7 cm, respectively, with longer duration of SD treatment (Table 3). SD treatment also reduced the numbers of leaves and nodes on the main stem especially when treated for 2 or 3 weeks at the cotyledon stage. The size of capitula tended to decrease with increasing SD duration. SD treatment significantly reduced the number of ray florets from 51.7 for the LD control to 22.2 to 33.5 for the rest of the treatments.

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4. Discussion

Our sunflower plants were responsive to SD treatment for as little as one week at an early developmental stage such as the cotyledon stage (9 days after sowing) that resulted in accelerated flower bud initiation as shown by the reduction in the numbers of leaves and nodes. Similar results were obtained for several other quantitative SD sunflower cultivars such as ‘Sunrich Pine’ and ‘Fire Cracker’ (data not shown). These results indicate that the juvenile phase of sunflower is very short. Only a few days of juvenility period is also described for LD plants *Chenopodium* (Cumming, 1959) and *Brassica campestris* (Friend, 1968). Damann and Lyons (1993) reported for a LD plant *Coreopsis lanceolata* that days to flower from start of LD reached the minimum when transferred to LD at the end of juvenility. In sunflower ‘Sunrich Orange’, the number of days to flower was shortest when SD treatment was applied from the cotyledon stage (Table 2). This also suggests its short juvenility period.

Hayata and Imaizumi (2000) reported ‘Sunrich Orange’ that SDs accelerated both floral induction and all subsequent events such as flower bud differentiation, development and flower opening. In our study, however, earlier flower budding and opening are more likely due to accelerated flower induction, since growing conditions were changed back to LDs after the respective SD treatment. The size of capitula tended to be smaller with SD duration. This result is inconsistent with the results of

Hayata and Imaizumu (2000) and also seems to be attributed to the difference in cultivating conditions after SD treatment.

Our results showed that the duration of SD treatment affected growth and flowering of sunflower more than the stage that SD treatment was applied. The SD treatment was applied as a limited inductive photoperiod (LIP) treatment in that the plants were transferred back to LD until flowering after the treatment had been given. In a different study, when the number of LD inductive cycles applied as a limited inductive photoperiod (LIP) treatment increased, days to flower decreased and stem length increased for *Coreopsis lanceolata* (Damann and Lyons, 1993). In the present study using sunflower 'Sunrich Orange', however, as the number of SD inductive cycles applied as a LIP treatment increased, days to flower decreased as in *Coreopsis*, but stem elongation decreased unlike in *Coreopsis*. In addition, weight, stem and capitulum diameter, and leaf and node number also decreased with an increase in the number of SD inductive cycles. A similar decrease in node number was also found in celosia exposed to inductive SD beginning immediately after germination (Warner, 2009).

Considering the small differences in the results of 2- versus 3-week SD treatments and labor for cultural practice, we conclude that SD treatment for 2 weeks beginning at

the cotyledon or 2 true leaves stage reduced the number of days to flower without affecting cut flower quality. Similar experiments simulating actual summer cultivation were conducted from June and August with similar results to those described above, except that SD treatment starting from the 2 or 4 true leaves stage
5 was more practical than starting at the cotyledon stage (data not shown).

Conclusion

The present study suggests that under LD conditions such as found in summer, SD treatment for 2 weeks beginning at the cotyledon or two true leaves stage can promote
10 earlier flowering of quantitative SD sunflower plants without reducing the quality of cut flowers.

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Highlights

- SD treatment promoted flower bud initiation of quantitative SD sunflower plants.
- The juvenile phase was very short.
- Flower bud differentiation and development were also accelerated by SD treatment.
- SD treatment for 2 weeks from the cotyledon or two true leaves stage was desirable.
- The SD treatment promoted LD-delayed flowering without reducing cut flower quality.

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Table 1

Effects of time and duration of short-day (SD) treatment on time to flower bud initiation and the number of plants found at a particular stage of flower bud development of sunflower 'Sunrich Orange'^a

SD treatment		First observance of flower bud initiation		Flower bud developmental stage ^c								
Time (stage) ^b	Duration (weeks)	Days after sowing	Number of true leaves	0	1	2	3	4	5	6	7	8
Control	0	28	8	6	3	1						
Cotyledon	1	24	6			1	2	6	1			
	2	20	4								1	9
	3	20	4							1	2	7
2 leaves	1	20	4					2	3	5		
	2	20	4							1	6	3
	3	20	4							1	6	3
4 leaves	1	24	6				4	5	1			
	2	24	6				8	2				
	3	24	6				8	2				

^aSown on October 8, 2003 and grown under greenhouse conditions.

^bTime (Developmental stage) when SD treatment was started.

^cData were taken for 10 plants when 8 true leaves had fully expanded. Based on Marc and Palmer (1981), flower bud developmental stages are defined with slight modification as follows. 0: Undifferentiated; 1: Apex dome-shaped (flower bud initiation) ; 2: involucre bract primordia at the flanks; 3: Numerous involucre bract primordia; 4: Formation of a flat disk with elevated rim at periphery; 5: Several rows of floret primordia; 6: Floret primordia occupy about 1/2 of radius of receptacle; 7: Differentiation of 5-lobed corolla; 8: Disc florets

Table 2

Effects of time and duration of short-day (SD) treatment on time to visible flower bud (VFB) and days to flower (FL) of sunflower 'Sunrich Orange'^a

SD treatment		Days to VFB	Days to FL
Time (Stage) ^b	Duration		
Control	0	54.4a ^c	98.6a
Cotyledon	1	33.0cde	75.7bc
	2	26.7f	66.4de
	3	27.3f	59.9f
2 leaves	1	34.3be	77.1b
	2	30.0def	68.5d
	3	29.3ef	60.5ef
4 leaves	1	37.2b	79.5b
	2	33.9bcd	70.4cd
	3	34.0bc	65.8def
Significance ^d			
	Time	**	**
	Duration	**	**
	Time x Duration	**	**

^aSown on October 8 and grown under greenhouse conditions.

^bTime (Developmental stage) when SD treatment was started.

^cValues in each column followed by the same letter are not significant at $P < 0.05$ by Scheffe's multiple range test.

^d**; Significant at $p < 0.01$ by ANOVA.

Table 3

Effects of time and duration of short-day (SD) treatment on growth and flowering characteristics of sunflower 'Sunrich Or

SD treatment		Stem length (cm)	Weight (g)	Stem diameter (cm)	No. of leaves	No. of nodes	Capitulum diameter	No. of ray florets
Time (Stage) ^b	Duration (Weeks)							
Control	0	154.4a ^c	459.1a	1.25ab	29.1a	26.6a	18.4a	51.7a
Cotyledon	1	119.9b	248.3b	1.09ab	18.2bc	15.1bc	14.5b	33.5b
	2	95.1d	132.9d	0.78d	14.6d	11.7d	9.9de	22.2d
	3	73.6e	67.9e	0.69d	13.5d	10.9d	9.1e	22.3d
2 leaves	1	115.6bc	202.9bc	0.81cd	15.9cd	13.3cd	12.9bc	26.6cd
	2	88.1de	118.3de	0.80cd	15.8cd	12.7cd	9.4e	23.5d
	3	74.8e	74.1e	0.68d	14.8d	12.1cd	9.5e	25.0d
4 leaves	1	124.9b	259.6b	0.91bc	20.2b	17.3b	12.4bcd	31.1bc
	2	99.6cd	154.0cd	0.86c	20.1b	17.2b	10.0de	31.3bc
	3	86.3de	101.1de	0.69cd	20.1b	17.1b	10.1cde	31.3bc
Significance ^d								
Time		**	**	**	**	**	**	**
Duration		**	**	*	**	**	NS	**
Time x Duration		NS	NS	**	**	**	*	**

^aSown on October 8, 2003 and grown under greenhouse conditions.^bTime (Developmental stage) when SD treatment was started.^cValues in each column followed by the same letter are not significant at $P < 0.05$ by Scheffe's multiple range test.^dNS, *, **; Nonsignificant, significant at $p < 0.05$ or 0.01, respectively by ANOVA.