AN APPRAISAL OF THE USE OF RECIPROCAL TRANSFER EXPERIMENTS: ASSESSING THE STAGES OF PHOTOPERIOD SENSITIVITY IN *ANTIRRHINUM MAJUS* L.

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Abstract

A new model to analyse reciprocal transfer experiments to assess stages of photoperiod sensitivity in *Antirrhinum* has been validated in the present study. Flowering time and leaf numbers data of *Antirrhinum* cultivars Chimes White, Liberty White, Ariane, Winter Euro Rose, Sonnet and Rocket Orchid were used for the validation of the model. Six plants of each cultivar were transferred from LD to SD and *Vice versa* at four days interval from emergence until first flower appearance. Plants at juvenile phase (initial phase of development) were insensitive to photoperiod in both inductive (LD) and non-inductive (SD) environment. After juvenile phase when plants were transferred from LD to SD, they recognised the stimulus under inductive environment and induced flowering. However, plants transferred from non-inductive environment to inductive showed a continuous phase of photosensitivity. Rate of flower development was less sensitive to photoperiod. The duration of photoperiod sensitive phases varied with the cultivars. Hence, it is concluded that Antirrhinum cultivars are not sensitive to photoperiod during their entire course of growth and development which is mere wastage of energy. These cultivars require 4-8 days of photoperiod at critical phase to flower that will minimise the production cost of cut flower industry.

Introduction

The control of flowering is still relatively poorly understood despite its importance both physiologically and as a major regulator of fruit and seed yield in agriculture and horticulture. Among the environmental influences, light is one of the most important factors determining the time and magnitude of flowering as well as the rate and position of flower development. The light climate itself is complex, fluctuating as it does diurnally or seasonally in intensity, quality and duration (Thomas & Vince-Prue, 1997). A significant research has been carried out on the control of flowering in *Antirrhinum*, which is commonly grown as bedding and cut flower plant. Early work showed a promotion of flowering in *Antirrhinum* by photoperiod extension, but this was usually at the expense of flower size or stem length (Laurie & Poesch, 1932; Post & Weddle, 1940). *Antirrhinum* emerged as a facultative long day plant (LDP); SD's delaying flowering (Haney, 1953; Flint, 1958; Buchanan, 1984; Cockshull, 1985). A 28 days earlier flowering was obtained in cultivar Jackpot by extending 18h day length (Maginnes & Langhans, 1960) Similarly, Cremer *et al*., (1998) observed that increased photoperiod reduced flowering time and leaf number in Sippe-50 and S-412 inbreds.

It has been envisaged that *Antirrhinum* does not require photoperiod for the whole flower developmental phase. Flowering is greatly influenced when photoperiod is provided during the 'critical phase' (Langhans & Maginnes, 1962). Plant physiologists believed that many plants have very distinct photo-sensitive/insensitive phases during their development. These phases can be determined using reciprocal transfer technique

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between inductive and non-inductive photoperiods at regular intervals until the opening of first flower (Robert *et al.*, 1986). Many researchers used this technique in plants like maize, barley, soybean, *Arabidopsis*, opium poppy, chrysanthemum and petunia (Kiniry *et al.*, 1983; Roberts *et al.*, 1988; Wilkerson *et al.*, 1989; Mozley & Thomas, 1995; Wang *et al.*, 1997; Adams *et al.*, 1998, 1999). Like other seed-raised crops, many Antirrhinum cultivars also showed distinct phases of photoperiod sensitivity (Adams *et al.*, 2003). To analyse the complex data obtained from reciprocal transfer experiment Ellis *et al.*, (1992) and Adams *et al.*, (1999) introduced analytical approaches. In these approaches only flowering time data is used to quantify photoperiod sensitive phases. However, Adams *et al.,* (2003) modify these approaches and introduced a novel model, which can quantify photoperiod sensitive phases using flowering time and leaf numbers. This approach is further validated in the present study on some contrasting Antirrhinum cultivars (early, mid and late) at a four days regular interval from LD to SD and *vice versa*.

Ellis *et al.*, (1992) introduced a novel technique to quantify flower development phases using four parameters; a_1 (the photoperiod-insensitive pre-inductive phase), I_S and I_L (the photoperiod-sensitive inductive phase in SD and LD), and a_3 (the photoperiod-insensitive post-inductive phase in LD and SD) in a reciprocal transfer experiments. The analytical approach presented by Ellis *et al.*, (1992) assumes that at the end of the photoperiod-insensitive, pre-inductive (juvenile) phase an immediate change in time to flowering will be seen in plants transferred from both inductive to non-inductive and non-inductive to inductive conditions for flowering. However, this method does not consider any time lag from the onset of photoperiod sensitivity before an effect of photoperiod on the time to flowering can be observed in plants transferred from an inductive to a non-inductive environment (Adams *et al.*, 1998). The duration of this phase, which can be determined provided a short transfer interval is used, coincides with the number of inductive cycles needed for flower commitment. It has also been reported in 'Opium Poppy' (a LDP) that minimum number of inductive cycles for flowering can be separated from the duration of the juvenile phase (Wang *et al.*, 1997). Therefore, the analytical approach presented by Ellis *et al.*, (1992) can confound the effects of juvenility with the number of inductive cycles required for flower commitment after plants become sensitive to photoperiod, and so may be inaccurate where a large number of cycles are required for flower commitment. Therefore, Adams *et al.*, (1999, 2001) separated the effects of photoperiod on flower induction and development, hence re-labelled the photoperiod sensitive phase in LD (I_L) as $P_I + P_d$ (photoperiod sensitive flower induction (P_I) and flower development (P_d) phases). These models analyse only flowering time data, however, Adams *et al.*, (2003) have developed a new model (Fig. 1A,B) which analyse flowering time and leaf number simultaneously and quantify both time to flowering and leaf number at various photoperiod-sensitive and insensitive phases (Adams *et al.*, 2003) which is further validated in the present study for its appraisal.

Materials and Methods

Reciprocal transfers between LD and SD were carried out to quantify photoperiod sensitive and insensitive phases of flower development. Seeds of six *Antirrhinum* cultivars were obtained from three seed companies viz., Colegrave Seeds Ltd., UK, (Chimes White and Liberty White); Walz Samen GmbH, Germany, (Sonnet and Ariane) and PanAmerican Seed Co., USA, (Winter Euro Rose and Rocket Orchid). Seeds were sown into P40 plug-trays (volume per cell 55ml; LBS, Horticulture, U.K.) containing peat-based modular compost (William Sinclair Horticulture Ltd., U.K.). Seeds were then germinated in a controlled-environment growth room at $20 \pm 2°C$ providing lighting using a mixture of warm white fluorescent and tungsten bulbs (6.3% tungsten calculated by nominal wattage) 72μmol m^{-2} s^{-1} (PPF) at plant height with a 16 hour photoperiod.

A

B
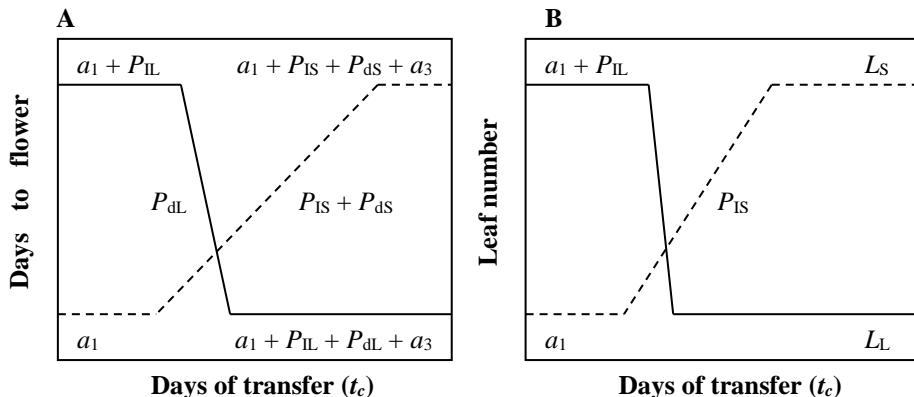
Fig. 1. Schematic representation (not to scale) of the model for a LDP transferred from LD to SD (———) and from SD to LD (--------) at regular intervals from seedling emergence to first flowering. The response of the plants being described by five developmental phases, a photoperiod-insensitive juvenile phase (a_1), photoperiod-sensitive flower induction (P_{IL}) and flower development (P_{dL}) phases in LD, a photoperiod-sensitive phase for flowering in SD (P_{IS}) and a photoperiod-insensitive flower development (a_3) phase (from Adams *et al.*, 2003).

After 75% seed germination (10 d of seed sowing), the seedlings were transferred to either 8 or 17h.d^{-1} photoperiod chambers within a glasshouse compartment. At 08:00h they were moved out into the glasshouse (7.3m × 11.3m) at a set point temperature of 20°C. Ventilation occurred automatically 3°C above the set point temperature. Plants remained here until 16:00h under natural day light. At 16:00h each day, all plants were moved into the respective photoperiod chambers where they remained until 08:00h the following morning. Photoperiod within each of the chambers was extended by three 60W tungsten light bulbs (60% tungsten calculated by nominal wattage) and two 36W white fluorescent tube lights, which provided a light intensity (PPF) of 5μmol m^{-2} s^{-1} (60:40). In all chambers the lights were switched on at 16:00h for a duration dependents on the desired photoperiod (8 and 17h.d^{-1}). Night temperature in photoperiod controlled compartments was set at 20 ± 2°C. Air conditioning units inside the photoperiod chambers switched on whenever night temperature exceeded above 20°C. In the glasshouse and photoperiod compartments K type thermocouples were connected to a Campbell CR10 (Campbell Scientific Inc, U.K.) data logger to record temperature after every 15sec and stored the hourly average. Tube solarimeters (in house manufacture, Szeicz *et al.,* 1964) were positioned three meters above the ground to measure the light transmission into the glasshouse. Hence, the plants spent 8 hours in the glasshouse and 16 hours in the photoperiod chambers at approximately 20°C diurnal temperature.

Six plants were transferred from SD to LD and *vice versa* on every fourth day from emergence until first flower appearance. Twenty plants were maintained in continuous LD and SD for the duration of the experiment as control. Plants were potted into 9 cm pot (volume 0.37 L) containing a 3:1 (v/v) mixture of a peat based potting compost and perlite (William Sinclair Horticulture Ltd., U.K.) at visible flower bud stage. Plants were initially fed twice weekly with a soluble fertilizer, Sangral 1:1:1 @ 0.9 g L^{-1} (William Sinclair Horticulture Ltd., U.K.) at a conductivity of 1500 to 1600μS.cm^{-2} (182ppm N; 78ppm P; 150ppm K), at pH 5.7 to 5.8. To avoid *Pythium*, water was applied manually after every two or three days as required.

Plants in each treatment were observed daily until flower opening (corolla fully opened). The number of days from the date of transfer to the glasshouse to flower opening was recorded and the leaf numbers below the inflorescence were counted. Data were analysed using the FITNONLINEAR sub-routine of GenStat-8 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK.).

Results

Plants grown under continuous LD flowered earlier than those grown under continuous SD. However, time taken to flower varied with the cultivars such as Chimes White, Liberty White, Ariane, Winter Euro Rose, Sonnet and Rocket Orchid flowered 22, 29, 30, 29, 42 and 56 days earlier in LD respectively. Similarly, plants grown in LD produced almost half number of leaves as compared to SD grown plants (Table 1).

In both environmental conditions (SD or LD) plants were insensitive to photoperiod when they were in juvenile phase (a_1) of their development and hence failed to affect flowering time or leaf number (Table 2 and Fig. 2 and 3). Similarly, photoperiod did not affect flowering time when plants were transferred from LD to SD or *vice versa* during the juvenile phase. After the completion of juvenile phase, SD delayed flowering time and increased leaf number whereas LD induced flowering. This could be easily observed in all cultivars when plants were transferred from LD to SD (P_{IL}) and from SD to LD (P_{IS} + P_{dS}). Early flowering cultivar Chimes White took minimum time (4 days) to induction (P_{IL}) whereas others took 9 to 20 days to complete this phase. Plants when transferred from SD to LD (P_{IS} + P_{dS}) after juvenile phase showed a sensitive phase of development by hastening of flowering and decreasing leaf number. Similarly, after the completion of juvenile phase in SD, plants became competent when transferred to LD (P_{dL} – photoperiod-sensitive phase). This inductive environment suddenly induced flowering and did not increase leaf numbers. After completing P_{dL} and P_{IS} + P_{dS} photoperiod-sensitive phases, plants entered into last phase of flower development (a_3 – photoperiod-insensitive phase). At this phase no significant effect of inductive and non-inductive environment was observed regarding flowering time and leaf number.

Table 1. The effects of long days and short days on the number of days to flower and the number of leaves below the inflorescence of six *Antirrhinum* cultivars.

Cultivar	Days to flower			Leaves below the inflorescence	
	LD	**SD**		**LD**	**SD**
Chimes white	61.60 (± 0.34)	83.80 (± 0.30)		10.07 (± 0.21)	18.27 (± 0.21)
Liberty white	81.47 (± 0.42)	111.47 (± 0.40)		21.87 (± 0.38)	39.87 (± 0.26)
Ariane	75.87 (± 0.31)	105.80 (± 0.30)		18.00 (± 0.20)	37.33 (± 0.30)
Winter euro rose	77.47 (± 0.35)	107.20 (± 0.41)		19.33 (± 0.23)	38.93 (± 0.25)
Sonnet	85.60 (± 0.29)	128.20 (± 0.37)		20.20 (± 0.20)	46.07 (± 0.25)
Rocket orchid	88.80 (± 0.52)	142.20 (± 0.61)		25.80 (± 0.22)	52.20 (± 0.41)

Standard errors of means are shown in parenthesis.

Table 2. Cultivar differences in the duration (days) of the phases of sensitivity to photoperiod.

Cultivar	a_1	P_{IL}	P_{IS}	a_3	L_L	L_S	r^2
Chimes white	15.54 (± 0.49)	3.71 (± 0.49)	24.88 (± 0.62)	41.59 (± 0.19)	10.24 (± 0.07)	18.23 (± 0.09)	0.99
Liberty white	25.74 (± 0.60)	13.54 (± 0.60)	42.72 (± 0.75)	41.68 (± 0.21)	22.32 (± 0.11)	39.87 (± 0.13)	0.99
Ariane	26.31 (± 0.59)	8.82 (± 0.59)	37.88 (± 0.77)	39.74 (± 0.23)	18.41 (± 0.12)	36.19 (± 0.51)	0.99
Winter euro rose	30.90 (± 1.30)	9.04 (± 1.30)	37.16 (± 1.67)	38.36 (± 0.50)	20.82 (± 0.27)	38.68 (± 0.33)	0.98
Sonnet	35.84 (± 1.02)	11.23 (± 1.06)	51.38 (± 1.42)	37.17 (± 0.47)	20.50 (± 0.24)	44.74 (± 0.30)	0.98
Rocket orchid	31.71 (± 1.09)	20.18 (± 1.09)	71.25 (± 1.48)	37.81 (± 0.44)	26.66 (± 0.25)	52.49 (± 0.29)	0.99

Standard errors of means (in parenthesis) derived from the FITNONLINEAR analysis of GenStat. The model was fitted with P_{dL} and P_{dS} set to a nominal 1 day duration.

Discussion

All cultivars of *Antirrhinum* flowered in both inductive and non-inductive environments. However, long days hastened flowering because of facultative LD nature of *Antirrhinum* as it evolved in the Mediterranean region (Hedley, 1974; Hedley & Harvey, 1975; Cockshull, 1985). Early flowering cultivar Chimes White flowered 22 days earlier whereas mid flowering cultivars such as Liberty White, Ariane and Winter Euro Rose flowered 29-30 days earlier. Similarly, late flowering cultivars such as Sonnet and Rocket Orchid flowered 42 and 56 days earlier, respectively in LD environment (Maginnes & Langhans, 1961, 1967). The commercial benefits of these findings could be obtained from the use of day extension at particular time when plant is fully committed to perceive the stimulus. Otherwise extending day length when it does not require by the plant will be mere waste of resources. Langhans & Maginnes (1962) already reported that *Antirrhinum* is sensitive to photoperiod during the 'critical phase' (at the 5-10 leaf pair stage of development) and LD given before the 'critical phase' did not promote flowering.

To quantify the flower development phases, Ellis *et al.,* (1992) adopted reciprocal transfer approach. Many scientists applied Ellis's model in their studies using flowering time data only (Collinson *et al*., 1992; Adams *et al*., 1999). However, in 2003 Adams and co-workers modified it and introduced a novel model using flowering time and leaf number data simultaneously (see Introduction). Flowering time and leaf number data of present investigation were successfully fitted in the new model (Fig. 2 and 3).

Present results clearly distinguished five phases of flower development in *Antirrhinum*. Plants of *Antirrhinum* cultivars were incapable to perceive the photoperiod signal during photoperiod-insensitive juvenile phase (a_1) hence unable to ripe/flower (Thomas & Vince-Prue, 1997). The reason why a plant in juvenile phase is unable to recognise the signal could be the low rate of assimilates partitioning, as during this phase carbohydrates are produced by the leaves, stored and may later play a role in flowering times (Thomas & Vince-Prue, 1997). The other possible reason could be that a minimum plant size is necessary before a plant is able to flower (Hackett, 1980). In *Antirrhinum* it has been

A. Chimes white
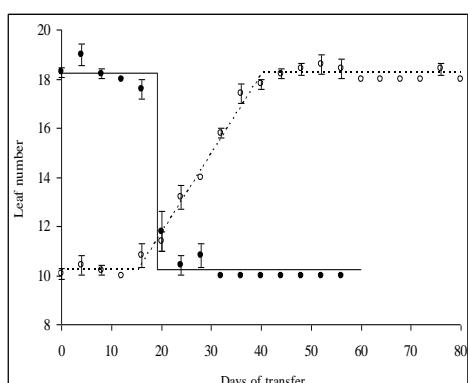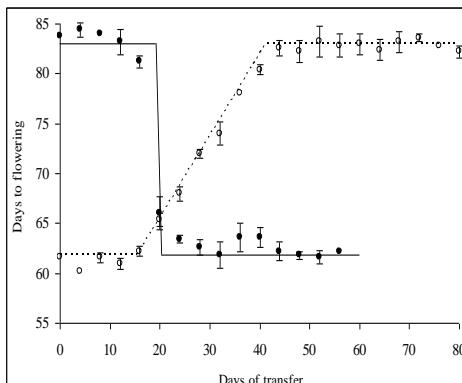

B. Liberty white
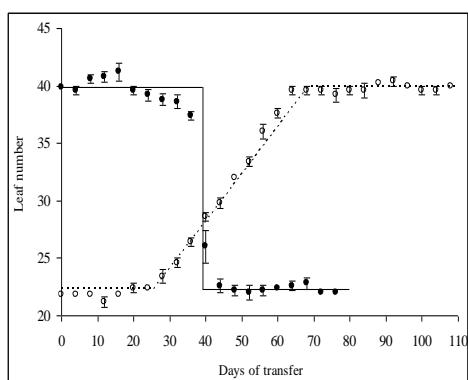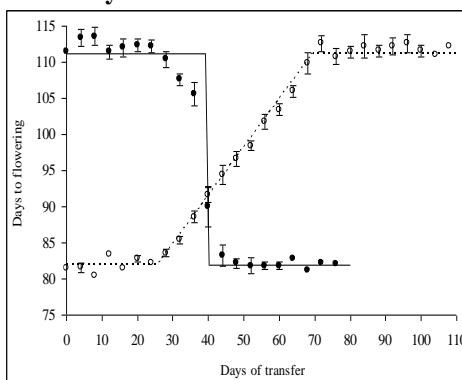
C. Ariane
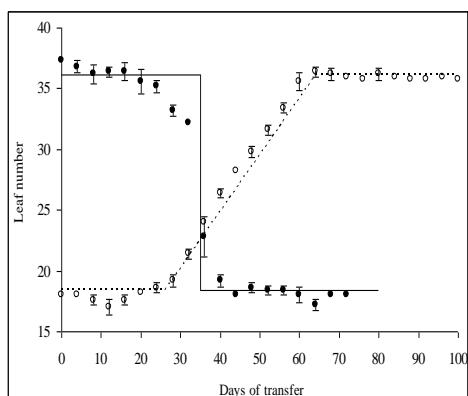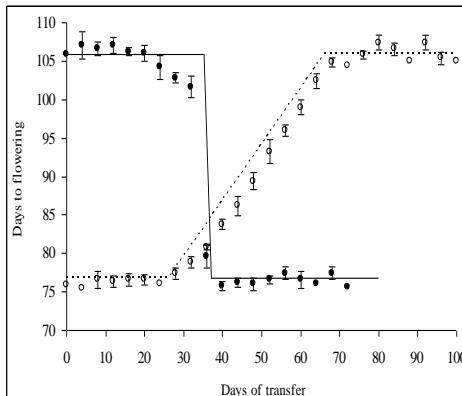
Fig. 2. The effect of transferring *Antirrhinum* cultivars Chimes White (A), Liberty White (B), Ariane (C) at 4 days interval from LD to SD (●) and from SD to LD (○) on the time to first flower opening and the number of leaves below the inflorescence. Vertical bars represent standard errors of the means. Solid and broken lines show the fitted relationship (see Table 2 for parameter estimates) for plants transferred from LD to SD and from SD to LD, respectively.

A. Winter euro rose
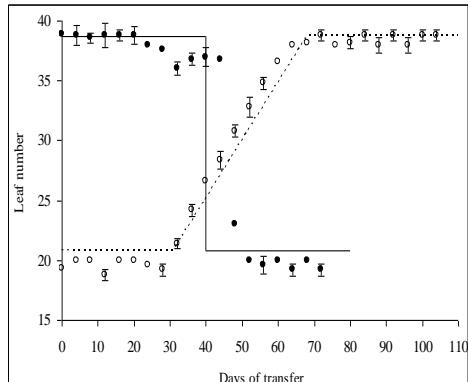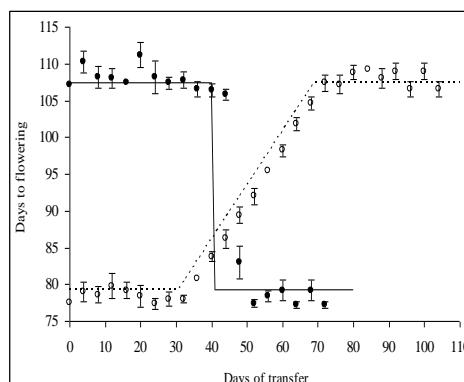
## B. Sonnet
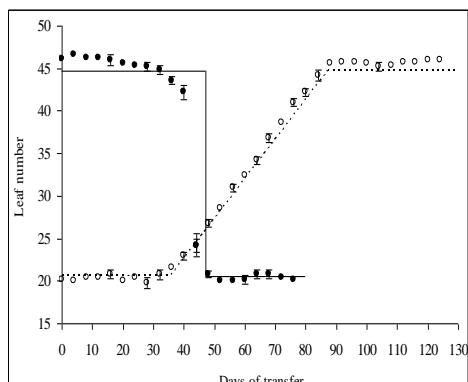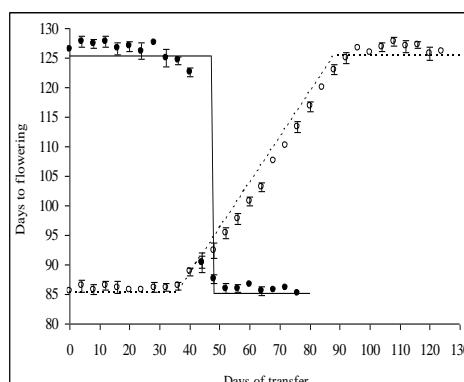
C. Rocket orchid
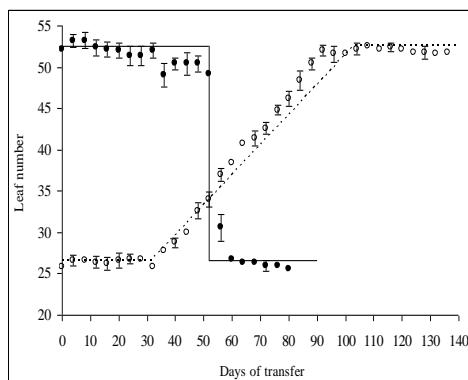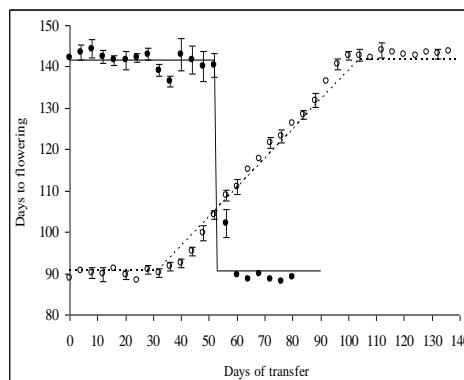
Fig. 3. The effect of transferring *Antirrhinum* cultivars Winter Euro Rose (A), Sonnet (B) and Rocket Orchid (C) at 4 days interval from LD to SD (●) and from SD to LD (○) on the time to first flower opening and the number of leaves below the inflorescence. Vertical bars represent standard errors of the means. Solid and broken lines show the fitted relationship (see Table 2 for parameter estimates) for plants transferred from LD to SD and from SD to LD, respectively.

reported that a particular plant size of around 5-10 pairs of leaves is necessary before the plant becomes sensitive to photoperiod (Langhans & Maginnes, 1962). This means that prior to reach this size, plants will remain juvenile. The alternate view is that the apical meristem behaves independently and separately undergoes the transition from the juvenile phase (Robinson & Wareing, 1969). Endogenous gibberellins (GA) on the other hand, may play a role in this phase transition and their action may be controlled by photoperiod (Pharis *et al.*, 1976). GA is thought to function as a juvenile hormone in many plants and photoperiod may affect its synthesis, which ultimately may prolong the juvenile phase (Poethig, 1990). The role of GA however, is very complex and can either inhibit or promote flowering (Thomas & Vince-Prue, 1997). However, after juvenility plants transferred from LD to SD or *vice versa* perceived the signal and switched on the inflorescence and floral genes consecutively. Three phases of flower development were determined after juvenile phase. These are photoperiod-sensitive flower induction (P_{IL}) and flower development (P_{dL}) phases in LD, a photoperiod-sensitive phase for flowering in SD (P_{IS}). After these photoperiod-sensitive phases plants again entered into a photoperiod-insensitive flower development (a_3) phase. During former three photoperiod-sensitive phases plants required few inductive cycles (4 days) to induce flowering. In other words, plants became competent to respond to the developmental signal when exposed to sufficient LD. Bradley *et al.*, (1996) have reported that the floral meristem gene *flo* can be induced by a single LD pulse. The induction of *flo* is discrete, with only the young nodes at the apex responding, suggesting that there are few competent leaf primordia and axillary meristems. Older nodes do not respond to the inductive stimulus suggesting that the action of *flo* is limited to those leaves most recently produced, presumably as a result of these having passed through the juvenile phase. This may explains why the plants in SD developed the ability to respond to the transfer to LD promptly.

Conclusion

Reciprocal transfer experiments approach is a powerful tool to understand how photo-thermal environment affects the flowering process. Using this technique and new analytical approach most sensitive flower developmental phases have been quantified, which were ignored in some previous studies as many investigations have concentrated on flower induction, the biochemical changes and genetics of *Antirrhinum*. It is emerged from the present piece of work that *Antirrhinum* cultivars required only 4-8 LD during critical phase and extending day length before and after this phase is mere wastage of resources. Therefore, the ornamental industry growing *Antirrhinum* can significantly reduce its energy cost and is able to maintain the supply of this crop in the market at proper time year-round production.

Acknowledgements

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