AN APPRAISAL OF THE USE OF RECIPROCAL TRANSFER EXPERIMENTS: ASSESSING THE STAGES OF PHOTOPERIOD SENSITIVITY IN PANSY, SNAPDRAGON, PETUNIA AND COSMOS

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Abstract

To assess photoperiod sensitive/insensitive phases of 3 long day plants (LDPs) viz., Pansy cv. Baby Bingo, Snapdragon cv. Coronette and Petunia cv. Dreams and one short days plant (SDP) viz., Cosmos cv. Sonata Pink a non-linear statistical model was used for its validation. 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 for LDPs and SD for SDP) and non-inductive (SD for LDPs and LD for SDP) environment. After completing the juvenile phase when plants were transferred from LD to SD (Pansy, Snapdragon and Petunia) and SD to LD (Cosmos), they showed a coherent recognition of the stimulus carry forward from their respective inductive environment and induced flowering. However, plants transferred from non-inductive environment to inductive showed a continuous phase of photosensitivity. The duration of photoperiod sensitive phases varied with the cultivars. Hence, it is concluded that LDPs and SDP are not sensitive to photoperiod during their entire course of growth and development. Therefore, providing light during whole growing span is mere wastage of energy. These cultivars require 5-10 days of photoperiod at critical phase to flower that will minimize the production cost of cut flower industry.

Introduction

All plants go through definite photoperiod sensitive and insensitive phases during their development. The duration of these phases can be determined using reciprocal transfers where plants are moved between inductive and non-inductive photoperiods at regular intervals until flower opening (Roberts et al., 1986; Adams et al., 2003). Many researchers have used this technique with a range of plants including poppy (Wang et al., 1997), chrysanthemum (Adams et al., 1998a), petunia (Adams et al., 1999) and Antirrhinum (Adams et al., 2003; Munir et al., 2010). Studies have shown that the early (juvenile) and late (flower development) phases of growth are insensitive to photoperiod (Collinson et al., 1992; Ellis et al., 1992). For example, most cultivars of Antirrhinum showed sensitivity to photoperiod after 40-65 days of germination or 5-10 leaf pair stage of development (Langhans & Maginnes, 1962). However, Adams et al., (2003) and Munir et al., (2010) using reciprocal transfer experiment tool reported that the critical phase of photoperiod sensitivity/insensitivity varies among cultivars i.e., dwarf or early flowering cultivars have short duration of juvenile phase hence become more responsive after minimum number of days (16 days in cv. Chimes White). Long days given before this critical phase did not promote flowering and mere wastage of resources. After completion of this phase, plants entered into a long phase of photoperiod sensitivity under non-inductive short day environment such as 25 days in Chimes White. Final phase of development is believed as photoperiod insensitive where reserved food is used for the development of floral parts.

Flowering in other annuals such as petunias (Baloch et al., 2009b) and pansy (Adams et al., 1997) is also hastened by long days (LD), but little is known about when the plants are most sensitive to photoperiod. An attempt was made previously to investigate the effect of light integrals and temperature on time to flowering of petunia by Adams et al., (1999) using reciprocal transfer experiments between long (16h) and short days (8h). The length of photoperiod insensitive juvenile phase of development was sensitive to light integrals (low light integrals prolonged this phase from 23 days at 2±6 MJ m-2 day-1 t o 36 days at MJ m-2 day-1). The length of this development phase was shortest (12±5 days) at 21°C; it was longer under cooler (21 days at 13±5°C) and warmer temperatures (17±6 days at 28±3°C). After this phase, time to flowering was influenced greatly by photoperiod as long days hasten flowering compared with short days.

Reciprocal transfer experimental approach gave valuable information on the phases of sensitivity to photothermal environment during the flowering process, and could provide the basis of a more physiologically-based quantitative model of flowering. The information is also seemed useful in the scheduling of lighting treatments to give optimal flowering times of high quality plants (Ellis et al., 1992; Adams et al., 2003). In our experiments, the effects of ambient day length (Baloch et al., 2009a), photoperiod (Baloch et al., 2009b) and light intensity (Baloch et al., 2009c) on flowering time were determined. Results obtained from these experiments illustrate how environmental factors affect flowering process but these experiments did not show whether any of the annual ornamental is sensitive or insensitive to photoperiod during their development. In present study a reciprocal transfer experiment was designed to examine photoperiod sensitivity in 3 LDPs (Pansy, Petunia, Snapdragon) and one SDP (Cosmos) using a novel statistical model (Adams et al., 2003; Munir et al., 2010).

Materials and Methods

This piece of work was carried out at Agricultural Research Institute, Dera Ismail Khan, Pakistan during the
year 2007. Seeds of Pansy cv. Baby Bingo, Snapdragon cv. Coronette, Petunia cv. Dreams and Cosmos cv. Sonata Pink were sown on 15th of June 2006 into seed trays which were kept at room temperature at night and they were moved out during the day (08:00–16:00 h) under partially shaded area. After 70% seed germination, plants were potted into 9cm pots containing leaf mould compost and river sand (3:1 v/v). These seedlings were then transferred to the LD (17 h.d⁻¹) and SD (8 h.d⁻¹) photoperiods chambers. Plants remained for 8h (from 08:00 to 16:00h) in the field (outside the photoperiod chambers) where they were exposed to natural daylight and temperature (Table 1). At 16:00h each day, all plants were moved into the photoperiod chambers where they remained until 08:00h the following morning. Photoperiod within each of the chambers was extended by two 60Watt tungsten light bulbs and one 18Watt warm white florescent long-life bulb (Philips, Holland) fixed above 1m high from the trolleys providing a light intensity (PPFD) of 7µmol.m⁻².s⁻¹. In all photoperiod chambers, the lamps were switched on automatically at 1600 h for a duration dependents on the day length required. These chambers were continuously ventilated with the help of micro exhaust fan (Fan-0051, SUPERMICRO® USA) with an average air speed of 0.2m.s⁻¹ over the plants when inside the chambers, to minimize any temperature increase due to heat from the lamps. Temperature and solar radiation were measured in the weather station situated one kilometre away from the research venue. Temperature was recorded with the help of Hygrothermograph (Nova Lynx Corporation, USA) while solar radiation was estimated using solarimeters (Casella Measurement, UK). Six plants were reciprocally transferred from LD to SD and vice versa on every fourth day from emergence until the appearance of first flower whereas 20 plants were kept as controls in either chamber. Plants were regularly watered by hand and a nutrient solution [(Premium Liquid Plant Food and Fertilizer (NPK: 8-8-8); Nelson Products Inc. USA)] was applied twice a week. Plants were observed daily until the end of experiment. Time to flowering (corolla fully opened) from emergence was counted. The analytical approach applied has been described in ‘Introduction’ (Adams et al., 2003). Data were analyzed using the regression statistical technique of GenStat-8 (Lawes Agricultural Trust, Rothamsted Experimental Station, U.K. and VSN International Ltd. U.K.).

Results

Pansy cv. baby bingo: Pansy cv. Baby Bingo (LDP) flowered 58 days after emergence under continuous LD whereas plants under continuous SD took 71 days to flower (Fig. 1A, Table 2). The durations of the development phases of photoperiod sensitivity are shown in Table 3. The duration of juvenile phase of development (a₁) was recorded 16 days. However, the duration of other photoperiod sensitive phases were much less affected than the juvenile phase such as the duration of photoperiod sensitive phases in LD (Pᵢ and P_d) was recorded only 5 days. The duration of photoperiod sensitive inductive phase in SD (Pᵢˢ) was 18 days photoperiod insensitive flower development phase (a₃) was the extended up to 38 days.

Snapdragon cv. coronette: Snapdragon cv. Coronette (LDP) flowered 92 days after emergence under continuous LD whereas plants under continuous SD took 127 days to flower (Fig. 1B, Table 2). The durations of the development phases of photoperiod sensitivity are shown in Table 3. The duration of juvenile phase of development (a₁) was recorded 30 days. However, the duration of other photoperiod sensitive phases were much less affected than the juvenile phase such as the duration of photoperiod sensitive phases in LD (Pᵢ and P_d) was recorded only 6 days. The duration of photoperiod sensitive inductive phase in SD (Pᵢˢ) was 41 days photoperiod insensitive flower development phase (a₃) was the extended up to 55 days.

### Table 1. Environmental detail of the experiment.

<table>
<thead>
<tr>
<th>Growing Season</th>
<th>Maximum (ºC)</th>
<th>Minimum (ºC)</th>
<th>Average (ºC)</th>
<th>Daily light integral 08:00-16:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2007</td>
<td>41.33</td>
<td>27.47</td>
<td>34.40</td>
<td>10.12 MJ.m⁻².d⁻¹</td>
</tr>
<tr>
<td>July 2007</td>
<td>38.32</td>
<td>26.13</td>
<td>32.23</td>
<td>9.76 MJ.m⁻².d⁻¹</td>
</tr>
<tr>
<td>August 2007</td>
<td>37.61</td>
<td>27.06</td>
<td>32.34</td>
<td>9.50 MJ.m⁻².d⁻¹</td>
</tr>
<tr>
<td>September 2007</td>
<td>36.53</td>
<td>23.10</td>
<td>29.82</td>
<td>9.69 MJ.m⁻².d⁻¹</td>
</tr>
<tr>
<td>October 2007</td>
<td>34.16</td>
<td>15.39</td>
<td>24.77</td>
<td>8.64 MJ.m⁻².d⁻¹</td>
</tr>
</tbody>
</table>

### Table 2. Effect of long days and short days on flowering time of Pansy cv. Baby Bingo, Snapdragon cv. Coronette, Petunia cv. Dreams and Cosmos cv. Sonata Pink. Standard errors of means are shown in parenthesis.

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Days taken to flower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD</td>
</tr>
<tr>
<td>Pansy cv. Baby Bingo</td>
<td>58.20 (± 0.29)</td>
</tr>
<tr>
<td>Snapdragon cv. Coronette</td>
<td>92.00 (± 0.54)</td>
</tr>
<tr>
<td>Petunia cv. Dreams</td>
<td>57.90 (± 0.38)</td>
</tr>
<tr>
<td>Cosmos cv. Sonata Pink</td>
<td>88.40 (± 0.52)</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>Pansy cv. Baby Bingo</td>
<td>71.20 (± 0.49)</td>
</tr>
<tr>
<td>Snapdragon cv. Coronette</td>
<td>126.90 (± 0.43)</td>
</tr>
<tr>
<td>Petunia cv. Dreams</td>
<td>80.60 (± 0.31)</td>
</tr>
<tr>
<td>Cosmos cv. Sonata Pink</td>
<td>60.20 (± 0.29)</td>
</tr>
</tbody>
</table>
Table 3. The durations of the phases of photoperiod sensitivity of three LD annual ornamentals, Pansy cv. Baby Bingo, Snapdragon cv. Coronette, Petunia cv. Dreams and one SD annual ornamental Cosmos cv. Sonata Pink. Values in parenthesis are the standard errors of the estimates of the parameters of the model fitted using the Fitnonlinear directive of GenStat-8.

<table>
<thead>
<tr>
<th>Name of LDPs</th>
<th>(a_1)</th>
<th>(P_{IL})</th>
<th>(P_d)</th>
<th>(P_{IS})</th>
<th>(a_3)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pansy cv. Baby Bingo</td>
<td>15.70 (±0.79)</td>
<td>2.03 (±4.29)</td>
<td>2.79 (±3.21)</td>
<td>17.60 (±1.45)</td>
<td>37.63 (±0.92)</td>
<td>0.98</td>
</tr>
<tr>
<td>Snapdragon cv. Coronette</td>
<td>30.12</td>
<td>6.54 (±0.67)</td>
<td>0.12 (±0.94)</td>
<td>41.32 (±1.11)</td>
<td>55.33 (±0.39)</td>
<td>0.97</td>
</tr>
<tr>
<td>Petunia cv. Dreams</td>
<td>15.54 (±0.67)</td>
<td>5.44 (±0.56)</td>
<td>0.28 (±0.73)</td>
<td>28.25 (±0.93)</td>
<td>36.62 (±0.38)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of SDP</th>
<th>(a_1)</th>
<th>(P_{IS})</th>
<th>(P_d)</th>
<th>(P_{IL})</th>
<th>(a_3)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmos cv. Sonata Pink</td>
<td>15.38</td>
<td>6.33 (±0.48)</td>
<td>3.96 (±0.76)</td>
<td>37.06 (±0.96)</td>
<td>34.79 (±0.34)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Student t-test was used to compare means.

Fig. 1. Effect of transferring plants from LD (17h.d\(^{-1}\)) to SD (8h.d\(^{-1}\)) (○) and from SD to LD (●) at regular intervals from seedling emergence of (A) Pansy cv. Baby Bingo, (B) Snapdragon cv. Coronette, (C) Petunia cv. Dreams and (D) Cosmos cv. Sonata Pink. Vertical bars (where larger than the points) represent the standard error within replicates. The solid lines show the fitted relationships (Table 3 for parameters estimates) for plants transferred from LD to SD and from SD to LD respectively.

**Petunia cv. dreams:** Petunia cv. Dreams (LDP) flowered 58 days after emergence under continuous LD whereas plants under continuous SD took 81 days to flower (Fig. 1C, Table 2). The durations of the development phases of photoperiod sensitivity are shown in Table 3. The duration of juvenile phase of development \((a_1)\) was recorded 16 days. However, the duration of other photoperiod-sensitive phases were much less affected than the juvenile phase such as the duration of photoperiod sensitive phases in LD \((P_{IL} \text{ and } P_d)\) was recorded only 5 days. The duration of photoperiod sensitive inductive phase in SD \((P_{IS})\) was 28 days photoperiod insensitive flower development phase \((a_3)\) was the extended up to 37 days.

**Cosmos cv. sonata pink:** Cosmos cv. Sonata Pink (SDP) flowered 88 days after emergence under continuous LD
whereas plants under continuous SD took 60 days to flower (Fig. 1D, Table 2). The durations of the development phases of photoperiod sensitivity are shown in Table 3. The duration of juvenile phase of development \((a_1)\) was recorded 15 days. However, the duration of other photoperiod sensitive phases were much less affected than the juvenile phase such as the duration of photoperiod sensitive phases in SD \((P_{IS} \text{ and } P_d)\) was recorded only 10 days. The duration of photoperiod sensitive inductive phase in LD \((P_{IL})\) was 37 days photoperiod insensitive flower development phase \((a_1)\) was the extended up to 35 days.

**Discussion**

Photoperiod, light integrals and irradiance, either independently or in combination have a decisive role in the development of many plant species. The results of our previous studies showed that LDPs and SDPs produced earlier flowering when grown in their respective ambient day length i.e., April to mid of June for LDPs and September to end of November for SDPs (Baloch et al., 2009a). In another study, some LDPs and SDPs produced earlier flowers when grown under respective inductive (LD for LDPs and SD for SDPs) environment (Baloch et al., 2009b; Baloch, 2009; Baloch et al., 2011). The difference in days taken to flowering between the two studies was assumed to be the difference in light integrals. Therefore, another experiment was designed to test flowering behaviour of these LDPs and SDPs under ambient light integrals (using shades) and artificial light integrals (irradiance). Findings of this study showed that the flowering time was delayed in LDs when grown under 40% shade or received minimum \((42\mu\text{mol.m}^{-2}.\text{s}^{-1})\) irradiance however SDPs behaved opposite as expected (Baloch et al., 2009c; Baloch, 2009; Baloch et al., 2012).

These studies were based on an assumption that all cultivars are equally sensitive to photoperiod throughout their development. This assumption was tested in present experiment using Pansy, Snapdragon, Petunia (LDPs) and Cosmos (SDP) for their photoperiod sensitivity. It was revealed that these annuals showed a distinct response towards their inductive environment and five flower development phases were estimated when a non-linear model (Adams et al., 2003) was applied. However, the duration of these flower development phases varies in different annual species. Plants grown under inductive environment flowered after 58 days (Pansy cv. Baby Bingo and Petunia cv. Dreams), 92 days (Snapdragon cv. Coronette) and 60 days (Cosmos cv. Sonata Pink) i.e. 13 (Pansy), 23 (Petunia), 35 (Snapdragon) and 28 days (Cosmos) earlier flowering than those grown in non-inductive environment. Findings of some previous studies on Pansy are in line with the results of present experiment i.e. plants grown in LD flowered earlier. For example, Pansy cv. Crystal Bow flowered after 74 days from sowing when grown under 16 h.d\(^{-1}\) LD environment at 20ºC (Karlsson, 1996). Similarly, Adams et al., (1997) reported that rate of progress to flowering increased significantly under LD \((17 \text{ h.d}^{-1})\) in Pansy cv. Universal Violet. It is also reported that Petunia cv. Express Blush Pink flowered 30 days earlier when received 16 h.d\(^{-1}\) LD at an average temperature of 28.7ºC as compared to 8 h.d\(^{-1}\) SD environment. In present study the difference in flowering time of Petunia cv. Dreams was 23 days. This difference could be due to the increase in photoperiod \((17h.d^{-1})\) and temperature \((31.6ºC)\) and cultivar variability. In another study Petunia cv. Midnight Madness required 8-10 more days for flowering in SD \((8h.d^{-1})\) compared to plants grown in 16 h.d\(^{-1}\) (Karlsson, 1996). Munir (2003) obtained 23 days earlier flowering when Snapdragon cv. Chimes was grown in LD \((17 \text{ h.d}^{-1})\) at 19.4ºC. However, in present study this difference was 35 days between LD and SD environment. The reason could be the variation in cultivars and their genetic makeup as cv. Coronette is a mid-flowering cultivar while cv. Chimes is an early-flowering one. High temperature \((31.6ºC)\) could be another cause of this difference. Cosmos cv. Sonata Pink flowered earlier in SD environment and Warner (2006) obtained a similar response also. However, Kanellos & Pearson (2000) obtained an opposite response in *Cosmos arosanguineus* and reported that plants in LD \((17 \text{ h.d}^{-1})\) flowered 33 days earlier than those at 8 h.d\(^{-1}\). The LD response of this plant could be due to the difference in the species as *Cosmos bipinnatus* species was tested in the present study. It is also reported that LD environment delayed flowering in SDPs such as Chrysanthemum cv. Snowdon (Adams et al., 1998a), *Oryza sativa* (Collinson et al., 1992) and *Glycine max* (Collinson et al., 1993).

Previous analyses of the phases of photoperiod sensitivity (Collinson et al., 1992, 1993; Ellis et al., 1992, 1997; Adams et al., 1998a, b, 1999, 2003; Bertero et al., 1999; Yin et al., 2008; Munir et al., 2010) have successfully analyzed all of the flowering data simultaneously to quantify the duration of flower development phases using reciprocal transfer technique. In present study, flowering time data were effectively estimated using the same technique in Pansy, Snapdragon, Petunia and Cosmos. Results of this research revealed that the duration of photoperiod sensitive juvenile phase \((a_1)\) was shorter than the photoperiod sensitive phase in inductive environment \((P_{IS} \text{ in LDPs Pansy, Snapdragon and Petunia and } P_{IL} \text{ in SDP Cosmos})\) and photoperiod insensitive flower development phase \((a_1)\). Although one of the previous studies reported that light integrals caused a dramatic increase in the duration of juvenile phase in Petunia cv. Express Blush Pink (Adams et al., 1999) but no such factor was studied in present investigation. However, the duration of juvenile phase was short as compared to \(P_{IS}\) and \(a_1\) phases and varied within the cultivars in Snapdragon cvs. Chimes, Liberty, Annabel, Bells, La Bella, Pirouette, Ribbon and Sonnet (Adams et al., 2003). In other studies on Snapdragon cv. Chimes reported that the duration of these phases are not only affected by photoperiod (Munir et al., 2010) but light integrals and temperature also had a significant effects i.e. low light integrals and low temperature enhanced the duration of \(a_1, P_{IS}\) and \(a_1\) (Munir, 2003). Munir (2003) also compared *CENTROTRADIALIS* (*CEN*) mutant of Snapdragon with wild type using reciprocal transfer tool and reported that *CEN* gene present in wild type reduced the duration of juvenile phase up to 10 days. *CEN* gene is expressed in the inflorescence apex a few days after floral induction (after perceiving the LD inductive signal), interacting with the floral meristem identity gene *FLORICAULA* (*FLO*) to regulate flower position and morphology at the spike and carries on its
determinate inflorescence growth. However, mutant CEN first terminates the inflorescence growth (determinate inflorescence) and then FLO genes produce flowers. The size of CEN mutant is obviously shorter than the Wild type one. Its counterpart, TERMINAL FLOWER 1 (TFL1) plays a similar role in Arabidopsis. But, unlike CEN, TFL1 is expressed during the vegetative phase and therefore affects vegetative and reproductive phases of development (Bradley et al., 1997; Ratcliffe et al., 1998, 1999).

In Pansy (LDP) and Cosmos (SDP) no such type of research has been reported previously. However, the photoperiod sensitivity response of Pansy was similar to Snapdragon and Petunia and the reason for shorter duration of juvenile phase (a1) could be the same as described above. Cultivars of these LDPs were most sensitive under non-inductive SD environment (PIS phase). However, this duration was shorter than the flower development photoperiod insensitive phase (a1) in all 3 LDPs. The reason could be that during PIS phase plants remain vegetative (produce leaves) in non-inductive environment (SD) while in a1 phase of flower development plants use the reserved assimilates because floral parts are incapable to do photosynthesis hence the duration of this phase is longer than PIS phase (Munir, 2003).

In Cosmos the duration of photoperiod sensitive juvenile phase was shorter (15 days) than the photoperiod sensitive phase in LD non-inductive environment (PIS) and photoperiod insensitive flower development phase (a1). In contrary to LDPs, the duration of PIS was two days longer in LD environment (37 days) as compared to a1 phase (35 days) however this difference seems to be non-significant statistically. Similarly, Collinson et al., (1993) reported that the duration of the photoperiod insensitive juvenile phase varied three-fold between cultivars of Glycine max, i.e. from 11 to 33 days and the duration of the photoperiod sensitive phase (PIS) was greater in LD (non-inductive environment). However, there was little variation in the photoperiod insensitive post-inductive phase (a2); it ranged from 15 to 20 days. Working on Chrysanthemum cv. Snowdon (SDP) Adams et al., (1998a) observed that plants were capable of responding to SD immediately after pinching. When they had received a sufficient number of SD (5 SD) they became induced to flower, although the leaf number of plants could be increased by the subsequent use of LD, suggesting LD could still delay inflorescence initiation. Plants needed a further 2-3 SD before the meristem was committed to flower. However, in present study Cosmos was raised through seeds therefore showed distinct phases of floral development as compared to Chrysanthemum.

Conclusion

In present study, reciprocal transfer experiment has been shown to be a useful tool in understanding how photoperiod environment influences the flowering process. Although the effects of photoperiod and light integral on time to flowering have been investigated in previous studies, the data presented here have shown which developmental phases are most sensitive particularly in Pansy and Cosmos as no attempt has been made previously to quantify their flower developmental phases. General flowering models tend to ignore the phases of sensitivity to photo-thermal environment. The model applied here provides the basis of a more physiologically-based quantitative model of flowering. Many flowering studies have concentrated on flower induction, the biochemical changes that occur within the plant at this time, and the associated genetics. Consequently, juvenility and the later phases of flower development tend to be ignored, despite their importance in the overall flowering process. Therefore, the commercial benefits from the use of day extension at a particular time could be significant.

References


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