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Dependence of *Arabidopsis thaliana* floral stem growth and architecture on photoperiod

L. JOUVE*, Y. CHARRON, C. COUDERC, H. GREPPIN and R. DEGLI AGOSTI

Laboratory of Plant Biochemistry and Physiology, University of Geneva,
3 place de l'Université, CH-1211 Geneva 4, Switzerland

Abstract

The influence of the applied photoperiod during floral stem development has been studied. A clear relationship between the length of the photoperiod, the frequency of light/dark switches, and the dynamic of growth and the subsequent morphology and architecture has been found. An increase in the day length (L/D = 16/8) or an increase in frequency of light/dark transitions (L/D = 7/7) stimulated the inflorescence growth. The two treatments induced the same enlargement in the early inflorescence, but the L/D = 7/7 condition causes a greater increase in the late one. The treatments induced also a diminution in the number of lateral inflorescences, principally with the high frequency light-on/light-off condition. Moreover than differences in the growth along the early inflorescence, at the level of the internode, a gradual growth was observed from the basal part to the upper part of the internode. Finally, the floral stem growth dynamic showed that it could be adapted to the environmental light/dark signalling by a good extension rate synchronisation.

Additional key words: elongation rate, expansion, inflorescence.

Introduction

In the last decade considerable efforts have been made towards an understanding of the mechanisms underlying floral evocation and morphogenesis of *Arabidopsis thaliana*. During the vegetative phase of *Arabidopsis thaliana*, the apical meristem gives rise to a rosette. Leaf size, shape, and arrangement change gradually throughout rosette development (Medford *et al.* 1992, Martínez-Zapater *et al.* 1995). These changes in leaf shape and phylotaxis could characterise, by analogy to what is known in other species (Poethig 1990), the transition between juvenile and adult vegetative

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Abbreviations: L/D - light period/dark period; LVDT - linear voltage differential transformer.

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* Corresponding author, fax: (+41) 22 3297795, e-mail: laurent.jouve@bota.unige.ch

phases. Such transition has generally been associated with the acquisition of meristematic competence in response to a floral stimulus (Shannon and Meeks-Wagner 1991, McDaniel *et al.* 1992). Lateral meristems in the axils of the rosette leaves remain quiescent until inflorescence development, after which they can give rise to secondary (lateral) inflorescences (Müller 1961, Hempel and Feldman 1994). Based on the morphology, the primary inflorescence can be divided into early (rosette proximal) and late (rosette distal) inflorescence. The early inflorescence nodes bear leaves referred as cauline leaves or bracts. The bracts sustained axillary lateral inflorescences that are very similar in morphology to the primary ones (Haughn *et al.* 1995, Martínez-Zapater *et al.* 1995). Indeed, the most obvious morphological change between the rosette and the early inflorescence is the elongation of the internodes that normally occurs after formation of the first floral buds (Smyth *et al.* 1990, Hempel and Feldman 1994). Thus the early inflorescence could be considered to be part of the vegetative phase (Haughn *et al.* 1995). After several coflorescence-bearing nodes of the early inflorescence, all subsequent nodes have a flower as a lateral shoot that is not supported by a leaf (late inflorescence).

Growth of several plant stems has been studied using linear voltage differential transformer (LVDT) method (Lecharny and Wagner 1984, Kristie and Jolliffe 1986, Ruiz Fernandez and Wagner 1994) and particularly *Arabidopsis* floral stem in which growth was clearly under the control of an endogenous circadian rhythm (Jouve *et al.* 1998). Moreover, a photoperiodic treatment which was not in phase with the 24 h daily period (L/D = 7/7) triggered a significant increase in the first internode length.

Inflorescence growth and the subsequent architecture are poorly understood and require investigation. The present work is a description of the relationships between increasing length of the photoperiod and morphology or architecture of the inflorescence and the growth rate.

Materials and methods

Plants and growing conditions: *Arabidopsis thaliana* (L.) Heynh., Landsberg *erecta* ecotype seedlings were grown under a L/D = 12/12 in a potting compost for 3 weeks after sowing. Afterwards they were transplanted in jars, as a single plants, and cultured for 3 weeks until inflorescence primordia initiation. Then, the photoperiod was changed for part of the plants to L/D = 16/8 or 7/7 for 1 week before floral stem elongation and measurements. During growth, light was provided by *Sylvania* 36 W *Luxline-Plus* fluorescent lamps, irradiance was 75 μmol(PAR) m^{-2} s^{-1}. Experiments were done in controlled-environment chambers: temperature 22.5 ± 0.5 °C and relative humidity 80 ± 6 %. During measurements irradiance was 45 μmol m^{-2} s^{-1}. *Arabidopsis* shoot morphogenesis is represented in Fig. 1 to show the different parts of inflorescence analysed in this work.

Whole inflorescence growth: A systematic measurement of the primary inflorescence length, internode number and length, lateral inflorescence number and length, primary axillary inflorescence number and length were done after approximately

3 weeks after the beginning of the inflorescence growth. These measurements were done after the end of the whole inflorescence growth on 105 different plants.

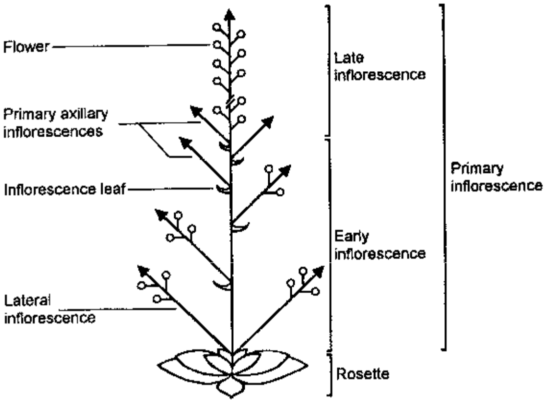

Fig. 1. Diagrammatic representation of *Arabidopsis thaliana* inflorescence morphology, according to Haughn *et al.* (1995).

Early inflorescence growth: In order to discriminate the growth of the whole internode and the relative growth of the internode, depending on the position in the internode, growth was measured as follows. The first, second and third internodes were marked with india ink to divide each of them in 4 equal segments at the beginning of growth (internode: 5.1 ± 1.0 mm long; mark: 1.3 ± 0.4 mm intervals). The length of an elongating zone between two marks was measured every day during 10 d on 30 different plants.

First inflorescence node growth: a computerised data acquisition and analysis: The measurement of the first inflorescence node elongation was carried out with a custom designed plant growth measuring apparatus (Degli Agosti *et al.* 1997, Jouve *et al.* 1998). Data were collected each 180 s. The data were processed as described in Jouve *et al.* (1998).

Statistical analysis: *ANOVA* and Student-Newman-Keuls multiple comparison tests were used for data treatment using *Instat for MacIntosh 2.01* software.

Results

The whole inflorescence: The incidence of the applied photoperiod during inflorescence growth on the subsequent morphology and architecture is clearly visible (Fig. 2). An increase in the day length L/D = 16/8 and even more significantly an increase in the frequency of light/dark switches (L/D = 7/7) stimulated inflorescence growth as well as in the early inflorescence (Table 1). Indeed, the growth extension as a function of the photoperiodic treatment was not homogeneously distributed in the primary inflorescence. The increase in the light period induced an equal distribution between early inflorescence (Σ internodes) and late inflorescence (± 1.6 cm greater than in L/D =12/12). However, the increase in the frequency of the light-on and light-off promoted an important increase in the late inflorescence (3.7 cm greater than in L/D =12/12) and a smaller one in the late inflorescence (1.8 cm greater than in L/D =12/12) (Table 1).

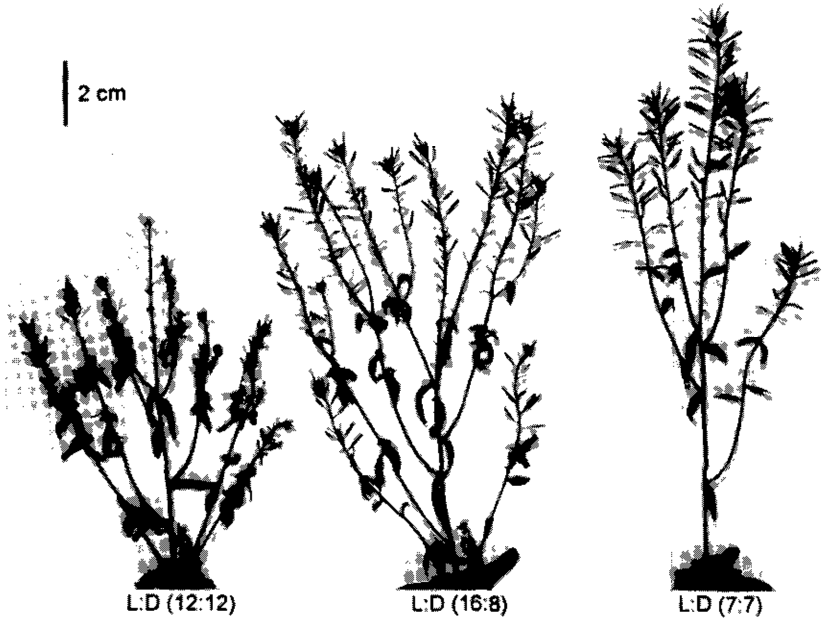

Fig. 2. *Arabidopsis thaliana* inflorescence after growth at different photoperiod (L/D = 12/12 (*left*), L/D = 16/8 (*centre*) and L/D = 7/7 (*right*)).

Table 1. *Arabidopsis* floral stem length as a function of photoperiod. Means ± SD; the different letters indicate significantly different values (n = 105 excepted lateral inflorescence length and primary axillary stem length where n = 30). *** - $P \leq 0.001$, ** - $P \leq 0.01$, * - $P \leq 0.05$, ns - $P > 0.05$.

	L/D (12/12)		L/D (16/8)		L/D (7/7)		*P*
Inflorescence length [mm]	176 ± 24	a	208 ± 26	b	23.1 ± 2.6	c	***
Σ internodes length [mm]	90 ± 13	a	107 ± 17	b	10.8 ± 1.9	b	***
Σ internode/inflorescence	0.52± 0.09	a	0.51± 0.06	a	0.47± 0.07	b	***
Internode number	4.3 ± 0.8	a	4.2 ± 0.7	a	4.3 ± 0.8	a	ns
Primary axillary stem length [mm]	111 ± 37	a	124 ± 31	b	128 ± 38	b	ns
Lateral inflorescence number	2.6 ± 1.4	a	2.1 ± 1.0	b	0.7 ± 1.0	c	***
Lateral inflorescence length [mm]	131 ± 34	a	125 ± 26	ab	92 ± 64	b	*

The modification of the photoperiod also induced changes in the number of developed lateral inflorescences (Table 1). Increase in the light period slightly decreased the number of developed lateral shoots, while the extension in the light-on/light-off frequency inhibited strongly their development. Moreover, these treatments induced similar, but not significant, modifications in the length of the lateral inflorescence. Light regime modifications did not change either primary axillary stem length or the internode number in the early inflorescence (Table 1).

The early inflorescence: The early inflorescence displays different changes with respect to the control (L/D = 12/12) when the flowering plants were grown in different photoperiods (Table 2). The most important change was the significant expansion of the first, second and third internodes during L/D = 7/7. The L/D = 16/8 induced the same extension in the second and third internodes but not in the first one. There was no variation in upper internodes (4th, 5th and 6th).

Table 2. *Arabidopsis* floral stem internodes length [mm] as a function of photoperiod. Means ± SD; the different letters indicate significantly different values (n = 105). *** - $P \leq 0.001$, ** - $P \leq 0.01$, * - $P \leq 0.05$, ns - $P > 0.05$.

	L/D (12/12)		L/D (16/8)		L/D (7/7)		*P*
1st internode	3.6 ± 1.8	a	3.9 ± 1.9	a	4.4 ± 1.8	b	**
2nd internode	2.7 ± 1.1	a	3.4 ± 1.1	b	3.2 ± 1.3	b	**
3rd internode	1.5 ± 0.6	a	2.0 ± 0.9	b	1.9 ± 0.8	b	***
4th internode	1.2 ± 0.5	a	1.3 ± 0.5	a	1.3 ± 0.7	a	ns
5th internode	0.7 ± 0.2	a	0.8 ± 0.4	a	0.7 ± 0.3	a	ns
6th internode	0.6 ± 0.2	a	0.5 ± 0.2	a	0.5 ± 0.2	a	ns

The relative growth rate of the internodes was followed until the stem growth has stopped. The stem elongation displayed a non-homogenous growth along the internodes (Fig. 3). The growth increased progressively from the lower part to the upper part of the internodes. This internal organisation of growth, inside the internode, could be observed in each measured stem segment (Fig. 3*A,C,E*), where segments S1 < S2 < S3 < S4, S5 < S6 < S7 < S8 and S9 < S10 < S11 < S12. A major increase in the S3 and S4 sections was observable when the floral stem grew in L/D = 7/7 (Fig. 3*E*), while all other segments did not statistically change as a function of the photoperiodic treatment. The resulting sum of the 12 segments represent the cumulated growth (Fig. 3*B,D,F*) of the three first internodes. The extension of these internodes is a sigmoidal function of time. Floral stems grown in L/D = 16/8 or 7/7 displayed a greater cumulated extension than the control. There was no difference in the sum of the three first internode lengths in plants cultured under L/D = 16/8 or 7/7 (Table 1).

The first internode: The cumulated growth was not different between the long day condition (L/D = 16/8) and the control (Fig. 4*A*). Nevertheless, the L/D = 7/7 regime induced a higher elongation than the two other conditions (Table 2).

Measurements of the extension rate (Fig. 4*B,C,D*) indicate that growth was well synchronised with the light/dark regime. The maximum growth rate occurred during light periods and the minimum during dark periods. The modulation in growth rate during light periods was different depending on the photoperiod. Plants in control condition (L/D = 12/12; Fig. 4*B*) displayed two peaks in extension rate, one at the beginning and one at the end of the light period. This had a real regularity all along the growth duration. The lengthening of photoperiod during inflorescence growth (Fig. 4*C*) causes two rate peaks in light during the first 3 d of growth and 3 peaks afterwards. The increase of the frequency of light/dark switches (Fig. 4*D*) displayed a simple peak during the first 4 d. Then, an additional peak during light time appeared but it seemed to be less regular. These changes between the beginning and the end of the growth duration occurred approximately at the same time, when growth extension reached its inflexion point.

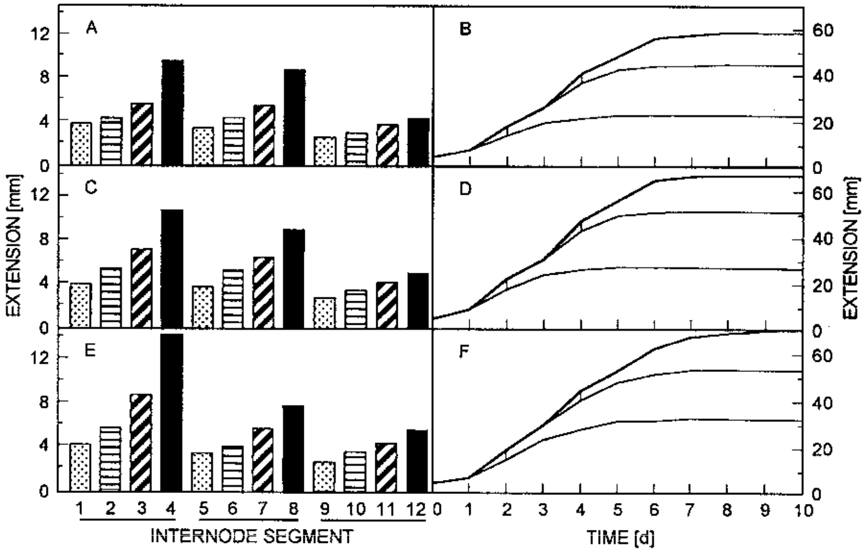

Fig. 3. Growth of the early inflorescence of *Arabidopsis thaliana*. Effect of photoperiod on extension of each segment of the three first internodes *A* - L/D (12/12), *C* - L/D (16/8), *E* - L/D (7/7), and on sum of the 12 different segments *B* - L/D (12/12), *D* - L/D (16/8), *F* - L/D (7/7).

Discussion

The incidence of the photoperiod on *Arabidopsis* vegetative or floral organ initiation was intensively studied (for reviews see Telfer and Poethig 1994 and Martínez-

Zapater *et al.* 1994). However, the specific influence of the photoperiod during floral stem growth has not been checked particularly. Our results show a major incidence of this light/dark regime on the inflorescence growth. We have seen that L/D =7/7 induced a more intensive primary inflorescence growth than L/D = 16/8 which itself caused a larger growth than the L/D = 12/12. Moreover, we saw that L/D = 16/8 and L/D = 7/7 treatments induced the same expansion in the early inflorescence (internode sum). These results support the idea that an increase in light duration or in light/dark switches frequency has a positive effect on inflorescence growth. The incidence of the photoperiod on the whole plant morphology has been partially described by Haughn *et al.* (1995). These authors showed several differences in *Arabidopsis* shoot morphology of plants grown under continuous light or short day conditions, but a precise description of them was not done. It appeared that short-day regime induced a greater number of internodes, which elongate after flower

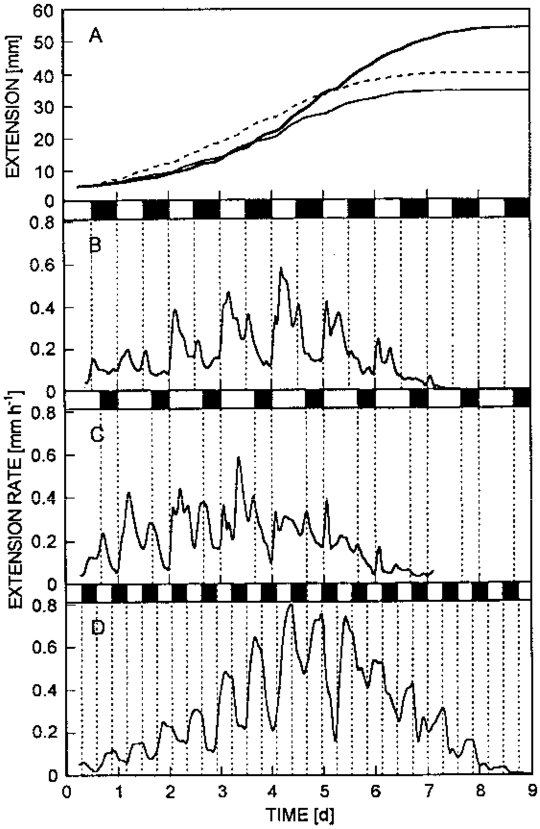

Fig. 4. Growth of the first internode of *Arabidopsis thaliana* early inflorescence. *A* - elongation measured in L/D 1(2/12) (*bold plain line*), L/D (16/8) (*plain line*) and L/D (7/7) (*dashed line*). *B, C, D* - extension rate measured in L/D (12/12) (*B*), L/D (16/8) (*C*) and L/D (7/7) (*D*). Results are the means of at least 4 experiments performed on different plant. We have considered time 0 as the beginning of the photoperiod of the first measurement day.

induction, and so a greater number of developed secondary inflorescences. We have not observed any changes in the internode number depending on the photoperiod used in our work. Nevertheless, we have monitored major changes between inflorescences grown under longer photoperiod or under increased light/dark frequency. Increase in the light period slightly decreased the number of developed lateral shoots but the increase in the light/dark frequency inhibited strongly this development. We have also shown a significant extension of the first, second and third internode during the L/D = 7/7. The L/D = 16/8 induced the same result on the second and third internode but not on the first one. Moreover, the late inflorescence was also stimulated by the two conditions, especially by the L/D = 7/7. Altogether these results may be explained by the fact that during the long day the leaves of the rosette (source) could provide more photosynthates to the floral stem (sink) than in the control conditions. Furthermore, L/D = 7/7 may mimic, in a certain way, an etiolation process inducing an increase of the length of internodes and the late inflorescence. This change in photoperiod may also induce an increase in the apical dominance (Tamas 1995). L/D = 7/7 led to an inhibition of axillary stem growth similarly as during the control of the vegetative development by the shoot apex (Phillips 1975, Cline 1991). Nevertheless, after flower induction an increase of axillary shoot growth can be usually found (Beever and Woolhouse 1975, Harrison and Kaufman 1980, Krekule *et al.* 1989). This suggests that the augmentation in the light/dark frequency can reverse the decrease of the level of dominance expressed in the apical reproductive structures.

In addition tô the changes mentioned so far, we have shown that the relative growth displayed a non-homogenous growth along the internode. The internal organisation of growth, inside the internode and the sequential growth along the internode may be referred to an internal control of the growth in space and in time.

Arabidopsis first inflorescence node growth was measured during several light/dark exposures. Under successive photoperiodic cycles the pattern of extension rate was modulated by the successive light-on and light-off. That kind of response was already observed in *Chenopodium rubrum* (Ruiz Fernandez and Wagner 1989) and *Arabidopsis* (Jouve *et al.* 1996, 1998) stem elongation rates. These properties observed *e.g.* by Bünning (1973) are typical for biological rhythms.

Our aim was to describe the behaviour of the flowering stem in *Arabidopsis thaliana* depending on the photoperiod used during the growth after flowering initiation. Results clearly pointed out the major involvement of the light/dark conditions in regulation of the development and the subsequent architecture of the inflorescence. The implication of the light/dark rhythm on the future development of the plant, and in the numerous factors implicated in the information transfer could be discussed. It would be interesting to check the implication of auxin (Krekule and Macháčková 1986) and cytokinin (Coenen and Lomax 1997), generally associated with the apical dominance, and also the phytochrome involvement in the detection of the light-on/light-off signal (Kerckhoffs *et al.* 1997). The use of several hormonal or phytochrome mutants, associated with classical physiological tests, could help to understand the underlying mechanisms.

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