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FLORAL STEM GROWTH OF ARABIDOPSIS ECOTYPES. I. DIFFERENCES DURING SYNCHRONIZED LIGHT REGIME AND CONTINUOUS LIGHT FREE RUN

BY

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ABSTRACT

Floral stem growth *of Arabidopsis* ecotypes. I. Differences during synchronized light regime and continuous light free run - The primary inflorescence architecture and the dynamic of the floral stem development has been studied in 4 ecotypes of *Arabidopsis*. Clear differences have been found between ecotypes. Lansberg *erecta* (Ler) ecotype has a short inflorescence and a progressive decreasing in the internode length train. The three other ecotypes showed an organization with successive alternation of long and short internodes. At the level of the internode, floral stem growth dynamic showed only a well synchronization with light and dark alternation with Ler ecotype. Moreover, circadian oscillations in growth rate were observable with Ler and Columbia (Col) ecotypes but not with C24 or Wassilewskija (Ws).

Key-words: Floral stem, architecture, elongation, synchronization, circadian rhythm, ecotypes, *Arabidopsis thaliana*.

Abbreviations: Ler = Landsberg *erecta*; Col = Columbia. Ws = Wassilewskija. LVDT = linear voltage differential transformer.

INTRODUCTION

Plant growth is slow, and sensitive methods are required to resolve the short-term dynamics of growth. When analyzed on a slow time scale, plant growth appears as a linear phenomenon but increasing the sampling frequency and the accuracy of the measuring apparatus reveals changes in the elongation rate. Linear voltage differential transformers (LVDTs) are among the most appropriate devices to obtain high-resolution measurements of stem segment (PRAT AND PARÉSYS, 1995) and intact plant elongation (PENNY *et al.*, 1974; KERCKHOFFS *et al.*, 1997). Plant growth exhibits many rhythms and pseudo-rhythms with very different periods, such as the annual and circadian rhythms (RUIZ-FERNANDES & WAGNER, 1994).

Arabidopsis thaliana (L.) Heynh. is a small annual weed that belongs to the *Brassicaceae* family one of the most extensively used in research. Although of no inherent economic value, *Arabidopsis* offers many advantages for rapid genetic and molecular analysis including its small size, rapid life cycle, small simple genome, prolific seed production and the availability of numerous mutations. Most of the commonly used laboratory strains have a single seed as the original source and have been inbred for many generations. They are thus homozygous at most loci. Some common laboratory strains,

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such as Landsberg erecta (Ler; RÉDEI, 1970), are not true wild-type in that they have been selected to be homozygous for mutations from Landsberg population that result in a growth habit favorable for laboratory/greenhouse conditions. RÉDEI (1970) selected the Columbia ecotype (Col) from the non-irradiated Landsberg population, as it was particularly fertile and vigorous plant that responded well to changes in photoperiod. C24 has also been referred to in the literature as Columbia. This must however be considered to be an independent ecotype (BOWMAN, 1994).

A fourth well-studied ecotype, totally independent from the Landsberg population, is also used for *Arabidopsis* biological plant model: Wassilewskija (Ws). All these four ecotypes are well genetically and morphologically characterized. Here we attempted to characterize they dynamical behavior at low time scale during alternative or continuous light exposure. Our aim was also to compare the four different ecotypes in a global point of view considering the final architecture of the floral stem.

MATERIEL AND METHODS

Plant material and growth conditions

Four *Arabidopsis thaliana* (L.) ecotypes (Ler, Col, C24 and Ws) seedlings were grown in potting compost for 3 weeks after sowing. Afterwards they were transplanted in a new pot (7 x 7 x 6 cm), as single plants, and cultured for 3 weeks waiting for inflorescence primordia initiation. All four ecotypes were cultivated at the same time. During growth, light (L:D 12:12) was provided by Sylvania (OSRAM GmbH, Munich, Germany) 36 W Luxline-Plus fluorescent lamps (75 $\mu mol.m^{-2}.s^{-1}$ PAR). Temperature was 20 ± 5 °C and humidity 70 ± 15 %.

Experimental conditions

Experiments were done in thermo- and hygro-regulated culture chambers: temperature was 22.5 ± 0.5 °C and relative humidity 80 ± 6 %. During growth measurements, the photon flux was decreased to 45 $\mu mol.m^{-2}.s^{-1}$ PAR using Sylvania 36 W Gro-Lux fluorescent lamps in order to increase inflorescence growth.

Two measurement conditions were tested: L:D (12:12) and continuous light . The duration of the experiment was 10 d. The four ecotypes benches were used for experiment in parallel.

Data acquisition and treatment

The measurement of the first inflorescence internode elongation was carried out with a custom designed plant growth measuring apparatus (DEGLI AGOSTI *et al.*, 1997; JOUVE *et al.*, 1998b). Data were collected each 60 seconds. In order to reduce mechanical and electronic noise, a first basic treatment is performed as following: 3 successive points are averaged. From the resulting time series a moving mean over 3 points is calculated on it. Sampling is reduced to 1 point per 3 min. After 3 such successive treatments on the original data series the sampling is thus reduced to 1 point per 27 min. The resulting time series represents the elongation of the plant, and to obtain the stem extension rate, measurements are differenced (DEGLI AGOSTI *et al.*, 1997; JOUVE *et al.*, 1998b).

Floral stem architecture study

A systematic measurement of the primary inflorescence length, internodes number and lengths were done after 4 weeks after the beginning of the inflorescence growth. These measurements were done after the end of the whole inflorescence growth on 15†different plants. Then, an assay of internode successive arrangement comparison in-between plants was done.

Statistical analysis

ANOVA and Student-Newman-Keuls multiple comparison tests (NEWMAN, 1939; KEULS, 1952) were used for data treatment using Instat for MacIntosh 2.01 software.

RESULTS

Architecture of the inflorescence

Measuring the inflorescence length after the end of growth reveals that Ler ecotype has a statistically smaller (n = 20, $P \leq 0.001$, ***) inflorescence than the three other ecotypes (Table I). Nevertheless, Ler ecotype has the same ratio between the sum of all internodes and the whole inflorescence than the C24 ecotype. This ratio shows that internodes represent about 50% of the length of the inflorescence. The other ecotypes, Col and Ws, display a smaller proportion (± 40 %) of the internodes in the whole inflorescence (Table I) showing a major importance of the late inflorescence in Col and Ws ecotype than in C24 (n = 20, $P \leq 0.001$, ***).

After shifting arrangements of the internode length measurement series between plants, results show that there was a significant ordering in the internodes train (Fig. 1). In one hand, from the basal internode to the upper ones Ler floral stem internodes decreased in length (Fig. 1A). On the other hand, the tree other ecotypes show a non linear order in

TABLE I.

Arabidopsis floral stem length as a function of the ecotype. Ler = Landsberg *erecta*; Col = Columbia; C24; Ws = Wassilewskija. Presented data are means with the calculated standard deviation. The different letters indicate significantly different values (n = 20, $P \leq 0.01$).

	Inflorescence length (cm)		**Σ Internodes length (cm)**		**Σ Internodes / Inflorescence**	
Ler	18.71 ± 3.46	a	9.84 ± 1.46	a	0.54 ± 0.12	a
Col	36.46 ± 4.13	b	13.25 ± 2.51	b	0.37 ± 0.08	b
C24	34.94 ± 4.15	b	18.82 ± 2.07	c	0.54 ± 0.08	a
Ws	37.82 ± 6.57	b	15.20 ± 3.43	b	0.41 ± 0.11	b

length of the internodes (Fig. 1BCD). The organization seems to exhibit an alternative disposition between long and short internode along the floral stem. These successive alternations are present in about 90 % (n = 20) of the measured plants. Nevertheless, only a certain number of plant display exactly the same disposition; i.e. considering C24 ecotype, only 7 measured plants show three alternations between long and short internodes disposed at the same distance (Fig. 1C).

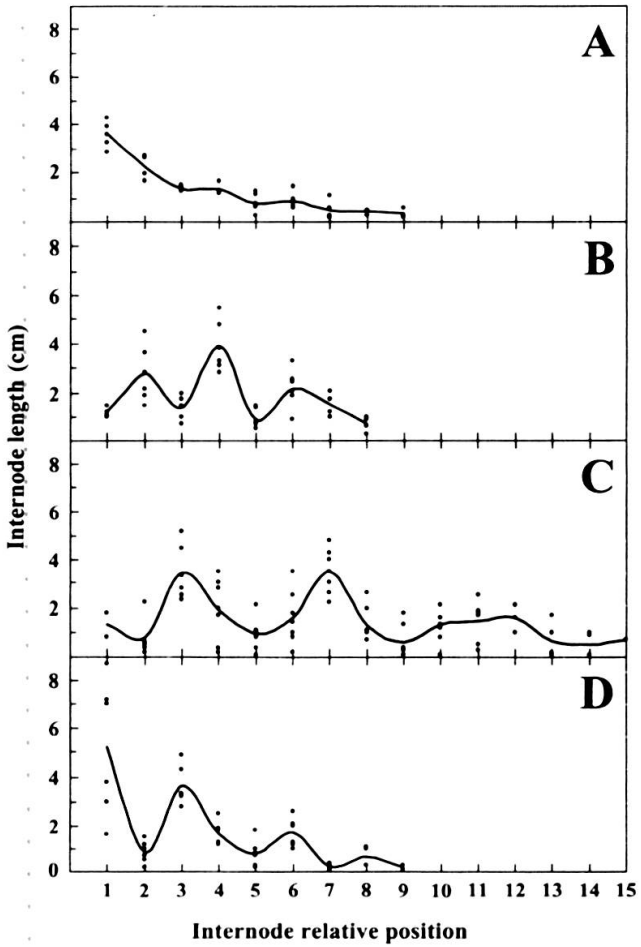

FIG. 1

Four ecotypes of *Arabidopsis thaliana* internodes length as a function of their relative position along the stem. A: Ler = Landsberg *erecta*. B: Col = Columbia. C: C24. D: Ws = Wassilewskija. Results are the mean of at least 5 measured plants, points represent individual measurements.

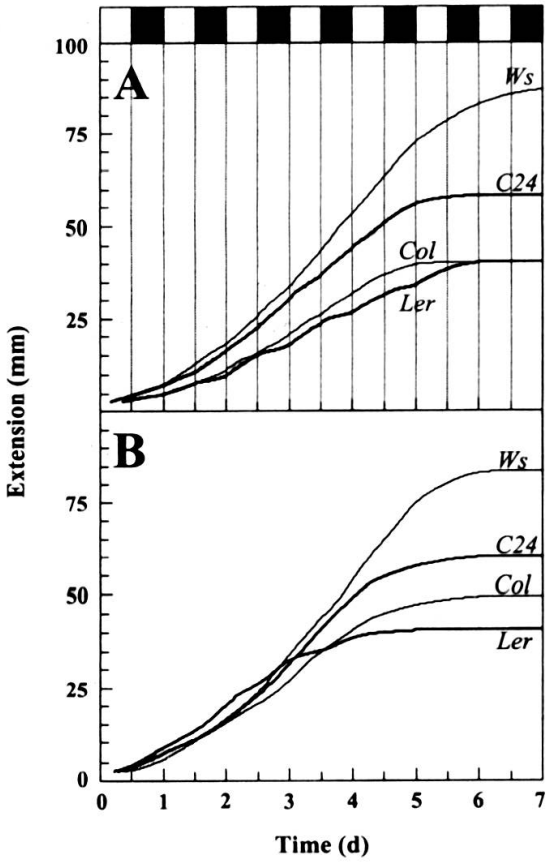

FIG. 2.

Elongation of the first floral stem internode in four ecotypes of *Arabidopsis thaliana* measured by the LVDT sensor in L:D (12:12) (A) and continuous light (B). Ler = Landsberg *erecta*; Col = Columbia; C24; Ws = Wassilewskija. The upper insert shows the photoperiodic environment during growth measurement (white = light; black = dark). Time 0 was considered as the beginning of the photoperiod of the first measurement day. Results are the mean of at least 5 repetitions, extension is expressed in mm.

Growth extension and rate

Figure 2 shows elongation of the first inflorescence internode of the four studied ecotypes during light/dark (Fig. 2A) and continuous light (Fig. 2B) regimes. It appeared the all stem elongation growth follow a sigmoidal like curves. Ler and Col ecotypes displayed the lower, non-significantly different, first internode elongation in the two photoperiodic conditions. On the contrary, the Ws ecotype shows the statistically largest extension and C24 an intermediate one.

When measurements were done in light/dark conditions, the inflorescence extension rate fluctuates considerably over the whole growth span (Fig. 3). Looking to Ler elongation rate, results show that the rate was really well synchronized with the photoperiod with higher extension rate during light than during dark exposure (Fig. 3A). The three other ecotypes did not show a well-synchronized pattern (Fig. 3BCD). Nevertheless, it seems clear that light and dark interruptions induced modifications in the behavior of the growth rate but without evidence for a clear repetitive response all along the growth period, at least for the C24 and Ws ecotypes (Fig. 3CD). Col response (Fig. 3B) was more repetitive along the light and dark period, but great modification in the growth rate and the synchronization was not as well marked than in Ler ecotype (Fig. 3A).

In continuous light exposure periods of successive low and high extension rate were observed with Ler and Col ecotypes. Time gaps between peaks or valleys showed a consistent regularity all along the stem elongation: they seemed to be of about 24 h (Fig. 4AB). The measured period, which are not statistically different, are 23.51 ± 1.23 h for Ler and 24.21 ± 1.68 h for Col. No clear and repetitive oscillation was observed with C24 or Ws ecotype when they were measured under continuous light condition (Fig. 4CD).

DISCUSSION

Some studies focused onto the differences between *Arabidopsis thaliana* ecotypes have been done. Differences in the chemical composition of epicuticular wax were found (RASHOTTE *et al.*, 1997). Moreover, early development, growth and flowering time measurement and comparisons have been done in some ecotypes (NORDBORG & BERGELSON, 1999; KISS *et al.*, 2000). Genetical comparisons, using QTL (SWARUP *et al.*, 1999), have also been done but this kind of study are not numerous and might to be promoted. In contrast to *Pissum sativum* (SINGER *et al.*, 1999), *Arabidopsis* ecotype inflorescence growth and architecture are poorly studied, nevertheless some works (RÉDEI, 1970; HAUGHAN *et al.*, 1995, JOUVE *et al.*, 1998a) have reported differences between ecotypes and inside the ecotype. Majors variations of *Arabidopsis* floral stem architecture, inside the ecotype group, have be found when plants were cultivated under different photoperiodic regimes (HAUGHAN *et al.*, 1995, JOUVE *et al.*, 1998a). Our results did not show that kind of thing because all plants are cultivated under L:D (12:12) photoperiod. Nevertheless, majors differences have been found between ecotypes. Ler have a significant smaller inflorescence than the Col, C24 or Ws ecotypes, but its ratio between internodes and whole inflorescence is the same than C24. The organization of the train of the internode length exhibits also a great difference between Ler and the other ecotypes. A continuous decrease in length occurred with the acropetally order of the internodes. The

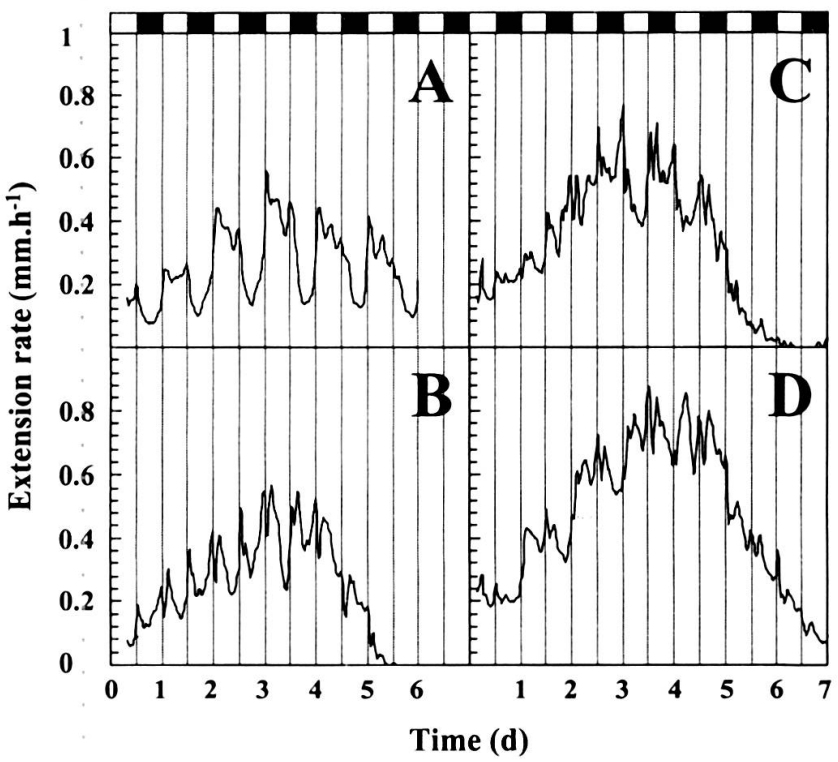

FIG. 3.

Arabidopsis thaliana first inflorescence internode extension rate of four ecotypes in L:D (12:12) as a function of time. A: Ler = Landsberg *erecta*. B: Col = Columbia. C: C24. D: Ws = Wassilewskija. The upper insert shows the photoperiodic environment during growth measurement (white = light; black = dark). Time 0 was considered as the beginning of the photoperiod of the first measurement day. Results are the data displayed by a representative measured plant, extension rate is expressed in $mm.h^{-1}$.

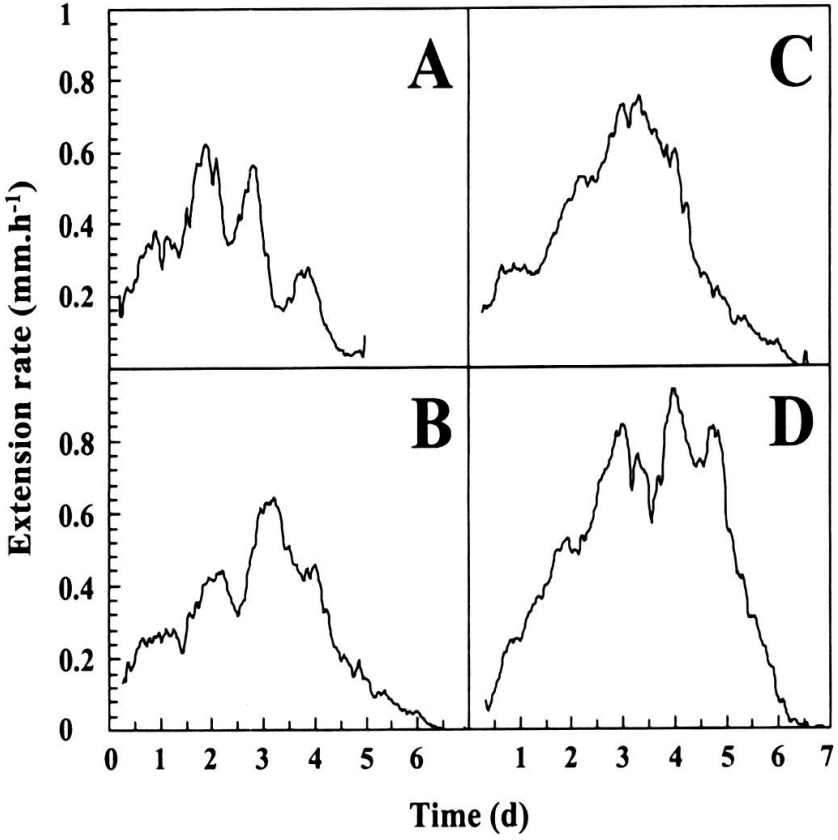

FIG. 4.

Arabidopsis thaliana first inflorescence internode extension rate of four ecotypes in continuous light as a function of time. A: Ler = Landsberg *erecta*. B: Col = Columbia. C: C24. D: Ws = Wassilewskija. Time 0 was considered as the beginning of the photoperiod of the first measurement day. Results are the data displayed by a representative measured plant, extension rate is expressed in $mm.h^{-1}$.

three other ecotypes showed fluctuations in length with alternation of small and longer internodes.

Arabidopsis first inflorescence node growth was measured during light/dark or continuous light. Under a L:D (12:12) cycle the Ler floral stem extension rate was modulated by the successive light-on and light-off. This entrainment property was usually observed as indicated by BÜNNING (1973) for biological rhythms. Nevertheless, this ability to synchronize the growth rate rhythm to the L:D (12:12) photoperiod seem no to be the case for all the ecotype studied. It has been shown in a previous paper (JOUVE *et al.*, 1998a) that Ler ecotype was really well synchronized under L:D (12:12) photoperiod but less or almost not under L:D (16:8) regime. This could be in relation with the ecological environment origin of the ecotype inducing a greater affinity with the photoperiod for expressing the rhythm and the synchronization property. Furthermore than synchronization, it could be seen in each ecotype a certain modulation of the growth rate linked to the light to dark or reciprocally transitions. This point will be developed in a following article of this issue (JOUVE *et al.*, 2000).

In continuous light exposure, periods of low and high extension rate were persisting and showed a circadian rhythmicity with Ler and Col ecotypes. This response has been already observed in *A. thaliana* for several parameters (JOHNSON *et al.*, 1995; MILLAR *et al.*, 1995a, 1995b; HICKS *et al.*, 1996, SWARUP *et al.*, 1999). Results observed with C24 and Ws did not display growth rate oscillation, and particularly circadian one.

Taken together, these results showed that in a same species gender, depending on the ecotype, the morphological and dynamical behavior is different. Moreover, results clearly displayed an expressed circadian rhythm in Ler and Col ecotypes but not in C24 and Ws. In view of the large interest of this species, and the possible genetical control of the circadian growth rhythm, it would be interesting to check more extensively the differences between *A. thaliana* ecotypes as SWARUP *et al.* (1999) did.

RÉSUMÉ

CROISSANCE DE LA HAMPE FLORALE DE PLUSIEURS ÉCOTYPES D'*ARABIDOPSIS*.
I. DIFFÉRENCES OBSERVÉES EN LUMIÈRE ALTERNÉE ET EN LUMIÈRE CONTINUE

L'architecture primaire de l'inflorescence et la dynamique du développement de la hampe florale de 4 écotypes d'*Arabidopsis* ont étés étudiées. Des différences claires ont été montrées entre les écotypes. L'écotype de Ler a une inflorescence courte et une distribution décroissante de la longueur des entre-nœuds. Les trois autres écotypes ont montré une organisation avec des alternances d'entrenœuds longs et courts. Au niveau de l'entrenœud, la dynamique de croissance de la tige florale a montré une bonne synchronisation avec l'alternance lumière et obscurité uniquement avec l'écotype Ler. De plus, des oscillations circadiennes dans la vitesse de croissance ont été observées avec les écotypes de Ler et Col mais pas avec C24 ou Ws.

Mots-clés: Hampe florale, architecture, élongation, synchronisation, rythme circadien, *Arabidopsis thaliana*.

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