The shoot apex as a marker of the responsivity to photoperiodic treatment inducing flowering of *Chenopodium rubrum* L.

A. BLAŽKOVÁ, Z. VONDRÁKOVÁ* and J. KREKULE

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 135, 165 02 Praha 6 - Lysolaje, Czech Republic

Abstract

Two maxima in flowering response to one inductive dark period of 13 h were found in the short day plant *Chenopodium rubrum* within three weeks of cultivation under continuous illumination either *in vitro* or *in vivo*. These maxima correlated with the number of leaf primordia and their relation to the size of the apical meristem. The first maximum in flowering responsivity corresponded with the stage when primordia of the second leaf pair had not yet overtopped the apical meristem, the second one when the primordia of the fourth leaf overgrew the meristem. Maximum responsivity to flowering reached by a mother plant was reflected in explants derived from it. The above morphological markers of responsiveness to floral induction were not linked to plant age and/or to general growth habit. The explants flowered only when part of the stem was present.

Additional key words: apical meristem, leaf primordia, photoperiodic flower induction, short-day plant.

Introduction

Responsivity to photoperiodic signals in floral induction usually changes with plant age (*e.g.* Bernier *et al.* 1981). This important developmental trait has generally been interpreted in terms of the juvenility concept (*e.g.* Podolnyi *et al.* 1989) or seen in gradual structural changes accompanying the development of the apical meristem (Bernier *et al.* 1981). These views are not mutually exclusive since the lack of sensitivity of an apical meristem to the florigenic stimulus, reflecting its developmental state, is considered to be one aspect of juvenility. However, in our previous experiments two maxima of high responsivity to photoperiodic flower induction were observed in the short-day plant *Chenopodium rubrum* (Ullmann *et al.* 1985). The first, the early one, occurs already at the cotyledonary stage, the second after four visible pairs of leaves have been formed. Thus the juvenility concept cannot be applied to the control of flowering in *Chenopodium* which is characterized by early (neotenic) flowering, bypassing juvenility. This trait focuses attention upon structural changes *per se* and on the role of individual organs. The aim of these experiments was to find criteria and/or markers of fluctuation in responsivity to floral inductive treatment. The problem was addressed using both *in vitro* and *in vivo* systems, enabling the comparison of photoperiodic sensitivity in plants with different rates of development and with different morphological characteristics. Investigation of the role of individual organs involved in changes of photoperiodic responsiveness may be better tackled *in vitro*.

Material and methods

Cultivation *in vitro*: Seeds of the short-day plant *Chenopodium rubrum* L., ecotype 374, were sterilized in a 0.1 % solution of $HgCl_2$ for 10 min, rinsed extensively in sterile distilled water, spread in Petri dishes on 0.7 % agar medium(*Difco Bacto* agar) supplemented with 0.5 % glucose and transferred to growth chambers under the

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* To whom correspondence should be addressed; fax: (+420) 2 20390419, e-mail: krekule@ueb.cas.cz

following regime: light (12 h, 30 °C), darkness (12 h, 10 °C), light (28 - 30 h, 30 °C). The spreading of the seeds was designated as time 0.

Germinated seeds were transferred to 100 cm^3 flasks with 25 cm^3 of basic cultivation medium (half-strength Knop's nutrient solution, 0.7 % agar, 2 % sucrose, pH 5.6). The plants were then grown in small-volume growth chambers at 21 ± 2 °C under continuous light (8 white fluorescent tubes, *Tesla* 811, 18 W, irradiance 184 μmol m^{-2} s^{-1} at plant level).

Cultivation *in vivo* has already been described by Ullmann *et al.* (1985). Briefly: Selected plantlets from seeds germinated *in vivo* or *in vitro* were cultivated in perlite in small plastic vessels and watered with half strength Knop's solution. The conditions of germination and cultivation were the same as described for the *in vitro* system.

Cultivation of explants: The terminal explants were taken from intact plant cultivated *in vitro* under continuous illumination and transferred to fresh basic medium. Successive explantation started with those plants in which the 2nd leaf pair was unfolding and the fourth leaf pair had already been originated at the shoot apex. Four types of explants at different morphological stages of the shoot apex were used *a*) shoot (plant without roots), *b*) shoot without cotyledons, *c*) shoot without cotyledons and the first leaf pair, the stem bellow the leaves is presented on the explant, *d*) shoot without cotyledons, the first leaf pair and the stem. Six experiments of this type were performed and the values given represent the average of 60 explants.

Photoperiodic induction *in vitro* and *in vivo*: Plants were successively exposed at one day intervals to one inductive dark period of 13 h during the three weeks of cultivation. All types of explants were subjected to one inductive dark period of 13 h immediately after their explantation from mother plant. Flowering of intact plants and explants was always determined 7 d after the end of the inductive treatment, using a stereomicroscope. The percentage of plants with initiated floral organs was evaluated. Control plants (explants) were grown under continuous illumination. Morphological parameters such as the length of the shoot, the hypocotyl, the cotyledons and the leaves, the total number of leaves (leaf primordia on the shoot apex included) and the dry mass of the shoot and the roots were evaluated prior to each photoperiodic treatment. The experiment was repeated five times; each value is the average of 50 plants ± SE.

Results

In *Chenopodium rubrum* grown *in vitro*, the first maximum of flowering induced by dark period of 13 h occurred at the age of 4 - 5 days, the second one from the age of 17 ± 2 d. Full flowering of plants between 4 - 5 d and 17 ± 2 d was obtained only after 2 or 3 inductive cycles (Fig. 1*A*). The first maximum of flowering appeared, when the primordia of the second leaf pair had not overgrown the shoot apex, the second one when the primordia of the fourth leaf pair had overgrown the apex. Besides these morphological parameters no other growth characteristics measured, such as length of the shoot, the hypocotyl, the cotyledon or the leaves, nor the number of leaves, could be correlated with the maxima of responsiveness to photoperiodic induction of flowering (Fig. 1*B*). Also, changes in dry matter of the shoots and of the roots showed predominantly continuous characteristics, not pointing to any links with the maxima of flowering (Fig. 1*C*).

We compared the pattern of photoperiodic responsivity of plants grown *in vitro* and *in vivo*. The growth rate in the first case is slower, and this provided an opportunity to compare photoperiodic sensitivity in plants differing in age and in growth habit (number and shape of the leaves). The highest responsiveness to photoperiodic treatment occurs at the two stages of the development of leaf primordia as already mentioned (2nd and 4th leaf pair), irrespective of the chronological plant age and general growth habit.

In explants flowering occurred only in those excised from plants with the 4th leaf pair (excision is not possible before third leaf stage) overgrowing the apex and also with later time of excision (Fig. 2). No flowering was observed on explants with the stem removed.

Discussion

Our results summarizing the experiments with intact plants indicate that the two maxima of higher sensitivity to dark period inducing flowering in *Chenopodium rubrum* are reflected by two distinct stages in shoot apex development. Readiness to flower is sometimes linked with critical size of the apex (*e.g.* Horridge and Cockshull 1979) or duration of the plastochron (Jacobs 1972). This does not apply in our case as the respective stages of

higher responsivity do not differ markedly either in apical meristem size, nor is the plastochron changed within the given period of recording the response. The problem of changing capacity for photoperiodic induction in *Chenopodium rubrum* has already been addressed by Seidlová and Opatrná (1978). They concluded that photoperiodic sensitivity is linked with the amount of available meristematic tissue of the apical meristem,

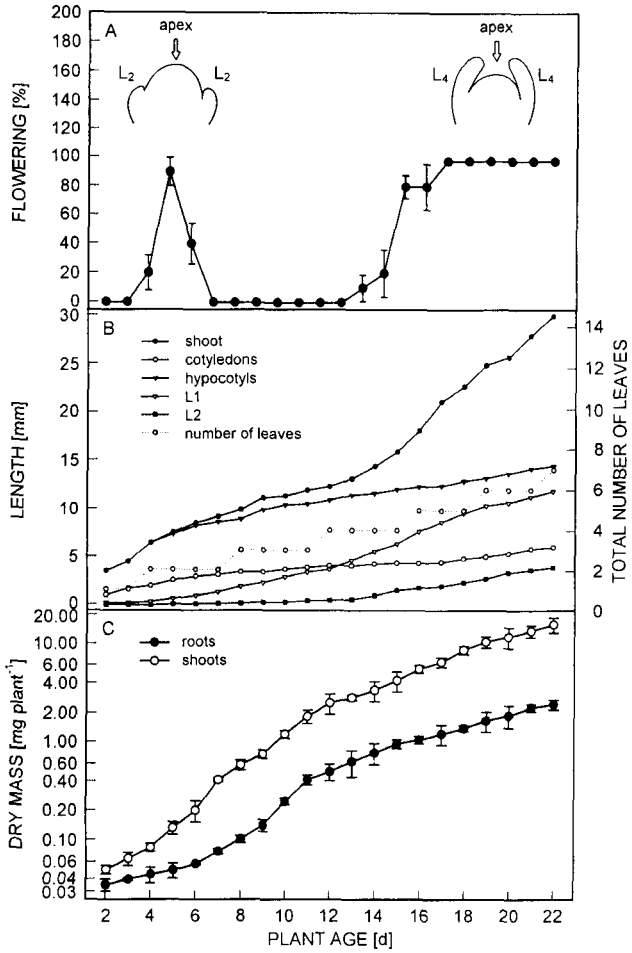

Fig. 1. *A* - The flowering of intact plants of *Chenopodium rubrum in vitro* induced to flower by one inductive darkness (13 h) at the age of 1 to 22 d. Above: schematic drawing of the growth pattern of leaf primordia (L_2 - second leaf pair, L_4 - fourth leaf pair) of the vegetative shoot apex at two periods of maximal responsivity to photoperiodic treatment. *B* - The morphological parameters (the length of the shoot, the hypocotyl, the cotyledons, the leaves and the total number of the leaves with leaf primordia at the shoot apex included) of intact plants of *Chenopodium rubrum* estimated at one day intervals beginning with the 2 nd day. In all cases the SE did not exceed 10 % of given values. *C* - The shoot and root dry mass of intact plants of *Chenopodium rubrum* estimated at one day intervals beginning with the 2 nd day.

which reflects the stage of the plastochron. Hovewer, some other possibilities should be considered when interpreting our results. The first maximum of flowering is characterized by the differentiation of the second leaf pair and coincides with the high rate of cotyledon growth: at that time these are the only receptors of the photoperiodic signal (Ullmann *et al.* 1985). The second leaf pair primordia, which are positionally formed directly above the cotyledons (two successive leaf pairs are initiated in the same perpendicular plane), have not yet developed vascular bundles (preliminary results, data not shown). Hence, the florigenic signal transported from the cotyledons (phloem is the generally accepted transporting path) may reach the target tissue of the apex without being "attracted" by the second leaf primordia. In further development, characterized by the reduced photoperiodic sensitivity, the vascular bundles of the leaves are already well developed and the florigenic stimulus may be transported to them from cotyledons, without reaching the meristematic tissues to be evoked. The restoration of the responsivity to one dark period already reported by Ullmann *et al.* (1985) would then coincide with the replacement of the cotyledons by leaves as receptors of the florigenic signal. It could be that even the second maximum of capacity to flowering might be linked with formation of vascular bundles along the shoot, but

only detailed histological analysis can elucidate this question.

TYPE OF THE EXPLANT	PERCENTAGE OF FLOWERING [%] MORPHOLOGICAL STAGE OF APEX OF THE MOTHER PLANT				
	L_3	L_3	L_4	L_4	L_5
a	0	0	0	90 ± 10	90 ± 10
b	0	0	0	90 ± 10	90 ± 10
c	0	0	0	90 ± 10	90 ± 10
d	0	0	0	0	0

Fig. 2. Schematic drawings of the terminal explants of *Chenopodium rubrum* and of their shoot apex at the moment of inductive treatment by 13 h of darkness. The degree of flowering was estimated 7 days later: *a* - shoot (plant without roots), *b* - shoot without cotyledons, *c* - shoot without cotyledons and the first leaf pair, the stem bellow the leaves is presented on the explant, *d* - shoot without cotyledons, the first leaf pair and the stem (C - cotyledons, $L_{3,4,5}$ indicate serial number of the leaf pair; 50 explants were evaluated in each treatment).

The importance of the transport system in responsiveness to induction is further emphasized by the observation that the smallest shoot segment cut above the 1st leaf pair and comprising only the 1st internode and the three youngest leaves could be induced to flowering, while the isolated segment without internodes never flowered. A similar phenomenon has been reported for hypocotyl segments in *Chenopodium rubrum* by Josefusová (1985) and for the stem segment at the base of isolated shoot apices of *Lolium temulentum* by McDaniel *et al.* (1991). The stem may play, at least in *Chenopodium rubrum*, a role of receptor of the photoperiodic signal, but principally also assist in transport of the compounds participating in floral induction (Vondráková and Krekule 1997/8). We should take into account both the substances which have to arrive to the apex and those which are possibly to be transported away (*e.g.* inhibitors). In this connection the role of auxin and its basipetal movement and that of cytokinins, with their acropetal movement, should be considered (Bangerth 1994).

Comparison of the flowering patterns of *in vivo* and *in vitro* intact plants reveals strong similarity with respect to the growth pattern of the apex but a delay in time when the maxima are reached *in vitro* after 2 days for the first peak and 5-7 days for the second one. This again provides evidence that the fluctuation of the degree of responsiveness does not reflect chronological age. Moreover, flowering occurred only in the segments excised at the moment when the 4th leaf pair overgrew the apex, irrespective of the number of the leaves on the segment, providing that part of the stem was present. Other times of excision did not bring about flowering. Thus, the shoot segments are able to fix and further express the developmental state of the mother plant, despite any dislocation caused by removal from it.

The morphological markers of high responsivity to floral induction which we have described may be used for precise timing of the treatments affecting flowering.

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