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THE PHOTOPERIODIC CONTROL OF GROWTH AND DEVELOPMENT OF *CHENOPODIUM RUBRUM* L. PLANTS *IN VITRO*

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Abstract – Influence of the photoperiod on growth, flowering, and seed development *in vitro* of *Chenopodium rubrum* L., a short day annual, was examined. *Chenopodium rubrum* plants modify their growth and reproductive development in accordance with the photoperiod. With an increase of day length, growth was stimulated, flowering was delayed, seed development occurred earlier, and the plants produced more seeds. By altering photoperiods during induction and evocation of flowering, it is shown that the photoperiod experienced by seedlings during early reproductive development determines the pattern of plant growth to the end of ontogenesis, the time to flowering, and the course of seed development. It is therefore concluded that growth and reproductive development of *C. rubrum* are photoperiod-sensitive to during a precise short part of its life cycle.

Key words: Chenopodium rubrum, flowering, growth, photoperiod, seed development.

UDC 582.661.15 : 581.1 : 581.14

INTRODUCTION

Being sessile organisms, plants cannot choose their surroundings and have to modify their growth and development according to the environment. They often respond to environmental variation with phenotypic plasticity (G a l l o w a y, 2004). The developmental cycle of annuals, starting with seed germination and ending with seed maturation, is well synchronized with seasonal changes and is completed before the start of the growth limiting season. Light is one of the most important environmental signals regulating plant development. Plants register the quantity, quality, periodicity and direction of light, according to which they modify many physiological processes, from germination to the architecture of adult plants and reproductive development (F r a n k l i n and W h i t e l a m, 2004). For example, in 22 desert annuals from Israel, great differences in the number of leaves, leaf shape, type of branching, seed size, and seed coat color resulted from exposure to different day lengths (G u t t e r m a n and E v e n a r i, 1972). Previous results (M i t r o v i ć et al., 2002) showed that the photoperiod affects *C. rubrum* growth (Ž i v a n o v i ć et al., 1995) and seed size (M i t r o v i ć et al., 2002).

Chenopodium rubrum L. sel. 184 is a qualitatively short-day weedy annual sensitive to small changes in day length with a defined critical night length of 8 h (T s u c h i y a and I s h i g u r i, 1981). It is sensitive to photoperiodic stimulation of flowering as early as at the cotyledonary stage (S e i d l o v á and O p a t r n á, 1978), when six adequate photoperiodic cycles are sufficient for photoperiodic flower induction. As an early-flowering species (C u m m i n g, 1967), it is a suitable model plant for studying ontogenesis *in vitro*. *In vitro* culture method was used because of the precise control over environmental (surrounding) conditions (S c o r z a, 1982).

The aim of this study was to evaluate the effects of different photoperiodic conditions on *C. rubrum* growth, flowering, and seed development.

MATERIAL AND METHODS

Seed propagation

Plants for seed propagation were grown in a greenhouse of the Siniša Stanković Institute for Biological Research in Belgrade (44º 49′ N, 20º 29′ E) under conditions of natural day length from February (10 h of

light/14 h of darkness) to May (13 h/11 h) at temperatures that varied in the range of approximately 15 – 20ºC.

Plants in vitro

Experiments were carried out with intact *C. rubrum* plants derived from seeds sown *in vitro*. Seeds were surface-sterilized with 4% Na-hypochlorite for 2 min, washed with sterile distilled water, and aseptically sown on moistened filter paper in Petri dishes. Uniform germination was attained by exposure to temperature and dark/light cycles (24 h of darkness at 32°C, 24 h of darkness at 10°C and 48 h white light at 32°C). Four-day-old seedlings were transferred to glass jars containing 100 ml of MS (M u r a s h i g e and S k o o g, 1962) mineral solution supplemented with sucrose (5%) and gelled with agar (0.7%).

Photoperiodic treatments

Seedlings (48 per treatment) with fully developed cotyledons (4 days old) were exposed to different photoperiodic treatments for 10 weeks: continuous light (24 h/0 h), continuous darkness (0 h/24 h), on 8 h/16 h photoperiod, a 14 h/10 h photoperiod, a 16 h/8 h photoperiod, 6 days of an 8 h/16 h + 9 weeks of a 16 h/8 h photoperiod, or 6 days of a 14 h/10 h photoperiod + 9 weeks of a 16 h/8 h photoperiod. Irradiance was about 70 µmol m^{-2} s^{-1}. Temperature in the growth chambers was 25 ± 2ºC.

Measurements and statistics

Every 7 days during the 10 weeks, height of the plants was measured and the number of leaves, number of fully developed flowers, and number of plants with matured seeds were determined. At the end of the 10th week, matured seeds were collected, dried for one month, and measured (four replicates of 100 seeds). Treatment effects were determined using analysis of variance (ANOVA) combined with multiple range tests (significance level of $p<0.05$).

RESULTS AND DISCUSSION

Effect of the photoperiod on growth

With an increase of day length, growth is stimulated (Fig. 1a). At the end of their life cycle (after 10 weeks of culturing *in vitro*), plants grown under conditions of 24 h long-day photoperiod (noninductive for flowering of *C. rubrum*) were approximately twice as high as plants grown under a 16 h/8 h photoperiod and even seven times higher than plants grown under a 14 h/10 h photoperiod, both of the latter being inductive for flowering of *C. rubrum*. This is in agreement with the results of C o o k (1975), showing that *C. rubrum* plants grown under a 15 h/9 h photoperiod were higher and had more nodes than plants grown under 12 h/12 h photoperiod. The only exception in the trend of the ratio between day length and plant height (Fig. 1a) was exhibited by plants grown under continuous darkness (inductive for flowering of *C. rubrum*) due to etiolation. The most intensive growth during the first 8 weeks is noticed under a 16 h/8 h photoperiod (Fig. 1a), which corresponds to natural photoperiods for *C. rubrum*.

As already stated, six adequate photoperiodic cycles at the cotyledonary stage of development are sufficient for photoperiodic flower induction of *C. rubrum* (O p a t r n á et al., 1980). The pattern of *C. rubrum*

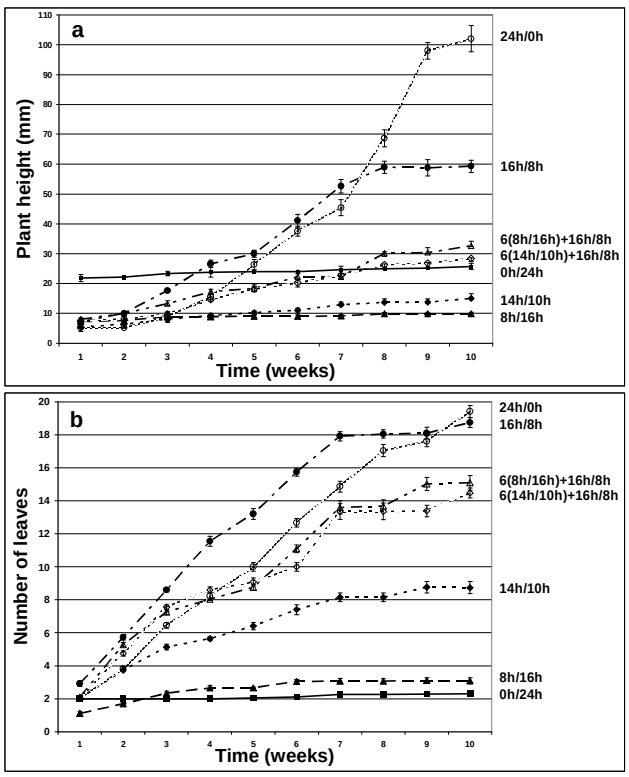

Fig. 1. Effect of different photoperiods (10 weeks of continuous light - 24 h/0 h, 10 weeks of continuous darkness - 0 h/24 h, 10 weeks of an 8 h/16 h photoperiod, 10 weeks of a 14 h/10 h photoperiod, 10 weeks of a 16 h/8 h photoperiod, 6 days of an 8 h/16 h + 9 weeks of a 16 h/8 h photoperiod, or 6 days of a 14 h/10 h + 9 weeks of a 16 h/8 h photoperiod) on *C. rubrum* growth during ontogenesis *in vitro*: plant height was (a) measured and number of leaves (b) was determined every 7 days for 10 weeks; means ± SE, n = 48.

Fig. 2. *Chenopodium rubrum* plants grown *in vitro* under different photoperiodic conditions (10 weeks on an 8 h/16 h photoperiod, 10 weeks of a 14 h/10 h photoperiod, 10 weeks of a 16 h/8 h photoperiod, 6 days of an 8 h/16 h photoperiod + 9 weeks of a 16 h/8 h photoperiod, 6 days of a 14 h/10 h photoperiod + 9 weeks of a 16 h/8 h photoperiod or 10 weeks of continuous light - 24 h/0 h), after 10 weeks of culturing.

growth to the end of ontogenesis is also affected by the photoperiod applied during flowering induction, but is affected as well by the photoperiod following shortly after (evocation of flowering) (Fig. 1a). A significant difference in height is noticeable among plants grown under different photoperiods for only the first 6 days of the total of 10 weeks (and under the same photoperiod for the following 9 weeks), while plants grown under different photoperiods for 9 of the total of 10 weeks (and under the same photoperiod only during the first 6 days) are similar in height (Fig. 1a). This points to importance of the photoperiod during induction and evocation of flowering for determination of final plant height. Plants grown for the first 6 days under a 8 h/16 h photoperiod and then transferred to a 16 h/8 h photoperiod for the remaining 9 weeks were about two times shorter than plants grown under a 16 h/8 h photoperiod for all 10 weeks and three times higher than plants grown continuously under an 8 h/16 h photoperiod (Fig. 1a, Fig. 2). Similarly, plants grown for the first 6 days under a 14 h/10 h photoperiod and transferred to a 16 h/8 h photoperiod for the remaining 9 weeks were about two times shorter than plants grown under a 16 h/8 h photoperiod for all 10 weeks and at the same time twice as high as those grown continuously under a 14 h/10 h photoperiod (Fig. 1a, Fig. 2).

Transient inhibition of growth at the time of flowering (O p a t r n á et al., 1980; U l m a n n et al., 1980; M i t r o v i ć, 1998) was noticeable (Fig 1a). The longer the inductive day length was, the more delayed was the flowering (Fig. 3a) and the flowering-related transient inhibition of growth (Fig. 1a). Under 8 h days, growth stopped at the time of flowering (Fig. 1a, Fig. 3a) and plants tips started to dry up as early as the 7th to 8th week

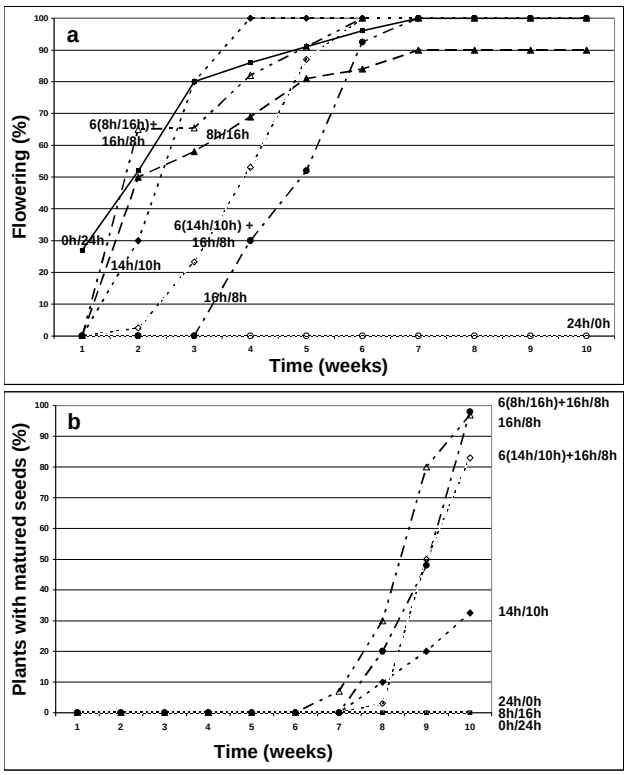

Fig. 3. Effect of different photoperiods (10 weeks of continuous light - 24 h/0 h, 10 weeks of continuous darkness - 0 h/24 h, 10 weeks of an 8 h/16 h photoperiod, 10 weeks of a 14 h/10 h photoperiod, 10 weeks of a 16 h/8 h photoperiod, 6 days of an 8 h/16 h photoperiod + 9 weeks of a 16 h/8 h photoperiod, or 6 days of a 14 h/10 h photoperiod + 9 weeks of a 16 h/8 h photoperiod) on *C. rubrum* flowering and seed development *in vitro*: the number of fully developed flowers (a) and number of plants with matured seeds (b) were determined every 7 for during 10 weeks; means ± SE, n = 48.

of culturing (Fig. 3b). Plants grown under noninductive continuous light did not flower (Fig. 3a) until the end of the 10th week of culturing, and stem elongation was relatively linear (Fig. 1a).

Different photoperiodic conditions also affected the number of leaves (Fig. 1b). Extension of day length (shortening of night length) stimulated leaf development. C o o k (1975) noticed an increase in the number of leaves of *C. rubrum* plants grown under 15-h days compared to ones grown under 12-h days. Like stem elongation (Fig. 1a), leaf development (Fig. 1b) was also affected by the photoperiod to which the seedlings were exposed during the first 6 days (flowering induction) and the photoperiod following immediately after (evocation of flowering).

Leaf development was inhibited (Fig. 1b) with the start of seed development (Fig. 3b). In plants grown in continuous darkness, leaf development stopped with flowering (around the 3rd week), and plants tips started to dry after the 7th to 8th week of culturing. In plants grown under noninductive continuous light, there was no flowering and the number of leaves increased linearly throughout all 10 weeks (Fig. 1b). A high correlation was found between the number of leaves and flowering percentage in *C. rubrum* grown in continuous darkness and *C. murale* grown under inductive photoperiodic conditions (M i t r o v i ć, 1998; M i t r o v i ć et al., 2000).

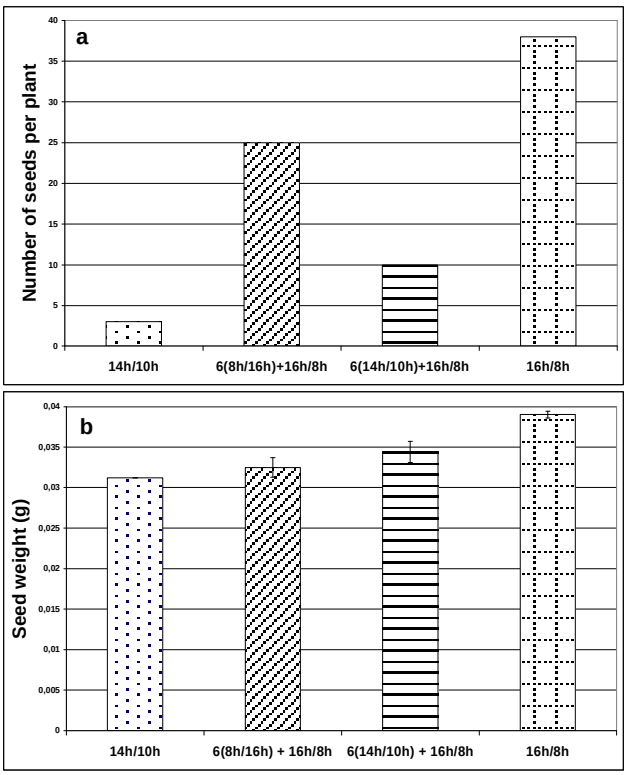

Fig. 4. Effect of different photoperiods (10 weeks of a 14 h/10 h photoperiod, 6 days of an 8 h/16 h photoperiod + 9 weeks of a 16 h/8 h photoperiod, 6 days of a 14 h/10 h photoperiod + 9 weeks of a 16 h/8 h photoperiod, or 10 weeks of a 16 h/8 h photoperiod) *in vitro* on the number of produced seeds and seed weight: matured seeds were collected from each plant and their number (a) counted at the end of 10th week of culturing and seeds were measured (b) after one month of drying (four replicates of 100 seeds); means ± SE.

Effect of the photoperiod on flowering

The shorter the inductive day was, the earlier flowering occurred (Fig. 3a). In plants grown in continuous darkness, flowering occurred as early as after the 1st week of culturing, while in plants grown under a 16 h/8 h photoperiod, flowering did not start until the 4th week. Adequate day length during the first 6 days is sufficient for flowering induction. However, the time to flowering (and growth, as already shown) is also determined by the photoperiod applied during evocation of flowering (Fig. 3a). Plants grown continuously under a 14 h/10 h photoperiod flowered during the 2nd week of culturing. Transferring plants grown under the same photoperiod after 6 days to a 16 h/8 h photoperiod delayed flowering to the 3rd week, while plants grown continuously under a 16 h/8 h photoperiod flowered after 4 weeks. A similar trend in flowering is evident in comparing three groups of plants: 1 - grown continuously under an 8 h/16 h photoperiod, 2 – grown 6 days under an 8 h/16 h photoperiod and transferred to a 16 h/8 h photoperiod, and 3 – grown continuously under a 16 h/8 h photoperiod (Fig. 3a).

Effect of the photoperiod on seed development

The photoperiod affected *C. rubrum* seed development *in vitro* in regard to both the time of seed maturation (Fig. 3b) and the number of matured seeds per plant (Fig. 4a). The longer the day is the earlier the seed maturation occurs (Fig. 3b) and the higher the number of matured seeds is (Fig. 4a).

Seed maturation started 4–5 weeks after flowering and like flowering responded to day length during flowering induction and evocation. Plants grown the first 6 days under a 14 h/10 h photoperiod and then transferred to a 16h /8 h photoperiod for the remaining 9 weeks produced about three times more seeds compared to plants grown continuously under a 14 h/10 h photoperiod and

about for four times fewer seeds compared to plants grown continuously under a 16 h/8 h photoperiod (Fig. 4a). Plants grown continuously under an 8 h/16 h photoperiod flowered (Fig. 3a), but did not produce seeds (Fig. 3b). Plants grown under an 8 h/16 h photoperiod during the first 6 days and then transferred to a 16 h/8 h photoperiod for the remaining 9 weeks produced approximately half of the total number of seeds produced by plants grown continuously under a 16 h/8 h photoperiod (Fig. 4a).

The highest number of seeds matured under conditions of the longest inductive photoperiod (16 h/8 h), while approximately 12 times fewer seeds matured under a 14 h/10 h photoperiod (Fig. 4). This is in agreement with C o o k's (1975) finding that *C. rubrum* plants produced a significantly greater number of seeds under a 15 h/9 h photoperiod compared to a 12 h/12 h photoperiod.

Chenopodium rubrum plants grown in continuous darkness (conditions inductive for flowering) flowered (Fig. 3a), but did not produce seeds (Fig. 3b). *Centaurium pulchellum* plants grown under continuous darkness *in vitro* did produce seeds, but those seeds were not viable (C v e t i ć et al., 2004).

Seeds collected from plants 10 weeks old, were dried for one month at room temperature and examined. Like seed number (Fig. 4a), seed weight (Fig. 4b) was affected by the photoperiod applied during flowering induction and evocation. This is in agreement with C o o k (1975), who showed that in 10-day-old *C. rubrum* seedlings, seed weight is determined by the photoperiod from the 4th to 8th day of reproductive development. He also showed that plants grown under a 15 h/9 h photoperiod produced a greater number of small seeds compared to plants grown under a 12 h/12 h photoperiod. Similar results were obtained on the quantitatively short day plant *C. quinoa* (B e r t e r o et al., 1999) and on different species of the genus *Chenopodium* (B e w l e y and B l a c k, 1982): plants grown under short days produced larger seeds compared to those grown under long days.

In our *in vitro* conditions, seeds collected from plants grown under a 16 h/8 h photoperiod were significantly heavier than those collected from plants grown under a 14 h/10 h photoperiod (Fig. 4b). As in previous work (M i t r o v i ć et al., 2002), we obtained opposite results in *C. rubrum* plants grown in a greenhouse and ones grown outdoors (under conditions of shorter photoperiods, winter-grown plants produced a small number of 4.3 times heavier seeds compared to plants grown during the summer under long photoperiods). We believe that temperature plays a significant role in determining the weight of seeds produced under conditions of the same photoperiod. *In vitro* culture provides an optimal supply of mineral nutrients, optimal humidity, and a temperature of 25ºC, while in the greenhouse a 15 h/9 h photoperiod was accompanied by high temperature and low humidity. In *C. quinoa* (B e r t e r o et al., 1999), the effect of the photoperiod on seed weight was strongly influenced by temperature, while growth temperature affected seed coat weight in *Plantago lanceolata* (L a c e y et al., 1997).

In agreement with C o o k (1975), our results indicate that *C. rubrum* seed weight is determined early during reproductive development. In nature, *C. rubrum* plants receive photoperiodic induction for flowering when summer days become shorter. Since the size and number of seeds is determined during early reproductive development, natural flowering induction works in line with minimizing seed weight and maximizing seed number, favoring physiological mechanisms that work under suboptimal photoperiods, thereby maximizing the probability of survival (C o o k, 1975).

We showed that *C. rubrum* plants modify their growth and development in accordance with the photoperiod. With an increase of day length, plant height increased, flowering was delayed, seed development occurred earlier, and the plants produced more seeds. The obtained results showed that the growth pattern to the end of ontogenesis, flowering, and seed development are all determined by the photoperiod experienced by seedlings during the early phases of reproductive development - induction and evocation of flowering. *Chenopodium rubrum* shows crucial sensitivity to the photoperiod during a short, precise, and very early period of its life cycle, when key processes in its development are determined.

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ФОТОПЕРИОДСКА КОНТРОЛА РАСТЕЊА И РАЗВИЋА *CHENOPODIUM RUBRUM* L. *IN VITRO*

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Испитиван је ефекат фотопериода на растење, цветање и сазревање семена *in vitro* краткодневне једногодишње биљке *Chenopodium rubrum* L. Показано је да је растење и развиће биљке *C. rubrum* одређено фотопериодом коме су изложене. Са продужењем дужине дана, стимулирано је растење, одлаже се цветање, скраћује се време потребно за формирање семена и биљке производе више семена. Променом фотопериода током индукције и евокације цветања, показано је да фотопериод коме су клијанци изложени током најранијег репродуктивног развића одређује њено растење до краја онтогенезе, као и цветање и сазревање семена. Према томе, вегетативно и репродуктивно развиће *C. rubrum* осетљиво је на фотопериод у тачно одређеном, и кратком периоду животног циклуса.