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# Is the Root Effect on Flowering of *Chenopodium rubrum* Mediated by Cytokinins?

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**Abstract.** Root removal enhances flowering in the short day plant *Chenopodium rubrum*. The extent of this effect depends on the de-rooting time with respect to photoperiodic induction. The largest promotive effect is observed when de-rooting coincides with the start of the inductive treatment or, to a lesser extent, when performed before it. De-rooting 24 h after induction has no effect on flowering. The flower-inducing action of de-rooting 24 h before the start of induction is increased by benzylaminopurine (BAP), whether applied simultaneously with de-rooting or 24 h later. At the beginning of darkness, BAP inhibits flowering slightly when applied simultaneously with de-rooting but inhibits it strongly when applied 24 h later. Flowering in plants de-rooted 24 h after induction is inhibited strongly by BAP. Root removal at the beginning of inductive darkness does not change the level of endogenous cytokinins in induced shoot explants, but under continuous light the level of cytokinins in shoot explants decreases during the same period compared with the level in the shoots of intact plants. BAP does not affect the level of endogenous cytokinins in light but causes an apparent increase in induced segments. Thus, two phases of the de-rooting effect and cytokinin treatment may be distinguished: one in which flowering is enhanced by both treatments and which is linked directly to photoperiodic flower induction, and the other in which both treatments are inhibitory to flowering and which is related to morphogenetic events following induction. The time courses of the effectiveness of de-rooting and BAP treatment differ slightly, suggesting that the effect of de-rooting cannot be attributed solely to cytokinin deprivation.

**Key Words.** *Chenopodium rubrum*—Cytokinins—Photoperiodic flower induction—Root removal—Short-day plant

Numerous experimental data indicate an important, if not indispensable, role of roots in the control of flowering. Most of the evidence comes from experiments with root removal or various root treatments. However, as indicated in a recent review by Kinet et al. (1993) the results are still highly controversial because both promotive and inhibitory effects of roots on floral transition have been observed depending on the developmental state of the plants and the timing of the treatments with respect to flower induction. Such a statement holds true for both photoperiodic groups of plants. The root effects on flowering are commonly interpreted in terms of cytokinin control.

Cytokinins are believed to represent one of the components in the multifactorial regulatory system of flowering (Bernier et al. 1977, 1993) and are synthesized predominantly in the roots with subsequent transport to the shoots (for review, see Hoad 1995). However, the analysis of cytokinins has been mostly neglected in experiments designed to investigate the floral role of roots. On the other hand, treatment with exogenous cytokinins has often been used to mimic root effects (Miginiac 1978).

In the short day plant *Chenopodium rubrum* L., the promotive effect of root removal on flowering has been demonstrated, provided the treatment is performed after photoperiodic induction (Krekule and Přívratský 1976, Krekule and Seidlová 1973). The rise in flowering caused by de-rooting is, to a great extent, canceled by cytokinin application, which has been interpreted as enhancement of vegetative morphogenesis at the apex (Krekule and Seidlová 1977). The inhibitory action of benzylaminopurine (BAP) on the flowering of de-rooted

Abbreviation: BAP, benzylaminopurine.

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explants was explained in a similar way by Blažková et al. (1998). Further, we observed that the short day regime, inductive for flowering, brings about inhibition of root growth (Josefusová et al. 1985). Also, the short day regime enhances temporarily the cytokinin content in root exudates of *Chenopodium* at the end of the dark period. This process is regulated by phytochrome (Macháčková et al. 1996).

Considering the above results we decided to investigate the role of roots in flowering and their possible action via cytokinins. We addressed the following questions: (1) What is the effect of the timing of root removal with respect to the short day treatment? (2) Can the root effects be mimicked by cytokinin application? (3) How do these two treatments affect cytokinin levels in shoots?

To minimize the stress caused by de-rooting and to enable standard application of cytokinins via medium, an in vitro system of cultivation was adopted.

## Materials and Methods

### *Plants*

The experiments were performed using the short day plant *C. rubrum* L., ecotype 374, which can be induced to flower by 1 short day (Ullmann et al. 1985). The seeds were sterilized with 0.1% $HgCl_2$ for 15 min and washed thoroughly in distilled water. The germination took place on 0.7% agar medium with 0.5% sucrose and 0.5% glucose. The seeds were exposed to temperature and light dark fluctuation to ensure uniform germination (12 h of light, 30°C; 10 h of darkness, 10°C; 12 h of light, 30°C). Further cultivation proceeded in Erlenmeyer flasks on LS medium (Linsmaier and Skoog 1965) with 2% sucrose and without phytohormones. The flasks were placed in small volume growth chambers under continuous illumination (white fluorescent tubes, Tesla 811, 8 × 18 watts, 184 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level) at 20°C ± 2°C for 2 weeks.

Two-week-old plants consist of roots, hypocotyl, five pairs of leaves and leaf primordias, and the shoot apex. The first pair of leaves finished maturation, and the second one is fully grown. The terminal bud contains all other leaves and the shoot apex.

### *Root Removal*

The roots were removed under sterile conditions in a set of plants that were transferred to fresh medium of the same composition as described above. After root removal the whole shoots were used in experiments with intact plants as the controls. The surgical treatment was done (1) 24 h before the beginning of the photoperiodic treatment (12 h of darkness); (2) immediately before the photoperiodic treatment; or (3) 24 h after the beginning of it.

### *BAP Application*

The shoots were transferred to the medium with BAP at a concentration of 0.02, 0.2, or 0.5 mg/liter immediately after de-rooting or 24 h later in the first experiment. In the following experiments only the 0.2 mg/liter concentration was used. The control intact plants were transferred to the medium with BAP at the same time as isolated shoots.

### *Flowering*

Both the intact plants and the isolated shoots on cultivation medium with or without BAP were cultivated further under continuous illumination in small volume growth chambers for 1 week. The degree of flowering was scored using a stereomicroscope.

All experiments were performed three times. The results represent a mean of 15–20 plants from one experiment. The other experiments gave similar results.

### *Cytokinin Analyses*

The material from photoperiodically treated explants was sampled 24 h after explantation (immediately after inductive darkness), and at the same time shoots from intact control plants were sampled. Photoperiodic treatment started 12 h after explantation. The second set of control intact plants and isolated shoots was cultivated under continuous illumination and sampled at the same time as mentioned above. All plants and segments were cultivated on medium with or without BAP (0.2 mg/liter). The transfer to fresh medium was done at the time of explantation.

The analyses of cytokinins were performed according to Strnad et al. (1990) as described with some modifications by Macháčková et al. (1993). The experiments were done three times. The trends in cytokinin levels were always the same, but individual values differed. Therefore, the results of one representative experiment are given.

## Results

### *Effect of Root Removal*

Root removal before photoperiodic treatment enhances flowering providing the control plants are not fully induced (Fig. 1). The highest effect was reached with de-rooting immediately before the inductive darkness (an increase about 70–90%), whereas the same treatment 24 h earlier led only to a moderate increase in flowering (about 30%). Root removal after inductive darkness was practically without effect.

### *Effect of BAP Application*

Cultivation of shoots isolated 24 h before induction on media with different concentrations of BAP led to an enhancement of flowering, and the positive effect of root removal on flowering became more pronounced (Fig. 2). Such an effect was apparent both in the segments that were grown at first for 24 h on the medium without cytokinin and only then transferred to cytokinin-containing medium and in those that were put there immediately.

Such a positive floral effect of cytokinin was not observed in segments grown immediately before inductive darkness. The highest concentration of BAP leveled the percentage of flowering to that of control intact plants on medium without BAP. The transfer of shoots to medium containing cytokinin (in higher concentrations) 24 h later

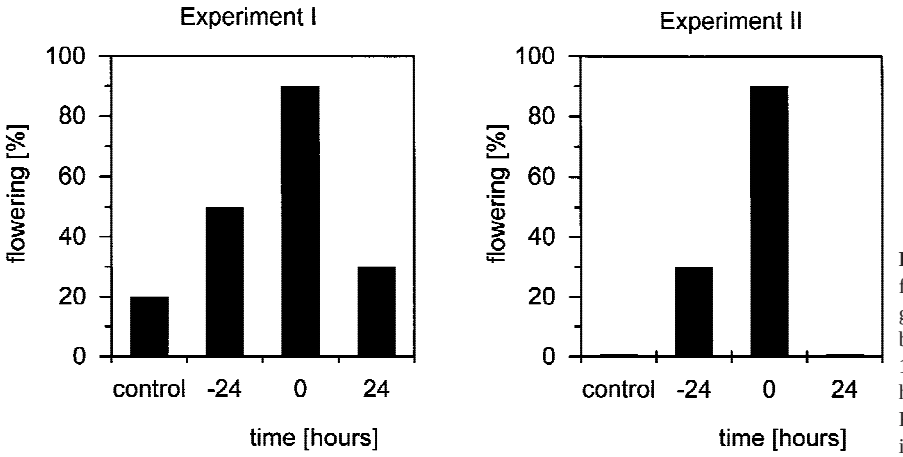


**Fig. 1.** Effect of root removal on the flowering of 14-day-old *C. rubrum* plants grown in vitro. Roots were removed 24 h before the start of the inductive darkness of 12 h (*−24*), just at its beginning (*0*), or 24 h later (*24*). *Control* indicates intact plants. Results represent the mean of 15–20 plants in one experiment.

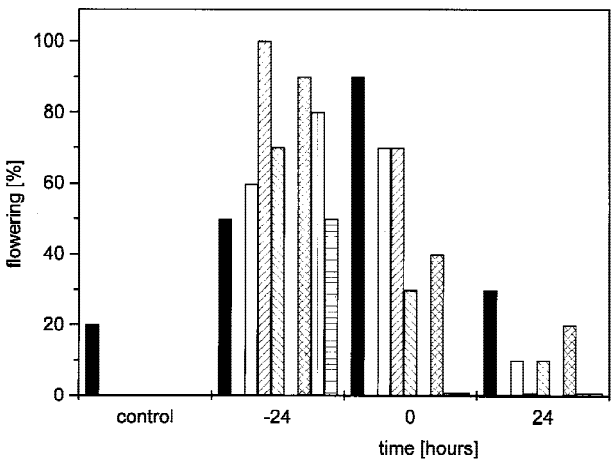


**Fig. 2.** Effect of BAP on the flowering of shoots isolated from 2-week-old *C. rubrum* plants grown in vitro. Roots were removed at time −24, 0, or 24 (see legend to Fig. 1), and shoots were then transferred to BAP-containing medium (0.02, 0.2, or 0.5 mg/liter) either immediately after root removal or 24 h later. *Control* indicates intact plants without BAP application. Results represent the mean of 15–20 plants in one experiment. For shoots cultivated on BAP-containing medium immediately after de-rooting, the symbols representing concentrations of BAP (in mg/liter) are: ▭, 0.02; ▨, 0.2; and ▧, 0.5. For shoots cultivated on BAP-containing medium 24 h after de-rooting, the symbols (in mg of BAP/liter) are: ▩, 0.02; ▬, 0.2; and ▤, 0.5.

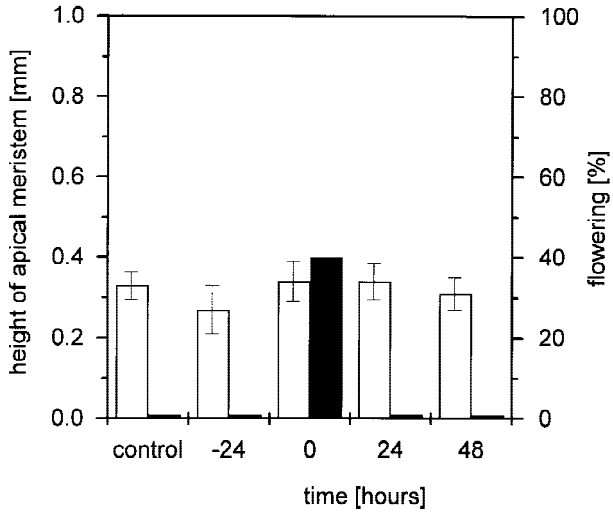


**Fig. 3.** Effect of BAP on flowering (*black columns*) and height of apical meristem (*white columns*) on intact 2-week-old *C. rubrum* plants grown in vitro. Plants were transferred to BAP-containing medium (0.2 mg/liter) 24 h before the start of the inductive treatment (*−24*), at its beginning (*0*), or 24 or 48 h later (*24, 48*). *Control* indicates plants without BAP application. Results represent the mean of 15–20 plants in one experiment.

resulted in a drop of the degree of flowering compared with that of control intact plants.

The same holds true for the shoots explained 24 h after the beginning of induction. The presence of BAP in the cultivation medium is inhibitory for flowering in all cases (Fig. 2).

BAP applied to intact plants 24 h before photoperiodic treatment and 24 and 48 h after had no positive effect on flowering (Fig. 3). Stimulation of flowering (40%) was found only after intact plants were transferred to cytokinin-containing medium at the beginning of inductive darkness (Fig. 3).

### *Endogenous Level of Cytokinins*

The endogenous level of cytokinins in our experiments was changed by root removal, by photoperiodic treatment, and by exogenous application of BAP via cultivation medium (0.2 mg/liter) (Fig. 4).

Root removal brought about a drop in the cytokinin level under continuous illumination on medium with and

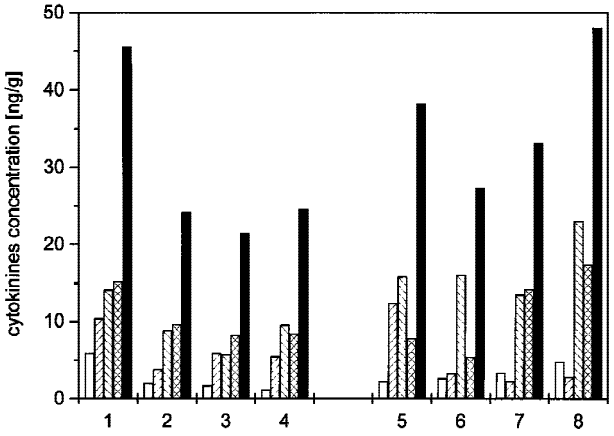


**Fig. 4.** Cytokinin content in isolated shoots and above ground parts of intact 2-week-old *C. rubrum* plants grown in vitro and subjected to de-rooting at the beginning of the inductive darkness and/or transfer to the BAP-containing medium (0.2 ml/liter) at the same time. Control plants and shoots were kept in the light. Results are from one representative experiment. Columns *1–4* indicate experiments without BAP: *1,* intact plants under continuous illumination; *2,* shoots under continuous illumination; *3,* intact plants after photoperiodic treatment/12 h of darkness; *4,* shoots after photoperiodic treatment. *Columns 5–8* indicate experiments with BAP at 0.2 mg/liter: *5,* intact plants under continuous illumination; *6,* shoots under continuous illumination; *7,* intact plants after photoperiodic treatment/12 h of darkness; *8,* shoots after photoperiodic treatment. □, Z; ▨, ZR; ▧, iP; ▩, iPA; ■, total cytokinins.

without BAP. De-rooting did not change the cytokinin content in photoperiodically treated plants on medium without BAP and increased it on medium with BAP.

The level of endogenous cytokinins of intact plants cultivated on the medium with or without BAP was decreased by photoperiodic treatment, whereas the cytokinin content of shoots on the medium without BAP was not affected by photoperiod. The cytokinin level in shoots cultivated under the photoperiod and on BAP-containing medium increased significantly.

The exogenous application of BAP had no great effect on the endogenous level of cytokinins in shoots and intact plants under continuous illumination. On the contrary, a rise in endogenous cytokinins was observed in photoperiodically treated shoots because of BAP, and in intact plants BAP lowered the decrease of the cytokinin level compared with plants grown in light.

## Discussion

The data clearly indicate that the effect of root removal on flowering depends on its timing with respect to the photoperiodic treatment. Flowering is stimulated only when surgical treatment is done at the beginning of darkness or before it, whereas de-rooting 24 h later is without effect. At the conditions used (20% of control plants flower), the presence of roots inhibited the processes at the beginning of the photoperiodic treatment to the photoperiodic induction of flowering, and during this time cytokinin applications does not substitute roots, as reported in other papers (for review, see Kinet et al. 1993). The effect of exogenous BAP is also strongly time dependent; it is synergistic with the effect of root removal before the beginning of the photoperiodic treatment, but it inhibits the flowering of shoot explains when applied 24 h later. It promotes the flowering of intact plants only when applied at the beginning of inductive darkness.

Our results show that the discrepancies reported in the review by Kinet et al. (1993) may be caused by their strong dependence on the effects of BAP and/or de-rooting on their timing. Most of the published papers used different timing and plants in various developmental stages, which led to different results. Thus, cytokinins were reported to enhance flowering in *Sinapis alba* (Havelange et al. 1986) and in *Pharbitis nil* (Ogawa and King 1979), whereas they usually inhibit flowering in *C. rubrum*, both in intact plants and in segments. (Blažková et al. 1998, Krekule and Seidlová 1973, Vondráková 1992). The stimulation of flowering after root removal just before the photoperiodic treatment was observed in *C. rubrum* in an in vitro system by Josefusová and Opatrná (1985), whereas Krekule and Přívratský (1976) demonstrated an increase in flowering on de-rooting 24, 48, 72 h after the end of the photoperiodic treatment and an inhibition of flowering on de-rooting before or at the beginning of induction. Thus, at least two situations should be distinguished considering the effects of de-rooting and/or cytokinin application: one that is linked to photoperiodic flower induction and the other linked to subsequent flower differentiation.

Another factor of importance is the sensitivity of tissue to photoperiodic treatment. In plants flowering after receiving one inductive cycle to 20%, de-rooting and BAP may stimulate flowering as reported here, but similar treatments in plants flowering after the same photoperiodic treatment to 90% are without any effect (Blažková et al. 1998a, 1998b). The segment size also plays an important role. Whole shoots seem to be less sensitive to BAP treatment than small segments that consist of a developing second leaf pair, second internodium, and terminal bud. In such segments, BAP inhibited flowering (Blažková et al. 1998). A similar correlation of segment size and its sensitivity to BAP treatment was reported by Vondráková (1992). The reason for this difference in sensitivity is not known; the older leaves (or cotyledons) may contain some substances affecting the response, or a difference in BAP transport in bigger and smaller segments might be of importance.

Endogenous cytokinins are considered a part of a multicomponent system of flowering regulation (Bernier et

al. 1993). If this were true, some of the discrepancies described above may be explained on the basis of changes in the levels of endogenous cytokinins, which is why we determined the levels of endogenous cytokinins as part of our experimental material. We know from our previous studies with 15-day-old *C. rubrum* plants grown in vivo that cytokinin levels decrease during inductive darkness in all organs of the plants except the terminal bud, in which it increases (Macháčková et al. 1993). This is also true in intact plants in vitro. Photoperiodic treatment induces a drop in the cytokinin level, irrespective of the presence of BAP in the medium, but on BAP-containing medium the drop is much lower than in the control. In contrast, the inductive photoperiod has no effect on the cytokinin level in shoots; interestingly, however, on medium with BAP, a very significant rise in the levels of endogenous cytokinins was observed in induced shoots. De-rooting caused an unexpected effect: a strong drop in the level of cytokinins in the resulting shoot in light, which is reduced greatly by BAP. This effect may be tentatively explained as an influence of BAP on the metabolism of endogenous isoprenoid cytokinins. Thus, the attempt to correlate the effects of de-rooting and/or BAP treatment on flowering with changes in the levels of endogenous cytokinins showed that there is no such obvious correlation. Also, we have to keep in mind that the expected morphogenetic effect of endogenous cytokinins may be influenced by a direct morphogenetic effect of BAP, which we have reported recently (Blažková et al. 1998a, 1998b).

We may conclude that two phases of de-rooting and BAP treatment may be distinguished: one in which flowering is enhanced by both treatments and which is linked to photoperiodic flower induction, and the other in which both treatments are inhibitory to flowering and which is related to morphogenetic events after induction. The difference in the time courses of de-rooting and the BAP effects and the results of analyses of endogenous cytokinins suggest that the effect of de-rooting cannot be ascribed solely to cytokinin deprivation.

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