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Organ Correlations in *Chenopodium rubrum* L. Shoots Studied by Means of ^{32}P Distribution

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Abstract. 21-day old plants of *Chenopodium rubrum L.* ecotype 374 were used. Organ relationships in the shoots were investigated by ^{32}P distribution, which indicated different organ correlations in plants grown in continuous light and in plants treated with flower-inducing and non-inducing dark periods. Dark periods were associated with a low ^{32}P distribution in young leaves and a high one in axillary buds. In the following light period the high ^{32}P distribution in axillary buds continued whereas the ^{32}P distribution in the leaves on the main axis increased and was similar to that in plants grown in continuous light. The high ^{32}P distribution in axillary buds was brought about by both, flower-inducing and non-inducing dark treatments. Decapitation resulted in a high ^{32}P distribution in buds, in continuous light an increased ^{32}P distribution was also found in leaves. These effects were not fully cancelled by IAA application.

The results are discussed with respect to an assumption that decrease of apical dominance represents a step in a sequence of events leading to flowering.

Many authors, for example Lang (1965), Zeevaart (1976), Krekule (1979a, 1985), Bernier (1988), mention the role of organ correlations in the regulation of flowering. It has also been found that correlative effects may limit flowering in some plants without an obligate photoperiodic requirement (Miginiac 1978). Photoperiodic conditions which induce flowering can bring about marked shifts in the growth rate of different organs such as young leaves and axillary buds (Seidlová and Opatrná 1978, Opatrná *et al.* 1980) or roots (Josefusová *et al.* 1985). Effects such as those involving changes in organ volume and rates of their initiation necessarily have an impact on organ correlations. In flowering control of *C. rubrum* the most conspicuous correlative effects were root-shoot relationships (Josefusová *et al.* 1985) and apical dominance. Effects which cancel the release from apical dominance due to photoperiodic induction of flowering,

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such as auxin or ethylene application, were inhibitory to flowering (Seidlová and Khatoon 1976, Krekule 1979b).

This study was aimed at clarifying shoot organ correlations with special regard to apical dominance under flower-inducing and non-inducing photoperiodic conditions. Relationships between organs were investigated by ^{32}P distribution. Using this method, the early effects of photoperiodic regimes can be observed in both decapitated and intact plants.

MATERIAL AND METHODS

Plant Material

21-day old *Chenopodium rubrum* L. ecotype 374 plants were used, the conditions of seed germination and plant cultivation on perlite being as described by Ullmann *et al.* (1985). At the age of 21 d, five to seven leaf pairs were developed on the main axis (the fourth leaf pair and younger ones formed an apical bud), and further leaf primordia had been initiated at the 0.3 to 0.4 mm long shoot apex. Two to three pairs of leaves had been developed in the axillary buds of the 1st and 2nd leaf nodes (node 1 was the first above the cotyledons). Both intact and plants decapitated above the 1st leaf node were used. Decapitation was performed immediately before ^{32}P application.

Light Regimes

Plants were grown in continuous light or under one of the following light regimes: flower inducing 12-h dark period; 12-h dark period interrupted with two red light breaks (30 min at the beginning of the 7th and 10th hour, red fluorescent tubes Philips TL 2.6 $\mu W\ cm^{-2}\ nm^{-1}$) which nullified flowering; 45 h of continuous darkness (the 2nd maximum of the endogenous rhythm of flowering).

Application of ^{32}P

Labelled sodium orthophosphate (Isocomerz GMBH Berlin GDR) was substituted for "cold" phosphate in half strength Knop's solution. This nutritive medium was applied *via* the roots during the whole experiment (usually 72 h) in place of the previously used nutritive solution. Labelled orthophosphate was applied at the beginning of the dark period; plants grown in continuous light were treated at the same time.

IAA Application

Three-μl droplets of IAA (indole-3-acetic acid, water solution 10^{-3} to 10^{-9} M) were applied to cut stumps immediately after decapitation. IAA application was repeated every four hours during experiment (36 h).

Estimation of ^{32}P Distribution

Samples were taken 12, 24, 36, 48, 60 and 72 h after ^{32}P application, in continuous light and uninterrupted darkness also after 4 and 8 h.

Plants were cut into parts: the 1st, 2nd, 3rd leaf pairs, all younger leaves overtopping the shoot apex and forming a substantial part of apical bud, the shoot apex (apical meristem with leaf primordia) and axillary buds of the 1st and 2nd leaf node. The parts were dried, weighed and their radioactivity measured using a liquid scintillant and Packard Tricarb 300C counter. Radioactivity (dpm) per 1 mg of dry matter was counted. The radioactivity in 1 mg of dry matter of the 1st leaf pair of intact plants at every treatment was considered as 1 and the relative ^{32}P distribution was calculated for the other analyzed parts. This procedure permitted comparison of the results of repeated experiments: eight experiments are summarized in this paper. One type of experimental treatment was repeated in at least three experiments, one to six samples were taken from each experimental treatment and five to ten plants were collected in each sample. Differences were evaluated using t-tests.

RESULTS

The radioactivity in 1 mg of dry matter of the 1st leaf pair was found to increase during all experiments. The amount found in darkness was substantially lower than that in light: twelve hours after the beginning of ^{32}P application it was about 40% of that found in light-grown leaves. After 45 h of continuous darkness radioactivity had accumulated only to about 20% of the controls. This difference disappeared during the following light period. Distribution of ^{32}P in the shoot apex showed the same trend as in youngest leaves. No significant difference in relative ^{32}P distribution was found between youngest leaves only and complete apical bud as the shoot apex represented only a very small part of the apical bud.

Intact Plants

The patterns of ^{32}P distribution in plants grown in continuous light and with dark treatments are compared in Fig. 1.

Continuous Light

During the first hours of continuous light ^{32}P was distributed mainly to the 1st leaf pair. Relative distribution of ^{32}P in the 3rd pair of leaves and in the youngest leaves increased eight hours after the beginning of ^{32}P application. The relative distribution of ^{32}P in all the axillary buds remained at about 1 (Fig. 1A), but that in the apical bud increased considerably.

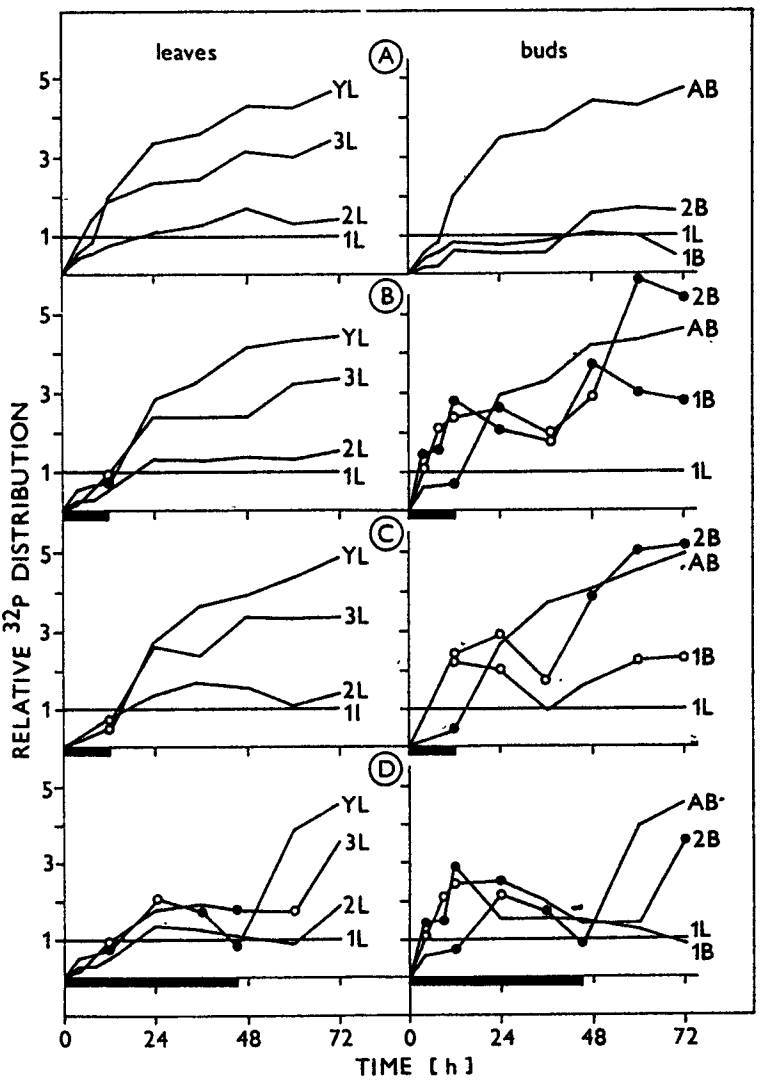

Fig. 1: Effects of light and dark on the relative ^{32}P distribution in leaves and buds of intact *Chenopodium rubrum* plants. A – plants grown in continuous light (control), B – 12-h dark period. C – interrupted 12-h dark period, D – 45-h dark period. ○, ● – difference from control significant, $t > P0.05$, $t > P0.01$ respectively. 1L, 2L, 3L, YL – the 1st, 2nd, 3rd leaf pair, younger leaves resp., 1B, 2B – axillary buds of the 1st and 2nd nodes resp., AB – apical bud, h – time since the beginning of ^{32}P application.

Darkness

With a single 12-h period of darkness, relative ^{32}P distribution in the 3rd pair of leaves and the younger ones was markedly lower than in continuous light. The difference between light and darkness, obvious already four hours after

the beginning of ^{32}P application, was significant after twelve hours (Fig. 1B). In contrast the relative ^{32}P distribution in axillary buds was higher in darkness then that in light, the difference being already significant 4 h after the beginning of ^{32}P application. During long continuous darkness the low ^{32}P distribution in young leaves continued for longer, while the relative ^{32}P distribution in axillary buds, high during the first hours of darkness, decreased after 12 to 24 hours (Fig. 1D). The ratio of relative ^{32}P distribution in apical bud/axillary buds was about 2 after 12 h of continuous light and about 0.3 after 12 h of darkness, about 4 after 48 h of light and about 1 after 45 h of continuous darkness.

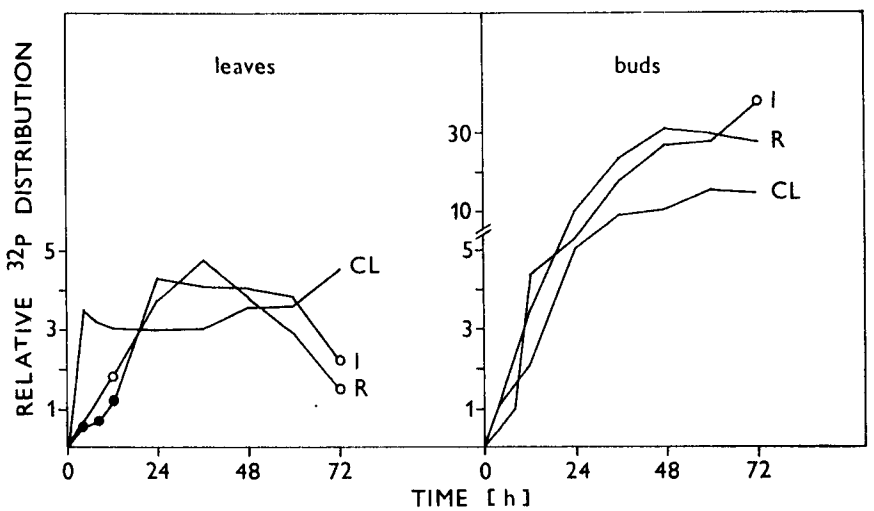

Fig. 2: Relative ^{32}P distribution in leaves and buds of *Chenopodium rubrum* plants decapitated above the 1st leaf node. CL – continuous light, I – 12-h dark period, R – 12-h dark period interrupted by red light. Plants grown under continuous light and dark treated ones are compared. ○ , ● – difference significant t > P0.05, t > P0.01 resp., h – time since the beginning of ^{32}P application.

Light Period Following Darkness

During light period following 12-h darkness the ^{32}P distribution in the 3rd leaf pair and youngest leaves increased and no significant difference was found between plants grown in continuous light and those that had experienced darkness. Throughout the following period of light, high relative ^{32}P distribution in axillary buds was found in dark-treated plants. Relative ^{32}P distribution in the buds of the 2nd node tended to be higher than that in the 1st leaf node. Distribution of ^{32}P in the light period following 45-h darkness was similar to that after 12 h dark period.

Effect of Red Light Breaks

Red light breaks, which cancell flowering, did not greatly change the pattern of ^{32}P distribution in leaves or buds (Fig. 1C).

Decapitated Plants

Leaves. In continuous light as early as 4 h after decapitation relative distribution of ^{32}P in leaves was 3.5 times higher that in intact plants (Fig. 2).

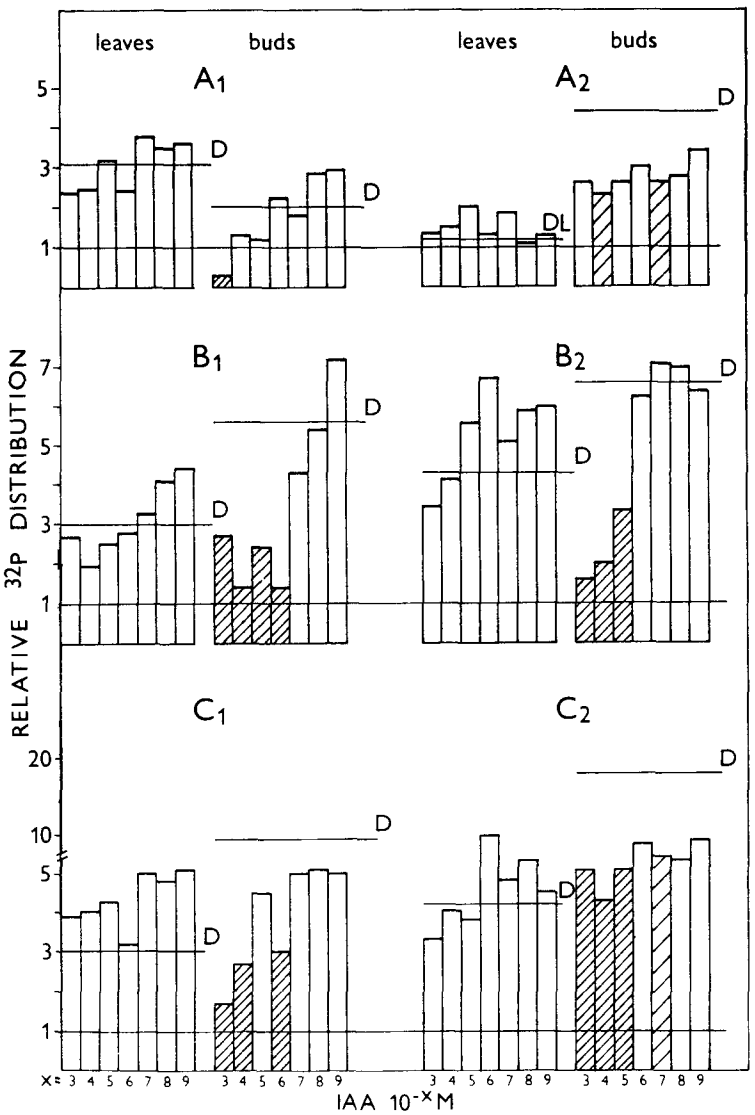

Fig. 3: Effect of IAA application on relative ^{32}P distribution in *Chenopodium rubrum* plants decapitated above the 1st leaf node. Plants treated with IAA (10^{-3} to 10^{-9} M) and non-treated plants (D lines) are compared, scarce hatching $t > P0.05$, dense hatching $t > P0.01$. A, B, C – 12, 24, 36 h since the beginning of experiment resp., 1 – continuous light, 2 – 12 h dark period.

In 12-h darkness no such rapid increase in ^{32}P distribution was found in the leaves of decapitated plants, the difference between light and dark grown plants being significant. A transient increase of ^{32}P distribution in leaves was found during the light period following darkness.

Buds. Distribution of ^{32}P in buds increased strongly after decapitation. The increase was more rapid in plants treated with a dark period, both interrupted and non-interrupted, that in plants grown in continuous light, although this difference did not reach significance for high variability.

Effect of IAA Application on ^{32}P Distribution in Decapitated Plants

The ^{32}P distribution in buds was generally reduced by IAA application; using a high IAA concentration (10^{-3} to 10^{-6} M), the effect of IAA application was often significant (Fig. 3).However IAA did not fully substitute for the removal of the apical part of the plant. IAA application did not affect ^{32}P distribution in leaves.

DISCUSSION

Correlations are often studied using surgical treatments, including microsurgical and *in vitro* techniques, or by following the distribution of radioisotopes, *e.g.* ^{32}P (Šebánek 1966), ^{42}K and ^{45}Ca (Miginiac 1974). In the case of potassium and phosphate ". . . there is some orderly relation between growth and nutrient absorption determined by the plants" (Sutcliffe 1976). The distribution of ^{32}P reflects only some aspects of the correlative system, rather then providing comprehensive information on organ interrelationships.

Different patterns of ^{32}P distribution suggest different shoot organ correlations in plants grown with periods of the dark compared with those kept in continuous light. The dark period was characterized by a low relative ^{32}P distribution in young leaves of the main axis and by greater distribution to axillary buds. The low ^{32}P distribution in young leaves throughout the whole dark period was not accompanied by any increase in ^{32}P distribution in the 2nd or 3rd leaf pair. This finding does not seem to be accounted for by any inhibitory influence of mature leaves in darkness but may perhaps be attributed to the effect of transpiration being reduced in darkness (Sutcliffe 1962). The change of relationships between young and mature leaves was transient and probably connected with a temporary inhibition of young leaf growth after a flower-inducing dark period, as described by Opatrná *et al.* (1980).

The high ^{32}P distribution after decapitation in light demonstrates an inhibitory influence of apical part of the plant upon mature leaves at the lower nodes that may also be suggestive of apical dominance (Phillips 1976). The increase of ^{32}P distribution in leaves was not reduced by IAA application and therefore it

appears that IAA does not play a role in the relationships between the apical part of the plant and leaves of the lower nodes.

The high relative ^{32}P distribution in axillary buds in darkness continued or re-appeared in light following a dark period.

In analysing the patterns of ^{32}P distribution in intact plants, the significant increase of ^{32}P distribution in axillary buds was found much earlier than the significant decrease in relative ^{32}P distribution in the apical bud. Comparison of intact and decapitated plants grown in the same light – dark regimes shows that the apical part of the plant inhibits axillary bud development in light as well as in darkness. IAA application did not fully substitute the inhibitory function of the apical part of plant. The effect of light and darkness on ^{32}P distribution in decapitated plants shows an inhibitory influence of leaves on buds in continuous light, but the high ^{32}P distribution in buds was not affected by the transient increase of ^{32}P distribution in leaves found in light after the dark period.

Axillary bud activation during and after dark treatment is clearly the result of release from apical dominance and from the inhibitory influence of mature leaves; the latter might precede the former. The release from apical dominance persisted even after a partial restitution of apical dominance following the dark period as indicated by the pattern of ^{32}P distribution in leaves and buds of the 1st and 2nd leaf nodes in intact plants.

The inhibition of axillary buds in continuous light and activation of their development after induction of flowering was described in *C. rubrum* by Cook (1976) and Opatrná *et al.* (1980). Activation of axillary bud growth and development as an effect of photoperiodic induction of flowering is commonly known in both short-day (Krekule 1979a) and long-day plants (Pavlová *et al.* 1989).

Axillary bud activation occurred in response to both flower-inducing and non-inducing photoperiodic regimes. It follows that these changes accompany the transition to flowering but they are not connected only with the induction of flowering but may also brought about by non-inductive photoperiodic treatment.

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