

The effect of cytokinins and other plant hormones on the growth of cotyledonary axillars of flax (*Linum usitatissimum*), sunflower (*Helianthus annuus*) and pea (*Pisum sativum*)

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ABSTRACT

Flax seedlings were decapitated above the cotyledons. After one cotyledon was removed the growth of the bud of the remaining cotyledon was stronger in 90% of the plants. However, the application of the cytokinin benzyladenine (BA) to the bud of the removed cotyledon caused a growth correlative reversal and, by contrast, in 65% of the plants the bud of the removed cotyledon grew out. On the other hand, in sunflower seedlings, which have epigeal cotyledons similar to flax, after the removal of one cotyledon the growth of the axillary of the removed cotyledon was more intensive in 59% of the plants. Not even an application of BA to the remaining cotyledon of sunflower resulted in more intensive growth of the axillary of this cotyledon. When both cotyledons were left on the seedling, BA applied to one of the cotyledons of decapitated flax plants resulted in a highly significant stimulation of growth of the axillary of this cotyledon; in sunflower; however, the effect of the BA was insignificant. After decapitation of the stem of pea seedlings where both cotyledons remained, both axillaries grew out, but after a certain period of time one of them (the dominant one) achieved a growth correlative dominance over the other (inhibited). The present study is focused on whether an application of plant hormones onto the inhibited shoot is able to cause a growth correlative reversal, i.e. to change the inhibited shoot into a dominant one. The application of 0.12% BA can cause such a reversal virtually in all plants if the original difference in the length between the inhibited and dominating axillaries is 12–24 mm. A 0.12–0.5% concentration of gibberellin causes a reversal in 13–75% of the plants, but only if the difference between the dominant and inhibited shoot is 1–12 mm. A 0.03–0.25% concentration of IAA causes a reversal in 34–57% of the plants, if the difference in the length of the axillaries is 1–4 mm.

Keywords: flax, sunflower and pea seedlings; benzyladenine; gibberellin; indolyl-3-acetic acid; growth of cotyledonary axillaries

Through their buds in the axils of cotyledons (axillaries) seedlings are very good models used to study plant integrity, particularly apical dominance (Dostál 1967, Šebánek et al. 1991, Šebánek 2003). The model for plants with hypogeal cotyledons is the pea; after decapitation of the epicotyl the cotyledons inhibit the growth of axillary buds (*Pisum* type). The model for plants with epigeal cotyledons is flax; here, in contrast, the cotyledons have a stimulating effect on axillary growth (*Linum* type). The focus of our present study is on the effect of the applied cytokinin benzyladenine (BA) and other plant hormones on the growth of cotyledonary axillaries of pea and flax, and comparisons of the growth of flax axillaries with the growth of sunflower axillaries. In relation to plant-hormonal research of the regulating effect of cotyledons, no attention has so far been paid to plants with epigeic cotyledons.

MATERIAL AND METHODS

The seeds of flax (*Linum usitatissimum*), variety Atalanta, were sown into vegetative pots containing garden soil. The seedlings were grown under natural light, 13–16 hour day and $22 \pm 3^\circ\text{C}$ temperature. When 10–15 mm of the epicotyl grew out the plants were decapitated, i.e. the epicotyl was cut off immediately above the cotyledons. Both cotyledons were then either left on the plant or one was removed. Lanolin paste with benzyladenine BA (producer Lachema, Czech Republic), or indolyl-3-acetic acid IAA (producer Lachema, Czech Republic) was applied to the surface epidermis of the cotyledon or to the petiole of the removed cotyledon. After termination of the experiment the growth of cotyledonary axillaries was evaluated. Experiments with sunflower seedlings (*Helianthus annuus*), variety Apetil, were established under

the same cultivation methods and operations as flax.

Seeds of pea (*Pisum sativum*), variety Vladan, were swollen and sown into agro-perlite moistened with water and then germinated in darkness at $22 \pm 2^\circ\text{C}$ and 80% relative humidity. After 5 days, when the epicotyls of the seedlings had grown out about 25 mm, plants with an undamaged habitus were picked out and transferred into vessels filled with water. The epicotyls were cut off above the cotyledons and the plants were grown in darkness under the same temperature and humidity. Axillary buds grew out in the axils of the cotyledons. At first the rate of growth of all the plants was the same, but after a certain period of time always one of them (the dominating one) achieved a growth correlative dominance over the other (inhibited). In repeated experiments we selected plants where the differences between the dominating and inhibiting cotyledonary axillary in the first, second and third group was 1–4 mm, 4–12 mm and 12–24 mm, respectively. Different concentrations of lanolin paste with BA, IAA or gibberellin GA (producer Phylaxia Budapest) were applied to the apical part of the inhibited cotyledonary axillary. After termination of the experiment we evaluated the percent of plants where the application of plant hormones to the inhibited axillary shoot resulted in correlative reversal of growth; that is when the originally inhibited axillary shoot became dominant after the application of plant hormones and the originally dominating shoot became an inhibited one.

The light conditions during cultivation of model species, seedlings used in classical experimental morphology correspond with their character: darkness in the pea as a species with hypogeal germination and light in flax and sunflower as the species with epigeal germination. Further on we provide details of the respective experiments.

RESULTS

The effect of plant hormones on correlation between cotyledons and cotyledonary axillaries in flax

The effect of BA on the growth of cotyledonary axillaries after the removal of one cotyledon.

On the 26th of June the seedlings were decapitated and one cotyledon was removed. A 0.12% concentration of BA paste was applied either to the remaining cotyledon or to the petiole of the removed cotyledon. The growth of cotyledonary axillaries was evaluated on the 6th of July (Table 1). Table 1 shows that the remaining cotyledon affected the growth of axillaries of more than 90% of the plants and that even an application of BA paste to the remaining cotyledon did not change this effect. BA applications to the bud of the removed cotyledon resulted in a growth correlative reversal, since BA stimulated intensive growth of the bud in nearly 65% of plants. The average length of the axillary of the remaining cotyledon compared to the removed cotyledon agrees with this reversal. The results of experiments established on 6th and 11th of July were similar.

Comparison of the effect of BA and IAA on the growth of cotyledonary axillaries when both cotyledons remained.

The seedlings were decapitated above the cotyledons on the 8th of August and one cotyledon was treated either with 0.12% BA paste or 0.12% IAA paste. The growth of cotyledonary axillaries was evaluated on 22nd of August (Table 2). Table 2 shows that BA had a positive effect on the growth of 90% of axillaries of the treated cotyledon, while IAA inhibited this growth in one fifth of the plants. This corresponds with the length of axillaries in the axils of the BA- or IAA-treated cotyledons. The results of experiments established on 6th and 11th July were similar.

Table 1. The effect of 0.12% BA paste on the growth of cotyledonary axillaries of flax seedlings with removed epicotyl and one cotyledon

Series	Application of BA	% of plants with a more intensive growth of the cotyledonary axillary		% of plants with the same growth of both axillaries	Length of axillary (mm) of the cotyledon	
		remaining	removed		remaining	removed
1	–	90.9	9.1	0	23.7 ± 1.8	2.5 ± 0.7
2	to the remaining cotyledon	91.8	4.1	4.1	17.0 ± 1.3	3.1 ± 0.6
3	to the petiole of removed cotyledon	17.6	64.8	17.6	4.6 ± 0.5	9.6 ± 1.6

The difference in the length of the axillary of the remaining and removed cotyledons was highly significant in all experimental series

Table 2. The effect of 0.12% BA and 0.12% IAA paste on the growth of cotyledonary axillaries of flax seedlings, which have both cotyledons

Series	Application to one cotyledon	% of plants with a more intensive growth of cotyledonary axillary		Length of axillary (mm) of cotyledon	
		treated	untreated	treated	untreated
1	BA	93.2	6.8	28.5 ± 1.4	5.3 ± 1.1
2	IAA	20.7	79.3	6.0 ± 1.3	19.9 ± 1.6

In both series the difference in length of the axillaries of treated and untreated cotyledons is highly significant

The effect of BA on correlation between cotyledons and cotyledonary axillaries in sunflower

Achenes were sown out on 20th of July and on 6th of August the seedlings were decapitated above the cotyledons; either both cotyledons were left or one was removed. If both cotyledons remained, 0.12% BA paste was applied to one cotyledon or, if one cotyledon was removed, it was applied to the remaining cotyledon or to the petiole of the removed cotyledon. Evaluations were carried out on the 29th of August and are shown in Table 3. When both cotyledons were left, BA tended to have a positive effect on the frequency of more intensive growth of the axillaries of the BA-treated cotyledons, but the differences in the length of the axillaries were not statistically significant. After the removal of one cotyledon, in 59% did more axillaries grow out from the removed cotyledon, while the axillary of the retained cotyledon growing out in 41% of the plants. We see a stronger tendency towards the correlative Pisum type than Linum type, although sunflower cotyledons are epigeal; however, the differences in length between axillary shoots of the removed and remaining cotyledon were not

statistically significant. Sunflower never showed a definite type of stimulation effect of the epigeal cotyledon, so clearly evident in flax. Even BA applied to the remaining cotyledon cannot intensify the growth of the axillary of this cotyledon and the result is actually nearly the same as when BA was applied to the bud of the removed cotyledon.

The effect of plant hormones on dominance between cotyledonary axillaries of pea

Table 4 shows the results in terms of the frequency of the growth reversal of cotyledonary axillaries if plant hormones were applied to the inhibited cotyledonary axillary in the case of three different lengths between the dominant and inhibited axillary. It is obvious that of all plant hormones BA has the strongest effect on the growth correlative reversal, because a concentration of 0.12% results in a virtually complete correlative reversal in all cases of length differences between the axillaries. However, a 0.06% concentration of BA causes only a partial reversal (in 21–51% of the plants) and only in the shortest length differences. Even a high concentration (0.5%) of gibberellin causes

Table 3. The effect of 0.12% BA paste on the growth of cotyledonary axillaries of sunflower keeping both cotyledons or after removal of one cotyledon

Series	Cotyledons	Application of BA	% of plants of the most intensive growth of cotyledonary axillary		Length of axillary (mm) of cotyledon	
			treated	untreated	treated	untreated
1.	retained	to one cotyledon	61	39	26.4 ± 5.0	16.0 ± 4.3
			removed	retained	removed	retained
2.		to retained cotyledon	65	35	17.5 ± 3.7	9.8 ± 3.1
3.	one cotyledon removed	to bud of removed cotyledon	66	34	19.2 ± 4.4	8.6 ± 3.9
4.		–	59	41	27.4 ± 3.2	22.4 ± 3.0

Differences in the length of the axillary of the treated and untreated cotyledons, or the removed and remaining one were insignificant in all the series

Table 4. The ability of benzyladenine (BA), gibberellin (GA) and auxin (IAA) applied to the inhibited axillary shoot of decapitated pea plants to induce a growth correlative reversal

Applied plant hormone	Concentration (%)	Establishment of experiment	% of plants with growth correlative reversal between axillary shoots giving the initial difference between the dominant and inhibited shoot (mm)		
			1–4	4–12	12–24
BA	0.06	25.3.2002	23	0	0
		22.4.2002	51	7	–
		29.4.2002	21	0	–
	0.12	9.12.2002	100	94	100
		11.3.2003	100	97	92
GA	0.12	11.3.2003	43	13	0
		11.3.2003	48	12	2
	0.5	21.10.2002	77	45	0
		24.10.2002	22	43	4
		7.11.2002	27	13	0
IAA	0.03	1.4.2003	45	5	0
	0.06		37	2	0
	0.12		34	7	0
	0.25		57	8	0

a correlative reversal only in 13–77% of the plants if the length difference is 1–12 mm; if the difference is more than 12 mm it is virtually ineffective. It is remarkable that when the difference in the length of the axillaries is less than 4 mm, IAA causes a reversal in 34–57% of the plants regardless of the applied concentration, while if the difference ranges between 4 and 12 mm it causes a reversal only in 2–8%, and if the difference in length is more than 12 mm IAA is completely ineffective.

DISCUSSION

As early as 1930 Komárek came to the conclusion that the cotyledon in flax seedlings has a stimulating effect on the growth of its axillary if the plants are decapitated and one cotyledon is removed. In our present experiments we proved that BA applied to the remaining cotyledon did not change its stimulating effect, but that BA applied to the petiole of the removed cotyledon caused a correlative reversal because in 65% of the plants the growth of the bud of the removed cotyledon was stronger than of the bud of the remaining cotyledon. It is obviously related with the fact that the remaining cotyledon synthesises endogenous

cytokinins, because as soon as 12 hours after the removal of the cotyledon, a higher content of endogenous cytokinins could be detected in the bud of the remaining cotyledon than in the bud of the removed cotyledon (Šebánek et al. 1991). Darkness has the same effect as the removal of the cotyledon because when one cotyledon was left on the decapitated flax plants grown in darkness, it stimulated the growth of the buds of the cotyledon grown in light. Within 6 hours more cytokinins were synthesised in the illuminated cotyledon than in the darkened one (Tan et al. 1979). However, labelled ¹⁴C-BA applied to the roots of decapitated flax plants with one cotyledon removed is transported intensively both into the bud of the remaining and the removed cotyledon (Procházka et al. 1997). Despite this fact cytokinins have the capacity to accelerate the release of axillary buds from inhibition also in flax plants was confirmed by the fact that BA applied to the bud of the removed cotyledon in most cases caused enhanced growth of the bud of the removed cotyledon than of the remaining cotyledon. It was also confirmed in our experiments where the application of BA to one of the cotyledons of decapitated flax accelerated the growth of 93% of buds of this BA-treated cotyledon. We still do not know why IAA, as was

confirmed in our experiments, applied to one of the two cotyledons on the flax plants had a 20% inhibition effect on the growth of the bud of the IAA-treated cotyledon, while IAA applied to the remaining cotyledon (after removing one cotyledon) did not break the effect of the cotyledon on the growth of its axillary (Dostál 1960).

Komárek (1930) was the first author who incorporated sunflower plants into his studies on the growth correlative effects of cotyledons on the growth of axillaries. He discovered that the correlative regulating effect of sunflower cotyledons resembled pea seedlings. In our experiments, 59% of the sunflower cotyledons had an inhibitory growth effect similar to pea cotyledons, and in 41% a stimulating effect similar to flax cotyledons. This is probably associated with the fact that sunflower cotyledons, although they show photosynthetic activity, contain reserve substances, above all of a sucrose nature, like pea. According to our experiments sunflower differs from flax also in that even BA applied to the remaining cotyledon is not capable of inducing a stimulating effect of this cotyledon on growth of the axillaries. The model of sunflower seedlings shows how wrong it would be to generalise the stimulating effect of the cotyledons of the model of flax with epigeic cotyledons to all species showing epigeal germination.

Pea seedlings, which have hypogeal cotyledons, are model plants for studies of stem apical dominance and correlations between cotyledons and their axillaries (Procházka et al. 1997). Dostál (1937) discovered the inhibiting effect of the cotyledon on the growth of its axillary and he imitated this effect by applying IAA to the cotyledon (Dostál 1937). Auxin then appeared as a plant hormone preventing the release of the cotyledonary axillaries from inhibition. However, the growing cotyledonary axillary released from inhibition can be roused to more intensive growth by an application of IAA. Evidence of this is in the fact that cotyledonary axillaries treated with 0.03–0.25% IAA paste are capable of intensifying elongation growth compared to the other originally dominating axillary shoot. Even though this correlative reversal usually appeared only if the original length difference between axillary shoots was 1–4 mm, it is a surprising result, especially when a high concentration of 0.25% IAA was applied.

If a concentration of 0.25–0.5% of gibberellin was applied to the originally inhibited axillary shoot, a correlative reversal was observed within the length difference of axillaries of 1–12 mm; this is not surprising for gibberellin when we consider its well-known positive effect on elongation growth of the stem (Macháčková 1997).

Endogenous cytokinins accumulate in the buds of pea seedlings 6 and 24 hours after stem decapi-

tation (Li and Li 1996) and exogenous cytokinins release the buds from the inhibition of the cotyledon (Procházka et al. 1997, Turnbull et al. 1997). On a model of *in vitro* cultivated axillary pea buds, cytokinin synthesis appeared after separation of the buds from the mother plant (Balla et al. 2002). These results correspond with the fact that an application of 0.25% BA paste to the inhibited axillary cotyledonary shoot of pea transforms this shoot into a dominant one (Rahman et al. 1997). The same was confirmed in our experiments in 92–100% of plants even when the BA concentration was only 0.12%. A very low concentration of 0.06% BA still caused a reversal among the axillaries in as many as 51% cases, if only with the smallest differences among the axillaries. In this model it is a convincing demonstration of the exceptional role of cytokinins in reducing the apical dominance of the stem.

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ABSTRAKT

Vliv cytokininu a dalších fytohormonů na růst děložních axilárů lnu (*Linum usitatissimum*), slunečnice (*Helianthus annuus*) a hrachu (*Pisum sativum*)

U klíčnicích rostlin lnu dekapitovaných nad dělohami vyrůstá po odříznutí jedné dělohy u 90 % rostlin více pupen ponechané dělohy, ale aplikace cytokininu benzyladeninu (BA) k pupenu odříznuté dělohy působí růstově korelační zvrát, takže u 65 % rostlin pak naopak vyrůstá pupen dělohy odříznuté. Naproti tomu u klíčnicích rostlin slunečnice, ačkoli mají jako len epigeické dělohy, vyrůstá po odříznutí jedné dělohy v 59 % případů více axilár dělohy odříznuté. Ani aplikace BA na ponechanou dělohu nezpůsobí u slunečnice intenzivnější růst axiláru takto ošetřené dělohy. Při ponehání obou děloh podnutí BA aplikovaný na jednu z obou děloh dekapitovaných rostlin lnu vysoce průkazně intenzivnější růst axiláru takto ošetřené dělohy, avšak u slunečnice je v tom případě vliv BA neprůkazný. U klíčnicích rostlin hrachu s oběma ponechanými dělohami vyrůstají po dekapitaci lodyhy oba axilární výhony, ale po určité době jeden (dominující) získá růstově korelační převahu nad druhým (inhibovaným). Byla věnována pozornost tomu, zda je možno aplikací fytohormonů na inhibovaný výhon dosáhnout růstově korelačního zvrátu, tj. přeměnit inhibovaný výhon na dominující. V případě BA je tento zvrát možný v koncentraci 0,12 % téměř u všech rostlin tehdy, je-li původní rozdíl v délce inhibovaného a dominujícího axiláru 12–24 mm. Giberelin v koncentraci 0,12–0,5 % působí zvrát u 13–75 % rostlin, ale jen při rozdílu mezi dominujícím a inhibovaným výhonem v délce 1–12 mm. IAA v koncentraci 0,03–0,25 % působí zvrát u 34–57 % rostlin při rozdílu délky axilárů 1–4 mm.

Klíčová slova: klíčnicí rostliny lnu, slunečnice a hrachu; benzyladenin; giberelin; kyselina indolyl-3-octová; růst děložních axilárů

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