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Use of 2,3,5-Triiodobenzoic Acid in Studies on the Growth Correlation Differences between Epigeous and Hypogeous Seedlings (*Linum* and *Pisum*)

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Využití 2,3,5-trijodbenzoové kyseliny při studiu rozdílů růstových korelací mezi epigeickými a hypogeickými klíčními rostlinami (*Linum* a *Pisum*)

Dekapitované klíční rostliny lnu a hrachu, natřené pastou s trijodbenzoovou kyselinou buď nad dělohami nebo pod nimi, jeví zvláště na epikotylních pahýlech rozdílné morfogenetické změny v souvislosti s rozdílnými korelačními vlivy jejich epigeických, resp. hypogeických děloh, jež primárně rozhodují o rozdílné dominanci jejich pupenových základů.

V nejranějším období klíčení lze prvního internodia lnu, obyčejně velmi krátkého, a řapíků děloh hrachu užít k důkazu antagonismu mezi kyselinou trijodbenzoovou a indolyloctovou. První internodium lnu se prodloužilo působením trijodbenzoové kyseliny na semena, i když zrála na rostlině, a řapíky děloh hrachu, zadržené v růstu máčením semen v roztoku kyseliny trijodbenzoové, se zvětšily působením kyseliny indolyloctové zvnějšku. Tato kyselina naopak ruší morfogenetické účinky trijodbenzoové kyseliny na semena lnu.

Summary

Decapitated seedlings of *Linum* and *Pisum* treated with TIBA paste either above or below the cotyledons showed different morphogenetic changes especially on the epicotyl stumps, due to the differences in the correlations of their epigeous and hypogeous cotyledons respectively, these being also primarily responsible for the differing dominance of their shoot primordia.

At the earliest phases of germination, an antagonism between TIBA and IAA can be demonstrated on the first internode in *Linum*, which is usually very short, as well as on the petioles of the *Pisum* cotyledons. The former

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could be enlarged only by treating *Linum* seeds, even when ripening on the plant, with TIBA paste and the latter, if retained by soaking seeds of *Pisum* in a TIBA solution could be promoted by exogenous IAA. This, on the contrary, reversed the morphogenetic effects of TIBA upon *Linum* seeds.

Introduction

Growth correlations mediated by the transport of substances can be interrupted by blocking transport by various physical and chemical means. In the present report triiodobenzoic acid (TIBA) was applied to young seedlings of *Linum usitatissimum* L. and *Pisum sativum* L. for elucidating some growth correlation differences between these two plants with epigeous and hypogeous germination respectively. As shown earlier, they behave differently, if decapitated and deprived of one of the two cotyledons. In *Pisum*, the cotyledon left inhibits the growth of its own axillary bud (DOSTÁL 1908), in *Linum*, however, it exercises a certain stimulatory effect (KOŘÍNEK 1923). The inhibiting influence of *Pisum* cotyledons can be imitated by a very small amount of indoleacetic acid (PLCH 1936), this exogenous auxin being, however, ineffective on the *Linum* cotyledons (DOSTÁL 1955). Consequently, the epigeous cotyledons of *Linum* can be compared with the normal young foliage leaves, whereas the hypogeous cotyledons of *Pisum* correspond to the adult leaves with their inhibiting influence. The unequal amounts of food accumulated in the *Linum* and the *Pisum* seeds respectively is reflected in the opposite response of these seedlings to TIBA if it is applied to their various parts after certain operations in high concentrations which could not show the stimulatory effects described by THIMANN and BONNER (1947).

Materials and methods

Seeds of *Pisum sativum* L. var. Přebohatý were soaked for 24 hours in tap water and then sown in moist sawdust. The seedlings with roots 6—10 cm. long were transplanted into small glass containers filled with tap water and kept mainly in the dark. Seeds of *Linum usitatissimum* L. var. Record were sown directly in small pots of 8 cm. diameter filled with garden soil and exposed to natural light. 2,3,5-triiodobenzoic acid (from Imperial Chemical Industries Ltd. England) as well as the other chemicals used were applied in the form of 0.5—1% lanolin pastes by means of small scalpels. To eliminate the interaction of environmental conditions a large number of experiments were simultaneously arranged. The evaluation of the results bears exclusively upon the morphological changes, the aim of these studies being a further approach to the morphogenesis involved in plant totality at the earliest life phases.

Results

Seedling of *Linum usitatissimum* with the second internode stretched were decapitated below the nearly opposite third and fourth leaves and a TIBA paste ring applied, partly on the epicotyl stump, partly on the hypocotyl at distances of 5—10 mm. from the cotyledons. The control plants treated similarly with pure lanolin showed no changes in the epicotyl stump remaining alive. From the axillary buds only the cotyledonary buds were elongated. They prevented the growth of buds in the axils of the two first leaves situated closely above the cotyledons, the polarity of bud development being here overcome by the more advanced embryonic differentiation of the coty-

ledonary buds which in this plant exercise a certain basal dominance. In the seedlings with the TIBA paste ring below the cotyledons the epicotyl stump also remained healthy to the end of experiment and often swelled at the apical pole (Fig. 1a). The cotyledonary shoots in this case showed distinct morphogenetic effects of TIBA, their lower leaves being very narrow and the upper leaves fused into a ring fasciation, well known for other plants treated by TIBA from the work of ZIMMERMAN and HITCHCOCK (1942). These abnormalities indicating the acropetal transport of TIBA through the cotyledonary node may be due to the widely recognised interaction of TIBA with auxins and other substances (KUSE 1953, NIEDERGANG-KAMIEN & SKOOG 1956,

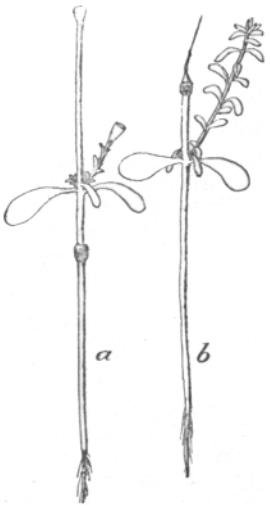

Fig. 1.

a) *Linum*. TIBA paste ring on the hypocotyl. Axillary shoots fasciated, epicotyl stump swollen.

b) TIBA paste ring on epicotyl stump. Upper part of epicotyl died, cotyledonary shoots normal.

AUDUS & LHRESH T 1965, IBBERT 1959, KENTZER & LIBBERT 1960), in these cases with the auxin activated from the materials stored in the cotyledons, both in the apical end of the epicotyl stump and in the cotyledonary buds. The elongation of the cotyledonary shoots appeared to be markedly retarded in comparison with the stronger growth of those on the seedlings with the TIBA paste ring placed above the cotyledons (Fig. 1b). The epicotyl stump of these latter plants began after some time to wither from the cut surface basipetally and at the end of the experiment only the epicotyl part below the paste ring remained alive. This effect of the upper paste ring may be connected with the blockage of the obvious movement of substances towards the apical pole of the epicotyl stump.

The parts below the TIBA ring, on the contrary, seemed not to be affected, since the cotyledonary shoots developed as normally as in the controls without TIBA, showing often no traces of ring fasciation. Decay of the epicotyl stump above the TIBA paste ring need not be connected with the development of the cotyledonary shoots, because it occurred already before their visible growth in plants deprived of the root and the greater part of hypocotyl, afterwards cultivated on the water surface.

When the cut surface of the epicotyl stump was treated simultaneously with gibberellic acid or indolylacetic acid pastes, the decaying of the epicotyl stump from the cut to the TIBA paste ring only proceeded more slowly. It cannot, therefore, be ascribed to the lack of some endogenous analogue of these substances. The same decay was observed when the epicotyl was decapitated above the third and the fourth leaves or when TIBA paste was smeared at the cut surface or when the TIBA paste ring in the middle of the epicotyl stump was combined with the similar ring below the cotyledons. Undoubtedly, the very restricted amount of reserve material in *Linum* seeds faciliated such a clear manifestation of unequal responses to TIBA if supplied below or above the cotyledonary node. In contrast to these responses of *Linum*

epicotyls, the epicotyls of *Pisum sativum*, if correspondingly decapitated closely below the first scale and similarly treated with TIBA in the middle of the first internode left, not only did not die above the paste ring but swelled considerably under the cut surface (Fig. 2a). Similarly operated seedlings of *Vicia faba* were previously shown to form a conical swelling of the upper end of the epicotyl stump (DOSTÁL 1959, Fig. 1). In these seedlings with their abundant material stored in the hypogeous cotyledons, TIBA appears to be unable to block its acropetal transport up to the cut surface. Similar spherical or conical swellings also resulted from the immediate application of TIBA paste to the cut surface, differing of course by the lack of a dventitious roots from the much larger tumourlike formations provoked by IAA treatment, both in *Pisum* and in *Linum*. The same TIBA paste ring applied to the quite short hypocotyl of the seedlings of *Pisum* also decapitated below the first scale induced no appreciable swelling of the epicotyl stump end in contrast to the *Linum* seedlings (Fig. 2b), except when their main root was completely removed. This latter could not be replaced as usual by adventitious roots from the hypocotyl which was covered with TIBA paste. Roots emerged in this case from the epicotyl stump, whose distal end became broader in some plants. In all of these experiments the cotyledonary shoots grew out and their removal undertaken from time to time, did increase the top swelling of the epicotyl stumps.

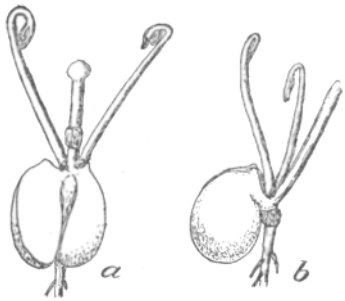

Fig. 2. a) *Pisum* TIBA paste ring one picotyl stump. Its apical part swollen.
b) TIBA paste ring on hypocotyl. Epicotyl unchanged.

On the whole, the difference between *Linum* and *Pisum*, as regards the decaying of the apical part of the epicotyl stump in the first plant and its swelling in the second, may correspond to the very pronounced difference in the correlation effects of their cotyledons, presenting the only source of materials in these earliest life stages. As mentioned above, the *Pisum* cotyledons inhibit the elongation of their own axillary buds and in this way primarily induce the apical dominance of the seedlings. The *Linum* cotyledons, however, promote the growth of their own axillaries, so that the cotyledonary buds only and not the axillaries of some 10 or still more epicotyl leaves may replace the terminal bud, if this is removed above these epicotyl nodes. (This "basal dominance" appears in some other *Linum* species to be so strong that it prevents their flowering on the main stem in the first year.) When applied above the cotyledons TIBA does not stress this peculiar correlation in the decapitated *Linum* seedlings. In addition to the cotyledons, the root may also participate in this morphogenesis, as shown by seedlings deprived simultaneously of the epicotyl, one of the cotyledons and almost the whole hypocotyl with the root. The preserved cotyledon in such plants grown afterwards on the water surface inhibited its own axillary bud, so that the bud in the opposite axil of the cotyledon removed became dominant. But the same growth relation appears in plants with the root left when the cotyledon has been treated with TIBA paste (Fig. 3a, b) or a quite small amount of 0.1% methylchlorophenoxyacetic acid paste, both substances being considered as antagonists of the endogenous auxin (DOSTÁL 1955).

The antagonistic influence of TIBA to IAA can be well demonstrated on the first internode of the stems of *Linum usitatissimum*, this internode in the material used here being 1—3 mm. long in contrast to the next much longer internode. But if the seeds were smeared with TIBA paste or soaked in TIBA solutions, the seedlings showed — besides the loss of the positive geotropism of the root and of the hypocotyl hook — a first internode of 5—20 or more mm. long, the cells of which being 6—10 times longer than those of the control plants (Fig. 3c) (DOSTÁL 1960). At the same time the next two usually opposite leaves were often displaced from each other by a 3—10 mm. long internode. This considerable elongation of the normally very short stem basis in *Linum* can be explained by TIBA decreasing the high level of endogenous auxins activated at the beginning of germination when the cotyledons are

still closed in the seed coats and the plumule has no visible internodes, the hypocotyl and the root only emerging out of the seed. None of the other similarly examined substances, namely indoleacetic acid, methylchlorophen-

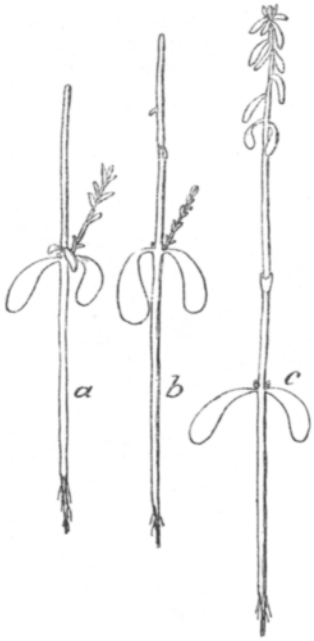

Fig. 3. a) *Linum* seedlings grown from normal seeds. First internode short.
b) Seedlings grown from seeds smeared with TIBA paste. First internode long and first leaf pair displ aced. In both fig. 5 and 6 one cotyledon (at left) smeared at upperside with TIBA paste. Neither epicotyl stump swollen and withered, cotyledonary buds at TIBA side completely inhibited.
c) Intact seedlings grown from a seed smeared with TIBA paste. First internode enlarged, ring fasciation of first leaf pair grown through them.

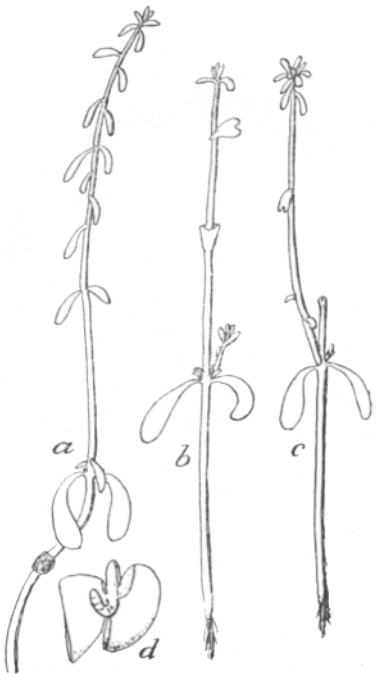

Fig. 4. a) Seedling grown from seed smeared with TIBA paste. Hypocotyl treated with IAA paste. First internode short and leaves free.
b) Seedlings grown from a seed gathered from a plant whose flower peduncles were smeared with TIBA paste. First internode long and lower leaves ring fascitaed.
c) Seedling grown from a seed treated with TIBA. Growth of epicotyl arrested by ring fasciation. Cotyledonary shoot with long basal internodes.
d) *Pisum*. Seed soaked in TIBA solution. Cotyledon at right treated with TIBA paste. Its petiole enlarged.

oxyacetic acid, gibberellic acid, maleic hydrazide, kinetin, had the same effect, the treated seeds or quite very young seedlings giving rise to stems with the first internode 0.5—3 mm. long like the controls. Even GA which provokes a strong lengthening of the hypocotyl and of the second internode, was ineffective as regards this first internode between the cotyledons and the two lowest leaves. The often supposed "synergism" of both IAA and GA is well supported by this fact. The effect of TIBA can easily be nullified by

the timely supply of endogenous IAA (Fig. 4a). For this, the young seedlings raised from the seeds treated previously with TIBA paste, whose cotyledons had not yet cast off the seed coats, were treated with IAA paste on the hypocotyl. This complementary treatment excluded the formation of ring fasciations as well as the stretching of the first internode otherwise induced by TIBA.

In all of these experiments the ring fasciations were restricted to the foliage leaves growing together. The cotyledons always remained free, even when the TIBA paste was applied already to the peduncle and (or) to the calyx of the flowers of to the basal surface of young fruit capsules. TIBA penetrated into the maturing seeds since 68 per cent seedlings raised from them had a markedly more elongated first internode than control seedlings originating from plants similarly treated below the flowers or young fruits, with pure lanolin. 34 per cent of the total of 55 seedlings formed ring fasciations (Fig. 4b). As usual, the greater part of these ring fasciations were afterwards broken through by the elongating stem apex and shifted aside or rarely, they embraced the stem at the place of their origin. In all of these cases the cotyledonary buds remained undeveloped and grew normally without forming ring fasciations and stretching their first internodes, when the main stem was removed. Two facts here may be of some interest: for first, TIBA does not influence the inhibited inactive primordia of the cotyledonary shoots, the dominant plumule being only susceptible to it. Secondly, the transport of the TIBA effect from the mother plant to the daughter could not be compared with lasting modifications because after forming one or two ring fasciations the TIBA plants develop quite normally. As regards the first fact, only when the plumule apex was more strongly affected by TIBA, so that soon afterwards the epicotyl stopped growth altogether, did the cotyledonary shoots grow out spontaneously, forming long basal internodes (Fig. 4c).

These experimental observations make it possible to elucidate some growin correlations between these organs in the first ontogenetic stages both th epigeous and hypogeous seedlings of *Linum* and *Pisum* respectively. The germinating seeds of *Pisum* do not present results similar to those of *Linum*, because their plumule already contains two scales and two completely differentiated foliage leaves. The scales in *Pisum* embryos reduce the inhibitory influence of the cotyledons which prevent the immediate formation of foliage leaf primordia on the plumule. This influence of scales also extends to the cotyledonary buds in *Pisum*, forming normal foliage leaves immediately, since the plumule dominance prevents only their extension growth but not their embryonic differentiation. Another difference between *Linum* and *Pisum* consists in the considerable elongation of the basal internodes in *Pisum* seedlings, especially if grown in the dark, in which *Linum* seedlings do not develop the epicotyl if not nourished e.g. by sucrose. Even after soaking seeds of *Pisum* in solutions of various growth regulators, their first epicotyl internode situated between the cotyledons and the lower scale elongated considerably (average length in IAA 30 mm., TIBA 26 mm., GA 38 mm., MH 13 mm.). On the other hand, the hypocotyl of *Pisum* could not be made longre when treated with these substances, even with TIBA, so very effective in the case of the short first internode of *Linum*. Seeds of *Pisum* soaked for 20 hours in a solution of TIBA 50 p.p.m. germinated very slowly and abnormally, curving up the main root dorsally and straightening the epicotyl hook. The petioles of both cotyledons also remained very short. The *Pisum* petioles used soon after soaking the seeds present a simple biological test for the presence of auxins (DOSTÁL 1936). They may also demonstrate the antagonism between TIBA and IAA in these abnormal TIBA seedlings. It suffices to reduce both the cotyledons to equal parts by a frontal cut and to smear the cut surface

of the one cotyledon with IAA paste and that of the other with pure lanolin. There is only the petiole at the IAA side which elongates markedly (Fig. 4d). On the contrary, seeds soaked normally in water instead of TIBA solution, but operated on in the same way, do not elongate the IAA petiole in contrast to the normal elongating opposite control petiole. Apparently, there exists a certain similarity between this quite short petiole arrested by exogenous IAA in *Pisum* and the quite short first internode inhibited very probably by endogenous auxin in *Linum*. The mechanisms of both these regulatory reactions in the earliest germination stages of these two different types appear to be the same consisting in the rapid activation of auxins in the germinating seeds.

Discussion

The morphogenetic experiments described in this paper were not intended to reveal the growth correlations occurring in the differentiating embryo. They, nevertheless, permit the conclusion that its directing regulatory organs are the cotyledons. This was shown by two quite different examples: *Linum* with its epigeous cotyledons practically without stored material and *Pisum* with hypogeous cotyledons very rich in reserve substances. The *Linum* cotyledons stimulate the growth of their axillaries, the *Pisum* cotyledons, however, inhibit it, as shown by young seedlings which have been decapitated and deprived of one of the cotyledons. Similarly, the growth regulators act differently: the inhibiting influence of *Pisum* cotyledons can be imitated by IAA, the stimulating influence of *Linum* cotyledons, however, can be nullified by TIBA (Fig. 3a, b). The usually quite short first internode of *Linum* can be lengthened by TIBA (Fig. 3c), the petioles of *Pisum* cotyledons, on the contrary, arrested by IAA. The antagonism of these substances is evident from the fact that the lengthening of the first internode as well as the formation of ring fasciation by TIBA in *Linum* may be nullified by IAA (Fig. 4a). Equally, the petiole in *Pisum* seedlings stopped growing by TIBA treatment can be lengthened by IAA (Fig. 4d). The large quantity of free auxin in the earliest germination phases which can easily be reduced by TIBA may be of great importance. This holds true also for the blockade by TIBA of material with auxin precursors transported from the cotyledons. If TIBA paste rings are applied to the decapitated seedlings above the cotyledons, the *Linum* epicotyl stump decays above the ring (Fig. 1b), in *Pisum*, however, this stump remains alive and even thickens at the apical end (Fig. 2a), as if the paste were smeared on the upper cut surface. A long time ago NĚMEC (1905) was the first to demonstrate the decisive role of specific influences in the correlation phenomena and later KOŘÍNEK (1923) demonstrated in *Pisum* cotyledonary shoots that correlation sensitivity depends upon the quantity of material, which may explain the different behaviour of the two seedlings examined here. The same TIBA paste ring applied below the cotyledons provokes in *Linum* thickening of the upper end of the epicotyl stump and the ring fasciating of the cotyledonary shoots proving in this way the acropetal transport

of the morphogenetic influences of TIBA, the basipetal one being in this respect invisible (Fig. 1b). Even in this case the *Pisum* stumps behave differently as they mostly do not enlarge (Fig. 2b). The cotyledonary primordia arrested by the epicotyl terminal bud (as we well as the cotyledons in the seeds ripening on the mother plant) escape this TIBA effect, which is manifested if the apical dominance is destroyed and the axillaries enter in the extension phase (Fig. 4c), the inhibited primordia being insensitive to this substance.

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Использование 2,3,5-трийодбензойной кислоты в деле изучения различий ростовых корреляций среди эпигеическими и гипогеическими прорастающими растениями (*Pisum* и *Linum*)

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Декапитированные прорастающие растения льна и гороха, на которые нанесена паста с трийодбензойной кислотой или сверху семядолей или ниже их, проявляют именно на остатках эпикотиля различия в морфогенетических изменениях в связи

с различиями корреляционных влияний их эпигеических, респ. гипогеических семядолей, которые первично решают судьбу различий в доминантности их почечных основ.

В самом раннем периоде прорастания возможно первого интернодия льна, обычно весьма короткого, и черешков семядолей гороха использовать для доказательства антагонизма между трийодбензойной кислотой и индолуксусной кислотой. Первый интернодий льна удлиняется вследствие действия трийодбензойной кислоты на семена, хотя и созревали на растении, и черешки семядолей гороха, приостановленные в росте замачиванием их в растворе трийодбензойной, кислоты увеличились вследствие действия индолуксусной кислоты. Данная кислота, наоборот, анулирует морфогенетическое действие трийодбензойной кислоты на семена льна.