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Symmetry in the *Pisum* Seedling and Growth Regulators

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Abstract. The rather equal development of the two cotyledons determines the bilateral symmetry in the pea seedling, just as their unilateral position results in the dorsiventrality of the epicotyl and root. The morphogenic manifestation of the symmetry relations conditioned, in the first place, by a different distribution of auxin out of the cotyledons may be enhanced by means of exogenous growth regulators, particularly IAA, GA_3, and TIBA.

The *Pisum* seedling exhibits two types of symmetry, first, the bilateral one, corresponding to the rather uniformly developed cotyledons and second, the dorsiventral symmetry of the plumule (stem) and radicle (hypocotyl and root) due to the particular attachement of the cotyledons at the cotyledonary node, which results from the campylotropic structure of the *Pisum* ovule. The main aim of the present paper is to stress the primary significance of the cotyledons for both these symmetry types in the pea seedling and for this purpose morphological experiments were performed, in which exogenous plant growth regulators were largely used to enhance the appearance of growth responses otherwise escaping attention, yet needed to prove this suggestion.

Materials and Methods

The experiments were carried out with etiolated seedlings of *Pisum sativum* (various cultivars) germinated from seeds soaked for 10—20 hours either in water or in solutions of plant growth regulators, such as IAA, GA_3, TIBA in convenient concentrations (10—50 mg. per litre). Such seedlings will be termed briefly as IAA seedlings and so on. Besides, the same substances in lanoline were used for localized application. The regulative factors coming out of the cotyledons will in these morphological experiments be called endogenous auxin, without their biochemical determination.

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The methods, on the whole rather simple, will be given in the following description of the experimental results divided into three sections, as dealing with the bilaterality in the pea seedling, the dorsiventrality in the epicotyl, and the same in the root.

Results

1. The bilateral symmetry in the pea seedling

In contrast to the rather equal development of both cotyledons their axillary buds, if released from the dominance of the epicotyl, grow out as usual only for a certain time equally and later one of them elongates faster and acquires a dominance upon the opposite, stopping further growth. No exact relation between small weight differences of both opposite cotyledons and the growth rate of their axillaries in decapitated plants could be found. Only when a sufficiently large difference between both cotyledons had been established by cutting off certain parts of one of them, regular growth promotion on the side with less cotyledon tissue or missing completely the latter, could be induced. Auxins (IAA, NAA, 2, 4-D . . .) appeared to be the only substances which applied to the cut surface of one of the two cotyledons presented the same correlative effect as do the endogenous inhibitive cotyledon factors, others, such as GA_3, TIBA, MH, coumarin, kinetin, being in this] respect ineffective, except when supplied to the buds immediately.

The bilateral symmetry manifesting itself in the growth of both opposite axillary buds of cotyledons in decapitated pea seedlings was studied by KOŘÍNEK (1921) also as regards the external conditions which may surely somewhat affect these correlations but are unable to exclude normal growth fluctuations lying in an inner asymmetry which shows no prevalence of a right or left type. It is certain that less favourable growth conditions result in an increase of this asymmetry which represents a measure of the so-called correlative sensibility formulated by the same author (KOŘÍNEK 1922): the ratio between the weight of the larger and the same of the smaller cotylaries presents a greater value if decapitated seedlings are grown in a poor medium or have been deprived of a considerable part of cotyledon tissue. These food factors possess, nevertheless, a secondary importance only, which may be supported by experiments using growth regulators. The correlative sensibility appears to be highly decreased by GA_3 treatment of *Pisum* seedlings or increased by IAA treatment of them. After 11 days the weight ratio of the larger shoots to the opposite smaller ones was in the GA_3 seedlings equal to 2, but in the normal water seedlings grown after decapitation equally in tap water was equal to 60. The maximal length of the GA_3 shoots was 150 and the maximal length of the water shoots 52 mm., but the root system of the GA_3 plants was visibly reduced. The correlative influence of cotyledons affects not only their axillaries, but also the plumule and the radicle in their bilateral symmetry. The cotyledons determine the formation of adventitious roots on the basal internodes, so that it occurs regularly at the side with the cotyledon left if the opposite one has been cut off. Fig. 1a shows such a root-forming influence of the cotyledon together with its inhibition as regards its own axillary bud when the epicotyl cut has been at the start of the experiment treated with IAA in lanoline. The same asymmetry occurs in the main root

if one of the two cotyledons has been removed or treated with IAA. The lateral roots appear in a greater number on the side with cotyledon left or enriched with IAA. Seedlings pretreated with IAA solution exhibite fasciations of these lateral roots in the row near the cotyledon left, the laterals in the other two rows being simple or missing (Fig. 1b).

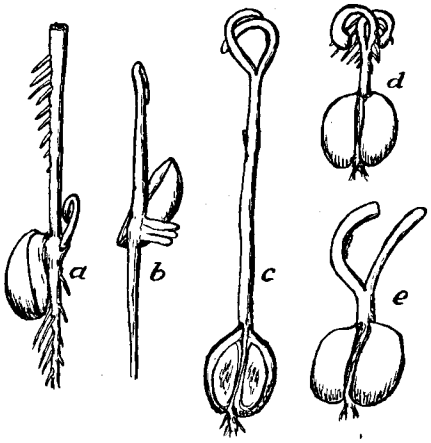

Fig. 1. a) *Pisum.* Asymmetry induced by the cotyledon preserved inhibiting its own axillary and provoking root formation unilaterally on the epicotyl stump treated at the cut with IAA in lanoline.
b) An IAA seedling showing fasciated lateral roots only below the cotyledon preserved.
c) A split epicotyl stump curves inward after treatment of the cotyledon cuts with IAA in lanoline.
d) A split epicotyl treated with IAA at the apical cut curves inward more strongly and forms adventitious roots.
e) IAA in lanoline applied laterally only to half of the split epicotyl does not change the normal outward curvature of the opposite half without IAA treatment.

The bilateral symmetry in the epicotyl axis can be proved by splitting its stump longitudinally, as is usual in Went's pea test, but without excising it from the parts of the seedling below. If kept thereafter in a rather moist atmosphere to prevent the splits from drying the normal outward curvature appears, because the cotyledon product supply moving upward in the stump is unable to provoke an inward curvature. This latter results, however, always from the application of exogenous auxin in lanoline (IAA, NAA, 2, 4-D . . .) either to the intact epicotyl base or to the cotyledon cuts (Fig. 1c) or even to the hypocotyl. But in spite of the relative high concentration of these auxins, no adventitious roots appeared on the split parts. These latter are, however, formed when the auxin pastes have been applied on the split itself either distally (Fig. 1d) or laterally. It may be remembered that auxin in lanoline has been used formerly by THIMANN and SCHNEIDER (1938) in the studies on Went's pea test to establish the sensibility difference between both sides of the split. In the present experiments the effect of IAA was proved as it moves acropetally over more nodes and internodes, of course, decreasing with the distance. The lateral application of IAA paste on the split seems to be, in general, less effective than the apical one, and when made only on one of both halves, the opposite one often retains its original outward curvature (Fig. 1e). GA_3 seedlings behaved differently. Their split internodes curved outward just as they do in the normal seedlings. A basal IAA application results, however, in an inward curvature, limited only to the middle and the basal part of the split, the upper one showing further its original outward curvature (Fig. 2a), probably not supplied with IAA from below. These distal ends might be compared with the decay of distal ends of shoots prolonged excessively after GA_3 treatment at the beginning of their growth.

On the contrary, TIBA seedlings growing otherwise very slowly show typical inward curvatures if treated similarly with IAA. In other experiments the effect of endogenous auxin activated in the terminal bud of epicotyl was examined, and for this purpose half of the split internode remained con-

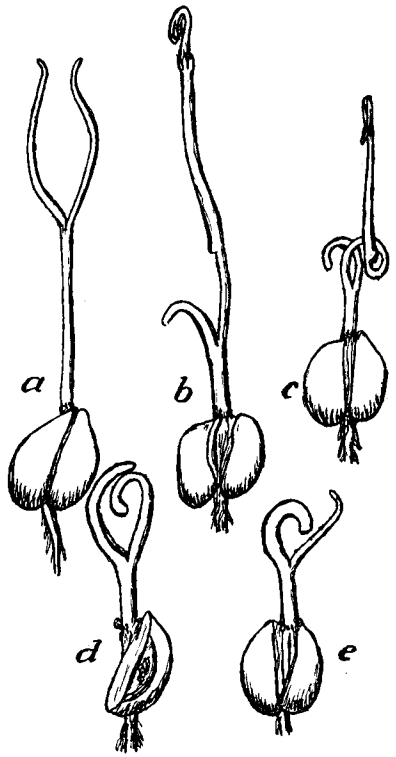

Fig. 2. a) A GA_3 seedling treated basally with IAA in lanoline shows an inward curvature only in the middle zone of the split.
b) The terminal bud preserved on of half the split epicotyl decreases its outward curvature.
c) The same but with IAA in lanoline showing an equal inward curvature of both halves.
d) A seedling with one cotyledon cut off and the other treated on the cut with IAA, showing a stronger inward curvature at the side of the cotyledon.
e) The thicker half of the split curves inward more strongly and along its entire length, the opposite thinner one, however, only at its apical end, IAA in lanoline having been applied basally.

nected with one of the two splits, as shown in Fig. 2b. Both opposite parts at first exhibited a similar outward curvature and only later a slight inward bending could be seen on the side with the epicotyl top growing out. On the other hand, when IAA in lanoline was administered either to the base of the epicotyl or above the transverse cut, both parts curved strongly inward (Fig. 2c), the terminal-bud auxin being here apparently ineffective. It is to be noted that the split epicotyl stump, if excised and placed basally in lanoline with IAA, also curves inward, but less strongly than when in connection with cotyledons. Therefore, also the influence of the cotyledons themselves on these curvatures was tried on seedlings deprived of one of them and treated with IAA paste either on the intact epicotyl base or on the cotyledon preserved, since without auxin supply no difference in the outward curvatures of both sides could be perceived. The split half adjacent to the cotyledon left was regularly that which curved more strongly inward than the opposite one. Of course, only strict median longitudinal cuts may be in such experiments conclusive, a thicker part curving more strongly and along its entire length, while the opposite thinner one bends less and at its distal zone only,

the lower zones remaining curved outward (Fig. 2e). The same precautions must be taken in similar experiments on the stem dorsiventrality.

2. Dorsiventrality in the *Pisum* epicotyl

As already mentioned above, the dorsiventrality of the plumule is evident in the development of its hook which could be, however, reversed by applying IAA in lanoline to the dorsal side of the extending epicotyl. The stem grows thereafter with the plumular hook oriented dorsally instead of ventrally, as is usual. The different distribution of auxin in the ventral and the dorsal side of the epicotyl as it diffuses out of the cotyledons is connected with the position of cotyledons which are not opposite but at an angle of 120° from each other, as was stated already by VAN TIEGHEM (1871). The plumule grows at the side of the cotyledons denoted here as a ventral one slower than at the opposite dorsal side, owing to the high sensibility towards auxin which may be compared with that of roots, the lower side of which if placed horizontally grows slower than the upper one. TIBA plants with their considerable decrease in the auxin content differences on both sides of the hook show even in the dark no hook curvatures (Fig. 5b), except in youngest stages with still high auxin level enabling even the root elongation in plants raised from seeds soaked in MH solutions. On the other hand, GA_3 seedlings increase their plumular hook so that a screw-like form develops, comparable with the well-known coiling of normally straight plants caused by GA_3 treatment. Even strong concentrations of IAA in lanoline used in the present experiments have not excluded the plumular hook, even when they led to considerable swellings of the adjacent lower stem parts. The thin internode within the hook does not swell at least for a certain time, even if treated with 0·5 per cent IAA in lanoline either intact or deprived of the apex (Fig. 3a). The hook remained preserved when treated with IAA on the upper (convex) or the lower (concave) side only, closing more in the former case and somewhat opening in the latter, the growth of the treated internodes being slightly enhanced in both cases.

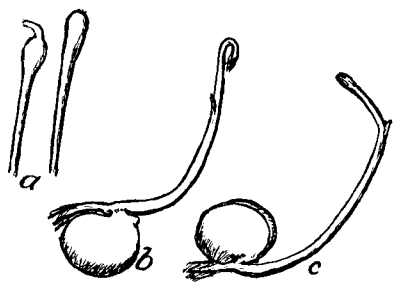

Fig. 3. a) The thin internode inside the hook does not swell if treated with IAA as soon as the adjacent straight epicotyl zone. To the right: the same 5 days later.
b) A seedling placed horizontally with the cotyledons below retains the plumular hook closed.
c) A seedling placed horizontally but with cotyledons above does open the hook.

Similar hook differences may be induced simply by gravity on intact seedlings if placed horizontally, oriented downwards either with the cotyledons or with plumule and the radicle. The stems bent thereafter geotropically upward, yet more strongly in the first position where also the hook became more closed (Fig. 3b), whereas it opened markedly in the other position and later began to turn away from the cotyledons (Fig. 3c). This different behaviour of the hook may be explained by the influence of gravity causing a certain accumulation of auxin in the lower side of the horizontal stem, interfering, however, with the different auxin distribution between the ventral and the dorsal epicotyl side resulting from their unequal connection with the cotyledons (DOSTÁL 1941). The auxin content difference may attain, therefore, a greater value in the position with cotyledons below than with cotyledons above so that the hook opens in this later position as if it would be altered by light in the experiments by GALSTON, TUTTLE, and PENNY (1964). The same light effect occurs in the parallel development of both opposite cotyledonary shoots, if decapitated seedlings with only one cotyledon left are exposed to a strong light which decreases the difference in the auxin distribution on both sides of their cotyledonary node.

Even the lower, already straight epicotyl parts manifest their dorsiventrality, particularly if plants are kept in an atmosphere with ethylene traces (Fig. 4a) or in plants rotated on clinostate, the influence of gravity being decreased or excluded in both cases. The close vicinity of the cotyledons contributes to this behaviour of the young epicotyl. It is to be noted that the first two internodes of the pea stem are differentiated under the cotyledon morphogenic inhibitions not only as regards their particular anatomical structure with exarch xylem (GOURLEY 1931), but also the formation of scales, organs evidently necessary for the initiation of foliage-leaf primordia already in the seed. The same morphogenic function of scales was established in the buds of woody plants (DOSTÁL 1964). It may be assumed that the dorsal position of the first scale may indicate the lower amount of inhibitions of auxin nature on this side.

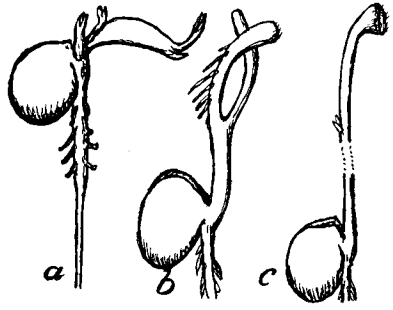

Fig. 4. a) A seedling grown in the atmosphere with ethylene traces bends the epicotyl horizontally toward the dorsal side. The cotylaries are increased.
b) An epicotyl splitted frontally shows the ventral half curved inward more strongly and forming more adventitious roots after equal IAA treatment of the distal cuts.
c) An epicotyl decapitated below the first foliage leaf and treated on the cut with IAA curves toward the dorsal side.

This may be supported by a different behaviour of the two stem sides if decapitated seedlings have been split longitudinally, yet not sagitally as in all foregoing experiments of this type but frontally, that is in ventral and dorsal halves. As a rule, there occurred a stronger inward curvature of the ventral half as compared with the dorsal one, when IAA in lanoline has been applied

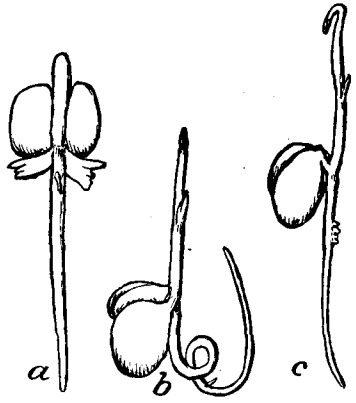

Fig. 5. a) An IAA seedling showing fasciated lateral roots only on the ventral side of the main root, near the cotyledons.
b) A TIBA seedling exhibits no plumular hook and curves the main root toward the dorsal side from which emerge the first laterals.
c) An IAA + TIBA seedling with the plumular hook preserved (effect of IAA) and lateral roots on the dorsal side of the main root, the ventral laterals having been suppressed (effect of TIBA).

as in other cases to the epicotyl base or the cotyledons or finally to the distal end of both split halves. Adventitious roots were developed only in this latter case, besides, more strongly at the ventral split half, proving the importance of its closer vicinity to the cotyledons (Fig. 4b). The *Pisum* stem dorsiventrality could be proved also in higher regions if supplied with IAA in lanoline. The upper end of already straight third internode smeared on the decapitation cut with IAA in lanoline curved towards the dorsal side, the ventral one growing more strongly (Fig. 4c). A similar dorsal concavity occurs normally in the upper region of the growth zone close beneath the apical hook as a result of growth inhibitions at the dorsal side which can be compared with a similar inhibiting influence of leaves upon the adjacent side of the lower internode. Of course, many of these symmetry phenomena could be observed only in plants with etiolated epicotyls, with their abnormal development taking place in the absence of light necessary for this aerial organ in contrast to the root.

3. Dorsiventrality in the *Pisum* root

The radicle presents no visible signs of dorsiventrality, as it is straight and lacks lateral organ primordia in contrast to the plumule with its two scales and two leaves differentiated already in the seed. Nevertheless, the dorsiventrality in the *Pisum* root may be established easily by means of plant regulators. Anatomical studies on the transition region between the radicle,

plumule, and cotyledons in *Pisum* made by GOURLEY (1931) showed that two (ventral) xylem strands of the triarch vascular bundle in the pea root extend into the cotyledons, whereas the third (dorsal) directs in the plumule. The close connection between both the ventral xylem strands and the cotyledons explains the occurrence of fasciated roots only in the two ventral orthostichs if seedlings have been raised from seeds soaked in IAA solutions (Fig. 5a), whereas the dorsal laterals are suppressed or only simple. Such IAA seedlings, if placed horizontally with one of the two cotyledons oriented downwards, show fasciated lateral roots only in the lower ventral orthostich, the gravity interfering here with the influence of IAA in cotyledons. A similar phenomenon may be often observed even without IAA pre-treatment of seedlings, when the main root has been cut off, except its very short base in which the endogenous auxin accumulates more in its ventral side producing thereafter fasciated laterals. TIBA seedlings behave quite differently as regards the branching of the main root. The basal, often more than 30 mm. long zone of this latter initiates no laterals. As the ventral side of these TIBA roots elongates as a rule faster than the dorsal one, a screw-like coiling can be observed here (Fig. 5b), which may be compared to a similar coiling of the plumular hook in the GA_3 seedlings, due, however, in this latter case to a faster elongation of the dorsal side of the stem containing presumably less auxin than the ventral one. But in the TIBA seedlings less auxin may be present in the ventral side of the root, which is nearer to the cotyledons, whose endogenous auxin is blocked by the seed pre-treatment with this auxin "antagonist". Correspondingly, the first laterals on such TIBA roots emerge from their dorsal side, in spite of its concavity (Fig. 5c). Furthermore, instructive seems to be in this respect the behaviour of seedlings germinated from the seeds soaked in a suitable mixture of IAA and TIBA solutions. They showed many short lateral roots only on the dorsal side of their straight main roots resulting, no doubt, as well as the hook structure preserved from the IAA effect, in both ventral orthostichs of lateral roots being completely suppressed by TIBA present in the cotyledons. Only the dorsal orthostich was un influenced by TIBA contained in cotyledons. Seedlings grown under common growth conditions do not reveal such pronounced differences between their ventral and dorsal sides in the main root as regards the above-stated different growth and the branching after growth regulator treatment. Their main roots grow directly downward and then branch uniformly from all three xylem strands of the triarch root structure. Nevertheless, if plants are grown under less favourable conditions, e.g. in less moist saw dust, the main roots, especially if plants are placed thereafter in water, exhibit a distinct apical curvation oriented toward the ventral side indicating that this side of the elongating region was somewhat inhibited by the cotyledons. Similarly, if the growing main roots strike against a horizontal obstacle, their tip avoids it mostly by curving toward the ventral side, as occurs rather regularly on roots till 50 mm. in length. The correlative influences of cotyledons may be proved very early if seeds soaked in water are deprived of the seed coat and one cotyledon, except its petiole. Not only this petiole rest but also the radicle elongate on the side lacking cotyledon more strongly (DOSTÁL 1936). The plumule grows, however, more strongly on the opposite side.

Discussion

The foregoing experimental results support the idea that the symmetry in the pea seedling, as well as in many other similar cases, is induced by auxin distribution. The regulative centre of this latter may be seen in the cotyledons controlling the development of all other parts of the seedling already in their embryonal stage. Other factors, such as postulated by TORREY (1956) for the initiation of lateral roots, or, on the other hand, activating the morphogenesis of the stem organs, could be omitted in this respect. Similarly, only secondary importance may be attributed to the food factors, as demonstrated already by NĚMEC (1905) in his classical regeneration studies. The correlative inhibitions in the first place responsible for the harmonious development of the plant body and its symmetry, too, decrease or even seem to fail completely when the food supply becomes abundant. This was evident from the experiments about the correlative sensibility established by KOŘÍNEK (1923) on pea seedlings. Therefore, in order to intensify the correlative influences, almost all the material of these experiments was kept in tap water and in the dark. Nowadays the experiments using largely exogenous auxins and their "anti-syn-ergists" support the explanations made long before the identification of these plant growth regulators. In this way auxins and above all the facility of their translocation in the pea seedlings depending on the development and the position of the cotyledons may help to elucidate also its symmetry relations.

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Stejnosměrným vývinem obou děloh je určena bilaterální symetrie klíční rostliny hrachu (*Pisum sativum*) tak jako jejich jednostranným postavením dorziventralita epikotylu a hlavního kořene. Morfogenetický projev těchto poměrů symetrie, podmíněných v prvé řadě distribucí auxinu z děloh, lze stupňovat použitím exogenních regulátorů růstu, zvláště indolyloctové, giberelové a trijodbenzoové kyseliny.

Р. ДОСТАЛ, Высшая сельскохозяйственная школа, Брно: **Симметрия сеянцев гороха и регуляторы роста.** — Biol. Plant. **7** : 308—317, 1965.

Одинаковым развитием обоих семядолей определена билатеральная симметрия сеянца гороха (*Pisum sativum* L.) также как их односторонним положением дорсивентральность эпикотиля и главного корня. Морфологическое проявление этих отношений симметрии, обусловленных в первую очередь распределением ауксина из семядолей, возможно интенсифицировать применением экзогенных регуляторов роста, особенно индолилуксусной, гиббереллиновой и трийодбензойной кислотами.