POTENTIATION OF LATERAL ROOT INDUCTION BY ROOT INITIALS IN ISOLATED FLAX ROOTS

By P. L. Goldacre*

[*Manuscript received April* 24, 1959]

Summary

When β-indolylacetic acid (IAA) (10^{-5}M) is added to isolated flax roots, lateral roots are induced to form adventitiously. If some lateral root initials are already present when IAA is added, newly induced primordia form preferentially immediately adjacent to them. It is suggested that a chemical stimulus, a kinin, originating from the existing root meristems interacts with IAA to induce further cell division in the pericycle. It is proposed that kinin formation may be a normal accompaniment of cell division.

I. Introduction

The production of a substance by the stem apex, and its transport to the stem, where it may induce adventitious root formation, is one of the earliest-known examples of physiological correlation (Went and Thimann 1937). This root-forming substance was identified as auxin (Thimann and Koepfli 1935).

It has been recognized that other endogenous metabolites may at times limit the induction of root initials by auxins. The rhizocaline hypothesis (Went 1938; Bouillenne 1950) proposes that although auxin is a prerequisite for the regenerative growth involved in the rooting process, it is a second material, rhizocaline, originating from stems, leaves, or cotyledons which specifically evokes root formation. Cooper (1936) and Van Overbeek and Gregory (1945) showed that in addition to auxin, some other substance is transported from the leaves and is necessary for adventitious root formation on stem cuttings. The cotyledons of the pea seed (Rippel 1937; Torrey 1950; Fries 1954) or the older portion of the isolated pea root (Peckett 1957) appear to contribute some substance which promotes lateral root formation. This factor can be diluted out by serial subculture of the main axis meristem (Torrey 1950). In other cases, known growth factors such as thiamine (Went, Bonner, and Warner 1938), nicotinamide and tryptophan (Galston 1948, 1949), biotin (Went and Thimann 1937), adenine (Torrey 1956), vitamins K and H (Hemberg 1953), and arginine together with tryptophan and histidine (Goldacre and Unt, unpublished data 1955) have been shown to enhance the formation or development of lateral roots.

The first observable act in the initiation of lateral roots is the occurrence of adventitious cell divisions in the tissue giving rise to the primordia, usually the pericycle in herbaceous plants (Van Tiegham and Douliot 1888; Crooks 1933). It is becoming increasingly clear that, for cell division to occur in many situations, it is necessary to have present both an auxin and a member of the class of compounds

* Division of Plant Industry, C.S.I.R.O., Canberra.

known as kinins (Steward and Shantz 1956; Braun 1957; Skoog and Miller 1957). It seems not unreasonable to suppose that root meristems may contain or produce kinins, and that the induction of lateral root primordia may require the participation of a kinin. This paper describes an observation which suggests that root meristems do contain a diffusible substance which, in the presence of auxin, promotes the formation of further root initials.

II. Materials and Methods

Seeds of flax (*Linum usitatissimum* L. 'Ventnor') were surface-sterilized by wetting with alcohol and immersing for 15 min in sodium hypochlorite (final concentration equivalent to 0·075N chlorine). The alkaline solution was washed off with 1 per cent. saturated bromine water. Seeds were spread onto sterile petri plates containing basal medium plus 0·7 per cent. agar and incubated at 25°C.

After 4 days, terminal 10-mm sections were excised from the radicles, transferred to 40 ml sterile medium contained in 150-ml conical flasks, and incubated in darkness at 25°C.

Basal medium contained Bonner's macronutrient salts (Bonner 1940), White's micronutrient salts (White 1943), with the addition of 0·05 mg/l $CuSO_4.5H_2O$, 0·13 mg/l $(NH_4)_6Mo_7O_{24}.4H_2O$, 0·1 mg/l thiamine, 0·5 mg/l nicotinic acid, 0·1 mg/l pyridoxine, and 2 per cent. sucrose. The pH was adjusted to 5·5 with $KHCO_3$.

After 3 days incubation, when the average length of the main axis was about 7 cm, there were present 8–12 unemerged lateral primordia ranging in length from 0·02 to 0·10 mm (Plate 1, Fig. 1). At this stage 0·4 ml sterile 10^{-3}M β-indolylacetic acid (IAA) was added (final concn. equals 10^{-5}M) and the roots were further incubated and sampled at time intervals.

Roots were fixed by vacuum infiltration with 2·4 per cent. formaldehyde in 70 per cent. aqueous ethanol. After 30 min they were washed in 70 per cent. ethanol, then cleared by immersion overnight in Sartory's clearing agent (33 ml glycerol, 17 ml lactic acid, 20 g phenol, and 20 ml water). Unemerged root initials were then photographed, or their positions measured along the main axis using a dissecting microscope and ocular micrometer.

Other roots, after fixation, were embedded in wax, sectioned at 10 μ, stained with ruthenium red, mounted, and photographed.

III. Results

The effect of adding IAA at this concentration (10^{-5}M) is to inhibit the growth of the main axis meristem and to promote the formation of lateral root primordia. When some primordia are already present at the time of adding the IAA, the new primordia which form consequently are not randomly distributed, but arise predominantly in the immediate vicinity of pre-existing root initials (Plate 1, Figs. 2 and 3). This results in clumps of root initials appearing at irregular intervals. The distribution of induced primordia in a typical root 20 days after adding IAA is shown diagrammatically in Figure 1.

In Figure 2 a measure is given of the relative frequencies of occurrence of new primordia near to and distant from the pre-existing laterals for four typical roots. Since the intervals between pre-existing laterals vary, they were each subdivided into the central half and two terminal quarters. Counts of primordia in the central half were subtracted from the pooled counts for the two terminal quarters for each interval (see Fig. 2, *inset*). This difference was then plotted against the interval length. First it is seen (Fig. 2) that almost all differences are positive, i.e. there are more laterals in the two terminal quarters than in the central half of each interval. Further, the longer the interval, the greater is the difference. This would

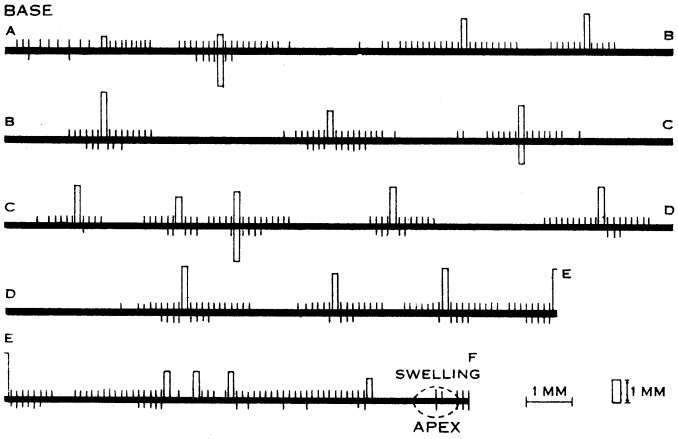


Fig. 1.—Distribution of induced lateral primordia on one typical root. IAA (10^{-5}M) added after 3 days growth; root fixed after a further 20 days incubation. For the sake of representation the main axis AF is here subdivided into AB, BC, CD, DE, EF. Open blocks represent pre-existing laterals, and their lengths represent the length at the time of fixing.

be expected if some influence originating from the pre-existing laterals were propagated a limited distance during the experimental period. The few cases in which the difference approximates zero or is negative are for very short or very long intervals. In the first case, induced primordia may occupy the whole interval; the original lateral, being thicker, occupies a significant portion of the space counted as terminal quarters: this leads to a negative difference in this form of representation. In the second case, where the interval is large, a large number of randomly disposed initials are induced along the central half.

Subsequent development of the induced primordia is inhibited by the presence of the IAA, but if the IAA is removed, the laterals grow out, giving a tufted appearance (Plate 1, Fig. 4).

Sampling at time intervals after adding IAA reveals that a wave of cell-division activity begins in the pericycle adjacent to the pre-existing primordia (Plate 1, Fig. 5; Plate 2, Fig. 1) and in the radial plane containing the protoxylem and moves along the root in both directions from these points (Plate 2, Fig. 2).

The tissue produced as a result of this cellular proliferation tends to segment and form organized structures which eventually develop into lateral root primordia

(Plate 1, Fig. 3; Plate 2, Fig. 3). The margins of such proliferating regions appear, in turn, to stimulate *neighbouring* (quiescent) pericycle parenchyma cells into dividing (Plate 2, Figs. 2 and 4). Thus the influence of the original meristem spreads consecutively in both directions, stimulating cellular proliferation which, upon further development, gives rise to many primordial root apices packed close to each other, and with centres approximately 0·13 mm apart (Plate 1, Fig. 3; Plate 2, Fig. 3). The old cortical tissue tends to disintegrate in these regions.

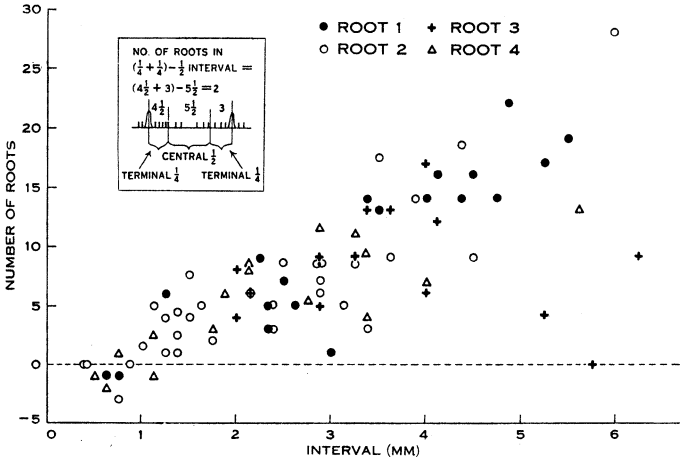


Fig. 2.—Distribution of induced lateral primodia on four typical roots. Each interval between pre-existing laterals has been divided into the central half and the two terminal quarters. For each interval the number of induced root initials in the central half has been subtracted from the sum of the initials in the two terminal quarters. This difference has been plotted against the interval length. Note that the differences are almost always positive; and that the longer the interval, the greater is the difference.

In cases where those lateral roots present at the time of adding IAA have already emerged, i.e. when the lateral root apex is separated from the stele of the main axis by an elongation zone, the influence of such roots in potentiating primordium formation at their bases is lost. This suggests that the stimulus arises in the meristematic zone itself and does not pass through the elongation zone.

IV. Discussion

These results indicate that some stimulus originating from the immediate neighbourhood of unemerged root initials potentiates the induction of new root initials by IAA. Time studies suggest that the stimulus is in some way propagated short distances in both directions along the root. The stimulus acts only upon the pericycle tissue lying in the same plane (i.e. in the radial plane containing the protoxylem), usually on the same side of the root, and to a lesser extent on the opposite side of this diarch root (Plate 2, Fig. 3).

This localization of response and indeed the almost total restriction of the normal formation of lateral primordia to the pericycle immediately adjacent to

the protoxylem (Van Tiegham and Douliot 1888; Crooks 1933; observations this paper) suggest that the protoxylem itself may play a part in the induction process.

It has been concluded by a number of authors (Rippel 1937; Geissbühler 1953; Fries 1954; Torrey 1956; Peckett 1957) that root branching is inhibited by the presence of the main axis meristem of peas, and extracts from such meristems have been shown to be inhibitory to the initiation of lateral root meristems (Libbert 1956; Torrey 1956). Contrarily to this antagonistic behaviour, the young meristems described in the present work *promote* the formation of their own kind. This suggests that the stimulus produced by the pre-existing primordia cannot be merely a nutrient metabolite specifically required for cell division, for then the meristematic region would demand it, rather than produce it. The substance postulated here has the property of being produced by dividing cells, and of stimulating cell division in the presence of IAA; i.e. given favourable conditions it can catalyse its own formation. The chemical identity of the stimulus merits further investigation. We have been unable to obtain any stimulation of lateral root formation by kinetin or by *N*,*N*′-diphenyl urea.

This concept raises the important question of the of mechanism self-perpetuation of meristems (cf. Sinnot 1956). One interesting possibility is that kinin production may be a normal accompaniment of cell division. Any such kinin leaking from a lateral root primordium into neighbouring pericycle tissues would, in the presence of added auxin, be expected to induce cell division there. One would further suppose that in the developing root apex, the cell extension zone somehow inactivates or limits the movement of this kinin with the result that division is confined to the apex and the whole organ does not become engulfed in a wave of cell proliferation. This supposition is in accord with the observation that lateral roots do not, in the presence of IAA, potentiate the formation of new primordia adjacent to their bases once an elongation zone has been established.

V. References

Bonner, J. (1940).—On the growth factor requirements of isolated roots. *Amer. J. Bot.* **27**: 692.

Bouillenne, R. (1950).—La rhizogenèse. *Année Biol.* (3) **26**: 597.

Braun, A. C. (1957).—A physiological study of the nature of autonomous growth in neoplastic plant cells. *Symp. Soc. Exp. Biol.* **11**: 133.

Cooper, W. C. (1936).—Transport of root-forming hormone in woody cuttings. *Plant Physiol.* **11**: 779.

Crooks, D. (1933).—Histological and regenerative studies on the flax seedling. *Bot. Gaz.* **95**: 209.

Fries, N. (1954).—Chemical factors controlling the growth of the decotylised pea seedling. *Symb. Bot. Upsaliens.* **13**: 1.

Galston, A. W. (1948).—On the physiology of root initiation in excised asparagus stem tips. *Amer. J. Bot.* **35**: 281.

Galston, A. W. (1949).—Indoleacetic acid–nicotinic acid interactions in the etiolated pea plant. *Plant Physiol.* **24**: 577.

Geissbühler, H. (1953).—Untersuchungen über die korrelative und hormonale Steuerung der Seitenwurzelbildung. *Ber. schweiz. bot. Ges.* **63**: 27.

HEMBERG, T. (1953).—The effect of vitamin K and vitamin H on root formation in cuttings of *Phaseolus vulgaris* L. *Physiol. Plant.* **6**: 17.

LIBBERT, E. (1956).—Untersuchungen über die Physiologie des Adventivwurzelbildung. II. Die korrelative Beeinflussung der Adventivwurzelbildung durch andere Organe, insbesondere durch die Wurzel. *Planta* **48**: 157.

PECKETT, R. C. (1957).—The initiation and development of lateral meristems in the pea root. I. The effect of young and mature tissue. *J. Exp. Bot.* **8**: 172.

RIPPEL, K. (1937).—Umkehr der Seitenwurzelgenese bei Leguminosen als korrelative Störung. *Ber. dtsch. bot. Ges.* **55**: 288.

SINNOT, E. W. (1956).—Botany and morphogenesis. *Amer. J. Bot.* **43**: 526.

SKOOG, F., and MILLER C. O. (1957).—Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. Exp. Biol.* **11**: 118.

STEWARD, F. C., and SHANTZ, E. M. (1956).—In "The Chemistry and Mode of Action of Plant Growth Substances". (Ed. R. L. Wain and F. Wightman.) (Butterworths Scientific Publications: London.)

THIMANN, K. V., and KOEPFLI, J. B. (1935).—Identity of the growth-promoting and root-forming substances of plants. *Nature* **135**: 101.

TORREY, J. G. (1950).—The induction of lateral roots by indoleacetic acid and root decapitation. *Amer. J. Bot.* **37**: 257.

TORREY, J. G. (1956).—Chemical factors limiting lateral root formation in isolated pea roots. *Physiol. Plant.* **9**: 370.

VAN OVERBEEK, J., and GREGORY, L. E. (1945).—A physiological separation of two factors necessary for the formation of roots on cuttings. *Amer. J. Bot.* **32**: 336.

VAN TIEGHAM, P., and DOULIOT, H. (1888).—Recherches comparatives sur l'origine des membres endogènes dans les plantes vasculaires. *Ann. Sci. Nat. Bot.* (7) **8**: 1.

WENT, F. W. (1938).—Specific factors other than auxin affecting growth and root formation. *Plant Physiol.* **13**: 55.

WENT, F. W., BONNER, J., and WARNER, G. C. (1938).—Aneurin and the rooting of cuttings. *Science* **87**: 170.

WENT, F. W., and THIMANN, K. V. (1937).—"Phytohormones". (The MacMillan Publishing Co.: New York.)

WHITE, P. R. (1943).—"A Handbook of Plant Tissue Culture." p. 105. (Jacques Cattell Press: Lancaster, Pa.)

EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1.—A cleared typical root after 3 days growth showing the development of lateral root initials before 10^{-5}M IAA was added. ×21.

Fig. 2.—Portion of a cleared root 10 days after 10^{-5}M IAA was added. ×2·4.

Fig. 3.—Enlarged portion of a cleared root 10 days after 10^{-5}M IAA was added. Note the extensive cellular proliferation in the pericycle and the beginnings of organization of root apices. ×60.

Fig. 4.—Resumed growth of induced primordia after removal of IAA by exposure to blue fluorescent light in the presence of 0·1 mg/l riboflavin. Note tufted appearance. ×0·95.

Fig. 5.—Median longitudinal section of root, 3 days after 10^{-5}M IAA was added. *P*, lateral root primordium present at time of adding IAA; *CD*, cellular division occurring in pericycle parenchyma immediately adjacent to the primordium. ×110.

PLATE 2

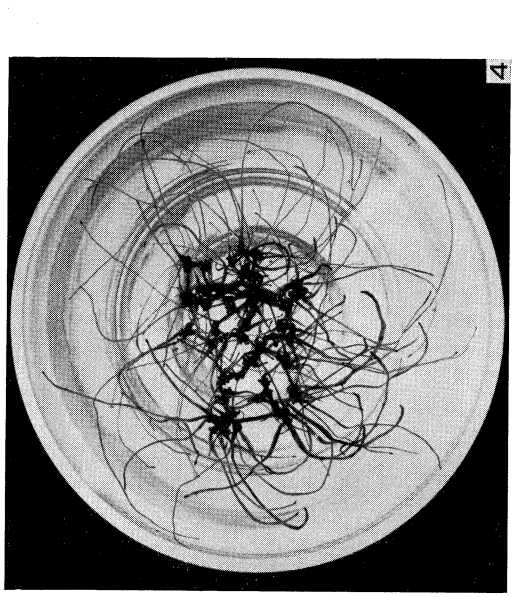
Fig. 1.—Enlargement of portion of Plate 1, Figure 5. ×350.

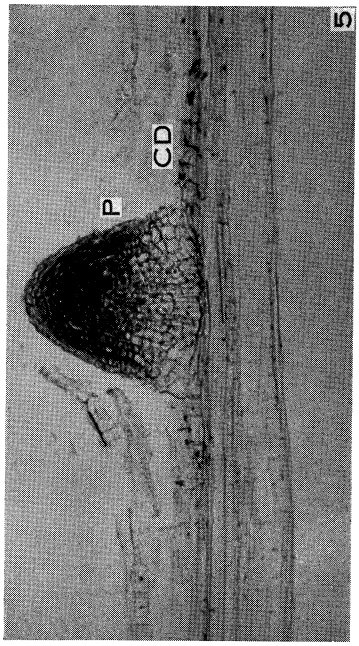
Fig. 2.—Longitudinal section of root 5 days after adding 10^{-5}M IAA. ×105.

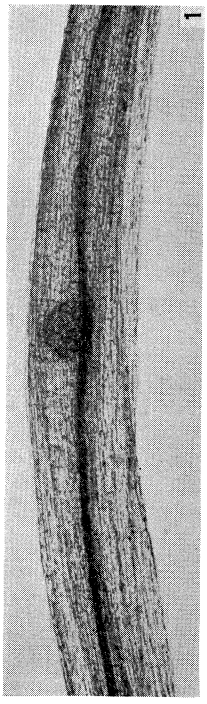
Fig. 3.—Longitudinal section of root 17 days after adding 10^{-5}M IAA. ×30.

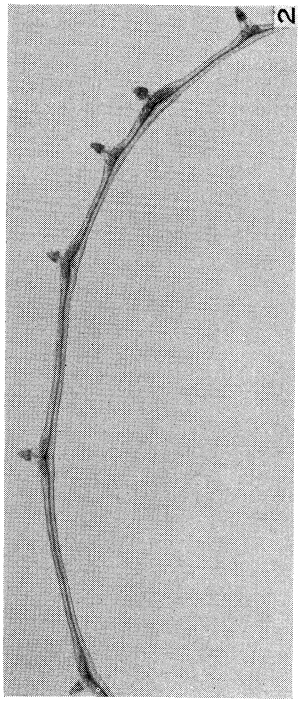
Fig. 4.—Longitudinal section of root 15 days after 10^{-5}M IAA was added, showing the front of the advancing wave of cell division in the pericycle parenchyma. *C*, cortex; *E*, endodermis; *PX*, protoxylem; *U*, original undivided pericycle parenchyma cells; *D*, pericycle parenchyma cells completing their first division; *P*, actively proliferating cells. The original lateral root is beyond the left of the picture. ×350.

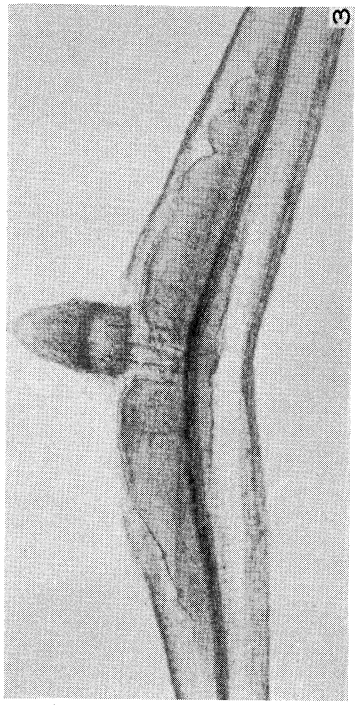
ROOT INDUCTION











ROOT INDUCTION