Chemical Factors Affecting Growth and Development of Lateral Root Formation in Isolated *Phaseolus vulgaris* L.

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العوامل الكيمياوية المؤثرة على نمو وتطور الجذور الجانبية في مزارع نبات الفاصوليا

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قسم علوم الحياة ، كلية التربية ، جامعة الموصل ، العراق

تنشأ الجذور الجانبية من الدائرة المحيطية وتظهر على مسافة صغيرة من المرستيم القمي للجذر الابتدائي أي أنها (داخلية المنشأ) . في هذه التجربة درس تأثير منظم النمو اندول حـامض الخليك على مزارع قطع الجذور بطول 1 سم والجذور الكاملة لنبات الفاصـوليا ، وقـد أبـدت الجذور المزروعة استجابة واضحة لهذه المعاملة حيث شجعت ظهور بادئات الكالس منذ الأيـام الأولى وتطورت خلال 144 ساعة إلى جذور جانبية . كما تم قياس معامل الانقسام المايتوزي في قمم الجذور المعاملة رغير المعاملة وحسب زمن تضاعف الخلية في كلا النوعين مـن الجـذور وكان للتراكيز العالية تأثير على معدل طول الجذر ، وعدد البادئات ، وعدد الجذور الجانبية فـي السنتمتر الواحد . .

Keywords: *Indol-3-yl acetic acid, Lateral roots , Phaseolus vulgaris, Primordium.*

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ABSTRACT

The initiation and subsequent emergence of lateral roots as secondary roots have been examined in attached and excised roots of *Phaseolus vulgaris* grown in the absence or presence of indol-3-yl acetic acid (IAA).   Treatments with high concentrations of IAA affected the point at which lateral emergence took place along the primary root. In addition, IAA treatments promoted lateral root emergence from cultured roots of *P. vulgaris*, which were 1-cm long at the time of excision ; such roots never formed in similar primaries grown in the absence of IAA. No correlation was found between proliferative activity in the meristem at the apex of primary or the rate of root elongation on the one hand and either the number of primordia initiated, or the number of laterals produced, per centimetre of primary, on the other hand.

Introduction

The development of all roots is similar in that they appear to undergo identical changes from their initiation until maturation [1].   Primary and lateral root apical meristems, both originate from the division of a relatively small number of cells, increase in size and cell number over on interval of several days and complete their development which involve cell proliferation and elongation following the growth of lateral through the tissues of primary root [2]. In both gymnosperms and angiosperms, branch roots are commonly initiated in the pericycle of the parent root [3, 4].   In lower vascular plants, and in some higher ones, the endodermis of the parent root also takes part in the formation of the primordium of lateral.

The pericyclic origin of most laterals places them in close juxtaposition with the conducting tissues of the parent root [3, 5, 6, 7, 8].   In diarch roots primordia usually occur between the xylem and phloem, intriarch and tetrarch roots in positions opposite to the protoxylem, and in many polyarch roots opposite to the protophloem [4, 9].

Cell division is then initiated in a group of pericyclic, and sometimes other cells of the parent root. These cells undergo periclinal and anticlinal divisions [3, 4, 5, 6], as a result, developing primordia increase in size and cell number. These increases are accompanied by changes in the organization of the meristem itself and in the kinetics of cell proliferation of the dividing cells [1, 2].

During the initiation of lateral root primordia in *P. vulgaris*, several changes take place in the cytoplasm and walls of a few pericycle cells as well as in those parenchyma cells lying close to the xylem poles. Such changes include an increase in the basophilic nature of the cytoplasm and increase in cytoplasmic and nuclear volume [10, 11].

In addition to sucrose, other factors including auxins, such as IAA , involve in the initiation and development of primordia. The transport of this hormone in roots is mainly acropetal, that is toward the tip of the root, and it is probable that its supply to the root apex is primarily from the shoot [12].

Exposure of excised roots of *P. vulgaris* to IAA for example indirectly facilitates lateral emergence by partially inhibiting meristematic activity in the root apex [13] .
The effects of IAA on the various stages of lateral root development in

P. vulgaris are also examined because considerable amount of information is available concerning the effect of IAA on lateral root initiation and emergence in dicotyledons [14, 15]. The results of these investigations were then correlated with the changes took place in the proliferative activity in the primary root apical meristem following excision. IAA being used as a tool to suppress such activity for extended periods of time.

Materials And Methods

Seeds of *Phaseolus vulgaris* L. Local variety were surface sterilized for 5 min in a 6 percent aqueous solution of sodium hypochlorite, washed several times in sterile distilled water and then scattered over surface of moist vermiculate or filter papers that had been autoclaved for 15 min at 103 KNm^{-2} and temperature of (121° C), and germinated in dark at 20° C.

Seedlings, which were kept as intact plants, were removed from the vermiculate in which they had been germinated 6d after the start of imbibition, washed and grown in aerated distilled water or 10^{-5} M solution of IAA ; each solution was changed at 48h intervals. Ten 1 cm root apical segment were excised into 150ml flask containing 50ml of White's (1943) [16] medium supplemented with 2% sucrose or 2% sucrose and 10^{-5} M IAA. After 72h following the onset of soaking, the pH of the medium was adjusted to 4.7 before autoclaving.

IAA being sterilized by Millipore filtration before being added to the autoclaved culture medium. The flasks were placed on an orbital shaker (60 cycles min') and the roots allowed to grow on. Twenty roots from each treatment were fixed at 24h intervals over the initial 6d culture period, the fixative being 3:1(V/V) absolute ethanol: glacial acetic acid. Each batch of roots was later hydrolysed in 1M HCl at 60° C for 6 min and then stained with feulgen. Measurements were made of the length of each primary root along with counts of the total numbers of emerged laterals and primordia [17]. Primordia were identified as densely staining clusters of cells under dissecting microscope at a magnification of X40.

Two additional experiments were carried out to investigate the changes took place in proliferative activity in the meristem at the apex of both excised and attached roots. Mitotic with time following the start of exposure to IAA 10^{-5} M). Fixations were made at 24h intervals up to 144h after experiment began.

Squash preparation were then made of the feulgen-stained apical millimetre of each of these roots. Mitotic index (MI), and the percentage frequency of cells in mitosis were determined by scoring 1000 cells from each of three slides for each fixation.

In the second experiment, cell doubling time (T_d) was calculated from the rate of accumulation of cell in metaphase in the root apical millimetre at intervals following the start of treatment with IAA. Control and IAA-treated attached roots were exposed to 0.025% solution of colchicine 72-75h after the commencement of auxin treatment. Exposure of the excised root to colchicine was effected by injecting 1ml of a 1.25% solution of colchicine into 49ml of the control and IAA containing culture media, to give

a final concentration of 0.025%, 8-11h or 72-75h following the start of culture. Control and IAA-treated roots were fixed at 30 min intervals over each 3h period of exposure to colchicine. The number of cells in interphase and in the various stages of mitosis was later determined by scoring 1000 cells from squash preparation of the apical millimetre of each of three feulgen-stained roots at each fixation time for each batch of primaries.

These conditions applied between 2 and 3h following the start of exposure to colchicine in each batch of roots could be determined as:

$$T_d = \frac{1n2}{c} \quad [18]$$

Statistical Tests

Comparison of mitotic index or numbers of primordia or lateral roots were made using t-test, while the statistical significance of each linear regression equation was determined using correlation coefficient and the appropriate statistical tables. The 5 per cent level was held to be significant.

Results And Discussion

The total number of primordia and lateral roots produced per centimetre of primary was always greater in the IAA-treated roots than in the corresponding controls (Figures 1B, 2B). IAA treatment stimulated primordium inception in roots of *P. vulgaris*. This results is in agreement with reports in the literature concerning the effects of IAA on the initiation of primordia in both attached and excised roots of dicotyledons [19, 20, 21].

The total number of primordia and laterals produced per centimetre of primary increased over the initial 2-3d of culture (Figures 1B, 2B). It is evident that the amount of 1AA synthesized by the excised controls was sufficient for anlage inception to take place at a rate comparable to that found in the corresponding attached roots (Figures 1B, 2B)> In the 1AA-treated attached root, for example, this number significantly increased between day 1 and 3 after treatment (p < 0.001) and then stayed constant (p>0.05; Figure 1B). Similar increases were found 1-2d and 1-3d after culture began in the control and IAA-treated excised roots respectively (p<0.001). Following this, the total number of primordia and laterals produced per centimetre of primary remained fairly constant (p>0.05; Figure. 2B). These increases may reflect one aspect of the recovery of root growth, following the shock of excision in case of cultured roots [1, 22], and also following transfer from growth in vermiculate to growth in liquid medium incase of attach roots ; being markedly affected by the conditions under which they are grown [23].

Root excision resulted in a marked reduction in the rate of excision growth (Figures 1A, 2A). Thus elongation took place at a significantly slower rate in both the control) (0.66 cm/d) and IAA-treated (0.12 cm/d) excised root than in the corresponding attached primaries (1.46 and 0.60 cm/d). However, the total number of primordial and lateral roots produced per centimetre of primary was similar (p > 0.05) in the excised controls and attached roots, at each time of fixation. A result similar to that found for IAA-treatment in excised and attach roots of *Pisum sativum* [17]. Short treatments of *Vicia faba* roots with IAA also led to extensive primordium formation through the

primaries [24].The pattern of increase in lateral number per centimetre of primary was examined in the attached roots in more detail by studying the changes took place at successive fixation times (Figure. 1B). This number of laterals increased significantly 2-3d and 2-4d following the start of treatment in the control and IAA-treated attached roots respectively (p<0.001), but not between any of the subsequent fixation time (p>0.05; Fig. 1B).

In IAA-treated excised roots MI decreased to level significantly lower than that obtained at the time of excision by 4h and it stayed low for the following 92h (p < 0.001-0.05; Table 1). By 120-144h, MI in these roots returned to values statistically similar to those of the excised controls (Table 1: p > 0.05). MI reflects the rate of cell formation in the apical meristem [25]. Similar results were found when IAA-induced stimulation of primordium and lateral initiation in excised and attached roots of *Pisum sativum* and *Zea mays* [17].

Table (1) : Mitotic index in the apical millimetre of attached and excised roots in the presence and absence of 10^{-5} M IAA.

0	5.50 ± 0.20	-	5.10 ± 0.30	-
4	5.30 ± 0.60	4.50 ± 0.40	3.01 ± 0.50	1.09 ± 0.60
8	6.59 ± 1.10	2.42 ± 0.75	2.25 ± 0.10	0.65 ± 0.10
12	5.88 ± 1.20	2.88 ± 0.73	4.90 ± 0.61	1.90 ± 0.50
24	6.10 ± 0.50	2.83 ± 0.12	4.89 ± 0.85	2.30 ± 0.30
48	5.90 ± 0.80	4.10 ± 0.21	5.85 ± 0.80	1.80 ± 0.08
72	5.80 ± 0.90	5.20 ± 0.70	5.08 ± 0.61	2.80 ± 0.10
96	5.88 ± 1.01	4.70 ± 0.62	7.20 ± 0.65	2.09 ± 0.90
120	6.20 ± 0.19	5.83 ± 0.65	4.80 ± 0.59	3.10 ± 0.95
144	5.70 ± 0.30	4.85 ± 0.11	6.60 ± 0.90	5.30 ± 0.60

Values are means ± SE
* Cultured in white's (1943) medium supplemented with 2% sucrose.

Some 8-11h following excision, T_d in the apical meristem of the cultured control roots (42.0h) was much longer than the value obtained for the untreated attached ones (17.2h). However, by 72-75h there was little difference in Td between the attached (17.2h) and excised (23.0h) control roots Td in the IAA-treated attached roots (21.5h) was similar to the value obtained for the corresponding controls (17.2h), 72-75h after treatment began. Exposure of the excised roots to IAA, however, resulted in longer values for Td between 8 and 11h (80.2h) and between 72 and 75h (99.0h) following the onset of treatment compared with the values obtained for the excised controls (42.0h) and 23.0h respectively).

It has been concluded that one or more inhibitors to lateral root emergence
originate in the root apex and the results of the experiments reported in the present work
suggest that such inhibitors are produced by the actively proliferating cells in the apical
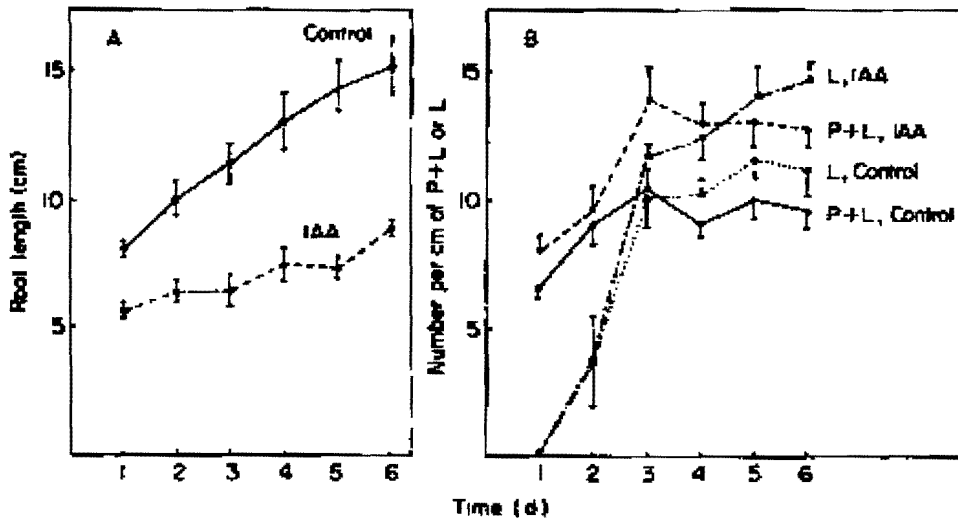meristem of the primary root and not in the apex as a whole.

Fig. 1. Change in (A) root length (cm) and (B) total number of primordia and lateral roots (P + L) or laterals (L) only, per centimetre of primary, with time (d) in control and IAA-treated attached roots. SEM bars are given for each point on A and B.
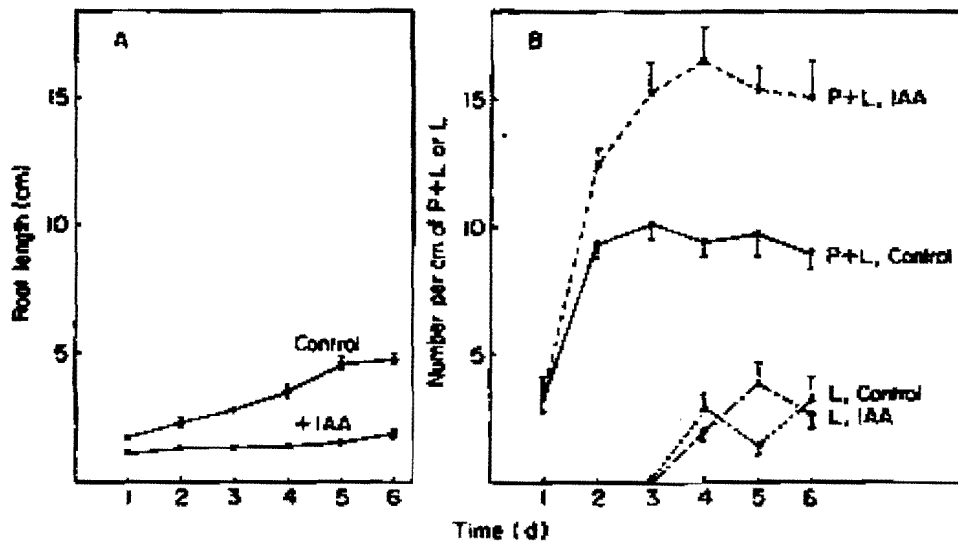

Fig. 2. Change in (A) root length (cm) and (B) total number of primordia and lateral roots (P + L) or laterals (L) only, per centimetre of primary, with time (d) in control and IAA-treated excised roots cultured in White's (1943) medium supplemented with 2% sucrose. SEM bars are given for each point on A and B.

REFERENCES

[1] Thompson A. and Macleod R.D. (1981) Increase in size and cell number of lateral root primordia in the primary of intact plants and in excised roots of *Pisum sativum* and *Vicia faba*. Amer-J. Bot., **68:** 955-964.

[2] Friedberg S.H. and Davidson D. (1971) . Cell population studies in developing root primordial. Ann. Bot., **35:** 523-533.

[3] Esau K. (1965) . Plant anatomy. 2nd edition John. Wiley and Sons, New York.

[4] Cutter E.G. (1971) . Plant anatomy: Experiment and interpretation part **2:** Organs 1st edition. Edward Arnold, London, pp. **343**.

[5] Esau K. (1953) . Development of lateral roots, in: Plant anatomy, 2nd edition. John Wiley and Sons, New York, pp. **509-513**.

[6] Esau K. (1960) . Anatomy of seed plants, New York, John Wiley and Sons, pp. 235-239.

[7] Foard D.E., Harber A.H. and Fishman T.N. (1965) Initiation of lateral root primordia without completion of mitosis and without cytokinesis in uniseriate pericycle. Amer. J. Bot., **52:** 580-590.

[8] Davidson D. (1972) . Morphogenesis of primordia of lateral roots. In: The dynamics of meristem cell populations, eds. M.W. Miller and C.C. Kuehnert, pp. 165-185. Plenum press, New York.

[9] Brook A.H (1964) . Lateral root formation. In: The living plant. p. 104-106, Edinburgh university press.

[10] Bell J.K. and Mccully M.E. (1970) . A histochemical study of lateral root initiation and development in *Zea mays*. Protoplasma, **70:** 179-205.

[11] Karas I. and Mccully M.E. (1973) . Further studies of the histology of lateral root development in *Zea mays*. Protoplasma, **77:** 243-269.

[12] Mohammad, A.A. and Raies A.H. (1995) plant physiology, 2nd edition. Baghdad press.

[13] Zeadan S.M. and Macleod R. D. (1984). Apical meristem activity and lateral root anlage development in excised roots of *Pisum sativum*. Ann. of Bot. **54:** 77-85.

[14] Pecket R. C. (1957) . The initiation and development of lateral meristems in the pea root. J. Exp. Bot., **8:** 181-194.

[15] Blakely L. M. and Evans T. A. (1979) . Cell dynamics studies on the pericycle of radish seedling roots. Plant. Sci. Letts., **14:** 79-83.

[16] White P. E. (1943) . A handbook of plant tissue culture, 1st ed., **275** pp. cattel, Lancaster, PA.

[17] Zeadan S. M. (1982). Some factors affecting lateral root initiation and emergence in *Pisum sativum* and *Zea mays*. Ph.D. thesis University of Newastte upon tyne, Egland.

[18] Dela Torre C. and Clowes F.A.L. (1974) . Thymidine and the measurement of rates of mitosis. The new phytologist **73:** 919-925.

[19] Torrey J. G. (1956) . Chemical factors limiting lateral root formation in isolated pea roots. Physiol. Plant., **9:** 370-388.

[20] Torrey J.G. (1962) . Auxin and purine interactions in lateral root initiation in isolated pearoot segments. Physiol. Plant., **15:** 177-185.

[21] Wightman F., Schneider E.A. and Thimann K. V. (1980) . Hormonal factors controlling the initiation and development of lateral roots. Physiol. Plant. **49:** 304-314.

[22] Van't Hof J. (1965) . Cell population kinetics of excised roots of *Pisum sativum*. J. of cell Biol. **27:** 197-189.

[23] Pengelly W.L. and Torrey, J.G. (1982) . The relationship between growth and indole-3 acetic acid content of roots of *Pisum sativum*. Bot. Gaze. **143:** 195-200.

[24] Davidson D. and Macleod R.D. (1968) . Response of meristems to indolacetic acid and colchicine: differences between primordia and root meristems. Bot. Gaze. **129:** 166-171.

[25] Ivanov V. B. (1973) . Growth and multiplication of cells in the root, pp. **7-42**. In Fiziologiya Rastenii, Moscow.