Chemical Control of Organ Formation in Root Segments of Convolvulus Cultured in Vitro[1, 2]

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Introduction

Plant parts with a plasticity of morphogenetic response have provided useful experimental material for study of the hormonal control of organ formation. Of particular value are cases in which alternate organ-forming potentials can be attributed to the same cell or group of cells. In the formation of endogenous buds and lateral roots from *Convolvulus arvensis* roots, both types of organs originate from a small number of pericycle cells at sites opposite the protoxylem poles. Several years ago, Torrey (14) reported that a clone of roots of this species had been established from the radicle of a seedling germinated in 1952, making possible controlled investigations of the physiology of organ formation in continuous root cultures.

The majority of physiological studies of bud and root formation in roots deals with thickened root cuttings. Organ formation in these cases is generally observed either in regions surrounding old lateral root traces or from callus tissue developing at cut surfaces. In either situation organ formation from root cuttings was found to be polar, with bud formation at the proximal[4] end and root formation or callus growth at the distal end. Plant (9), working with root cuttings of Crambe, Warmke and Warmke (17), with *Taraxacum* and *Cichorium* cuttings, Emery (4), with *Chamaenerion* cuttings, and Lindner (7), with *Cochlearia* cuttings, found that auxin application to the proximal end of the root cuttings resulted in inhibition of bud formation, promotion of root formation, and suppression of the polarity of organ formation. It was suggested that the polarity of organ formation in untreated cuttings resulted from differences in auxin concentration at the ends of the cuttings. Lower auxin concentrations at the proximal end promoted bud formation and higher concentrations at the distal end promoted root formation. Lowering the auxin level at the distal end should then result in bud formation at both ends. Stoughton and Plant (11) repeatedly excised 1-mm slices at 5-day intervals from both ends of Crambe cuttings to remove auxin accumulating at the distal end and to prevent new synthesis of auxin by buds which would otherwise form at the proximal end. After 8 weeks of this treatment, cuttings regenerated buds at both ends.

Warmke and Warmke (17) attempted to confirm the theory of auxin control over bud and root formation in *Cichorium* cuttings by extraction and bioassay of the endogenous auxin. Forty-eight hours after excision of the cuttings, they found that there was a somewhat greater amount of auxin activity in the neutral fraction extracted from the distal end than from the proximal end. By 96 hours there was more auxin activity in the free auxin fraction extracted from the distal end than from the proximal end.

An investigation of the chemical control of polar organ formation in *Convolvulus* root segments was undertaken to determine whether the same control mechanisms described for thickened root cuttings are operative in endogenous bud and root formation from the pericycle of cultured roots comprised of only primary tissues.

Materials and Methods

Stock cultures of *Convolvulus arvensis* roots were maintained in 125-ml Erlenmeyer flasks on a modified Bonner pea root medium (12) at an initial pH of 4.5. Root segments were cultured in the dark for 6 to 8 weeks, at which time the total length of the elongated lateral root was about 900 to 1300 mm. Each root was removed from the flask, placed in a dry, sterile petri plate, and cut into segments for use in the experiments. Segments were cultured either in Erlenmeyer flasks containing liquid medium or in petri plates containing medium solidified with 0.7 % agar. Root pieces which floated in liquid media could be cultured in stationary flasks. Successful culture of submerged pieces required constant agitation on a horizontal rotary shaker at 80 rpm.

For experiments in which either or both ends of segments were treated with growth substances, segments were placed vertically within a petri plate which contained solidified medium in both the bottom half and adhering to the top half. Ten 15-mm segments were placed upright in each petri plate, supported between the 2 layers of medium to a depth of 3 mm at each end.

Because conditions favoring organ formation may be markedly different from conditions favoring or-

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[4] The terms proximal and distal are defined with respect to the root apex; distal regions of the root axis are closer to the root tip than proximal regions.

gan elongation (13), segments were cleared by the method of Jacobs (6), so that all primordia could be examined. A dissecting microscope was used to record the number and position of buds and roots along the segment axis. The criteria for distinguishing buds and roots in cleared root segments have been discussed elsewhere.[5]

Stock solutions of all growth regulators were sterilized by Millipore filtration and added aseptically to autoclaved and partially cooled medium. Unless stated otherwise, segments were cultured in the presence of the growth regulators for the duration of the experiment. In no case was the growth regulator replenished during long-term culture. The growth regulators used in these experiments and the source from which they were obtained were as follows: indoleacetic acid (IAA), Nutritional Biochemicals Corporation; 2,3,5-triiodobenzoic acid (TIBA), Eastman Kodak Company; 6-phenylaminopurine (PAP), Mann Research Laboratories; kinetin (K), Cyclo Chemical Corporation; 6-benzylaminopurine (BAP), Shell Development Company.

Results

Organ Initiation in Intact Cultured Roots. Anatomical investigations of intact roots were conducted to determine the distribution of organ primordia along the root prior to segment excision and the location of any organs on the pathway to bud or root formation.[6] The study showed that in untreated intact roots organ initiation occurred in regions just proximal to the elongation zone. This observation was unexpected since the intact root axis showed no macroscopically visible organs for at least several hundred millimeters behind the tip. In sections derived from the apical 165 mm of the root axis, there were about 5 primordia in each 15-mm region of the root axis. Each primordium had developed to a stage in which no more than 3 radial rows of cells had formed by division of the pericycle and its derivatives. The presence of these small primordia in intact roots made it impossible to obtain segments which were without primordia at the outset of an experiment on organ formation.

Organ Formation in Untreated Segments. To obtain reliable results in experiments on polar phenomena, material should be used in which the disposition to form organs is the same along the axis. This was tested over the terminal 165 mm of the root, a region considerably distal to the first macroscopically visible organs. From each of 20 cultured roots a 15-mm tip portion was removed, and then ten 15-mm segments were excised. Corresponding segments, one from each root, were pooled and cultured in petri plates. After 6 weeks in culture, segments were cleared, and the number and position of organs were recorded. There was no striking change in either the number or position of roots or buds in segments within this region (table I). Root formation was always confined to the distal end of the excised segments. Because all segments excised from this portion of the root axis demonstrated the same capacity for polar organ formation, no special precautions were observed in subsequent experiments to distinguish segments with respect to their distance from the root apex.

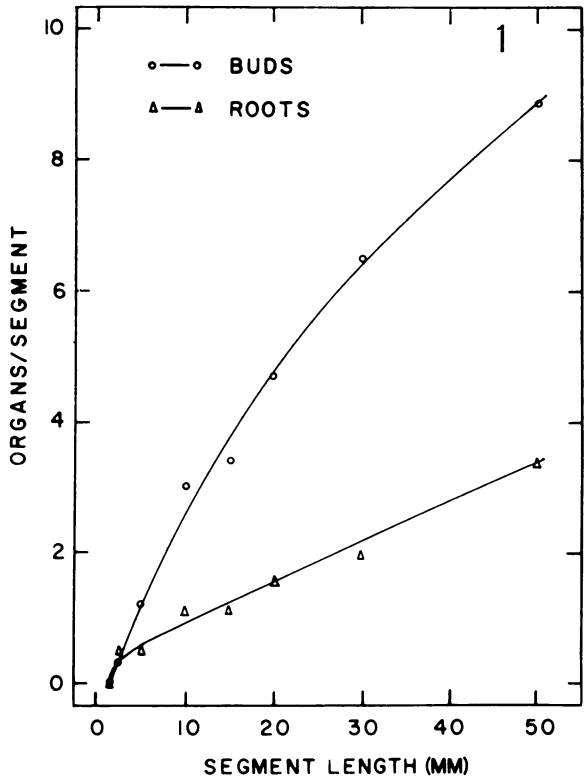
Investigations of the polarity and capacity for organ formation were extended to longer and shorter segments. After 6 weeks in culture, segments varying in length from 1.5 to 50 mm were cleared and the number and position of organs were recorded. The numbers of organs formed by these segments are shown in figure 1. Segments 1.5 mm long are practically incapable of organ formation. With increasing segment length, the number of buds increased, but the increase was not proportional to the

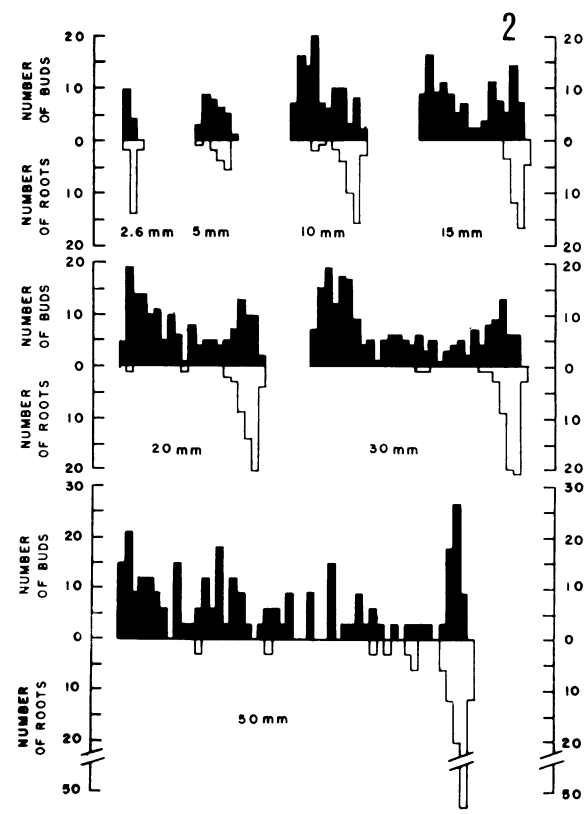
[5] Comparative Anatomy of Endogenous Bud and Lateral Root Formation in *Convolvulus arvensis* Roots Cultured in Vitro. Am. J. Botany. In press.
[6] Ibid.

Table I. *Organ Formation from 15-mm Segments Excised at Different Distances from the Root Apex*

Segment distance from root apex (mm)	Buds/ segment	Mean bud distance from proximal end (mm)	Roots/ segment	Mean root distance from distal end (mm)
15–30	4.2*	6.2 ± 5.1**	1.3*	2.7 ± 2.2**
30–45	4.9	6.4 ± 4.6	1.6	2.8 ± 3.0
45–60	4.5	6.9 ± 4.7	1.7	2.3 ± 1.6
60–75	4.8	7.2 ± 4.5	1.6	2.4 ± 2.6
75–90	5.6	7.6 ± 4.5	1.7	1.4 ± 0.8
90–105	4.7	7.3 ± 4.1	1.4	1.9 ± 2.0
105–120	4.5	7.2 ± 4.6	1.4	1.3 ± 0.7
120–135	5.4	7.1 ± 4.6	1.6	2.2 ± 3.2
135–150	4.7	6.3 ± 4.2	1.2	1.4 ± 0.7
150–165	3.9	5.7 ± 4.5	1.2	1.2 ± 0.7

* Average of 20 segments.
** Standard deviation from the mean.





increase in segment length. The number of roots increased in proportion to the increase in segment length.

The organ distributions observed in these segments are illustrated in figure 2. It is apparent that the polarity of root formation persists at all segment lengths tested. This result indicates that the sites of root formation along the primary root axis are determined by the position of the cuts made when excising the segment. Any portion of the axis capable of producing roots may be inhibited from this expression by its proximal position following segment excision. Bud formation, on the other hand, showed a different polar distribution. The average distance of buds from the proximal end increased proportionally with segment length, remaining about two-fifths of the way from the proximal end. A completely polar separation of organ types was never observed because at all segment lengths some buds developed near the distal end.

Experiments with Short Segments. Approximately 100 segments 1.5 mm long were excised from each cultured root and transferred to petri plates containing medium with various concentrations of IAA, or PAP, or both. About 25 segments were placed in each petri plate, with 3 or 4 plates in each treatment. After 6 weeks the segments were cleared, and the numbers of buds and roots were recorded. The results, expressed as the percent of organ formation, are shown in figure 3. The percent root formation is the total number of lateral roots divided by the total number of segments and multiplied by 100. Because some of the segments formed more than 1 root, the percent root formation is not equivalent to the percent of the segments which formed lateral roots. The same calculation was used for endogenous bud formation. In some treatments other buds appeared from a proliferation at the cut end of the segments. Because these buds are commonly observed to develop in longer segments in response to treatment with a cytokinin (14), they are referred to as kinin-induced buds. In many cases, a large number of these buds developed on 1 segment, so the percent formation of this bud type is the number of segments which formed these buds divided by the total number of segments and multiplied by 100.

FIG. 1. The influence of segment length on organ formation in root segments cultured for 6 weeks, cleared, and counted.

FIG. 2. The distribution of buds and roots in segments of different lengths cultured for 6 weeks, cleared, and counted. The proximal to distal direction runs from left to right. Each bar represents the total number of buds or roots recorded in 35 segments in each 1-mm interval. In 3 cases the data are based on fewer segments; 17 segments 2.6 mm long, 20 segments 15 mm long, and 12 segments 50 mm long. The number of organs in each millimeter portion in these cases was multiplied by the appropriate correction factor.

The results of figure 3 show that IAA alone was more effective in restoring the capacity of these short segments to form endogenous buds and roots than all combinations of IAA and PAP tested. The optimum concentration of IAA for root formation was 5×10^{-6} M; the optimum concentration of IAA for endogenous bud formation was 5×10^{-7} M. Root formation was inhibited by all concentrations of PAP tested and was totally suppressed at concentrations above 2.4×10^{-7} M. No combination of these growth substances significantly promoted endogenous bud formation in the absence of a concomitant promotion of root formation. There was no evidence for the control of root or bud formation by a critical balance of auxin and cytokinin. Although experimental separation of endogenous bud and root formation was not achieved by these treatments, promotion of lateral root formation occurred under different treatments than promotion of kinin-induced buds. High PAP concentrations coupled with little or no auxin were the most effective treatments in inducing these buds.

Effect of Cytokinins on Organ Formation in 15-mm Segments. Torrey (14) found that treatment of *Convolvulus* root segments with kinetin resulted in a proliferation from the distal cut surface and subsequent bud formation. Benzylaminopurine and phenylaminopurine were tested and found to have the same effect. Segments 15-mm long were cultured in liquid medium on a shaker. To facilitate distinguishing the ends of segments cultured in liquid medium, segments were cut so that the proximal cut surface was oblique and the distal cut surface was perpendicular to the long axis of the segment. At the end of 10 weeks the segments were cleared and the number of organs was counted. At 4.7×10^{-8} M PAP, there was a slight inhibition of lateral root formation but no effect on the number of buds. A concentration of 4.7×10^{-7} M PAP completely inhibited lateral root formation without inhibiting bud formation.

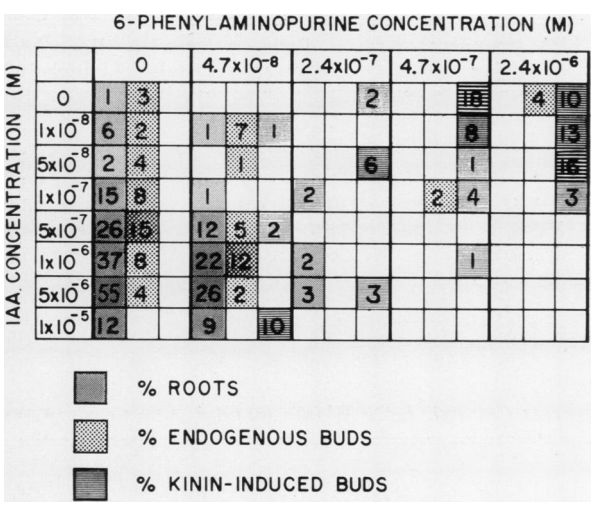


FIG. 3. Organ formation in 1.5-mm segments cultured for 6 weeks in various concentrations of IAA and 6-phenylaminopurine. The 3 columns under each of the PAP concentrations are referable to the legend at the bottom of the figure. The first column represents the percent root formation, the second column the percent endogenous buds, and the third column the percent kinin-induced buds.

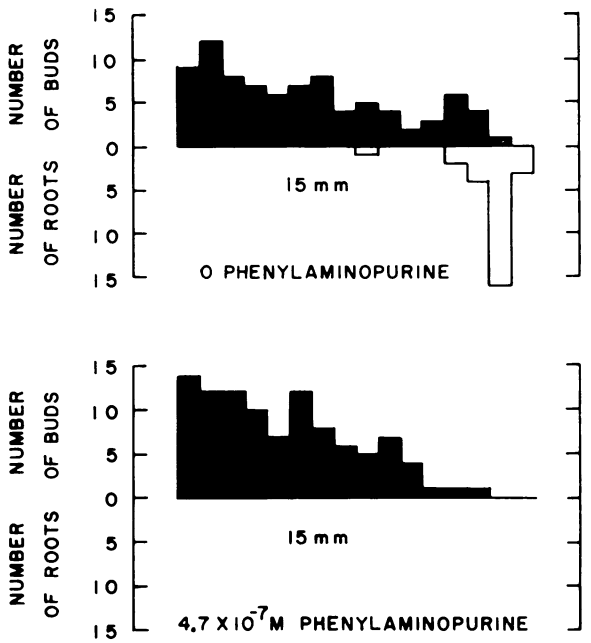


FIG. 4. The distribution of organs in 15-mm segments cultured in the presence or in the absence of 6-phenylaminopurine for 10 weeks, cleared, and counted. The proximal to distal direction runs from left to right. Each bar represents the total number of buds or roots recorded in 15 segments in each 1-mm interval.

Since concentrations of PAP can be found which will inhibit root formation without inhibiting bud formation, cytokinin treatment may cause primordia which would have developed into roots to develop into buds. Because of the strong polarity of root formation, the conversion of a large number of primordia into buds at the distal end would be readily apparent by a plot of the distribution of buds and roots. In figure 4 the distribution of buds and roots is shown for 15 segments treated with 4.7×10^{-7} M PAP and for 15 untreated segments. The distribution of buds and roots indicates that PAP acts to inhibit the formation of roots at the distal end of the segment without increasing the number of buds at the same end.

Treatment of 15-mm Segments with Increasing Concentrations of IAA. Segments were cultured on the shaker in liquid medium containing auxin in concentrations ranging from 10^{-8} to 10^{-5} M. At the end of 6 weeks the segments were cleared, and the number and position of organs were recorded. The

results are shown in table II. As the auxin concentration was increased, root formation was promoted and bud formation was progressively inhibited. At 10^{-5} M IAA, bud formation was completely suppressed; clearly the polarity of root formation was overcome as root formation occurred all along the segment axis.

TIBA Treatment of 15-mm Segments. Root segments were cultured in liquid medium on a shaker in various concentrations of TIBA. At the end of 6 weeks, the segments were cleared and the number of organs was determined. The polarity of distribution of organs could not be determined in this experiment because the segment ends were both cut perpendicularly to the root axis. Increasing TIBA concentrations progressively inhibited root formation without markedly inhibiting bud formation until, at a concentration of 10^{-5} M TIBA, root formation was reduced to an average of 0.2 roots per segment. Bud formation was only slightly affected (3.5 buds per segment).

If the only effect of TIBA was to prevent a local concentration of auxin from reaching a level high enough to promote the development of primordia into roots, it might be expected that the number of buds would rise as the number of roots declined. This result was not obtained in any of the TIBA treatments.

Treatment of 15-mm Segments with IAA during Portions of an 8-Week Span in Culture. In untreated 15-mm segments, development of all lateral roots to a stage recognizable in cleared root segments is nearly completed by the end of the first week in culture. Since auxin greatly promoted the number of roots, experiments were undertaken to determine when auxin application to segments was most effective. Segments were placed in liquid medium containing 10^{-5} M IAA and cultured on the shaker. After 1 day in culture, 14 segments were transferred to medium without auxin. After 2 days, 14 more segments were transferred to auxin-free medium. This was repeated at 3, 4, 5, and 6 days. Fourteen segments remained in IAA for the duration of the experiment, and 14 segments did not receive any auxin treatment. After the 8-week culture period, the segments were cleared and the number of organs was counted.

All auxin treatments promoted the number of roots per segment, but longer periods of auxin treatment were more effective than shorter periods (table III). Segments cultured continuously in auxin developed many more roots than any of the other treatments. Three of these segments are shown in figure 5. Bud formation was also strikingly promoted by treatments with auxin for several days. A 5-day auxin treatment resulted in 11.6 buds and 7.3

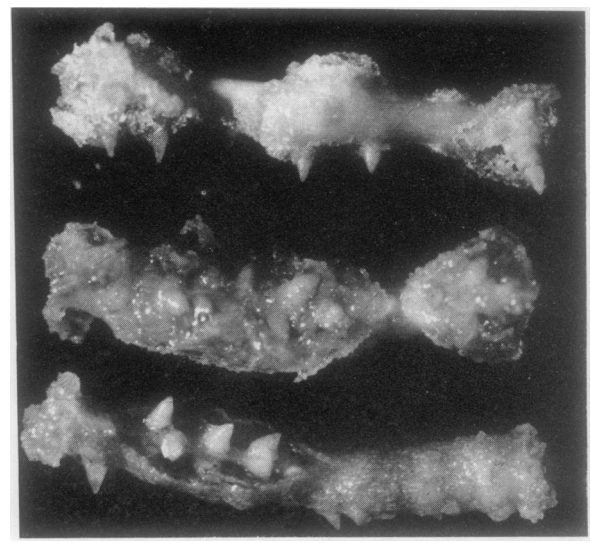


FIG. 5. Root segments 15 mm long cultured for 8 weeks in liquid medium containing 10^{-5} M IAA.

Table III. *Organ Formation in 15-mm Segments Treated with 10^{-5} M IAA during Portions of an 8-Week Span in Culture*

IAA treatment on day	Buds/ segment	Roots/ segment
No treatment	5.0 ± 2.2*	1.4 ± 0.7*
1	5.9 ± 3.1	2.2 ± 1.4
1, 2	8.5 ± 4.1	4.4 ± 2.3
1, 2, 3	7.6 ± 3.8	5.9 ± 3.9
1, 2, 3, 4	10.8 ± 4.2	5.7 ± 3.8
1, 2, 3, 4, 5	11.6 ± 4.4	7.3 ± 2.4
1, 2, 3, 4, 5, 6	8.0 ± 2.7	4.6 ± 1.3
1–56	0	16.8 ± 8.4

* Standard deviation of the mean. There were 14 segments in each treatment.

Table II. *Organ Formation in 15-mm Segments Cultured for 6 Weeks in IAA*

IAA conc (M)	Buds/ segment	Mean Bud distance from proximal end (mm)	Roots/ segment	Mean root distance from distal end (mm)
0	5.5 ± 2.2*	5.6 ± 5.1*	1.2 ± 0.4	1.4 ± 1.2
10^{-8}	4.6 ± 1.3	4.5 ± 5.3	1.3 ± 0.6	1.1 ± 0.6
10^{-7}	6.0 ± 3.2	6.4 ± 4.5	2.7 ± 1.0	2.8 ± 3.5
10^{-6}	1.1 ± 1.5	4.6 ± 3.1	8.1 ± 3.6	5.3 ± 4.6
10^{-5}	0	...	14.7 ± 5.8	7.2 ± 4.4

* Standard deviation of the mean. There were 15 segments in each treatment.

roots per segment, whereas in segments treated with auxin throughout the duration of the experiment, no buds and many more roots were formed. The maximum number of buds recorded in 1 segment was 22 for a 5-day treatment with IAA; in 1 segment cultured continuously in IAA, the maximum number of roots recorded was 30. These results show that to obtain a large number of roots, auxin treatment must be protracted. If auxin is withdrawn after a few days, a promotion of both buds and roots is obtained. No experiments were conducted in which segments were treated with auxin for periods longer than 6 days but shorter than 56 days.

Application of Growth Regulators to the Ends of 15-mm Segments. Petri plates were prepared so that root segments could be treated with various concentrations of IAA, BAP, or kinetin at either end. Ten 15-mm segments were excised from each root and placed upright in a petri plate so that their distal ends were always towards the bottom of the petri plate and down with respect to gravity. Experiments with untreated segments placed horizontally or with their distal ends up, or down, established that there was no significant effect of gravity on either the number or the distribution of endogenous buds and roots. After 6 weeks of culture in the dark, segments were cleared, and the number and position of organs were determined. The results are shown in table IV. Cytokinin treatment of the proximal end of segments was relatively ineffective in inhibiting root formation; cytokinin treatment of the distal end was as effective in inhibiting root formation as cytokinin treatment of the entire segment. As an inhibitor of lateral root formation, BAP showed more mobility, or more activity, or both, than kinetin. When segments were treated with auxin alone, a slight promotion of the number of roots was observed. The magnitude of the promotion was independent of the direction of auxin application. Auxin applied to the distal end did not influence the distribution of lateral roots whereas auxin applied to the proximal end slightly shifted the mean position of roots towards the proximal end.

When IAA and a cytokinin were supplied in combination, quite different results were obtained. Kinetin treatment of the proximal end of segments treated at the distal end with IAA did not alter the lateral root-forming response to the auxin treatment. Kinetin treatment of the distal end of segments treated at the same end with IAA suppressed nearly all lateral root formation. Neither of these treatments affected the locus of lateral root formation. When root formation at the distal end was inhibited by cytokinin (BAP) treatment and auxin was added to the proximal end, the inhibitory effect of BAP on lateral root formation could be fully reversed to control levels, but the site of lateral root formation was altered. The average distance of lateral roots from the distal end was progressively shifted towards the proximal end as a result of increasing auxin concentrations applied at the proximal end and increasing BAP concentrations applied to the distal end. By a treatment of 10^{-5} M auxin in combination with 1.0 mg/liter BAP, the number of lateral roots per segment (1.0) was nearly the same as the number in untreated segments (1.2); however, the average dis-

Table IV. *Effect of Cytokinin and Auxin Treatment on Lateral Root Formation in 15-mm Segments Cultured for 6 Weeks*

Treatment at proximal end	Treatment at distal end	Roots/ segment	Mean root distance from distal end (mm)
None	None	1.2	1.1 ± 0.3*
0.1 mg/liter K	None	1.3	1.5 ± 1.2
1.0 mg/liter K	None	1.0	1.5 ± 0.7
0.1 mg/liter BAP	None	1.0	1.4 ± 0.6
1.0 mg/liter BAP	None	0.6	1.0 ± 0.6
None	0.1 mg/liter K	0.7	2.0 ± 0.6
None	1.0 mg/liter K	0.0	
None	0.1 mg/liter BAP	0.1	1.5
None	1.0 mg/liter BAP	0.0	
None	10^{-6} M IAA	1.8	1.5 ± 2.1
None	10^{-5} M IAA	2.0	1.5 ± 1.0
10^{-6} M IAA	None	1.6	2.3 ± 3.2
10^{-5} M IAA	None	2.1	2.8 ± 3.6
1.0 mg/liter K	10^{-5} M IAA	2.5	1.1 ± 0.3
None	10^{-5} M IAA + 1.0 mg/liter BAP	0.2	1.5 ± 0.6
10^{-6} M IAA	0.1 mg/liter BAP	0.9	2.7 ± 3.6
10^{-5} M IAA	0.1 mg/liter BAP	1.9	4.4 ± 4.8
10^{-5} M IAA	1.0 mg/liter BAP	1.0	9.6 ± 3.6

* Standard deviation of the mean. There were 10 segments in each treatment.

tance of lateral roots from the distal end was shifted from 1.1 mm in the latter case to 9.6 mm in the former case.

In the experiment in which the ends of segments were treated with growth substances, all the differences in the number and distribution of buds were small and inconsistent in various treatments. The failure to achieve any control over bud formation by these treatments may possibly be attributed to the difference in the rate of development of endogenous buds and roots. In these experiments segments were left in the treatments for the duration of the experiment, but it is unlikely that the hormonal levels within the segments remained constant long enough to significantly influence bud formation.

Discussion

Two approaches to studying the chemical control of organ formation have been applied. The first of these involved the use of segments which were short enough so that the polarity of the tissue was of little importance and the endogenous hormone levels were too low for consistent organ formation. The second approach involved the treatment of longer segments with growth regulators at concentrations high enough either to overwhelm the endogenous hormonal regulation and thereby to inhibit or accentuate the normal response, or to effect a morphogenetic response which is identical with the response controlled endogenously, but which occurs at a different locus along the root axis. These approaches provide an indication of the endogenous control over organ formation to the extent that a full measure of control over the morphogenetic response is obtained.

Untreated root segments, 1.5 mm long, were almost entirely incapable of organ formation. Segments 2.6 mm long formed, on the average, nearly 1 organ per segment. Other workers have similarly noted a critical size for organ formation in root cuttings. Dore and Williams (2) studied formation of roots and buds from horseradish root cuttings. These organs appear from the region surrounding old lateral root traces. Only a few primordia and 1 bud developed from the many plugs of tissue, 1.24 mm in diameter and 2 mm deep, which were bored out of thickened roots so that they each included 1 old root trace. Slices 1 mm thick of roots several millimeters in diameter developed both roots and buds. Warmke and Warmke (17) reported that dandelion root cuttings would not regenerate if the diameter was less than 1.25 mm or the length less than 6 to 10 mm.

To restore bud and root formation in 1.5-mm *Convolvulus* root segments, addition of auxin was the only requirement. Phenylaminopurine inhibited the promotion by auxin of endogenous organ formation but induced production of buds from the cut surface of the segments.

In an approach similar to that of working with short segments, hormonal interactions in organ formation have been investigated in callus tissue. In many cases organization must also be induced by addition or withdrawal of growth regulators. It was with such a system that Skoog and Miller (10) showed that both a cytokinin and an auxin were required for organ formation, and the relative concentrations of the 2 hormones determined whether buds or roots developed. In cell colonies derived from cell suspensions of *Convolvulus* root callus, Earle and Torrey (3) were able to induce bud formation predictably with balanced concentrations of auxin and cytokinin in a synthetic medium. Although in a few instances roots were induced in these cultures, no predictable control of root initiation was achieved. In 1.5-mm *Convolvulus* root segments, control of endogenous bud and root formation by different interacting concentrations of IAA and PAP was not achieved. The formation of buds from the cut end of segments was more closely analagous to the response of tobacco callus tissue than was the formation of endogenous buds. Exogenously supplied cytokinins were not required for endogenous bud or root formation.

The simplest explanation for the difference between the distal 1.5 mm of a 15-mm segment, which nearly always is a site of lateral root formation, and the excised 1.5-mm segment is the presence of sufficient auxin to cause lateral root formation in the distal 1.5-mm portion of the longer segments. This high auxin level must be due to polar movement of auxin from the proximal region of the segment to the distal end. Experiments using C^{14}-IAA have already confirmed the existence of polar auxin transport in *Convolvulus* root segments (1). Addition of exogenous auxin would be expected to raise the level of auxin along the entire segment axis and result in a marked promotion of root formation, coupled with a suppression of the polar distribution. Such a response was obtained when auxin at a concentration of 10^{-5} M was supplied.

The only significant promotion of bud formation observed in 15-mm segments was in response to auxin treatment. Treatment of segments for several days in auxin at a concentration of 10^{-5} M, followed by removal of the segments to medium lacking auxin, led to a significant promotion of bud formation. When segments were allowed to remain in auxin instead of transferring them to medium without auxin, only roots developed. These responses may be due to the initiation of a large number of primordia by auxin, which can then develop into either buds or roots depending on subsequent auxin levels.

Whether organ formation in untreated segments represents continued development of preexisting primordia or initiation and development of new primordia, the act of excision resulted in differences of organ-forming capacity in regions which had been adjacent to each other prior to excision. Roots developed only at the distal end of segments. Although buds, which develop more slowly, were most often found near the proximal end, they also frequently ap-

peared near the distal end. This distribution of organ formation requires that the chemical environment at organ-forming sites be similar enough to bring about bud formation at both ends of excised segments yet dissimilar enough to cause restriction of root formation to 1 end only. An explanation for this distribution is suggested by the results of table III. If the development of primordia into roots reduces the ambient auxin concentration at the distal end of a segment to a level below that required for root determination, then other more slowly developing primordia would develop into buds. A similar explanation could be invoked for the development of primordia into buds instead of roots, which was found in the regions of the intact root nearer the root apex.

Polar lateral root formation in untreated segments is believed to result from acropetal polar movement and accumulation of auxin at the distal end. Polar transport of IAA has been shown to be inhibited by TIBA. Niedergang-Kamien and Skoog (8) found that TIBA at a concentration of 4×10^{-5} M prevented accumulation of endogenous free auxin at the basal end of excised tobacco stem segments. The restriction of bud formation to the basal end was also altered by TIBA, such that the region of most active bud formation shifted from the basal end towards the apical end as the concentration of TIBA was increased. Recently Hertel and Leopold (5) showed that 2×10^{-5} M TIBA inhibited polar transport of exogenously supplied C^{14}-IAA in corn coleoptiles. Similar concentrations of TIBA also inhibited lateral root formation in *Convolvulus* root segments. If root formation depends on an accumulation of auxin at the distal end, an inhibition of polar transport by increasing concentrations of TIBA would not decrease the polar distribution of root formation but, rather, suppress root formation. This result was obtained as the concentration of TIBA was increased from 2×10^{-7} to 2×10^{-5} M.

Further evidence for the polar movement of auxin and its role in root formation was obtained by auxin application to the ends of root segments. By manipulating the direction of application and the concentration of cytokinin (BAP or K) and auxin (IAA), it was possible to inhibit, promote, or alter the number and position of lateral roots. A segment could be cultured such that a root would develop near the proximal end of the segment and buds would develop in regions distal to the root. Such a segment, with regard to organ formation, exhibits a polarity opposite to that of an untreated segment. The polarity of the segment was not changed, but rather the gradients of organ-forming factors resulting from the polarity of the segment were experimentally modified by auxin and kinin applications. Alterations of the polarity of organ formation in root cuttings have also been reported by Warmke and Warmke (17).

Interactions of applied auxin and kinin resulted in root formation in the middle of segments, with regions of the segment axis in both the proximal and distal direction free from lateral roots. In intact roots, which develop lateral roots at specific sites along the root axis, localization of root formation to these sites, as in the case of root segments, may be the result of a balance of inhibiting and promoting factors. Torrey (13, 15, 16) developed the concept of control of lateral root formation in pea root segments by an interaction of several substances. Lateral root formation was found to be influenced by auxin, thiamin, nicotinic acid, naturally occurring inhibitors, micronutrient elements, adenine, and kinetin. He suggested that localized lateral root formation in the intact root could be brought about by interactions of these factors, with the root tip acting as a site of cytokinin production.

Gradients of interacting factors may be invoked to explain localization of lateral root formation to certain regions along the longitudinal axis, but these gradients do not readily explain either the localization of root formation to pericycle cells opposite protoxylem points, i.e., the radial localization, or the limitation of primordia to 1 or 2 of the poles at any transverse level. Although the number of protoxylem poles varies from 3 to 6 in cultured *Convolvulus* roots, no more than 2 or 3 primordia at a given level have been observed in sections of untreated segments.

Treatment of *Convolvulus* root segments with auxin resulted in promotion of lateral roots in 1.5-mm and 15-mm segments, in promotion of bud formation, in inhibition of bud formation, and in alteration of the site of lateral root formation. The interpretation of these results can best be predicated on the existence of an acropetal transport of auxin.

Summary

Isolated root segments of *Convolvulus arvensis* are capable of developing into plants by forming both endogenous buds and roots. Root formation in untreated segments was limited to the distal end of the segments, whereas formation of buds, although tending toward the proximal end, occurred anywhere along the segment axis. The contrasting behavior of the ends of root segments with regard to organ formation was interpreted as the result of differing hormonal regimes at organ-forming sites along the length of the segment. Several different types of experiments indicated that movement of auxin from the proximal to the distal end of segments might underlie polar root formation. This hypothesis was supported by the following observations: A) the position of lateral roots in segments was not altered by auxin application to the distal end of segments, whereas the position was influenced by auxin application to the proximal end, particularly in conjunction with cytokinin application to the distal end: B) lateral root formation was inhibited by 10^{-5} M triiodobenzoic acid, an inhibitor of polar transport of auxin; C) short root segments, about 1.5 mm long, rarely formed lateral roots in the unsupplemented culture medium. They required only the addition of auxin to this medium to form lateral roots.

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