ENDOGENOUS BUD AND ROOT FORMATION BY ISOLATED ROOTS OF CONVOLVULUS GROWN IN VITRO[1,2]

JOHN G. TORREY

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

Among the most successful weed species are numbered those which propagate themselves vegetatively by forming buds from underground tissues, especially root systems. Cultivation of the soil serves to break the root into numerous pieces, each capable of forming a new plant. Notable among the weeds of this type are members of the family Convolvulaceae, e.g., bindweed, *Convolvulus arvensis*, members of the Compositae such as the Canada thistle, *Cirsium arvense*, members of the Polygonaceae, e.g., sorrel, *Rumex acetosa* and many others. Bud formation by roots has been studied extensively from the morphological and anatomical standpoint, but little information is available concerning the physiological processes. In an extensive monograph on the subject, Beijerinck (1) described a large number of species in which bud formation by roots is the usual method of propagation. According to Priestley and Swingle (7) at least three types of bud formation by roots may occur. In the 1st type, bud formation is preceded by the formation of a callus tissue from cortical parenchyma or from deeper-lying tissues of the root and from this callus tissue mass are differentiated bud primordia. In the 2nd type, bud primordia develop from wound callus tissue which proliferates from the cut ends or damaged surfaces of the roots. Such buds may arise exogenously or have endogenous origin. The 3rd type of bud formation by roots involves the initiation of bud primordia within the deep-seated tissues of the root, i.e., from the pericycle. Such endogenous bud formation is closely comparable to endogenous lateral root initiation, occurring in pericycle cells usually located at the protoxylem points of the vascular cylinder.

Because of the importance of bud formation by root systems in the general problem of weed control and because of the fundamental problems involved in the determination of differentiating meristems, it was considered desirable to establish isolated root systems in culture as clonal materials to be used in studying the problem under controlled conditions.

Bud formation by isolated roots grown in vitro has been reported occasionally in the literature. White (14) illustrated bud formation from a callus formed on an isolated root segment of dandelion. This type of bud formation represents the 2nd type described above. Norton and Boll (6) also reported briefly on an isolated root system of a hybrid tomato which occasionally formed buds, again apparently after formation of a callus by the root. Bud formation by isolated root callus tissue grown in vitro is well known (5). Recently, Seeliger (8) illustrated cultured roots of *Robinia pseudoacacia* L., a woody species, whose roots produce endogenous buds in vitro.

In the present report is described the establishment in continuous culture of isolated roots of the common bindweed, *Convolvulus arvensis* L., which are capable of forming true endogenous buds in culture.

MATERIALS AND METHODS

Seed material was collected from the field in the late summer of 1952 in Northern California from the wild species, *Convolvulus arvensis* L. Seeds were surface sterilized by soaking for 15 minutes in a commercial preparation of sodium hypochlorite diluted to a final concentration of approximately 0.5 % by weight. Seeds were transferred aseptically to moistened sterile filter paper in Petri dishes and were allowed to germinate in the dark at 25° C for three days. At this time, 5-mm root tips were excised and transferred, one tip per flask, to 50 ml of liquid nutrient medium in 125-ml Erlenmeyer flasks. The flasks were placed in the dark at 25° C. Of the large percentage of roots which grew, one root showing particularly robust growth was selected for clonal propagation. All of the studies subsequently have been made with the same root clone which was first established in November 1952. The clonal material has been maintained in the dark, except for brief periods of fluorescent illumination during transfer, with continuous subculture approximately every four months. In certain experiments, roots in flasks were grown under fluorescent lights at approximately 100 and 200 ft-c at 25° C.

The medium used throughout these studies was based on the modified Bonner medium developed for the growth of pea roots (11). The medium contains the following constituents: 242 mg $Ca(NO_3)_2 \cdot 4\,H_2O$; 42 mg $MgSO_4 \cdot 7\,H_2O$; 85 mg KNO_3; 61 mg KCl; 20 mg KH_2PO_4; 1.5 mg $FeCl_3$; 40 g sucrose made up to one liter with glass distilled water. Usually the vitamins thiamin HCl at 0.1 mg/l and nicotinic acid at 0.5 mg/l were also added. In addition, trace elements were added as follows: 1.5 mg $ZnSO_4$; 4.5 mg $MnSO_4$; 0.25 mg $Na_2MoO_4 \cdot 2\,H_2O$; 1.5 mg H_3BO_3 and 0.04 mg $CuSO_4 \cdot 5\,H_2O$ per liter of solution. The pH of the medium was adjusted to give a final pH of 5.0 after sterilization. The complete medium was sterilized by autoclaving at 15 lbs/in.^{2} for 20 minutes. Flasks were capped over gauze-covered cotton plugs with unwaxed paper cups during the culture periods to decrease the rate of evaporation and protect against dust.

[1] Received March 1, 1958.

[2] This investigation was supported in part by grant number RG 2861 from the National Institutes of Health, Public Health Service. The author is indebted to Mr. Victor Duran for his photographic work.

Experimental Results

Establishment of the Clone and the Morphology of the Root in Culture: Isolated roots of Convolvulus grown in the medium described above show a marked apical dominance, which expresses itself in two ways—by the rapid elongation of the main axis tip and by an almost complete suppression of lateral root formation. Thus an isolated root tip excised from the radicle of a germinating seed elongates very rapidly. In the root tip used to establish the clone, the average elongation rate was 24 mm per day for the 1st three weeks. Upon transfer to fresh medium, the excised 10-mm tip continued to elongate at an average rate of about 25 mm per day. Lateral root formation was relatively infrequent. Such primary tips have been carried without transfer for up to 60 days in the same flask without any marked diminution in the elongation rate.

Establishment of a clone, however, must not depend upon propagation of the main radicle tip, but upon development of roots from segments cut from the radicle. It is with such segments that the experiments to be described are concerned. In establishing the clone, an excised primary root tip which had grown to a length of 480 mm by the 3rd week was used. The root was cut into 10-mm segments and each segment was transferred to fresh medium. In all experiments segments were chosen which did not possess macroscopically visible primordia, although the presence of either lateral roots or buds on the initial segment did not significantly change the course of development.

It is convenient to describe the sequence of events during the development of a root segment after its transfer to fresh medium continued in the dark. By the end of the 2nd week usually one lateral root has developed from the distal half of the root segment (i.e., distal with respect to the root from which it was derived). Occasionally a 2nd lateral root may be initiated, but it seldom elongates. The 1st lateral root to develop elongates slowly at first, then more rapidly, increasing in diameter until it achieves approximately the diameter of the segment from which it came; this diameter is maintained for the remainder of its elongation before sub-culture. The rate of elongation of lateral roots in the dark is lower than that for primary root tips, averaging between 10 and 20 mm per day over prolonged culture periods once the lateral root begins elongation. Usually concurrent with the initiation of the lateral root which will become the main root axis of the new culture, there are initiated on the inoculum segment one or more buds which at one week are just evident as swellings along the root segment and at two weeks are clearly evident morphologically as buds. By three weeks the buds may enlarge to 3 to 5 mm. Their distribution along the root segment is not fixed; they may occur near the lateral root or at the other end of the segment. Usually they occur proximal to the site of lateral root initiation (fig 1). Thus each root segment shows distinct polarity and becomes, in fact, a complete plant and no longer can be considered in the same category as isolated root segments, such as those of tomato, which in culture normally form only more root structure. Under these conditions in the dark, the bud on the initial root segment seldom elongates beyond 5 to 10 mm (table I). After the lateral root segment has reached the length of 500 mm or longer, endogenous buds may also be formed on the basal portions of the lateral root. In fig 2 is illustrated in flask culture a root started from a 10-mm segment after growing 20 weeks in the dark. In figure 3 is shown the extent of such a root after 20 weeks without transfer.

All of the buds formed by the root under these conditions are true endogenous buds, originating deep within the tissues of the root in the pericycle, usually opposite the protoxylem points of the primary vascular tissues of the main root axis (fig 4).

Development of 10-mm Root Segments in the Dark and in the Light: In table I are summarized the results of two experiments in which 10-mm root segments were grown in the dark on the control medium. These data illustrate quantitatively the se-

Table I

Development of Lateral Roots and Endogenous Buds by Convolvulus Root Segments Grown in Control Medium in the Dark and in the Light at 25° C *

	Second week		Fourth week		Seventh week		Fourteenth week	
	Dark	Light **	Dark	Light	Dark	Light	Dark	Light
Average number of lateral roots per segment	0.3	0.4	0.9	1.0	1.2	1.0	1.2	1.4
Average length of laterals per root in mm	1	5	35	128	244	181	1128	1207
Average number of buds per segment	0	0	0.5	1.0	2.0	1.0	2.0	1.0
Average bud length in mm	...	...	4	10	5	92	5	270

* Each figure is based on the average of 2 experiments each composed of 8 to 12 root segments.
** Illumination was about 100 ft-c from warm white fluorescent tubes on a 12-hr light-dark cycle.

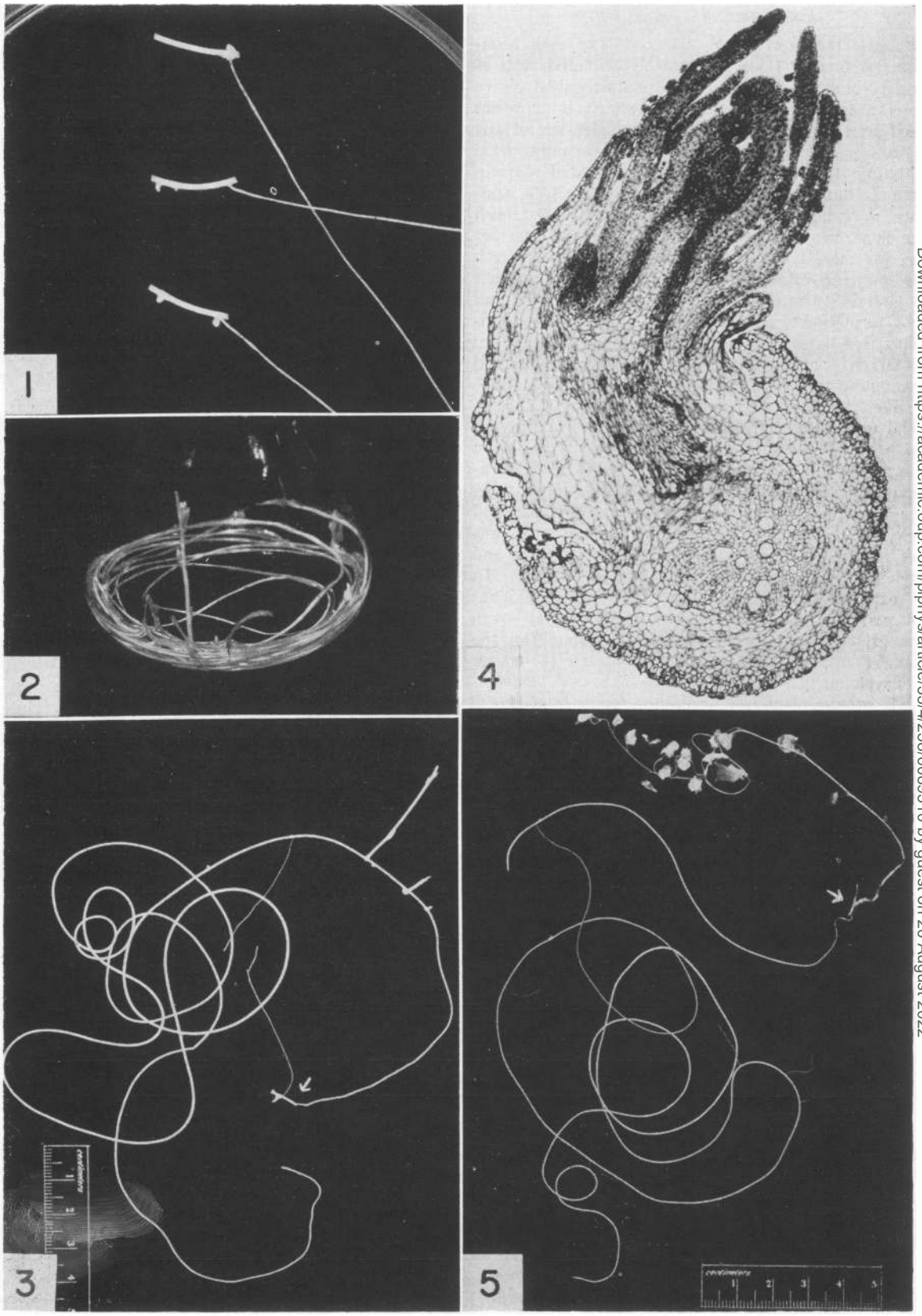

TABLE II

EFFECT OF KINETIN ON LATERAL ROOT AND BUD DEVELOPMENT IN CONVOLVULUS ROOT SEGMENTS GROWN IN THE DARK AND LIGHT AT 25° C*

CONCENTRATION OF KINETIN (MG/L)		AV NO. OF LATS/SEGT		AV LENGTH OF LATS (MM)		AV NO. OF BUDS/SEGT		% OF SEGTS WITH TERMINAL BUDS	
		WEEK		WEEK		WEEK		WEEK	
		7	20	7	20	7	20	7	20
0	Dark	1.3	1.4	198	1025	1.5	2.8	0	0
0	Light**	1.1	1.3	116	160	1.6	1.7	0	0
0.01	Dark	1.0	1.5	218	1252	0.6	2.2	0	11
0.01	Light	1.0	1.0	111	400	1.1	1.4	50	72
0.1	Dark	1.3	1.4	297	1591	0.1	2.2	11	50
0.1	Light	0.7	0.7	20	21	1.4	3.0	90	100

* Each figure is based on the average of 12 to 15 root segments.
** Illumination was about 200 ft-c from mixed warm white and daylight fluorescent tubes on a 12-hr light-dark cycle.

quence of events described above. It should be noted that lateral roots become apparent first and bud primordia develop shortly thereafter.

A parallel series of root segments grown in the control medium was illuminated with mixed warm-white and daylight fluorescent lights at approximately 100 ft-c. From a study of the data in table I it becomes apparent that, at these low light intensities, root development is similar in most respects to that in dark-grown roots. Whether in the light or dark, each segment usually produces only one lateral root and one or two buds. Lateral root elongation, while initially slightly accelerated in the light, later is much the same under the two conditions. The major difference in behavior is evident in the response of the buds, which elongate in the light, forming many leaves while buds in the dark usually are completely inhibited or elongate only slightly and develop no expanded leaves. In figure 5 is illustrated a plant after 20 weeks in alternating 12-hour periods of dark and light, showing bud elongation and numerous leaves. At somewhat higher light intensities (see table II), it has been found that lateral root elongation can be markedly inhibited, as has been observed in cultured pea roots (10). Comparison of figure 3 with figure 5 shows that roots grown in continuous dark achieve a larger root diameter than roots exposed to periods of light.

It is difficult from the experiments carried out to date to sort out any consistent correlation phenomena among bud and root initiation or elongation other than the fact that lateral root initiation precedes bud initiation under these conditions of culture and that organ formation by the root segments appears to be rather specifically limited.

EFFECTS OF GROWTH FACTORS ON BUD FORMATION IN THE LIGHT AND DARK: A number of experiments have been performed in which specific growth factors which might affect organ formation in these root segments have been added separately or together to the control medium. Indoleacetic acid (IAA) in concentrations between 10^{-8} M and 10^{-12} M was found to have essentially no effect on organ formation in roots grown in the dark. IAA at 10^{-6} M, while having no effect on root segments cultured in the light, produced extensive swelling and disruption of the cortical tissues at both ends of the root segments in the dark. At this concentration, all organ formation was suppressed by IAA in dark-grown roots. Adenine sulfate, when tested on roots grown in the dark over a range of concentrations between 0.4 and 40 mg/l, was found to have no evident effect on organ initi-

FIG. 1. Root segments of Convolvulus grown on agar medium in the dark for 7 weeks, each showing the development of a lateral root and one or more endogenous buds. ×1.2.

FIG. 2. Ten-mm root segment of Convolvulus grown in liquid medium in the dark for 20 weeks. Total length of lateral root was about 1600 mm. Note several elongate etiolated buds produced along the length of the lateral root. ×0.8.

FIG. 3. Root segment of Convolvulus grown in liquid medium in the dark for 20 weeks, showing the extent of root development. Original 10-mm segment (indicated by arrow) formed 2 lateral roots and 2 buds. Total length of lateral roots was 1130 mm; 5 buds were formed by the lateral root. ×0.6.

FIG. 4. Transverse section of root segment of Convolvulus grown in liquid medium in the dark for 20 weeks, showing the endogenous origin fom the pericycle opposite one of the primary xylem points, of a bud which has been cut longitudinally. ×70.

FIG. 5. Root segment of Convolvulus in liquid medium in alternating 12-hr periods of dark and light of 100 ft-c from white fluorescent tubes. Note the development of the shoot system from the original 10-mm segment (indicated by arrow). Compare the diameter of the lateral root with that in fig. 3. ×0.6.

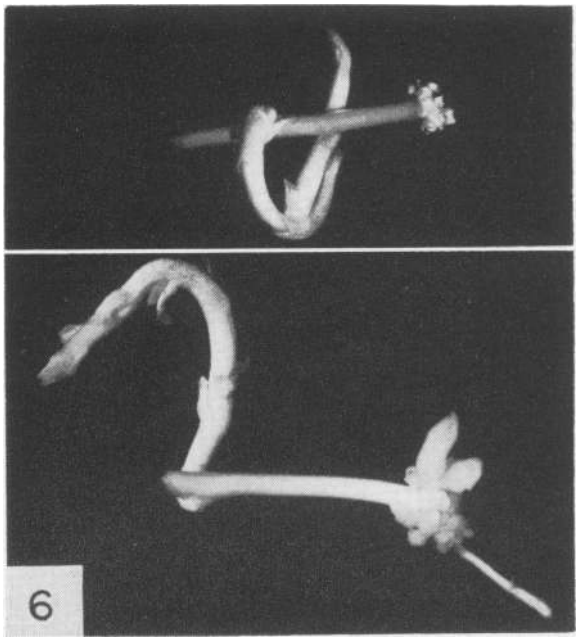

FIG. 6. Bud formation of segments of Convolvulus root grown for 8 weeks in liquid medium containing kinetin under alternating 12-hr periods of dark and 100 ft-c white fluorescent light. Top, 0.01 mg/l kinetin; bottom, 0.1 mg/l kinetin. ×3.5.

ation. The following substances, tested because of their physiological activity in a variety of plant tissue systems, were also found to have no effect on organ initiation at the concentrations tested: Difco Bacto yeast extract, casein hydrolysate (enzymatic), pyridoxine HCl, calcium pantothenate, choline HCl, folic acid, *p*-aminobenzoic acid, inositol, riboflavin, biotin, glycine, and 2,4-dichlorophenoxyacetic acid.

A positive controlling influence on organ initiation was found when kinetin (6-furfurylaminopurine) was tested. In table II are summarized the results of a typical experiment in which kinetin was added to the control medium at 0.01 and 0.1 mg/l and the roots were grown both in continuous darkness and in 12-hour periods of alternating dark and light at 200 ft-c. In the medium lacking kinetin, development was similar to that described in table I, except that at the higher light intensity lateral root elongation was markedly inhibited. This inhibition of root elongation was accentuated by the presence of 0.1 mg/l kinetin, which in the dark appears to stimulate root elongation. Root segments grown in the presence of 1.0 mg/l kinetin turned brown, whether in the light or dark, due apparently to toxicity.

At the concentrations shown in table I, kinetin does not significantly affect the number of lateral roots initiated either in the light or the dark. In dark-grown root segments, kinetin slows the development of buds but appears not to affect significantly the number of buds ultimately formed by the segments.

By far the most striking effect of kinetin on these root segments is a stimulus to the formation of bud primordia at the distal end of each root segment. These structures, which appear to be incompletely organized bud primordia, occur only at the cut surface of the root-end of the root segment. Their size and number are dependent on the concentration of kinetin and whether the root is grown in the light or the dark. In figure 6 are illustrated root segments grown for 8 weeks in the light in the presence of two concentrations of kinetin. At 0.01 mg/l kinetin, the distal end of the root segments appear swollen as if with a callus formation, but distinct structures are evident. At the higher concentration of kinetin, the bud primordia are larger in size and more numerous. An accurate count of these structures is difficult to make except on histological preparations. Some indication of the effectiveness of kinetin in the formation of these structures is given in table II by the percentage of segments which show this characteristic response to kinetin treatment. The greatest response occurs in roots cultured in the light, but with time a proportion of segments grown in the dark also responds. Careful histological analyses of these structures will be necessary before any relationship between them and normal endogenous bud formation can be established.

DISCUSSION

The capacity for endogenous bud formation is a distinctive character of a relatively few groups of plants and involves a mechanism about which we have very little physiological knowledge. In the present experiments, the inherent capacity of Convolvulus roots to form endogenous buds has been retained by isolated roots through successive sub-cultures for several years apparently without signs of diminution. Thus, under these conditions of culture, the root itself is capable of providing the metabolites and cell division factors which are essential for the initiation, organization and partial development of the buds. Light is required for the complete development of the shoot system. It is probable that bud initiation, like lateral root initiation (12), is dependent upon a number of specific chemical factors formed by the root in culture. The present experiments suggest that some factor of the kinin-type may be involved in bud initiation by Convolvulus roots. It is interesting that kinetin is much more active when roots are grown in the light than in the dark. This result suggests the possibility of an interaction of factors such as has been reported by Skoog and Miller (9) in tobacco pith tissue.

Danckwardt-Lillieström (2) has recently shown that isolated roots of *Isatis tinctoria*, which in first passage normally regenerate shoots from callus formed at the cut surface, lose this capacity on repeated sub-culture. If kinetin is supplied to roots in these later passages, shoot initiation takes place. In this case, it would seem that the root is depleted of a

kinetin-like factor during subculture which must then be replenished via the nutrient medium.

In studies with the thickened roots of Taraxacum and Cichorium, Warmke and Warmke (13) reported that auxin distribution within root cuttings seemed to control the capacity of the root tissues for organ formation; roots were initiated at the morphologically distal end of the root segments which were relatively high in auxin content, whereas treatment which reduced the total auxin content of the segments resulted in leaf initiation from the distal ends in a few cases. Emery (4) found that IAA treatment of root cuttings of the fireweed, *Chamaenerion angustifolium*, resulted in the initiation of large numbers of lateral roots, but inhibited bud initiation. Other studies with thickened root segments show a similar inverse relation between auxin level and bud initiation. Whether kinetin-like substances interact in these systems has not been studied. In the root segments of Convolvulus studied here, no simple interaction of kinetin with auxin was observed over the range of concentrations tested.

It will be of both theoretical and practical interest if, using such systems as described here, one can both stimulate and inhibit bud initiation by chemical treatment or convert potential lateral root primordia to bud primordia or vice versa. It remains to be investigated whether at some stage in Convolvulus an "indifferent" meristem is formed as has been reported, for example, in roots of horseradish (recently reviewed by Dore (3)). Such meristems can become either root or shoot meristems, depending upon the physical-chemical environment at the time of initial development. The situation described here offers an ideal system in which to study the physiology of these and related processes.

Summary

Excised roots of the common bindweed, *Convolvulus arvensis*, were cultured in a sterile synthetic nutrient medium containing the usual macro- and micronutrient elements, sucrose, and the vitamins thiamin and nicotinic acid. A clone of roots has been maintained in continuous culture in this medium with regular transfers approximately every four months for over five years. Ten-mm root segments grown in liquid medium in the dark usually produce a single lateral root which becomes the main root axis of the new culture. The lateral root develops slowly at first and then achieves an elongation rate of up to 20 mm per day. The initial root segments also typically form one or two endogenous buds which originate in the pericycle opposite the primary xylem poles in the same anatomical position that lateral roots originate. Low intensity white fluorescent light (100 ft-c) has relatively little effect on root elongation, but stimulates shoot development which does not proceed in the dark. At higher light intensities, root elongation is inhibited. Of a large number of growth factors tested by adding to the nutrient medium, only kinetin (6-furfurylaminopurine) influenced organ initiation by Convolvulus root segments. In the presence of 0.1 mg/l kinetin, root segments grown in the light produced a large number of bud primordia at the cut distal end (root end) of the segment. In the dark the stimulation to bud initiation by kinetin was much less marked. It is concluded that externally supplied kinetin induces bud initiation in cultured Convolvulus root segments and that light markedly augments this induction.

The author expresses his appreciation to Dr. H. B. Currier for provision of seeds of Convolvulus and to Dr. J. B. Wright of the Upjohn Company for a supply of kinetin.

Literature Cited

1. Beijerinck, M. W. Beobachtungen und Betrachtungen über Wurzelknospen und Nebenswurzeln. Verhandl. Koninkl. Acad. Wetenschap. Amsterdam 25: 1–146. 1887.
2. Danckwardt-Lillieström, C. Kinetin induced shoot formation from isolated roots of *Isatis tinctoria*. Physiol. Plantarum 10: 794–797. 1957.
3. Dore, J. Studies in the regeneration of horseradish. I. A reexamination of the morphology and anatomy of regeneration. Ann. Bot. (N.S.) 19: 127–137. 1955.
4. Emery, A. E. H. The formation of buds on the roots of *Chamaenerion angustifolium* (L.) Scop. Phytomorph. 5: 139–145. 1955.
5. Gautheret, R. J. Sur la variabilité des propriétés physiologiques des cultures de tissus végétaux. Rev. gén. bot. 62: 1–107. 1955.
6. Norton, J. P. and Boll, W. G. Callus and shoot formation from tomato roots in vitro. Science 119: 220–221. 1954.
7. Priestley, J. H. and Swingle, C. F. Vegetative propagation from the standpoint of plant anatomy. U. S. Dept. Agr. Tech. Bull. 151, Washington, D. C. 1929.
8. Seeliger, I. Über die Kultur isolierter Wurzeln der Robinie (*Robinia pseudoacacia* L.). Flora 144: 47–83. 1956.
9. Skoog, F. and Miller, C. O. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia Soc. Exptl. Biol. No. 9, pp. 118–131. 1957.
10. Torrey, J. G. Effects of light on elongation and branching in pea roots. Plant Physiol. 27: 591–602. 1952.
11. Torrey, J. G. The role of vitamins and micronutrient elements in the nutrition of the apical meristem of pea roots. Plant Physiol. 29: 279–287. 1954.
12. Torrey, J. G. Chemical factors limiting lateral root formation in isolated pea roots. Physiol. Plantarum 9: 370–388. 1956.
13. Warmke, H. E. and Warmke, G. L. The role of auxin in the differentiation of root and shoot primordia from root cuttings of Taraxacum and Cichorium. Amer. Jour. Bot. 37: 272–280. 1950.
14. White, P. R. A handbook of plant tissue culture. Jaques Cattell Press, Lancaster 1943.