INITIATION OF THE LATERAL ROOT IN THE ADVENTITIOUS AERIAL ROOT OF *LUFFA CYLINDRICA**

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Abstract: The initiation of lateral roots from both old-and young-parts of the aerial root of *Luffa cylindrica* (Linn.) Roem. is reported. The dormancy of the lateral root initial in the young aerial root is also discussed. The lateral root from the older part is initiated in the region near the vascular cambium in the ray, whereas in the younger part it originates from a group of pericyclic cells located opposite the protoxylem strand. Though the lateral root primordia are organized very early in a regular pattern in the young part, no lateral roots become emergent until they are dipped in water, or penetrate into the earth. If the air is not wet enough. the aerial root stops growing and its tip becomes hypertrophied and the pericycle-cortex complex retains a strong meristematic potential instead of a promeristem. The pericycle-cortex complex gives rise to numerous lateral roots soon after dipping in water.

INTRODUCTION

Lateral organs of the shoot system usually originate in the meristematic zone of the apex in definite sequence. This type of relationship has not been described in the root system. Lateral roots always arise at variable distances from the apical meristem. The majority of the species of both higher and lower vascular plants initiate their lateral roots very close to the apical meristem where only a few mature vascular elements can be recognized (Chiang, 1970; Chiang and Gifford, 1971; Chiang and Chou, 1974; Foard, Haber, and Fishman, 1965; Mallory, Chiang, Cutter, and Gifford, 1970; Seago, 1973). Most of the observations on the development of the lateral root have been carried out on underground or water cultivating roots. Several aspects of lateral root development have been mentioned by the workers listed above, including the initiation, pattern of cell division, the correlation between meristem and tissue ontogeny, and correlation of the lateral and the procamial arrangement of the parent root. A few have studied the arrangement and spacing of the lateral root on the parent root (Mallory *et al.*, 1970; Riopel, 1966). The dormancy of the adventitious root has been described (Baranova, 1951), but no reports concerning the dormancy of the lateral root primordia have been reported. The present investigation concerns observations of the early detectable stages of the lateral root primordia which remain dormant under certain conditions.

The fruits of *Luffa cylidrica*, have been an important vegetable for the people of Taiwan for a long time, but little work has been done on the root anatomy of this plant. One to several aerial adventitious roots per each node are constantly produced at the lower nodes under favorable conditions (Fig. 1). In addition to being an important vegetable, the present worker chose these adventitious roots for this study because they are: (1) free from soil particles; (2) lack of root hairs; (3) grow rapidly; and (4) can be easily to produce induced lateral roots by merely dipping them in water.

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MATERIALS AND METHODS

Aerial roots produced from the nodal region of *Luffa cylindrica* (Linn.) Roem. were used. The plants were grown from seed in earthen pots (25 cm in diameter) in a green house. At the proper stage the aerial roots were collected and placed in FAA. Sections were made by the traditional paraffin method. Some of the materials were stained in tannic acid and iron alum with safranin (Sharman, 1943), and others with periodic acid Schiff's (PAS) reacton (Jensen, 1962) for the detection of starch granules.

RESULTS

Induction of the Lateral Root:

Luffa cylindrica is an annual vine. The stem is angular. Aerial roots are constantly produced at the lower nodes when the air is not too dry. Shortly (about two days) after their emergence, the aerial roots grow very rapidly. On the average, they increased 2 cm per day. The majority of them bend downward until they penetrate the ground. Some of them trail over the soil surface for a distance before penetrating into the soil. Still others fail to reach the earth, becoming pendent, and hanging in the air. The tip of the aerial roots are blunt, whereas they became more tapered after growing in the soil (Fig. 2). The tips of the pendent members which fail to reach the ground become swollen or gradually hypertrophied, and look like a root tumor formed by colchicine. These adventitious aerial roots bear no lateral roots on the aerial part, but constantly produced numerous lateral roots on the underground portion (Fig. 1). The aerial portion of the young area, near the tip; the old part, near the stem (the secondary tissue being well developed), and the pendent hypertrophied root produced lateral roots after being submerged in water. One day after dipping in water, the root became swollen along the whole submerged surface (Fig. 3, right, arrow), and numerous lateral roots emerged from the parent roots (Fig. 3, left) in this area two days after dipping. Though the root originating from the more or less older parts of the root has been termed the adventitious root by some botanists (Esau, 1977), for convenience, the roots produced from both the young parts and the old parts of the growing aerial adventitious roots are described as lateral roots in the present report.

Initiation and Development of Lateral Root in Old Root:

The primary xylem in the root is either tetrarch or pentarch (Fig. 4). In some roots one single metaxylem element occupies almost the entire center, whereas in others, the metaxylem elements in the center enlarge very little (Fig. 5). The cambium became active before the complete maturation of the central metaxylem. The vascular cambium shows a circular ontline in transection, but its derivatives, both secondary xylem and secondary phloem, do not form a continuous cylinder (Figs. 4, 5, 6, 7). The secondary tissue is clearly interrupted by the wide rays located opposite the protoxylem poles. Though no starch granules can be identified in the cortical tissue, they were uniformly distributed in the rays including the cambial cells in the ray region, as well as the area near the rays (Fig. 5). The secondary

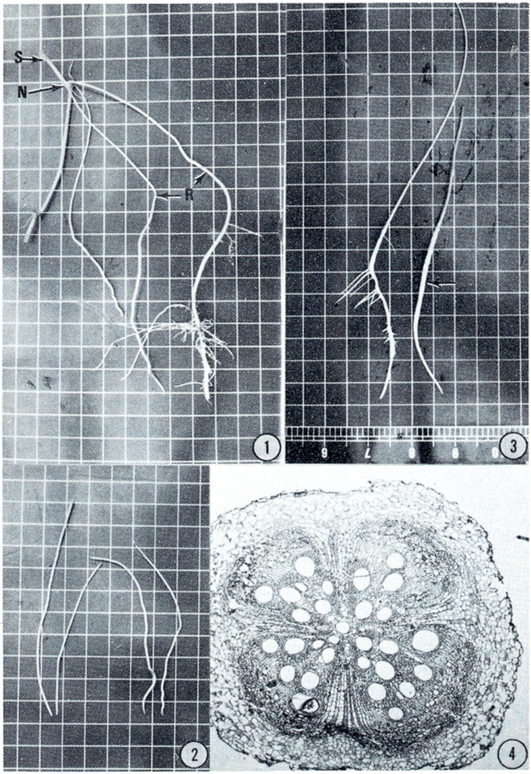
Fig. 1. Portion of stem with three attached aerial roots at the node, the lower portion of these root have already penetrated into ground. The length of the root regend to Fig. 3, arabic numerals indicate centimeter.

Fig. 2. Photograph showing the blunt tips (left two) and tapered tips (right two) of the roots growing in the air and soil respectively.

Fig. 3. The pendent aerial roots one day (right) and three days (left) after submerged in water.

Fig. 4. Transection of a well-developed aerial root, showing the tissue arragement, ×38.

Key to labeling: S-stem; N-node; R-aerial root: Ry-ray; CZ-cambial zone; LP-lateral root primordium; Px-protoxylem elements; Ph-protophloem; PCC-pericycle-cortex complex; Pe-pericycle.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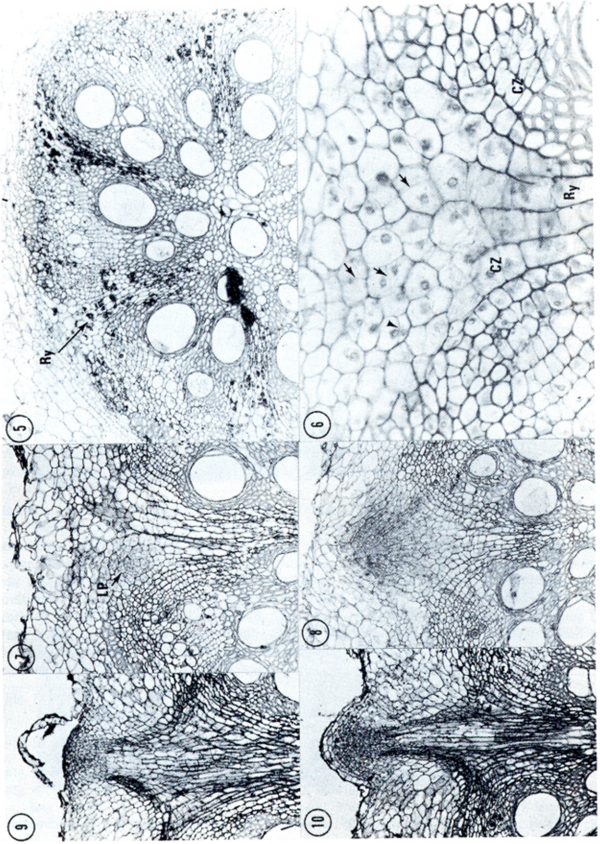
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vascular tissue contained only a few starch granules. After dipping in water the lateral root was initiated in the ray parenchyma located near and outside the cambial zone. The following sequential changes in this region were seen soon after the induction (dipping water) of the lateral root.

The starch granules disappeared from the ray, and both the cells and the nuclei were markly enlarged (Fig. 6). Various planes of the cell divisions occurred. Consequently these cells gave rise to the apical zone of the lateral root primordium located outside the cambial zone (Fig. 7). As soon as the apical region of the lateral root primordium was established, the cambial cells in the ray together with all ray cells located inside the cambium became extensively elongated, more likely pushing the apical zone of the primodium outward (Figs. 9, 10). The cortical cells adjacent to the young primodium became serially arranged, keeping pace with the elongation of the lateral root primordium. The elongation of the cells occurred along the whole ray from the newly formed lateral root, radially to the protoxylem in the center of the parent root (Fig. 10). Apparently the cell division in the ray parenchyma was involved in early development of the lateral root, but in the later stage, the elongation of the cambial and ray cells located between the primordium and the parent protoxylem played a more important role in the elongation and penetration of the primordium through the mid and outer portions of the parent cortex than the increase of cell number by division. No conspicuous increase in the width of the primordium was seen during the penetration stage (compare Figs. 8, 9, 10). Accompanying the penetration, most of the elongated cells located between the primordium and the parent protoxylem developed secondary walls and were transferred to be the central cylinder of the lateral root (Fig. 10). Furthermore it became continuous with the vascular cylinder of the parent root. The direction of the xylem maturation was centrifugal.

Histology of the Young Aerial Root:

All tissues of the root appear to arise from a common meristematic group of cells (Fig. 11). Guttenberg (1960) named this organizational pattern as the open type. Two regions, the columella and the peripheral cap can be identified in the root cap according to their cell arrangement (Fig. 11). The promeristem is wide and shows a continuous pattern with the cap columella due to cell lineage. Starch granules are abundant in the cap cells, especially in the columella and its adjacent peripheral cap cells. The central part of the root meristem i.e., the promeristem, central procambium and periblem show clearer in staining. Few starch granules are found. But the starch granules appear in the young cortical tissue in the region of about one centimeter from the extreme tip of the root where only the protophloem elements have matured (among the procambium) (Figs. 13, 14). They are more abundant in the inner cortical cells than in the outer portion. Almost no starch granules are distributed in the procambial zone. The developing cortical tissue does not maintain starch granules at a later stage of development. The cortical starch granules decrease gradually as the root grows. No starch granules can be recognized in the cortical cells in the region about 5–6 cm from the tip.

Though no lateral roots became emergent from the aerial root (Fig. 2), various developing

Figs. 5, 6, 7, 8, 9, 10. Transections of the aerial roots, showing the various developing stages of the lateral root after submerged in water (PAS stained).

Fig. 5. Note the accumulation of the starch grains in the ray parenchyma located opposite the protoxylem poles, ×94.

Fig. 6. Ray in the cambial zone, showing the increase in the newly formed cell walls (arrows) and the conspicuous nuclei in this area, ×375.

Fig. 7. Same region as in Fig. 6, note the more recently formed cells, ×94.

Figs. 8, 9, 10. Organization of the apical zone and the penetration of the lateral root, ×94.

stages of the lateral root primordia were found inside the root (Figs. 13, 14). The youngest primordium was found in the portion where only the first mature protophloem elements were recognizable in the central cylinder (Fig. 14). The pericycle consists of two to three layers of cells, mostly two. The lateral root primordium is a cluster of pericyclic cells, between two adjacent protophloem groups, opposite the procambial xylem strand. The primordial initials increased in both cell size and stainability. This cluster of cells of the primordium do not show an orderly lineage. Accompanying the initiation, the starch granules increase slightly in the root primordial cells, but still show less starch granules than their adjacent incipient cortical cells (Fig. 14). As the primordium enlarges, the incipient endodermal cells and their immediated adjacent cortical cells also participated in organizing the primordium (Fig. 13). The primordial cell group always contains less starch granules than the incipient cortical cells located outward. Apparently numerous lateral root primordia were organized before being dipped water. But they failed to develop into the emergent lateral roots. Once they were submerged in water, many of them started to grow and gave rise to the emergent members. Though the author has tried to induce the lateral root development by putting the roots in the dark, no trace of an emergent lateral root was observed. These pre-organized lateral root primordia appear to be more sensitive to water than to the dark in later development.

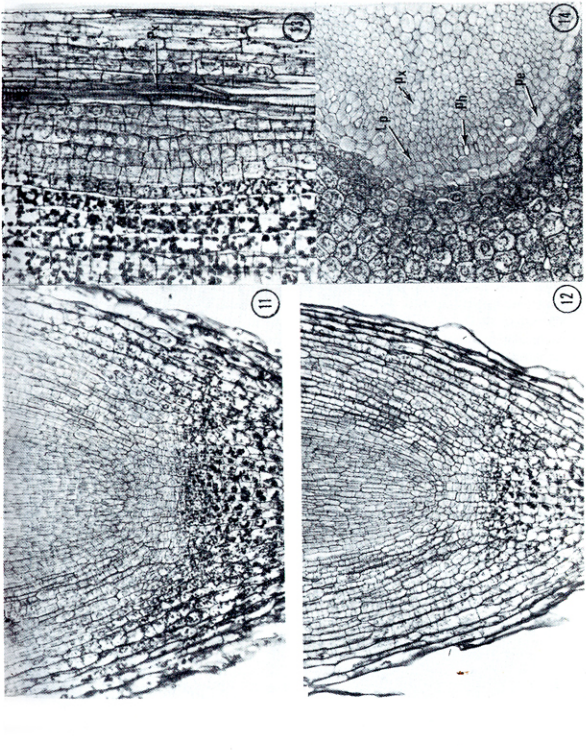
After being submerged in water, the starch granules decreased in both the root cap cells and the young cortical cells located 5-6 cm away from the root tip where the starch granules were conspicuously distributed throughout the cortical tissue. The cap columella was the only structure which maintained starch granules. The width and the cell number in the promeristem the cap columella were markly decreased after putting the root in water (Fig. 12). And the cells in this area became elongated in the direction parallel to root axis. Though the pre-organized lateral primordia were very commonly observed in the aerial root, yet they were merely a bunch of meristematic cells. No recognizable incipient root tissues such as: calyptrogen, periblem, or plerome could be identified.

The Tissue Variation and the Induction of the Lateral Root in the Hypertrophic Root:

As mentioned above, the adventitious aerial root that failed to reach the ground became hypertrophic (Fig. 15). The largest diameter was in the region 1.5-2 mm away from the extreme tip. The ordinary cellular arrangement of the apical meristem described in the preceding paragraphs disappeared (Fig. 17). The boundary between the cap and the root proper was obscure. The enlargement of the cortical and pith cells in radial direction was conspicuous (Fig. 16). The most interesting histological change in the hypertrophic root was the increase in cell number of pericycle cells together with tieir immediate neighboring cortical cells. These derivatives of the pericycle-cortex complex possessed high protoplasmic stainability, conspicuous nuclei and a compact arrangement (Figs. 16, 18). They formed as a band, located opposite the procambial xylem pole, from the region very close to the tip all the way up-ward to within one centimeter from the tip (Fig. 15). Evidently this pericycle-cortex complex corresponded with the place where many pre-organized lateral root primordia occurred in the young aerial root. Many irregular cracks could be seen on the root surface (Figs. 15, 17). The crack was formed by the lysis of epidermal and cortical cells, and was mainly caused by the enlargement and cell division in the pericycle-cortex complex. The expansion of the outer cortical cells also took part in the crack formation of the root surface.

Figs. 11, 12. Median longesections of the root tips (PAS stained) of the aerial root (Fig. 11), and the aerial root three days after submerged in water (Fig. 12), ×190.

Figs. 13, 14. Longisection (Fig. 13) and transection (Fig. 14) through the juvenile lateral root primordia. ×375.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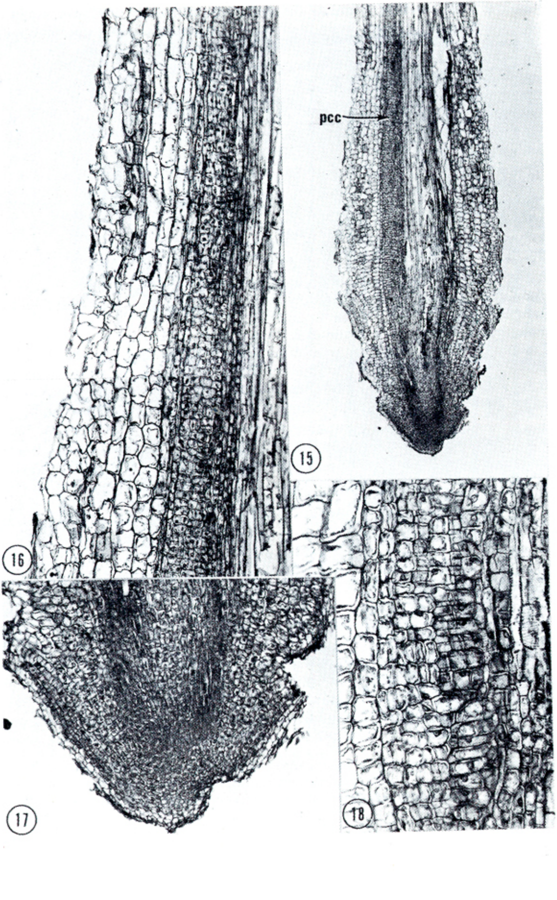
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As that occurred in the growing aerial root, the hypertrophic root produced numerous emergent lateral roots one day after being dipped in water. The hypertrophic root grew lateral roots nearer to the root tip than on the growing young aerial root. No indication of the redevelopment of the apical meristem of the hypertrophic root in *Luffa* was seen during its submergence.

DISCUSSION

Similar to what occurs in many plants, the lateral roots from the older parts of the root of *Luffa cylindrica* were initiated in the region near the vascular cambium in the ray (Esau, 1965), but not in the cambium itself. It is evident that the cambial cells near the lateral root initials became a part of the lateral root at a later stage (Figs. 9, 10), but no trace of cell division was observed in the cambial area. Therefore the site the of lateral root was in ray of parenchyma near the cambium rather in the cambial cells. In many aspects the lateral root development from the old root in *Luffa* is similar to that which occurs in other plants (Byrne, Collins, Cashau, and Aung, 1975; Steffen, 1952; Torrey, 1958). They are characterized by an increase in the size of nuclei, nucleoli, and associated with the cell division. However, the early differentiation of the lateral roots from both young and old parts was irregular. Cell divisions accompanying the initiation proceeded in various planes whereas in many other plants, they were more orderly (Byrne, 1973; Byrne *et al.*, 1975; Seago, 1973).

The fact that the disappearance of the starch granules from the ray after being dipped in water, may be considered to have some significant relationship with the lateral root initiation rather than simply being submerged in water. During the experiment, the author did not place the roots deep enough to prevent them from receiving sunlight which might affect the presence of the starch granules in the ray parenchyma. The increase of some histochemical contents in the formation of root primordium has been reported by many workers, such as: rRNA, protein and their related enzymes (Chang and Chan, 1975; Lee, Chen, and Lin, 1978). The changes of the starch granules during the lateral root initiation has not been described.

The young parts of the aerial root that bore no emergent lateral root possessed endogenous lateral root primordia. They were located opposite the procambial protoxylem poles. The primordium was merely a cluster of cells. Though the orientation of the plane of the cell division in the early stage was more or less irregular, yet it was obvious that in *Luffa* both meristematic pericyclic and endodermal derivatives were responsible for the lateral root histogenesis in the young part of the parent root (Figs. 13, 14). This phenomenon is very common in some other texa, but in others, endodermal derivatives were not incorporated into the lateral root formation although the endodermis divided actively at this early period (Byrne, 1973). The later development of these pre-existing primordia was not studied in the present investigation.

These pre-existing primordia seem to become dormant shortly after their initiation. It is very easy to induce the emergence of the lateral roots from both the young and old parts of the aerial root merely by them putting in water. Water, rather than darkness, is the important factor in breaking the dormacy of the pre-existing lateral root primordia in young roots,

Fig. 15. Median logisection through the hypertrophic tip of the aerial root which ceased the growth in length, ×28.

Fig. 16. Partly enlarged view from Fig. 15, note the increase in cell number of pericyclic region, ×94.

Fig. 17. Enlarged view of the extreme tip of Fig. 15. ×190.

Fig. 18. Portion of the enlarged view from Fig. 15, both enlargement in radial direction and the newly formed periclinal walls can be seen in the pericyclic region, ×190

and in the induction of the lateral root formation in the old part of the root. The formation of lateral roots on the aerial root was commonly observed in other texa after the root apex reached the soil and became anchored (Gill and Tomlinson, 1975).

The axillary (or lateral) buds are commonly formed very early in the second plastochron, and in close association with the shoot apical meristem (Esau, 1965; Sussex, 1955). Many lateral buds usually never grow into branches because of apical dominance (Greulach, 1973). Apical dominance is generally weak in the root. The factors involved in breaking the dormacy of the lateral root seems to be environmental rather than the activity of an auxin as is commoly found in the shoot. Water is apparently an important factor in this plant, for *Luffa* has very strong hydrophytic tendencies, as mentioned earlier some aerial roots trail over the soil surface from some distance before penetrating into the earth, and do not enter the ground immediately. This phenomenon seems to show that positive geotropism is not so important a factor for these aerial roots as hydrotropism.

The activity of the pericycle-cortex complex is stronger than the apical meristem of the parent root after it becomes hypertrophic (Figs. 15, 16, 17, 18). The pericyclic cells occupy a larger area than the cortical cells in the complex. Apparently the pericycle always retains strong tendencies of a meristematic nature. Though no indication of the organized lateral root initials are observed in the pericycle-cortex complex, many pronounced emergent lateral roots can be induced to develop from this complex after only one day of being submerged in water. The retaining of the capacity for meristematic growth in the pericycle has been found in many plants with reference to histogenesis of the lateral root, vascular cambium, phellogen, and also at the nuclear level (Esau, 1977; Torrey, 1965). The regeneration of the apical meristem in the roots of other species has also been observed after removing of the apices (Clowes, 1959, Feldman, 1976). No sign of the redevelopment of the apical meristem of the hypertrophic root in *Luffa* has been noted following submergence. The retaining of the meristematic nature in the pericycle-cortex complex possibly reduces the meristematic tendency in the distal area of the root tip.

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