The quiescent center and primary vascular tissue pattern formation in cultured roots of *Zea*

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Roots of *Zea mays* cv. Kelvedon 33 were cultured in nutrient media supplemented with varying concentrations of sucrose. In some instances an individual root was grown successively for periods of time in several different concentrations of sucrose. From this work it was shown that the complexity of the vascular patterns in cultured roots as well as the size of the quiescent center could be manipulated by altering the levels of sucrose in the nutrient medium. The simplest vascular patterns were found in roots in which the quiescent center was most reduced in size. In roots with relatively larger quiescent centers more complex vascular patterns were observed. Based on these studies a proposal is advanced concerning the relationship between the quiescent center and the initiation and maintenance of primary vascular tissue patterns in roots.

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Des racines de *Zea mays* cv. Kelvedon 33 ont été cultivées dans des milieux nutritifs additionnés de diverses concentrations de sucrose. Dans quelques cas, une racine donnée a été cultivée avec succès pendant plusieurs périodes successives dans plusieurs concentrations différentes de sucrose. A partir de ce travail, les auteurs montrent que la complexité des patrons vasculaires dans les racines cultivées ainsi que la dimension du centre quiescent peuvent être manipulées en modifiant le niveau de sucrose dans le milieu nutritif. Les patrons de vascularisation les plus simples se rencontrent dans les racines où le centre quiescent est le plus petit. Des patrons de vascularisation plus complexes ont été observés dans les racines ayant un centre quiescent relativement plus gros. En se fondant sur ces études, les auteurs proposent une relation entre, d'une part, le centre quiescent et, d'autre part, l'initiation et la persistance des patrons du tissu vasculaire primaire dans les racines.

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Introduction

Among the many studies that document the origin and early development of vascular tissues in roots, special note is often made of the variation in vascular pattern along the length of an individual root (Hayward 1938; Heimsch 1951; Jost 1932; Meyer 1930; Miller 1958). Little attention, however, has been directed toward the elucidation of factors which might account for this variation. Heimsch (1951) suggested that the physiological activities of the root, especially its rate of growth, might influence its internal structure, particularly "the localization of differentiating vascular tissues." Nevertheless, with the exception of a few reports (Bünning 1951, 1952; Esau 1940; Heimsch 1951; Torrey 1957; Wilcox 1962*a*, 1962*b*; Williams 1947), most workers have failed to relate any activities of the root to the occurrence of distinct stelar patterns.

The investigations of Reinhard (1954) and Torrey (1955), which demonstrated that the root apex (the terminal 0.5 mm) controlled the initiation and formation of vascular pattern in the root, suggested that any understanding of the morphogenetic processes that are involved with patterning necessarily must focus on events in the root apex itself. Attempts to correlate various parameters of the apex with the changing complexity of vascular tissues in the root (Torrey 1957; Wilcox 1962*a*, 1962*b*) have been largely unsuccessful. Clearly, a reexamination of events in the apex is now in order. Such a study is particularly timely in light of the demonstration of a quiescent center (QC) in the apex of most if not all roots (Clowes 1961), an apical structure which was not taken into account in the

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earlier studies on vascular pattern formation in roots.

The QC, a relatively inactive group of cells by most criteria, occupies a position in the root apex just proximal to the root cap junction, a site where vascular pattern determination is believed to occur (Bünning 1951, 1952). Thus far, no convincing role has been demonstrated for the QC. Because of its location, it is important to try to understand the relationship of this population of quiescent cells to the subsequently derived stelar patterns that are observed in more mature portions of the root.

The initiation and progressive differentiation of primary vascular tissues have been studied in a number of genera (*Humulus*, Miller 1958; *Hordeum*, Heimsch 1951; *Daucus*, Esau 1940; *Sinapis*, Bünning 1951 and Peterson 1967; *Pisum*, Torrey 1955 and Popham 1955; *Sorghum*, *Triticum*, and *Zea*, Luxova and Lux 1974). From such studies, monocot and dicot roots appear to be dissimilar when one compares mature primary root tissues in cross section. The monocot root is typically polyarchous with many prominent, enlarged, metaxylem elements in a ring around a central pith. Small inconspicuous protoxylem elements occur outside the metaxylem vessels, in some cases in radial rows aligned with the metaxylem elements and in other cases on different radii and in numbers unrelated to the number of metaxylem elements. In some monocot roots, a single enlarged metaxylem vessel occupies the center position in the root and may be separated from other metaxylem elements by parenchyma. In contrast, dicot roots usually possess fewer primary xylem strands, varying in number from 2 up to 8 or 10, which are prominent as radial rows of cells which extend from small-diameter protoxylem elements at the periphery of the central cylinder to the progressively larger elements which may or may not meet in the center of the root as enlarged late metaxylem elements. Small groups of primary phloem occupy the outer alternate radii in both types of structure.

In dicot roots in which transitions in primary vascular tissue patterns occur, either as an increase or as a decrease in the number of xylem strands, such a change has a profound effect that involves an adjustment to the new strand in the central as well as the peripheral portions of the central cylinder of the root. In monocot roots, in contrast, a change in the number of metaxylem elements involves a relatively small portion of the procambial tissues and is more readily accommodated by the root.

Although the mature vascular patterns differ in roots of monocots and dicots because of cell arrangements, the processes of vascular tissue pattern formation appear to be the same. In immature tissues of the central cylinder, which are immediately proximal to the root meristem, the patterns of metaxylem determination and blocking-out of vascular tissues in the procambium are remarkably similar. Thus, one feels reassured that ideas developed from studies on monocot roots may well be applicable to roots of dicots and thus to angiosperms in general.

The assumption was made at the outset of this work that changes in the size and (or) activity of the QC might be predicted to elicit a change in the primary vascular tissue pattern formed by the root. Van't Hof (1966) showed that removal of the sugar supply (starvation) of cultured roots of pea caused a cessation of mitotic activity in the root apex. Addition of sucrose allowed a resumption of deoxyribonucleic acid (DNA) synthesis and mitotic activity (Webster and van't Hof 1969). By using autoradiographic evidence, Webster and Langenauer (1973) demonstrated that the starvation effect in cultured pea roots was associated with a decrease in the size of the QC and that resumption of root growth after starvation led to QC activation, marked mitotic activity, and an increased size of the QC. Thus, manipulation of carbohydrate supply to cultured root tips offered a method for changing QC activity at will. This paper presents the results of experiments that were designed to induce changes in vascular patterns in roots of *Zea*. Cultured roots of corn were subjected to 'starvation' by reducing the sugar level in the medium. Changes in QC size and activity were monitored during starvation and recovery and the primary vascular pattern of the treated roots was analyzed by serial section histology. As a result of these studies, a proposal is advanced concerning the relationship between the QC and the initiation and maintenance of primary vascular tissue pattern.

Materials and Methods

Corn seed (*Zea mays* cv. Kelvedon 33) was obtained from Hurst, Ltd., Witham, Essex, England. The seed was

surface sterilized in half-strength commercial chlorox, rinsed three times with sterile distilled water, and aseptically germinated in the dark at 23 °C. After 3 days, seedlings with primary roots 25–35 mm in length were selected.

In some experiments the terminal centimetre of 3-day-old seedling roots was cultured initially in 50 ml of White's medium (White 1954) plus 2% sucrose (in 125-ml erlenmeyer flasks) on a rotary shaker, about 50 cycles per minute, in the dark at 23 °C. In 2 days they had more than doubled their length. After 48 h in White's medium plus 2% sucrose, the terminal centimetre of each of these roots was again excised, transferred to White's medium supplemented with either 0.5, 0.25, 0.15, 0.1, 0.05, or 0.0% sucrose, and returned to the shaker in the dark at 23 °C. Roots that were grown in these media were subsequently divided into three groups. Roots in one group were allowed to continue growth in the same media for an additional 2-week period, after which time the entire roots were fixed. Roots in a second group were permitted to grow in these media for 48–72 h and then the roots were transferred back to White's medium plus 2% sucrose, allowed to grow for an additional 48 h, and then fixed. Roots in a third group continued growth in these various media for an additional 48 h only. These roots were then transferred to White's medium plus 2% sucrose plus 5 μCi/ml methyl[^{3}H]thymidine (specific activity 6.0 Ci/mM) and were returned to the rotary shaker in the dark at 23 °C for an additional 8 h. These roots were then fixed and prepared for autoradiography as noted elsewhere (Feldman 1975).

For histological work, tissues were fixed either in freshly prepared formalin – acetic acid – alcohol or in chromium – acetic acid solution (Sass 1958), dehydrated, embedded in paraplast, sectioned at 5–20 μm, and stained either with fast green and safranin, or with safranin, orange G, tannic acid, and alum (Sharman 1943).

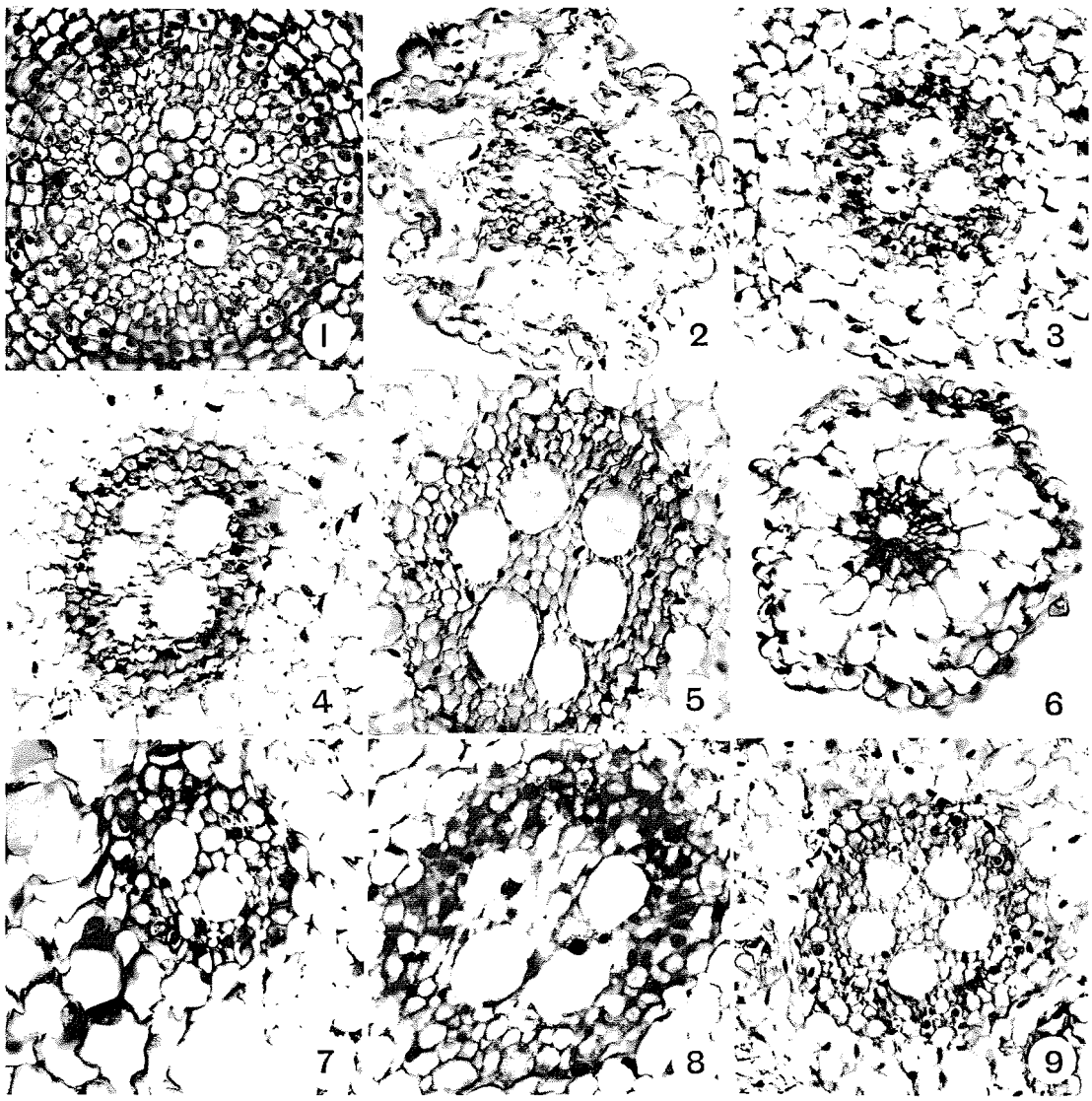
Results

Manipulation of Vascular Patterns in Cultured Roots by Varying the Constituents in the Nutrient Medium

Roots that were grown in medium that lacked sucrose (0%) had increased little in length after 2 weeks and would not resume growth when they were subsequently transferred to medium that was supplemented with 2% sucrose. Roots in 0.05% and 0.1% sucrose-supplemented media continued to grow slowly throughout the 2-week period and resumed a more rapid rate of growth when they were transferred to a medium that contained 2% sucrose. However, in roots that were grown in media that were supplemented with 0%, 0.05%, or 0.1% sucrose, the vascular patterns remained unchanged throughout the entire length of each cultured root. Usually six or seven discrete files of metaxylem elements were evident in basal as well as apical portions of the root (Fig. 1).

In contrast, in roots grown for 2 weeks in media supplemented with 0.15% or 0.25% sucrose, the vascular patterns differed at the proximal and distal ends of the respective roots. Figures 2 and 3 were taken from sections near the apex of two different roots that were grown for 2 weeks in media supplemented with 0.15% sucrose and showed a vascular pattern that was reduced to three or four metaxylem elements. Sections that were made in the second root proximal to those illustrated in Fig. 3 clearly show that a gradual transition in the number of metaxylem elements had occurred, with a reduction from six metaxylem elements at the base (Fig. 5) through five elements (Fig. 4) to four at the tip (Fig. 3). In roots grown for 2 weeks in medium that was supplemented with 0.25% sucrose, the metaxylem was most often reduced to a single element near the tip (Fig. 6). From serial sections that were made proximal to those which are shown for the root in Fig. 6, it is clear that a gradual transition in the number of metaxylem elements had occurred. From the proximal or basal end which showed five distinct elements (Fig. 9), there was a progressive reduction through four and two to one (Figs. 8, 7, 6). In roots grown in medium that was supplemented with 0.5% sucrose, the rate of growth was only slightly less than the controls that were grown in 2% sucrose and only a slight change in vascular pattern was observed. Table 1 summarizes the results of an analysis of the pattern of the metaxylem in 24 roots which were studied.

In the second group, roots were cultured for 48 h in medium that contained 2% sucrose, were subsequently transferred for 48–72 h to media supplemented with lower concentrations of sucrose, and were then allowed to recover in 2% sucrose for 48 h. Since, in the previously described experiments, transfer of roots to 0.25% sucrose-supplemented medium proved the most effective in altering vascular patterns, the results described here are for roots that were cultured for 48–72 h in a medium that was supplemented with 0.25% sucrose and were then subsequently transferred for 48 h to a medium that contained 2% sucrose. The vascular patterns that resulted from one root which was treated in this manner are shown in Figs. 10–13. Figure 13 which was cut at the root base represents tissue in which vascular differentiation had occurred before transfer of the roots into the medium that contained the lowered sucrose concentrations. Figure 12 represents tissue that was formed in



FIGS. 1–9. Transections of cultured roots of *Zea mays* cv. Kelvedon 33. Fig. 1. Transection of an excised corn root cultured in 2% sucrose medium; transection cut just promixal to the level of blocking-out of the late metaxylem elements. Note that there are six enlarged elements. × 220. Figs. 2 and 3. Transections taken near the apex from two different roots that have been grown for 2 weeks in media supplemented with 0.15% sucrose. Note three or four files of metaxylem. × 250. Figs. 4 and 5. Sections taken at progressively more proximal levels from the root shown in Fig. 3. × 250 and 400, respectively. Figs. 6, 7, 8, and 9. Series of transections beginning just below the apex and continuing in a proximal direction; transections taken from a root grown for 2 weeks in medium supplemented with 0.25% sucrose. Figs. 6 and 9, × 250; Figs. 7 and 8, × 400.

0.25% sucrose and Figures 11 and 10 show the tissue after recovery in 2% sucrose. Notice that in the lowered sucrose medium the number of metaxylem files was reduced from five to four and upon recovery returned to five. Sections of a second root are shown in Figs. 14–16 in which a similar reduction is noted, in this case from six files of metaxylem (Fig. 16) to four in the lowered sucrose concentration (Fig. 15) with a subsequent return to six (Fig. 14). This variation in pattern was observed in four of the seven roots that were treated in this manner. The other

TABLE 1

Vascular patterns observed in apices of roots grown for 2 weeks in media that were supplemented with varying concentrations of sucrose. The number of metaxylem files was determined from sections made proximal to the root tip about 1 mm from the root cap junction

% sucrose	No. files of future metaxylem elements at the apex (each number represents one root)	Average no. files at apex
0.00	6,6,6,7	6.25
0.05	6,7	6.50
0.10	5,6,6	5.70
0.15	3,4	3.50
0.25	1,1,1,2,3,5	2.20
0.50	4,5,6	5.00
2.00	5,6,6,7	6.00

three roots showed no alteration in the arrangement of the number of metaxylem elements.

Modification of the Size of the QC in Cultured Roots by Varying the Constituents in the Nutrient Medium

In the third group, roots were grown for 48 h in medium that was supplemented with 2% sucrose and were then transferred for 48 h to media that contained reduced concentrations of sucrose. If roots in media with the lowered sucrose concentrations were provided with [^{3}H]thymidine in the last 12 h of the 48 h in the second medium, little label was incorporated, with the exception of the roots that were grown in 0.5% sucrose-supplemented medium. For this reason, roots were allowed to grow 48 h in media with lowered concentrations of sucrose and then all the roots were provided with relatively high concentrations of label for 8 h in a medium that contained 2% sucrose. Exposure of roots to label for 8 h in 2% sucrose was considered a long enough time to allow the still slowly cycling non-QC cells to increase their rate of incorporation of label, but too short a time to allow any significant reactivation of cells in the QC.

Autoradiographs that were prepared from these roots showed that a QC was present in all roots (Figs. 17–20) except those previously cultured for 48 h in medium that lacked sucrose (Fig. 21). Measurements were made of the dimensions of the QC from the autoradiographs. The heights of the QC's, that is the distance between the root cap and the first uniformly labeled layer proximal to the QC, for 30 roots are summarized in Table 2. On the average, the QC reached its smallest measured size in those roots which were cultured in medium that was supplemented with 0.25% sucrose. Figure 21 shows that roots that were grown in medium which lacked sucrose and then were returned to 2% sucrose-supplemented medium have undergone activation of the QC.

TABLE 2

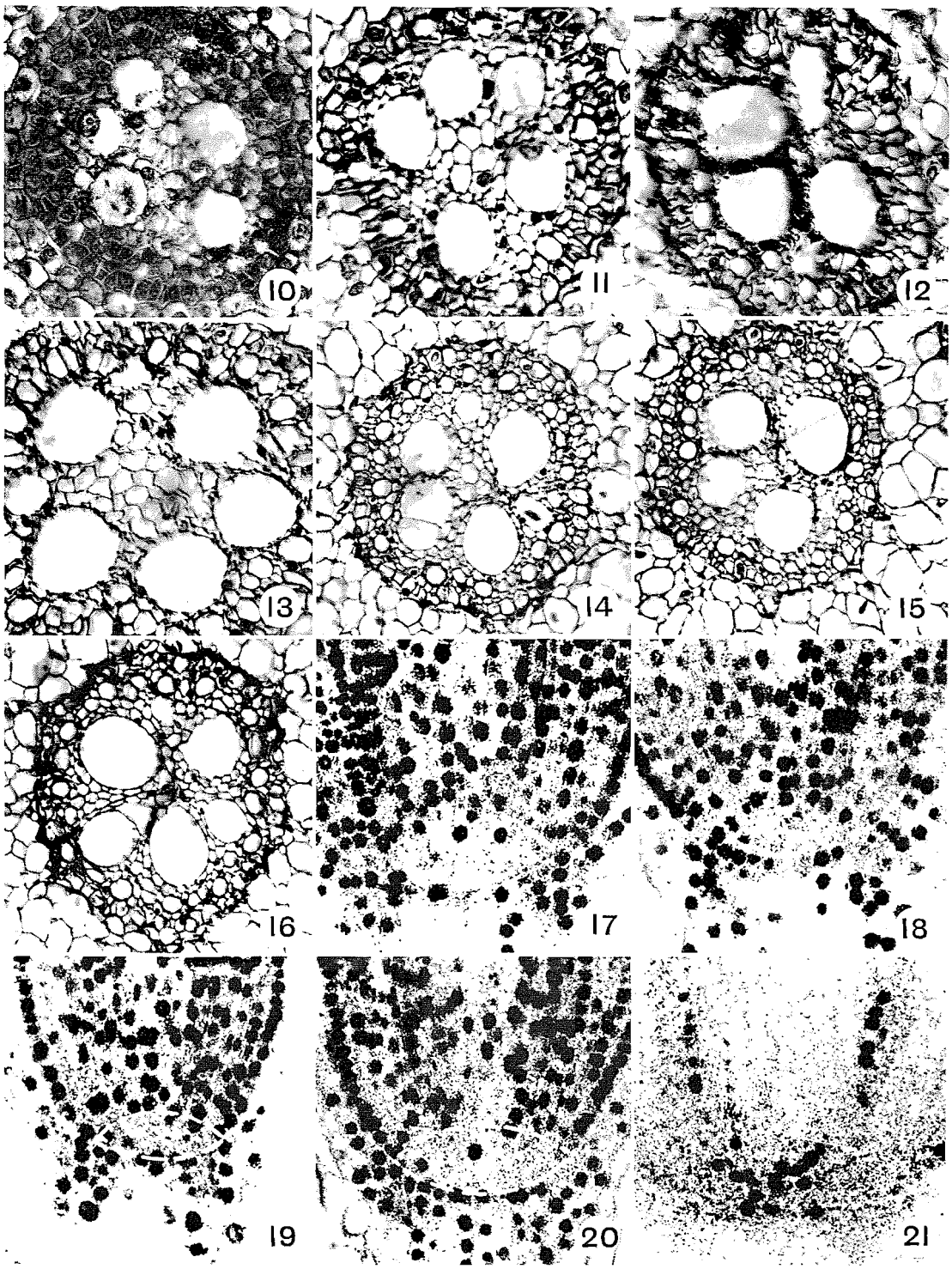
Height of the QC in roots that were grown in media which were supplemented with varying concentrations of sucrose. Recovery for 8 h in 2% sucrose-supplemented medium coincided with [^{3}H]thymidine labeling

% sucrose	Height of the QC (μm), each number represents one root	Average height, μm
0.05	40,55,55,60,60,60	55
0.10	40,50,50,55,55,55,60	52
0.25	30,35,35,40,45,45,55,60	43
0.50	45,45,50,50,55,70,70,85.90	60

Discussion

The observations by Esau (1965), Heimsch (1951), Jost (1932), Meyer (1930), and others of the changing vascular patterns within a single root prompted a number of investigations in which attempts were made to relate various

FIGS. 10–21. Sections of cultured roots of *Zea mays* cv. Kelvedon 33. Figs. 10–16. Series of transections through two different roots. Each root was grown initially in media supplemented with 2% sucrose, then transferred to media supplemented with 0.25% sucrose, and subsequently transferred to media containing 2% sucrose. Note the reduction and subsequent elaboration of the metaxylem pattern. × 400. Figs. 17–21. Longisections of apices from roots grown in media supplemented with varying amounts of sucrose. All roots were subsequently transferred to media containing 2% sucrose and methyl[^{3}H]thymidine. In Figs. 19 and 20, the proximal and distal edges of the quiescent centers are indicated. × 220. Fig. 17. Autoradiograph from root grown initially in media supplemented with 0.05% sucrose. Fig. 18. Autoradiograph from root grown initially in media supplemented with 0.1% sucrose. Fig. 19. Autoradiograph from root grown initially in media supplemented with 0.25% sucrose. Fig. 20. Autoradiograph from root grown initially in media supplemented with 0.50% sucrose. Fig. 21. Autoradiograph from root grown initially in media which lack sucrose.



parameters of the root (e.g., diameter of the root itself, diameter of the stele, etc.) to the changing vasculature. In cultured pea roots in which the primary vascular pattern had been changed after surgical treatment, Torrey (1957) showed that the complexity of the pattern changed in relation to the diameter of the procambial cylinder at the level where blocking-out of pattern occurred. Wilcox (1962*a*, 1962*b*) found no reliable correlations between the complexity of the vascular system and any other parameter that he observed in roots of incense cedar. Studies of this sort imply that the parameters heretofore measured need not alter for there to be a change in the vascular pattern within an individual root. Torrey (1955) attributed changes in pattern to "forces which control, either directly or indirectly, cell division and cell enlargement in the apical meristem." From numerous studies it is clear that components of the vascular tissue are already determined at distances of less than 100 μm from the root cap junction (Bünning 1951, 1952; Esau 1940; Heimsch 1951; Luxova and Lux 1974; Torrey 1955; Wilcox 1962*a*).

One of the difficulties in analyzing the events within the root apex concerns the three dimensionality of the system. Histological studies of pattern are limited to two dimensions, either transection or longitudinal section. The pattern-forming process occurs in three dimensions and spherical gradients, conical structures, and arched planes all need to be considered. In the present study, we have shown a relationship between the changed dimensions of the lens-shaped QC within the apex and modifications of the primary vascular tissue pattern in excised corn roots grown in culture. In these roots a shift in the size of the QC is paralleled by a concomitant shift in the number of files of metaxylem elements. With the formation of unusually small QC's (less than 45 μm in height), one can observe the differentiation of abnormally simple vascular patterns (one or two files of metaxylem). The fact that vascular patterns can be decreased and subsequently increased in complexity in the same root with attendant shifts in the size of the QC adds further evidence to the proposal that these two events are more than coincidentally associated.

That such a correlation might exist was suggested from investigations on roots of *Libocedrus* (Wilcox 1962*a*, 1962*b*). Although Wilcox did not fully apply the concept of a QC, he did note the presence of a group of inactive apical initials surrounded by hemispherical zones of meristematic initials. Of greater significance, however, Wilcox noted that vascular patterns may vary during periods of changing growth activity in the root. Particularly interesting was the observation that changes in the vascular pattern were correlated with fluctuations in the dimensions of the zones of active meristematic initials.

The work presented here leads to a more explicit proposal, that is, that the meristematic zone bordering the proximal end of the QC is not only the immediate source of new derivatives in a growing root (Clowes 1971), but it is also the site of regulation of vascular patterns in roots. We have called this meristematic region 'above the QC' (Barlow 1971) the proximal meristem. In a rapidly growing root of *Zea* the proximal meristem is defined as a layer or narrow zone of cells which borders the proximal face of the QC and functions as the immediate source of all new cells in the root, except those of the root cap. The term proximal meristem is applied to a functioning zone of rapidly dividing cells rather than a fixed layer of cells. This view fits well with Wilcox's observation that the 'zone of dividing cells' fluctuates in size and presumably in position also.

In cultured roots of *Zea* we have demonstrated that when the size of the QC is reduced, the distal limits of the proximal meristem move closer to the root cap junction (compare Figs. 19 and 20) and, alternatively, when the QC enlarges, the proximal meristem is displaced still further from the root cap junction. This displacement of the proximal meristem is accompanied by changes in the vascular pattern. This observation strongly suggests that it is within the proximal meristem that the pattern of the xylem is regulated and that the complexity of the vascular pattern is ultimately traceable to the size of the QC.

In the experiments which are reported here, we have yet to establish how changes in sucrose levels affect vascular patterns. Clearly, the nutritional status of an organ, as suggested from the work of Luxova (1971) with isolated corn embryos and the work of Feldman and Torrey (1975) with isolated QC's which were grown in culture, influences the complexity of the vascular tissues that are formed. Whether the sucrose affects the pattern indirectly, by causing a change

in the size of the QC and hence the extent of the proximal meristem, or more directly is not known. Of interest is the observation that alteration in vascular patterns and in the size of the QC was most dramatic at the intermediate and not the lowest concentrations of sucrose. It seems likely that the rates of growth of roots cultured at the lowest sucrose concentrations were so slow as to preclude any changes in pattern at the apex. Only when growth occurs can one expect to observe an expression of pattern change.

Webster and Langenauer (1973) suggested that changing the levels of available carbohydrates affects the 'proliferative activity' of the meristem and hence the size of the QC. Whatever the mode of action of the sucrose, it seems clear that the QC may alter its size in response to changes in the levels of available carbohydrates. It is also clear that changes in QC size, which in turn determine the position and dimensions of the proximal meristem, may lead to modification of the primary vascular tissue pattern of the root.

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