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Developmental studies on *Euphorbia esula*: Seasonal variation in the apices of long roots[1]

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Abstract:

Long-root apices of *Euphorbia esula* L. were supplied with [^{3}H]thymidine in the field on 11 occasions during the growing season. Autoradiographs of the root apices showed that the characteristic quiescent centre of long roots was absent in a large percentage of the roots in the early part of the growing season. It is suggested that the cells of the quiescent centre play an important role in the seasonal reactivation of the perennial roots of this species.

Introduction

In an earlier investigation (Raju *et al*. 1964) it was shown that the phenomenon of quiescence as indicated by low nuclear incorporation of tritiated thymidine is found in root apices of *Euphorbia esula* L. However, quiescence is restricted to the meristems of indeterminate long roots and is absent from the apices of short roots which have a limited or determinate growth. The condition in long root apices corresponds to what has been described as a quiescent centre in the roots of many species (Clowes 1967); but its absence from short root meristems, which have a similar structural configuration, suggests a variability which is related to the developmental potentiality of the root. Moreover, there were indications that the size of the quiescent region in long roots is variable; but no attempt to analyze this variation was undertaken in the original study. All of the roots analyzed were supplied with tritiated thymidine as they grew in the field during the early summer period of active elongation growth.

It now seems clear that the quiescent centre of roots is characterized not by an absence of deoxyribonucleic acid (DNA) synthesis and mitosis but rather by an extended

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cell cycle time, with much of the increased duration occurring in the G_1 phase (Clowes 1965). Moreover, there is variation in duration of the cell cycle among the cells of the quiescent centre and there may even be a radial gradient of decreasing cycle time from the central to the peripheral regions (Phillips and Torrey 1971). There are also indications that at certain times or under particular conditions DNA synthesis and mitotic activity may be greatly increased in the quiescent centre. Clowes (1959, 1970) has shown that cells of the quiescent centre become active after X-irradiation of root tips. Removal of the root cap in *Zea* or even the excision of the distal half of the cap stimulates mitosis in the quiescent centre (Clowes 1972; Barlow 1973). Clowes and Stewart (1967) have reported activity in the quiescent centre of seedling roots of *Zea* after a cold-induced period of dormancy. Finally, it has been shown that in cultured roots in which cell proliferation in the meristem has been arrested by carbohydrate starvation, DNA synthesis occurs in the quiescent centre when carbohydrate is added to the culture medium (Webster and Langenauer 1973; Langenauer *et al*. 1974).

These reports have a great importance for the understanding of the biological significance of quiescence in root meristems in suggesting that predominately quiescent cells may have periodic bursts of activity in response to stress or perhaps to seasonal change. One way in which this latter possibility could be explored would be an investigation of DNA synthesis and mitotic activity throughout the growing season in perennial roots where a natural periodicity of growth occurs. The perennial root system of *E. esula* offers a system in which such an analysis could be carried out under field conditions. It is already clear that quiescence is well established in long roots of this species during the summer period of active growth (Raju *et al*. 1964). The objective of the present investigation is to extend observation of long-root meristems throughout the period during which climatic conditions permit any growth activity to occur.

Materials and methods

The plants used in this study were growing at the same site near Saskatoon (S.W. 34-35-6 W3) at which the earlier investigation was carried out (Raju *et al*. 1964) and the methods used were the same. Tips of long roots attached to the parent plants in the field were carefully exposed and were treated either with [^{3}H]thymidine at a concentration of 0.5 μCi/ml or colchicine at a concentration of 0.05% for a period of 24 hours. The experiment was repeated on 11 occasions throughout the growing season from early May to October. After treatment the root tips were immediately fixed in the field in formalin - acetic acid - alcohol and prepared either for autoradiography or for histological study as described previously. Sections were cut longitudinally at 8 microns (μ) and were stained with safranin and orange G - tannic acid (Sharman 1943) before autoradiography or in Feulgen reagent with a counterstain of fast green (0.5% in 95% ethanol) in the case of colchicine-treated roots.

The analysis of each root was made on what was judged to be the median longitudinal section as indicated by the clarity of cell layers in the promeristem region. The root apex was divided into 26-μm arcs, with the centre located at the base of the root cap as in the earlier study (Raju *et al*. 1964). This was accomplished by means of a drawing tube by which a diagram of the arcs was superimposed upon the image of the root apex. Four

zones thus delimited were analyzed in each root since the area included exceeded the size of the quiescent centre encountered in any root in this study.

In the case of roots which had been treated with [^{3}H]thymidine, the number of labeled nuclei in each zone was recorded. To establish what should be considered a labeled nucleus, five roots were analyzed in detail, that is, by counting the grains above each nucleus. This analysis showed that there were two general populations of nuclei: those with grain counts ranging continuously from 1 to 10 and those with 30 or more. Very few nuclei were encountered with values falling between 10 and 30. Consequently, in the general analysis of the 79 roots collected throughout the growing season, a value of 30 or more grains was taken to indicate a labeled nucleus. In fact, most such nuclei had so many grains that it was impossible to count them accurately. It should be noted that in the earlier study of roots of *E. esula* (Raju *et al*. 1964), a value of 30 or more grains was also used as an indication of a labelled nucleus.

The apices of colchicine-treated roots were scored for the presence of mitotic figures in each of the four zones. However, the results of this phase of the study were generally unsatisfactory. In many roots collected during the growing season mitotic figures were few in number or absent throughout the root tip, while on other occasions many mitotic figures were observed. It is difficult to offer an explanation for these results. Thus, the data obtained from the colchicine-treated roots will not be presented in detail; but they will be cited for those instances in which enough roots showed mitotic figures to allow a meaningful interpretation.

Results

The analysis of long-root apices which had been supplied with [^{3}H]thymidine on each of 11 occasions throughout the growing season and had subsequently been autoradiographed is given in Table 1. For each date the number of roots which fall into each of three categories is given. Those which had labeled nuclei in the most apical zone (zone 1) as well as in the second in effect had no quiescent centre; those with labeled nuclei in zone 2 but not in zone 1 had a small quiescent centre; and those with no labeled nuclei in either zone 1 or 2 had a well developed quiescent centre. It was considered unnecessary to include data from the remaining zones since, almost without exception, the third and subsequent zones contained labeled nuclei. This is in agreement with the observations of Raju *et al*. (1964). The percentages of roots showing these three categories of distribution of labeled nuclei in their apices are also given in Table 1.

Table 1. Distribution of labelled nuclei in long-root apices of *E. esula* treated with tritiated thymidine.

	No. roots containing labelled nuclei in:		
Date	zones 1 and 2	zones 2 only	neither zone 1 nor 2
May 5	5 (71%)	2 (29%)	—
May 13	4 (50%)	3 (37%)	1 (13%)
May 26	4 (50%)	4 (50%)	—
June 10	2 (22%)	1 (11%)	6 (67%)
June 25	—	6 (54%)	5 (46%)
June 29	—	1 (25%)	3 (75%)
July 6	1 (12%)	5 (63%)	2 (25%)
July 22	—	2 (33%)	4 (67%)
August 10	1 (17%)	3 (50%)	2 (33%)
September 1	1 (17%)	3 (50%)	2 (33%)
October 7	—	4 (67%)	2 (33%)

It is evident from the data presented that in the very early part of the growing season (May 5 to June 10) a substantial proportion of the roots examined contain labeled nuclei in even the most apical zone of the apex. This situation is very different from that found in long roots of *E. esula* in the previous study (Raju *et al*. 1964), which did not include observations at the beginning of the growing season. The synthesis of DNA in the most apical zone of the long-root apex indicates that ordinarily quiescent cells are active at this season of the year or, in other words, that at the beginning of the growing season there is no quiescent centre in many roots. Figure 1 illustrates a long-root apex, supplied with [^{3}H]thymidine and autoradiographed early in the growing season, which shows no quiescent centre. As the growing season progressed, a well defined quiescent centre encompassing the first and often the second zone became the almost universal rule (Fig. 2). Of the 47 roots examined after June 10, only three, one on July 6, one on August 10, and one on September 1, had labeled nuclei in zone 1 (the most apical zone) and thus lacked a quiescent centre. These three exceptions are of interest in suggesting that there may be sporadic activity in the quiescent centre throughout the growing season.

For reasons already explained, the results obtained in the colchicine studies are not adequate to permit an overall picture of activity in the quiescent centre throughout the growing season. They do, however, provide support for some of the conclusions derived from the autoradiographic work. In the early part of the growing season, mitotic figures were observed in the most apical zone of the root apices of some roots. On both May 5 and May 13, three of the seven roots examined showed mitotic figures in the most apical zone, that is, the absence of a quiescent centre (Fig. 3). On May 25 the five roots which showed reasonable mitotic activity all revealed a quiescent centre in their apices. Throughout the rest of the season, most colchicine-treated roots showed few or no mitotic figures in the entire root apex. Wherever mitotic figures were reasonably abundant, a distinct quiescent region was noted except in two roots collected on July 6 and one collected on July 22. In view of the unexplained difficulty encountered in this phase of the investigation, the significance of these last observations is difficult to assess.

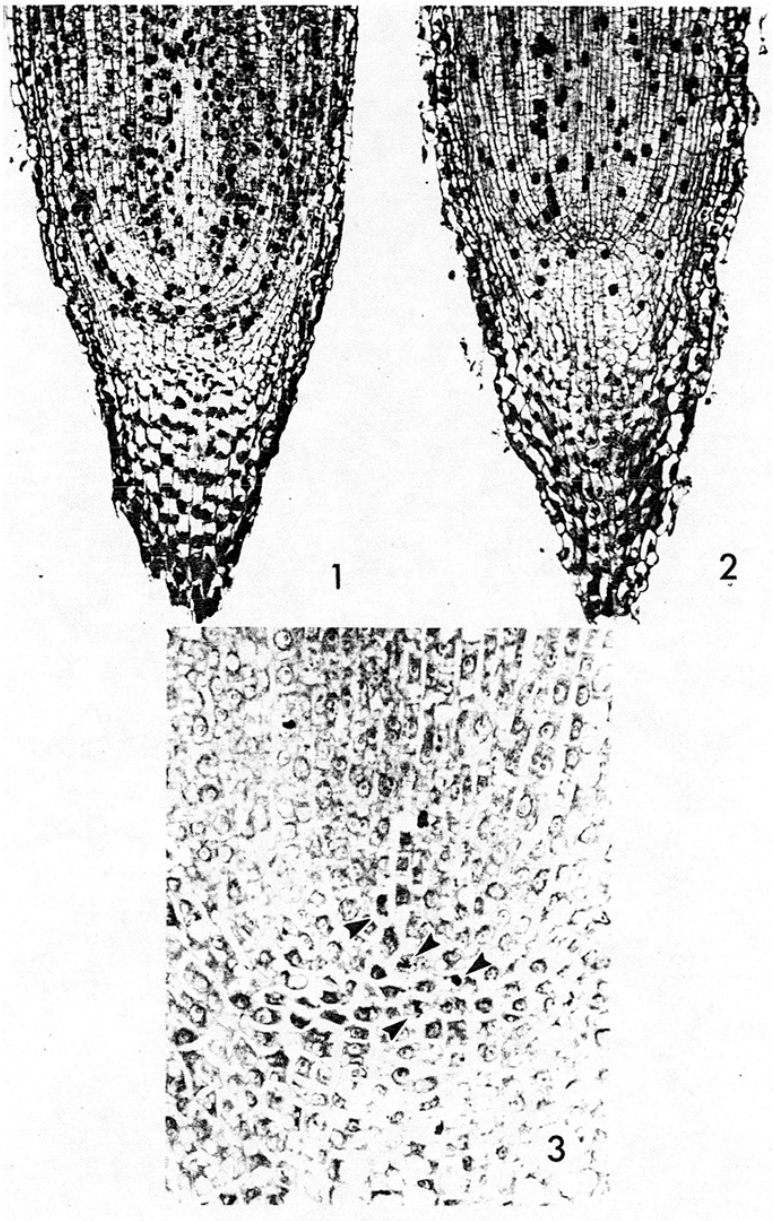

Fig. 1-3. Long-root apices of *E. esula.* Fig. 1. Median longisection of an apex supplied with [^{3}H]thymidine on May 26, showing the absence of a quiescent centre. × 130. Fig. 2. Median longisection of an apex supplied with [^{3}H]thymidine on July 6, showing a well defined quiescent centre. × 130. Fig. 3. Median longisection of an apex treated with chochincine on May 5, showing mitotic figures in the most apical zone (arrows). × 320.

Discussion

It has been determined that cells of the root quiescent centre can be induced to undergo greatly increased DNA synthesis and mitotic activity by treatments which arrest growth and subsequently allow it to proceed (Clowes and Stewart 1967; Webster and

Langenauer 1973; Langenauer *et al*. 1974). The work that bears most directly upon the present investigation is that of Clowes and Stewart (1967) in which young seedlings of *Zea mays* were put into a dormant state by exposure to low temperature and subsequently were restored to active growth by a return to normal temperature. During the return to normal growth, cells of the quiescent centre incorporated labeled thymidine to a greater than normal extent and subsequently showed a higher than normal mitotic activity. These increases were transitory, and a return to normal quiescence followed. Clowes and Stewart have noted the possible relevance of these observations to recovery from dormancy under natural conditions. The significance of this present report is that it provides evidence for a comparable activation of cells of the quiescent centre during the seasonal recovery from dormancy of perennial roots growing under natural conditions. Although experimental studies carried out in this way are difficult and lack laboratory precision, they do make it possible to view developmental phenomena in the context of natural plant growth.

A high percentage of root apices examined early in the growing season showed activity in the region of the quiescent centre. If this activity is a normal feature of seasonal growth, it is reasonable to ask why all roots examined at this time do not show it. All roots which were labeled were judged to be actively growing at the time; but there was no means of determining how long they had been active. Although the duration of the activation phase in this species is not known, it may be relatively brief; thus it is possible that some of the roots examined, even on the earliest date, had already passed through it. Root growth begins much earlier in the spring in this species than had been anticipated and certainly well before there is any evidence of renewed shoot activity above ground. If, however, the activation phase is relatively brief, one might not expect to find roots in this state during a period of more than a month in the spring. However, it is probable that roots are not all activated at the same time and that a succession of root apices become active during the early part of the season. The data reported suggest a declining percentage of such roots during this period, from 71% on May 5 to 22% on June 10; but the small number of roots examined on each occasion requires caution in the interpretation of this trend.

The sporadic occurrence of activity in the quiescent centre later in the growing season seemingly could have two explanations. On the one hand it may indicate that a few roots do not become active in the spring but are activated at a later time. On the other hand, it could represent roots which, after a period of growth, become inactive and are subsequently reactivated. General observations of root systems of this species have shown that individual roots do stop growing at various times throughout the season, but it has not been established that such roots do, in fact, resume growth during the same season. Presumably environmental factors could be involved in such a process, but if they did participate in the present instance, they must have been extremely localized since only a few roots gave evidence of what may have been reactivation in the latter part of the season. It would be of interest to look for this phenomenon in a season in which striking climatic factors could be identified; for example, a prolonged drought followed by heavy rainfall late in the season.

In spite of its widespread occurrence in root apices, there seems to be no general agreement as to the function of the quiescent centre or, indeed, whether it may be said to

have a function at all. Clowes (1971) has commented that it may be looked upon as a geometrical necessity in the root apex. Torrey (1972) has reviewed the evidence relating to the possibility that quiescence in the centre of the root meristem may be the result of a restriction in the supply of necessary substances for meristematic activity as a consequence of position or of the competition of actively dividing cells located proximally and peripherally. He has concluded that the evidence does not support the existence of such a physiological restraint and has proposed that the quiescent centre has an active function in root apical organization as a site of hormone synthesis. The role of the quiescent centre in the recovery of roots after severe radiation damage has also been noted (Clowes 1959, 1970); however, as Clowes and Stewart (1967) have remarked, it is unlikely that this is a major role which could have emerged through evolutionary processes.

The apparent participation of quiescent centre cells in the recovery from dormancy, both artificially induced and natural, suggests a very fundamental role for the quiescent centre, or perhaps of quiescence, in the root apex. Wilcox (1962) suggested that such cells, which he considered to be the apical initial cells, in roots of *Libocedrus decurrens* play a role in the periodic renewal of growth in the natural cycle of dormancy and activity. A similar concept is inherent in von Guttenberg's suggestion (1964) that the central region should be called an intermittent centre rather than a quiescent centre. In an earlier study of *E. esula* (Raju *et al*. 1964), it was shown that whereas long roots which are characterized by indeterminate or potentially unlimited growth possess quiescent centres (an observation confirmed for most of the growing season in the present study) short roots which have limited growth lack quiescent centres. On that basis, it was suggested that quiescence might play a role in the long-term retention of meristematic potentiality. The observations reported here, which suggest the involvement of the ordinarily quiescent region in the periodic renewal of growth, would seem to support this hypothesis.

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