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ANATOMY OF REGENERATING ROOT SEGMENTS OF *TARAXACUM OFFICINALE* WEB.

MOHAMMAD ISHAQUE KHAN*

Department of Botany, The University of Sheffield, Sheffield, U.K.

Abstract

A detailed study of the anatomy of the proximal and distal ends of regenerating *Taraxacum officinale* root cutting was undertaken. It was found that cell division began just below the cut ends of the root segments after 24 hr. of regeneration. At the proximal end, shoot meristems were distinguished at 48 hr. in a narrow zone around the xylem core. Later on callus growth followed, carrying the new shoots upwards. Xylem strands differentiated basipetally through the secondary phloem at seventh day. At the distal end, xylem nodule appeared at the fourth day of regeneration in a wide zone across the secondary phloem. Callus growth followed later and meristem appeared in the callus, distal to the xylem nodule, after two weeks. The young shoot and root meristem had identical appearance.

Introduction

The regeneration of shoots and roots from isolated root segments of different species can be grouped into (a) Regeneration from the sides, at some distance from the cut surfaces as in *Armoracia rusticana* and *Convolvulus arvensis*. (b) Regeneration from the cut surfaces through callus as in *Taraxacum officinale* and *Chicorium intybus*.

In the past some anatomical work has been done with the first type of regenerating roots (Lindler, 1940; Dore, 1955; Bonnett *et al*, 1966). However, relatively little is known about the anatomy of regeneration in the second type. Naylor (1941) gave a very brief account of the anatomy of regenerating root segments of *Taraxacum laevigatum* and described the formation of callus at the surfaces from which shoots and roots later originated. Buds were found to originate at the proximal end of the cutting from thin-walled storage cells of the secondary phloem. Roots developed at the distal end from phloem parenchyma several days or even weeks after the formation of leafy shoots.

The present paper deals with a detailed anatomical study of regenerating *Taraxacum* root cuttings. The early cell changes leading to the formation of shoot primordia at the proximal end and xylem nodules at the distal end is described. Similarly the period of observation was further extended and the origin and development of the root primordia was studied.

Materials and Methods

Roots of *Taraxacum officinale* Weber, were collected from neglected open ground in the vicinity of Sheffield University (U.K.) and brought to the laboratory in polythene bags as required. Before use the roots were thoroughly washed with cold tap water.

Following the observation of Warmke & Warmke (1950) that the rate and extent of regeneration varies with root diameter, roots for use in the present study were selected within the range of 7-10 mm diameter.

Long root pieces were marked with red ink and cut with a razor as described earlier (Khan, 1972). Root segments 2 cm long were washed with running cold tap-water for 30 min to remove the oozing latex. These cuttings were horizontally placed

*Present address: Department of Botany, University of Karachi, Karachi-32, Pakistan.

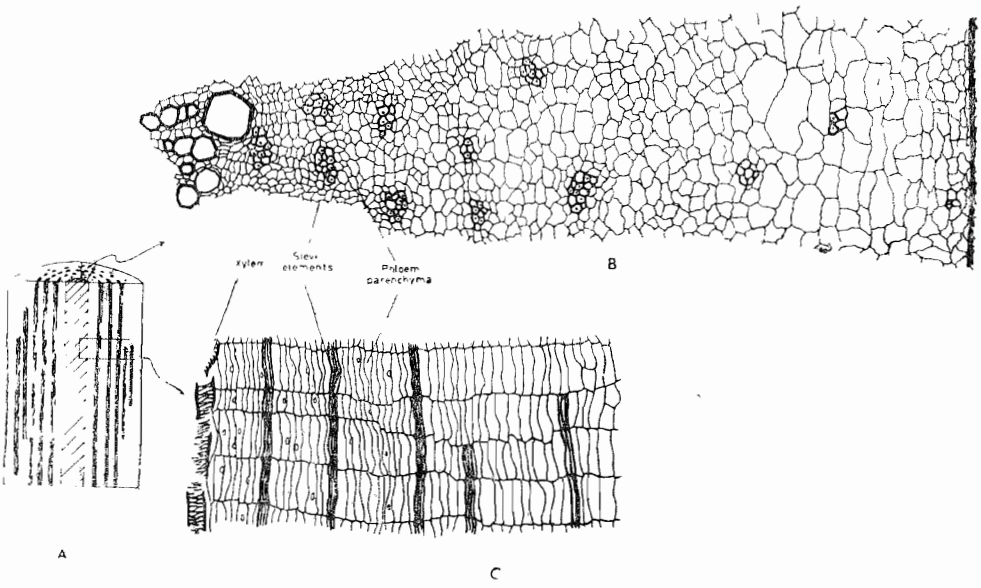

Fig. 1. Transverse (B) and radial longitudinal (A and C) sections of *Taraxacum* root.

approximately one centimeter beneath the surface of a mixture of five parts washed sand to one part of vermiculite in plastic seed trays kept at 25°C and watered every day. Five replicate samples were taken every 12 hr. up to 4 days and then on the 7, 16, 21 and 28th day after the start. Each sample was divided transversly into two, giving a proximal and distalend pieces. These were then fixed in formaldehyde: glacial acetic acid: 70% ethanol (5 : 5 : 100) for a minimum of 48 hr. dehydrated through an ethanol series and embedded in paraffin wax (Johansen, 1940).

Serial radial longitudinal sections were cut with a Unicam, Cambridge, rocking-type microtome at 12 μ thickness. Initially some sections were stained with crystal violet and erythrosine B, a quick method of staining described by Johansen (1940). However this did not differentiate the parenchymatous tissue sharply enough and the stain faded after some time. Subsequently safranin and fast green were used which gave more satisfactory results. The sections were first overstained by keeping them immersed in safranin solution overnight. They were then washed in running tap water and destained for 10-15 seconds with 95% ethanol containing 0.5% (W/V) picric acid. The sections were quickly dehydrated in a series of baths of increasing concentration of ethanol and counter-stained with fast green for 15-20 seconds.

Results

Anatomy of the root before regeneration

Taraxacum root consists mainly of secondary tissues due to the activity of a vascular combium. This adds rather more cells to the phloem than to the xylem. The primary tortical tissues are crushed early in development and some secondary phloem tissue to wards the periphery of the roots is sloughed off each year due to the development of new secondary tissues and the formation of a cork cambium between the old and the new tissues. Figure 1 (A—C) shows such a root in transverse and radial longitudinal section.

CL Cell becoming Lignified
CT Callus tissue
DC Dividing cell
DS Distal cut surface
DX Differentiating xylem
L Leaf
MT Meristematic tissue
PC Proximal cut surface

PP Phloem parenchyma
RA Root apex
SE Sieve element
SM Shoot meristem
SP Shoot primordium
XC Xylem core
XN Xylem nodule
XS Xylem strand
RP Root primordium

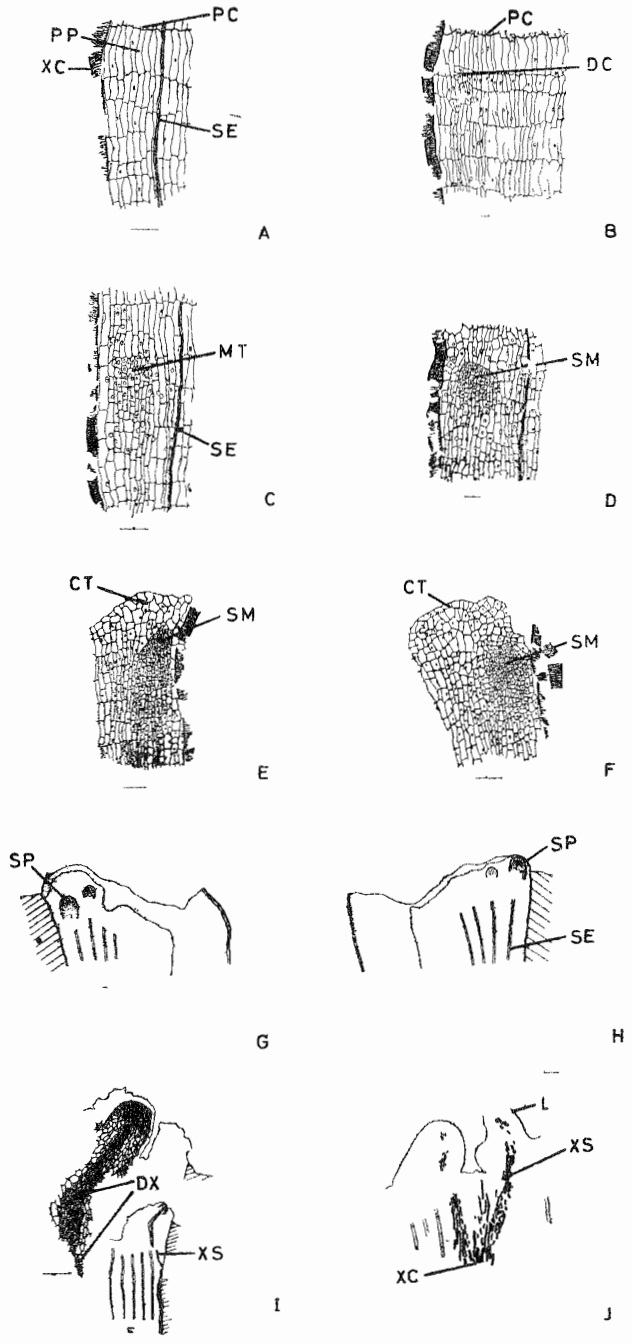

Fig. 2. Anatomy of the proximal cut end of *Taraxacum* root during regeneration. The measurements given in all the Camera Lucida drawings are 100 mμ. A, 12 hr; B, 24 hr; C, 36 hr; D, 48 hr; E, 60 hr; F, 72 hr; G, 84 hr; H, 4 days; I, 7 days; J, 21 days.

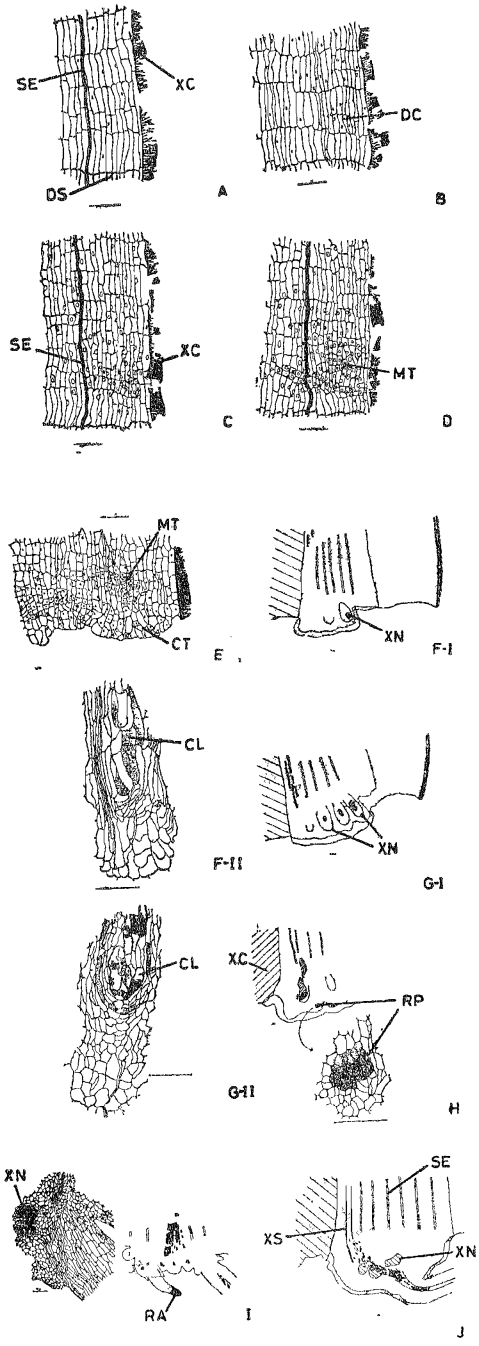

Fig. 3. Anatomy of the distal cut end of *Taraxacum* root during regeneration. A—J as in Fig. 2.

Each of the prepared set of serial sections was examined under a binocular microscope and for each regeneration time, the relevent part of a representative section was selected for presentation. Considerable care was taken in selection and in many cases the median section through an organ such as a shoot bud or a xylem nodule was taken. In all cases several sections on either side of the selected section were examined in detail so that a three dimensional structure of the material could be visualised. Camera lucida drawings of selected sections were made and photographed.

Internal to the outer corky layer of the root is a broad band secondary phloem, consisting of sieve elements in broken concentric rings (in T.S) surrounded by thin walled parenchyma. The sieve elements comprise of sieve tubes and companion cells joined up to form continuous vertical rows in longitudinal section. A net work of narrow latex tubes run alongside the sieve elements and are difficult to identify due to their thin structure. The number of sieve element rings increases with the age of the root. The xylem cylinder in the centre is composed of secondary tracheids, vessels and xylem parenchyma. Between the secondary phloem and xylem is a narrow layer of cambial cells.

Anatomy of the proximal cut end during regeneration

No anatomical changes were noted in the proximal cut end up to 12 hr. of regeneration (Fig. 2-A). By 24 hr. a few phloem parenchyma cells adjascent to the xylem core, and in the second file of cells below the cut surface, divided transversly to the longitudinal axis (Fig. 2-B). As the cells became transformed from storage parenchyma into a meristamatic state their cytoplasm and nuclei became densely stained and they were thus easily distinguihsed. This site of meristematic activity continued to enlarge (Fig. 2-C), and at 48 hr. a dome-shaped apical meristem was distinguished (Fig. 2-D). After 48 hr. of regeneration, cell division in the storage parenchyma cells surrounding the shoot primordium became apparent (Fig. 2-E & F). These lead to the formation of callus tissues which grew in size by cell division and cell enlargement. Transverse and longitudinal sections of the regenerating proximal end of the root cuttings were examined to visuliaze a three dimensional picture of the origin and development of shoot primordium. A discontinuous ring of shoot primordia encircling the central xylem core was first observed but later on a second ring of primordia originated adjacent to the first one (Fig. 2-G). The shoot primordia grew upwards and started to break through the callus (Fig. 2-H & I).

Xylem elements differentiating below the newly formed shoot primordia were first found at about the 7th day. In Fig. 2-I, the shoot primordium can be seen to have a densly-stained provascular strand. Towards the basal end of this a number of isolated xylem elements were found and lower down was presant a continuous file of xylem elements which had apparently differentiated basipetally from the developing shoot primordium. Each primordium was found to have such xylem differentiation associated with it. These lower xylem strands differentiated within the phloem parenchyma so that, in cross section, the central xylem core could be seen to be encircled by one or two discontinuous rings of xylem elements. The number of rows of xylem elements in each xylem strand continued to increase and this stage of development can be seen in Fig. 2-J. Here, after 21 days, the shoots were well formed. The section was slightly oblique and tangential to the edge of the xylem core. The later can be seen at the lower edge of the figure as a zone of dark and wide cells. Above are strands of xylem elements passing downwards from two shoots, the edge of the shoots only having being cut in this section.

Anatomy of the distal cut end during regenenation

As in the case of proximal end no anatomical changes occurred up to 12 hr. (Fig. 3-A), at 24 hr. cell division in the phloem parenchyma adjacent to the central xylem core were noted (Fig. 3-B). Cell division continued (Fig. 3-C & D 36 and 48 hours), but did not lead to the formation of primordia. Instead diffuse divisions just below the cut surface became more prevalent and these continued laterally away from the central xylem core forming callus across the whole of the exposed secondary phloem tissue, (Fig. 3-E & F), unlike the situation at the proximal end.

At 4 days discrete zones of cells, just internal to the dividing cells, which had shown considerable enlargement started to become lignified into short xylem elements, (Fig. 3-F). Such a group of xylem elements formed a slightly elongated xylem nodule, a later stage being shown in Fig. 3-G (seven days). At this time a little cell division occurred in the cells surrounding the xylem nodule, with cell walls forming periclinally to the nodule. One nodule appeared to be associated with the lower end of each sieve-tube strand, (Fig. 3-G), so that in cross section the central xylem core could be seen to be surrounded by four or five concentric rings of xylem nodules. Root primordia were found to develop just internal to the outer surface of the callus at some distance from the nearest xylem nodule. Such a root primordia is shown in Fig. 3-H, taken at 16 days. The distinctive dense cytoplasm and nuclei of a primordium being clearly shown. Well developed roots were found at 21 days, (Fig. 3-I).

In the description of regeneration at the proximal end the basipetal differentiation of xylem strands below the newly formed shoot primordia was described. These strands differentiated down to the distal end of the 2cm long cutting and can be clearly seen in the secondary phloem tissue of the distal-end sections at 7 days and later, (Fig. 3-G onwards). The connection of these strands with the xylem of the newly formed root is shown in Fig. 3-J and is seen to be independent of the earlier-formed xylem nodules.

Discussion

Results of the anatomical studies carried out in the present investigation show that initial stages of regeneration process is identical at the two poles of the root cuttings. At both ends indication of cell division was found after 24 hours. The site of dividing cells were adjacent to the central xylem core just below the cut surfaces. However, by 48 hours the paths of differentiation at two ends diverged. At the proximal end shoot meristems were initiated whereas at the distal end isolated pockets of dividing cells continued to appear, spreading laterally across the root away from the central xylem core.

Certain features of shoot primordium development are worth mentioning. The impression which one could get from the literature cited earlier, is that the shoots were initiated by 5-7 days from callus formed at the cut surface of the root cuttings. However the results of the present study show that shoots were initiated as early as 48 hours, after the formation of the callus.

An interesting comparision can be made between the present study and a description of root initiation in callus cultures of carrot phloem by Steward, *et al*, (1958). These workers have described xylem nodules surrounded by a narrow layer of "Cambium or pericycle like cells". These structures were closely similar to those described in the present study. They stated that root apex is formed in this cambium or pericycle

like region and it subsequently grows out through the tissue mass into the surrounding medium. Their interpretation is based on two photomicrographs, of sections through callus tissue. The first photomicrograph show a xylem nodule, while the second shows a well developed root associated with the xylem nodule. They have not shown the initial stages of root development as shown in the present study. It can be said, therefore, that the suggestion of Steward *et al*, (1958) regarding the site of root inita-tion is not based on substantial experimental evidence.

Satchuthananthavale (1966) has described the anatomy of *Taraxacum* root callus cultures. He has shown that callus, grown in a medium which leads to root initation contains large number of xylem nodules identical to those found at the distal end of the regenerating root cuttings. His description of toot development was rather similar to that of Steward *et al*, (1958) . In a further study (Satchuthananthavale & Booth-personal communication) the examination of several hundred sections of *Taraxacum* root callus failed to give any indication that the root apical meristem was initated in close association with the xylem nodule. Instead, young root primordia were distinguished in the callus at some distance from the nodule. This situation appears to be exactly the same as found in the distal region of root segments described in the present study.

Acknowledgements

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