CHAPTER 3

THE ORIGIN AND STRUCTURE OF ADVENTITIOUS ROOTS IN SWEET POTATO (*Ipomoea batatas* (L.) Lam.)

3.1 ABSTRACT

Morphological and anatomical studies demonstrated the root formation characteristics of sweet potato. In this study the presence and importance of preformed root primordia is recorded for the first time. On the vines preformed root primordia are present in sets of four to ten adjacent to the leaf bases. These roots originate from the procambium on both sides of the leaf gap. Macroscopically the root tips of preformed root primordia protruding through the cortex and epidermis of the stems are prominent. The preformed root primordia produce adventitious roots, which typically exhibit pentarch, hexarch or septarch steles. These nodal adventitious roots can develop into fibrous roots, pencil roots or storage roots. It is postulated that storage roots will under normal circumstances only originate from undamaged preformed root primordia on the nodes of cuttings, or on nodes of newly formed vines. "Secondary" adventitious wound origin roots with similar anatomical characteristics to the nodal adventitious roots can, however, originate in the absence of preformed root primordia. Such "secondary" adventitious roots with the potential to develop into strorage roots can be initiated from callus tissue at the cut end of the cutting; from nodes where preformed root primordia were excised or damaged; or on leaf petioles. Lateral roots originating from damaged root primordia, or directly from the adventitious roots, or from callus tissue, exhibit tetrarch steles and develop into fibrous roots without the potential to develop into storage roots. This understanding of the origin, anatomy and

morphology of sweet potato roots should improve production practices, which will contribute to improved crop establishment and increased yield.

3.2 INTRODUCTION

Sweet potato roots develop as adventitious roots (Togari, 1950). They normally arise from the under ground stem portion of a vine cutting used as planting material. Based on their external morphology Togari (1950) and Kays (1985) subdivided adventitious roots into “thick” and “thin” roots and also stated that adventitious roots on cuttings may differentiate into fibrous roots, pencil roots or storage roots. Kays (1985) indicated that if the environment is conducive “thick” roots develop into storage roots. Thickening growth of the storage roots occurs by the activity of the vascular cambium as well as anomalous primary and secondary cambial segments (Wilson, 1970 & 1982). Activity of all cambia results in the formation of thin-walled, starch storing parenchyma cells. The contribution of different cambia in the production of storage parenchyma varies among cultivars and appears to be a cultivar related characteristic. A high yielding cultivar will show extensive anomalous cambial activity compared to low yielding cultivars with limited anomalous thickening growth (Wilson & Lowe, 1973a). The proximal end of the storage root does not take part in the formation of the storage root and forms the storage rootstalk. The length of the stalk appears to be cultivar dependent. It is generally accepted in literature on this topic that “thin” adventitious roots develop into either fibrous roots which are generally less than 5 mm in diameter, or in some cases into pencil roots which are generally between 5 and 15 mm diameter. Based on their external morphology Lowe & Wilson (1974) sub-divided sweet potato adventitious roots into ‘thick’ pigmented storage roots, ‘thick’ pigmented pencil roots that do not form storage roots, and 'thin', white fibrous roots. All three types give rise to lateral fibrous roots. Pigmented roots were defined as roots distinguishable from white fibrous roots by the development of surface pigmentation (cream colored, pink or red) similar to those later occurring on tuberous roots (Lowe & Wilson, 1974). Apart from adventitious roots arising from the nodal part of the stem, wound roots develop from the callus tissue in response to the wounding effect after the cutting is made (Hartman et al., 2002). Spence & Humphries (1971) reported that leaves of sweet potato cut at the base of the petiole formed roots when planted in sand moistened with a dilute nutrient solution and kept in subdued light in the glasshouse.

Air and soil temperature, physical characters of the soil, and soil fertility influence sweet potato storage root formation and growth (Ravi & Indira, 1999). Night air temperatures seem to be the most critical factor for storage root growth, presumably due to greater translocation of sugar from the shoot to the roots at lower temperatures. Plants grown for 81 days with 2.5% oxygen in the root zone, produced more fibrous, non-storage roots (88.7% of the total dry mass) than the plants grown in a 21% oxygen root zone with only 10.9% (dry mass) non-storage fibrous roots (Chua & Kays 1981). Dry and compact soil hampered storage root formation (Watanabe *et al.,* 1968a, and 1968b; Sajjapongse & Roan 1982).

The factors determining whether adventitious roots will remain unthickened, develop into pencil roots, or develop into storage roots, remain unclear. Wilson (1973a & 1973b) conducted experiments using roots up to 3 cm long emerged from petioles. These rooted leaves were grown in a sand culture for ten to twelve days. Rooted leaves were then treated with four levels of nitrate-nitrogen (21, 42, 105, and 210 ppm). After eight weeks of growth in a greenhouse individual rooted leaves were scored for number of adventitious shoots, tubers and uniformly thickened tuberous roots. Those grown at 21 ppm NO_3-N produced tubers and uniformly thickened roots but no adventitious shoots. Rooted leaves grown at 42 and 105 ppm NO_3-N developed tubers on which single adventitious shoots were formed as well as uniformly thickened roots. Only a few adventitious shoots developed on the uniformly thickened roots in these plants. With the leaves grown at 210 ppm NO_3-N, very few tubers were produced, while adventitious shoots were formed in clusters of up to four on the uniformly thickened roots. This negative effect of high nitrogen levels on storage root development may be comparable to the negative effect of high nitrogen levels on tuberization in potato, which has been linked to the effect of nitrogen on levels of endogenous gibberellins (Hammes & Beyers, 1973).

According to Artschwager (1924) and Togari (1950) storage roots usually develop from thick roots with pentarch or hexarch steles and enlarged apical meristems. Roots which did not develop into storage roots included thin adventitious roots containing tetrarch steles with a central core of metaxylem elements and no pith (Artschwager, 1924) and a small apical meristem (Togari, 1950).

Although existing literature provide a reasonable amount of information on the classification and structure of sweet potato adventitious roots, some issues remain unclear. No information is available about the origin or the relationship between origin, structure and function of these roots. The objectives of the study were to:

- investigate the origin of adventitious roots of sweet potato;
- provide additional information on the structure of sweet potato roots; and
- find possible relationships between origin, structure and function of the roots.

3.3 MATERIALS AND METHODS

Mother plants of the cultivar Atacama were grown on the Experimental Farm of the University of Pretoria to provide stem cuttings for the observations on the origin and anatomy of the roots. The investigation consisted of three parts.

1. Observations were made on the undamaged root primordia on freshly cut vines. Transverse and longitudinal sections, using a sliding microtome with freezing equipment, were prepared for microscopic observations.

2. Terminal and middle parts of the vines were used to obtain adventitious roots. Freshly cut vines were placed in polyethylene bags and incubated at 20^{o} C. Roots obtained in this way were sampled regularly from day three to day 14. This technique ensured that the roots were free of sand or other substrate particles. In addition, pencil roots and storage roots from more mature plants in a greenhouse were also sampled for microscopic observation.

3. Leaf cuttings, and stem cuttings with the preformed root primordia removed, were planted in containers with sand. The containers were placed on a mist bed

for two weeks. Roots obtained from these cuttings were anatomically characterised.

For all the anatomical observations root segments were fixed in F.A.A., dehydrated in increasing ethanol concentrations and embedded in wax after substituting the alcohol with xylene (O'Brien & Mc Cully, 1981). Sections of 7 to 10 μ m, in thickness were made with a rotating microtome and stained in Safranin 0, counter stained in Fast Green, and mounted in Clearmount (O' Brien & Mc Cully, 1981).

3.4 RESULTS AND DISCUSSION

Preformed root primordia

Macroscopic longitudinal rows of small white protruberances are present on both sides of the leaf bases on the vines, even on young nodes close to the vine apex (Figures 3.1A & 3.1B). These protuberances occurred in sets of four to ten per node. It was confirmed microscopically that the protuberances were in fact root tips of preformed roots, protruding through the cortex and epidermis of the stem (Figures 3.1C & 3.1D). From the micrographs it is clear that the roots originate from the procambium on both sides of the leaf gap. Despite the macroscopic prominence of the preformed root primordia no reference to it could be found in the literature. The presence of the preformed root primordia is probably recorded for the first time. Hartman *et al*., (2002) reported the origin of preformed roots in a number of woody and herbaceous plant species, but sweet potato is not included. As in other plant species an outstanding feature of the preformed root primordia of sweet potato is that they will remain dormant until suitable growing conditions occur. Root primordia on older nodes on sweet potato vines are exposed to damage over a longer period of time, and necrotic root primordia can often be observed. Even the vines from plants produced in a glasshouse under favorable conditions exhibited preformed root primordia with necrotic tips as can be seen in Figure 3.1B. Microscopically there were indications that some root tips were actually aborted. In contrast with this the preformed root primordia on younger nodes near the vine apex are typically sound and healthy. This may partly explain the phenomenon that terminal cuttings tend to be more vigorous and productive than basal cuttings (Onwueme, 1978).

Root development from preformed root primordia

The root primordia of vines exposed to a humid atmosphere in polyethylene bags sprouted and produced clean white roots within 24 hours as illustrated in Figures 3.2A & 3.2B. This proved to be a valuable technique to obtain root material free from soil and ideal for microtome slides. The number of adventitious roots per node are determined by the number of performed root primordia per node, and the extent of damage and degeneration suffered by root primordia. The emerging adventitious roots were all more or less of the same diameter as illustrated in Figure 3.2, and lateral roots developing from the basal part of the adventitious roots can be confused with adventitious roots. In Figure 3.2C two lateral roots can be seen developing from the base of one of the adventitious roots. Adventitious roots can only emerge from healthy undamaged preformed root primordia, damaged preformed root primordia can not develop into adventitious roots as illustrated in Figure 3.2D. The presence of preformed root primordia and their ability to start growing within hours of being exposed to favorable conditions, explains the remarkable capacity of sweet potato cuttings to establish successfully in a short period of time.

Wound induced roots

Cuttings with wounds inflicted during the removal of preformed root primordia, as well as leaf cuttings, produced wound roots within two weeks as illustrated in Figure 3.3. Wound induced adventitious roots developing from the cut ends of the cuttings (Figure 3.3B) and from the cut ends of leaf petioles (Figures 3.3C & 3.3D) were found to be similar to the wound roots produced on the nodes where preformed root primordia were removed (Figure 3.3A). It is clear from the photographs that all wound roots were morphologically similar in appearance and originated from the callus tissue or directly adjacent to the callus. The development of wound roots on nodes with the preformed root primordia removed is probably reported for the first time. No reference to the capability of sweet potato cuttings to produce adventitious roots when the preformed root primordia are removed or damaged could be found. All adventitious roots have the potential to develop into pencil or storage roots. Some will remain without thickening throughout the plant’s growth period. The latter form of adventitious root was identified as thick roots by most authors, including Wilson & Lowe (1973a), Du Plooy (1989).

The typical root system of a sweet potato plant originating from a stem cutting is illustrated in Figure 3.4A, where the different types of adventitious roots, namely unthickend roots, a pencil root and a storage root are visible. Lateral roots develop on all the adventitious roots and even from the base of damaged preformed root primordia. Lateral roots of first, second and higher orders will develop to form the fibrous root system for absorbing and transporting water and nutrients.

In cultivars with pigmented storage roots, pigmentation of the root epidermis starts after approximately three weeks, with the result that in mature plants all the adventitious roots are pigmented, while lateral roots remain unpigmented.

Anatomical structure of sweet potato roots

It was observed from micrographs of transverse sections of adventitious roots and wound roots that storage roots and pencil roots arise from roots with poly-arch steles while the fibrous roots arise from lateral roots with tetrarch or pentarch steles. In Figure 3.4B a lateral root with central metaxylem element, typically pentarch in arrangement of the primary vascular tissue, is illustrated. The five xylem and phloem poles within the vascular cylinder can be seen. This arrangement of the vascular tissues and the beginning of a lignified stele indicate no potential to develop into a storage root. In the formation of the fibrous roots none or little normal secondary thickening growth with no anomalous secondary thickening growth with extensive lignification, occurs.

In this investigation it was clearly observed that all lateral roots developed from existing roots and were usually thin with tetrarch and infrequently pentarch steles. The primary laterals grew profusely downwards into the soil forming secondary laterals. In some cases tertiary laterals emerged from secondary laterals. These lateral roots of sweet potato form the water and nutrient absorbing system of the plant and are often referred to as the fibrous roots.

In Figure 3.4C an adventitious root, typically hexarch in the arrangement of the primary vascular tissues, (that is six xylem and phloem poles found within the vascular cylinder) and without the central metaxylem element, is illustrated. This anatomically structure is characteristic of roots with the potential to develop into storage roots. Adventitious roots, which is septarch in the arrangement of the primary vascular tissues (seven xylem and phloem poles found within the vascular cylinder), without the central metaxylem element, as illustrated in Figure 3.4D, also have the potential to develop into storage roots. In storage root development the cells between the protoxylem points and the central metaxylem cell do not become lignified, or only a small portion of these cells are lignified, as illustrated in Figure 3.5A. In storage roots, storage tissue formation occurs by normal secondary thickening growth, as well as anomalous secondary thickening growth, with little lignification. The development of storage roots from thick adventitious roots with poly-arch steles reported by Wilson & Lowe (1973a) is similar to the observations reported here. Wound roots with hexarch steles and with large central metaxylem elements is illustrated in Figure 3.5B. This is a typical structure indicating that the lignification of steles is not total and the root may develop into a pencil root. It was observed in this study that storage and pencil roots develop from both adventitious and wound roots with poly-arch steles. The development of adventitious roots from leaf cuttings reported by Spence & Humphries (1971) was also confirmed in this study. Pencil roots, as they were named by Wilson & Lowe (1973a), are derived from adventitious roots, with poly-arch steles.

According to Wilson & Lowe (1973a) thick roots are either pentarch, hexarch or septarch at the base and tetrarch nearer to the apical meristem, and contain a central pith with or without central metaxylem cells. However, in this study the number of xylem strands in the roots was found not to differ throughout the length of the root (Figure 3.5.C).

The morphological and anatomical observations in Chapter 3 emphasized the potential of sweet potato cuttings to quickly activate root formation, the literature revealed a gap of information on the rooting of cuttings, and factors affecting root establishment. The aspect of environmental factors such as temperature and soil moisture content on cutting establishment will be dealt with in Chapter 4.

3.5 CONCLUSION

Observations on the origin of sweet potato roots, their morphology and anatomy, indicated that adventitious roots normally develop from preformed root primordia on the nodes. Adventitious roots can also develop from wound callus on stems or petioles. All adventitious roots have poly-arch steles and can develop into pencil roots or storage roots. Lateral roots develop on unthickened adventitious roots, pencil roots and storage roots, mainly have tetrarch steles and comprises the fibrous roots. It is suggested that the confusing terminology in the literature referring to thick and thin roots resulted from superficial observations, with the “thin” roots being either lateral roots from the base of preformed root primordia, or young adventitious roots. Only by correctly identifying the origin of the root, or by considering the stele characteristics (tetrarch or poly-arch), can the young roots be identified as either adventitious roots (potential storage roots) or lateral roots (fibrous roots). More research is required to identify the factors determining whether an adventitious root will remain as it is, thickened into a pencil root, or develop into a storage root.

An understanding of the origin and nature of the sweet potato root system not only contributes towards scientific clarity, but should also assists agronomists to improve production practices. Thus the existence of preformed root primordia and their ability to produce adventitious roots within hours after exposure to favorable circumstances partly explains the remarkable capacity of cuttings to quickly establish even under less favorable circumstances. It also emphasises the advantage of using relatively young cuttings with undamaged root primordia as planting material. The optimum number of subterranean nodes when cuttings are planted will be affected by the number of undamaged preformed root primordia per node. With this new perspective on the origin of storage roots from preformed root primordia, basic studies to obtain more potential storage root sites by production practices like ridging, may yield interesting results.

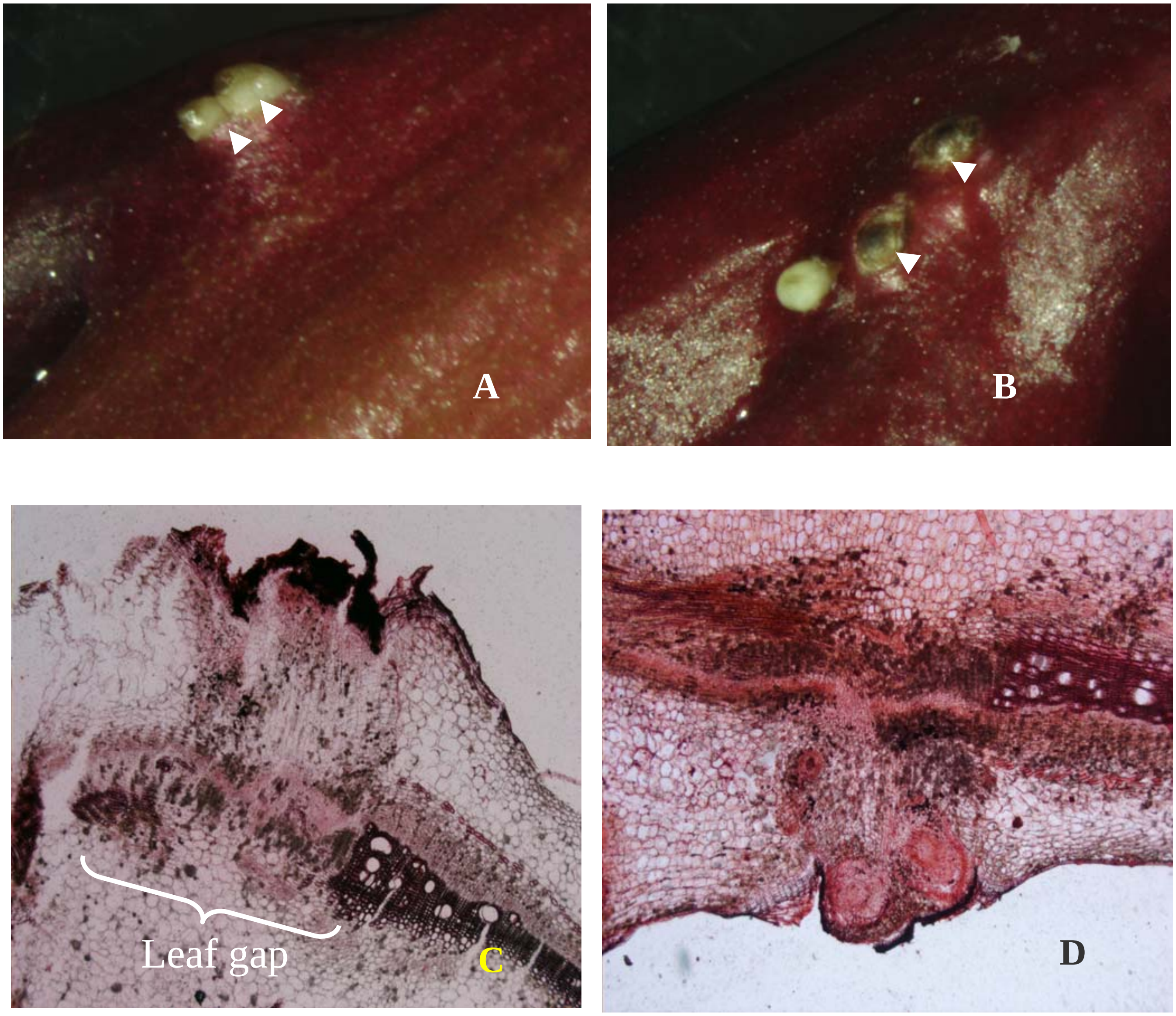

Figure 3.1. Nodal part of sweet potato vines showing preformed root primordia

A. Primordia on a young node close to the shoot apex.
B. Primordia on an older node near the base of the vine.
C & D Sections of nodes illustrating root tips of preformed root primordia protruding through the cortex and epidermis.

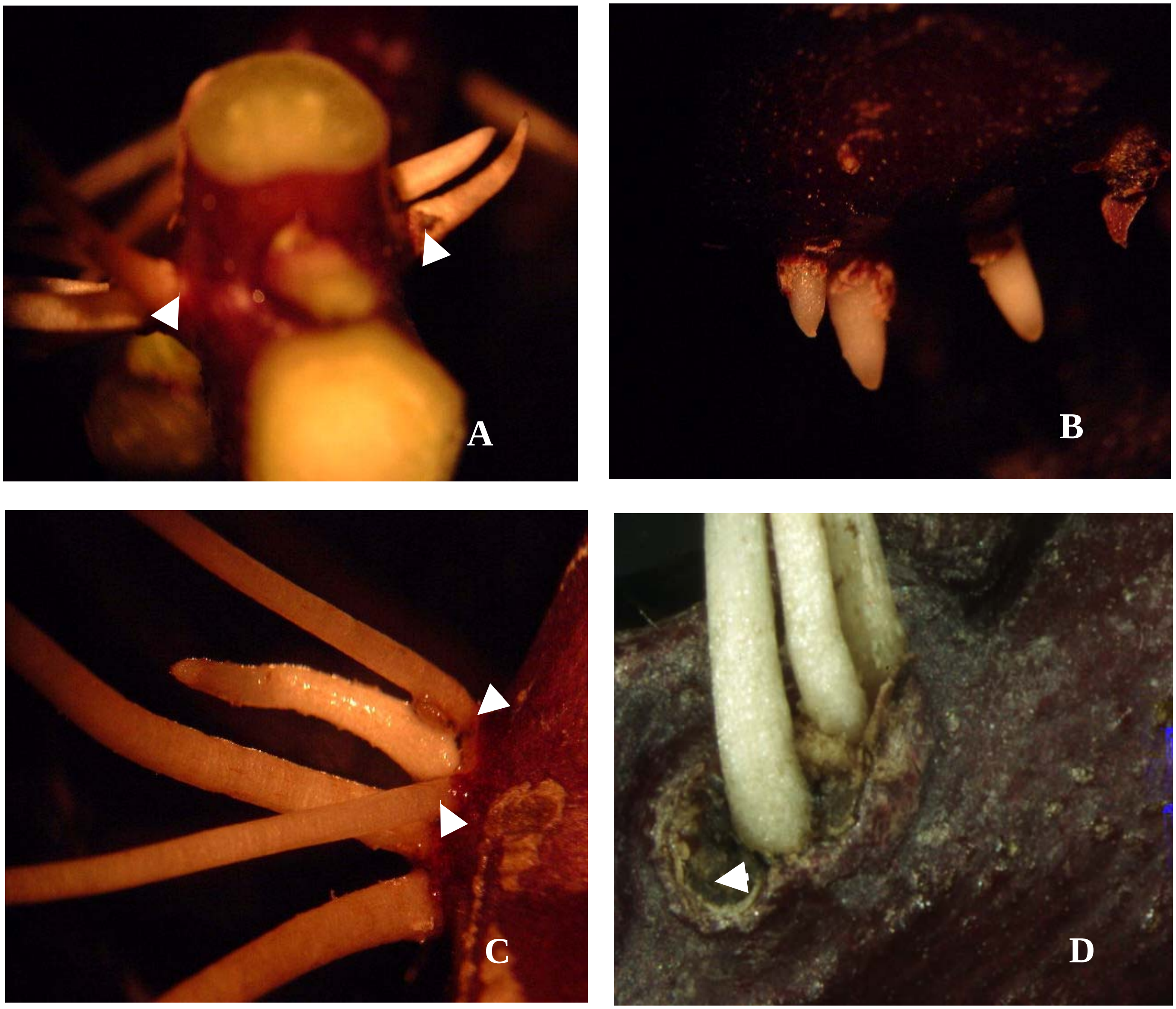

Figure 3.2 Root development from preformed root primordia

A & B. Exposed to a humid atmosphere in a polyethylene bag adventitious roots developed within 24 hours.

C. Three adventitious roots with two lateral roots growing from the base of an adventitious root after 72 hours in a moist atmosphere.

D. Three adventitious roots from undamaged primordia, and a damaged unsprouted primordium.

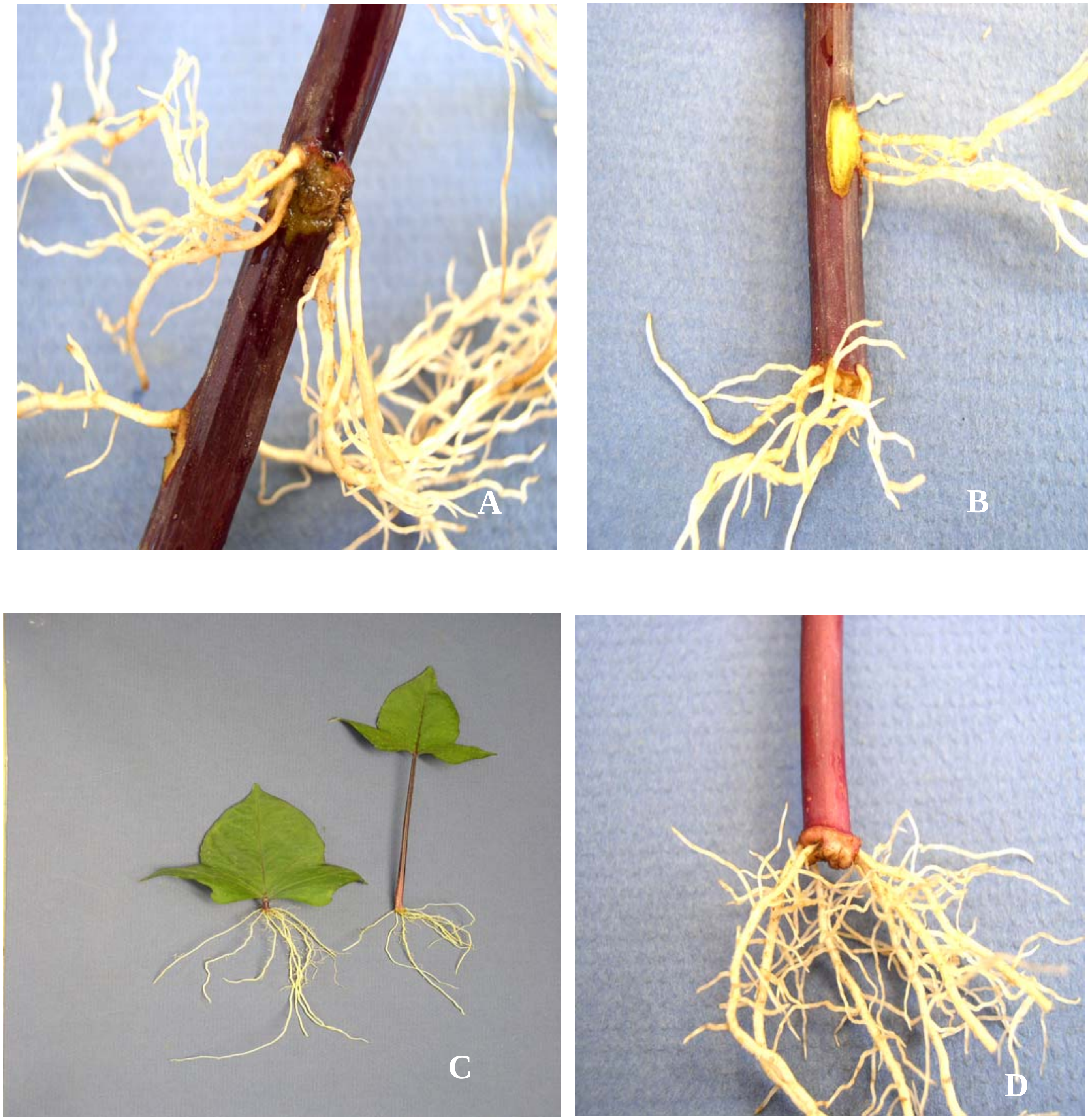

Figure 3.3. Cuttings of sweet potato showing wound induced adventitious roots

A. Adventitious wound roots where preformed root primordia were excised from cutting

B. Adventitious wound roots where the stem was cut (bottom).

C & D. Adventitious wound roots on leaf petioles

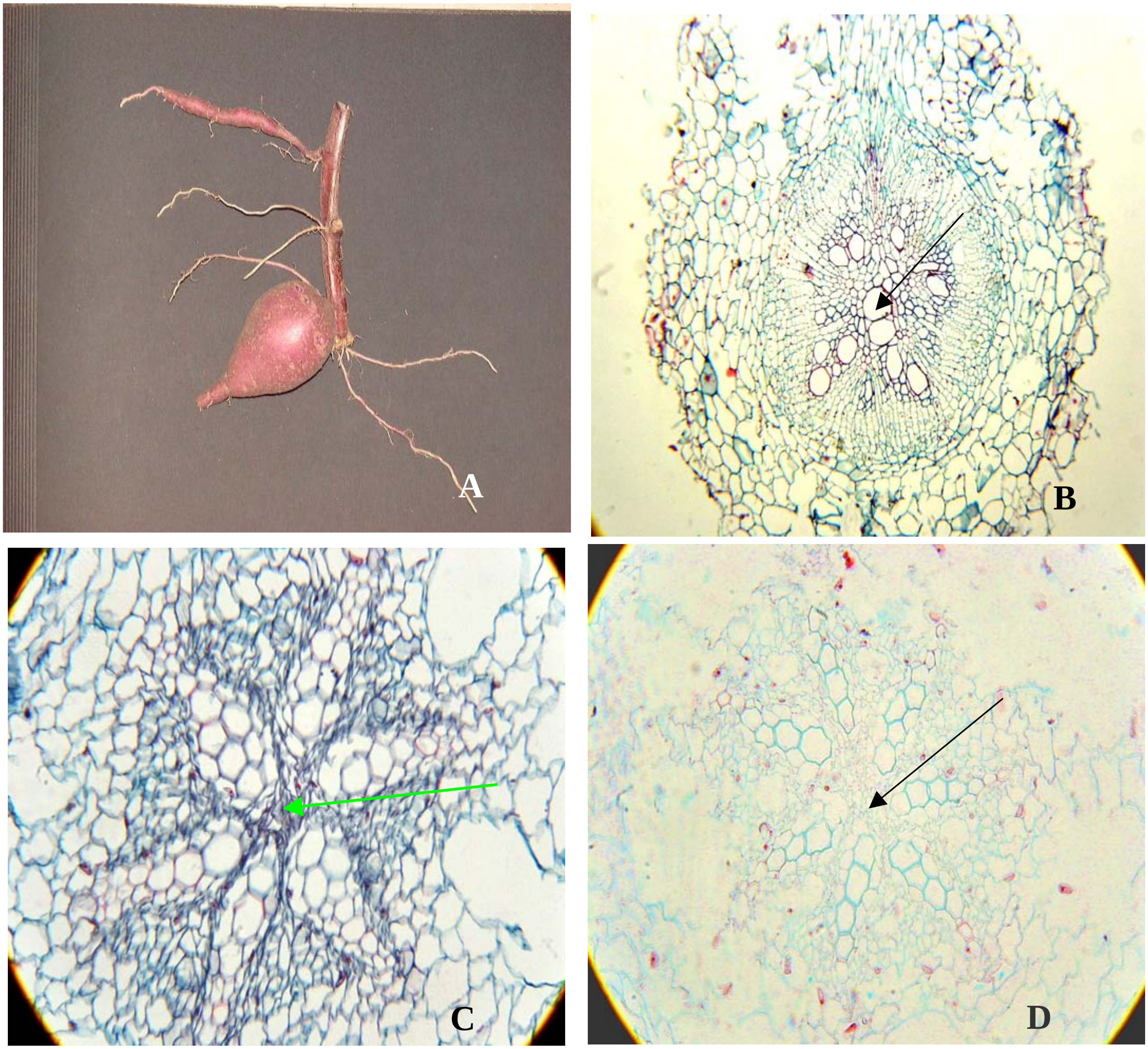

Figure 3.4. Illustration of the morphology and anatomy of the sweet potato root system

A. The general morphology of the roots. The root system of sweet potato consists of unthickened adventitious roots, pencil roots, and storage roots, all with lateral roots.
B. Micrograph of transverse section of a root with a pentarch stele and central metaxylem cell, indicating the beginning of a lignified stele. This is a typical structure of roots without the potential to develop into storage roots.
C. Micrograph of transverse section of a root with a hexarch stele without the central metaxylem. This is a typical structure indicating a root without potential to develop into a storage root.
D. Micrograph of transverse section of a root with septarch stele without the central metaxylem. Roots with this structure have the potential to develop into storage roots.

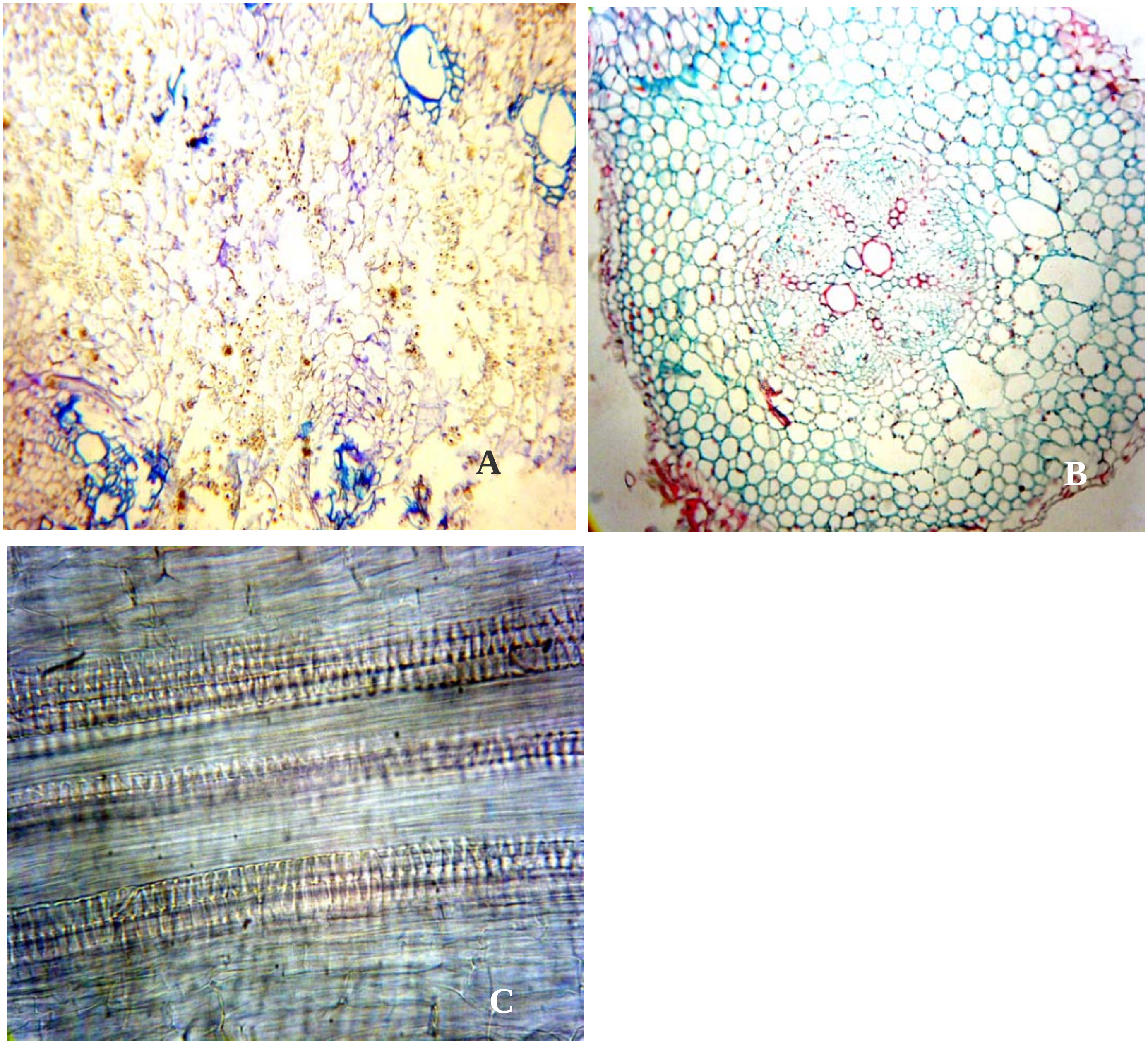

Figure 3.5. Micrographs of transverse sections of a young storage root, a wound root, and a longitudinal whole mount of a root

A. Magnified micrograph of transverse section of a young storage root illustrating storage tissue formation in the centre of the root
B. Micrograph of transverse section of a wound root with large central metaxylem elements and hexarch stele. Roots with this structure have the potential to develop into pencil roots
C. Micrograph showing portion of a clear whole mount of a longitudinal section root with three primary xylem strands parallel to each other. It was observed that the number of xylem strands remain unchanged throughout the length of the roots.

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