HISTOLOGY OF ADVENTITIOUS SHOOT AND ROOT FORMATION ON LEAF-PETIOLE CUTTINGS OF BEGONIA X HIEMALIS FOTSCH cv. 'APHRODITE PEACH'

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ABSTRACT

HISTOLOGY OF ADVENTITIOUS SHOOT AND ROOT FORMATION ON LEAF-PETIOLE CUTTINGS OF *BEGONIA* X *HIEMALIS* FOTSCH cv. 'APHRODITE PEACH'

By

Edward P. Mikkelsen

A histological investigation was undertaken to determine the origin of adventitious shoots and roots on leaf-petiole cuttings of the Rieger begonia, *B.* x *hiemalis* Fotsch cv. 'Aphrodite Peach'. The main objective was to ascertain whether shoots originated from single or multiple epidermal cells of the leaf petiole. Thus, the origin of non-chimeric plants from mutation induction might be properly interpreted.

Observations made with a light microscope of 10 micron sections of leaf petioles at various stages of shoot and root development showed that the epidermal and subepidermal parenchymal cells gave rise to a callus near the cut end of the petiole approximately two weeks after excision from the stock plant. Roots originated within this callus and also within parenchymal cells of the petiole three to four weeks after excision. Five to six weeks after excision, nodular protrusions, which gave rise to adventitious shoots, developed at the surface of the callus. On the basis of these observations, it was not possible to determine whether the cells

of the entire new shoots were the derivatives of single or multiple epidermal cells of the leaf petiole.

The histological information suggests that adventitious shoots may originate from more than one epidermal cell of the petiole; therefore, some chimeric plants from irradiated leaf-petiole cuttings would be expected. However, primarily non-chimeric plants have been obtained to date with such mutation breeding programs. The factors, aside from chance, which may be involved in ensuring non-chimeric adventitious plants above the soil line are 1) certain epidermal cells of the petiole are "chosen" to develop into adventitious shoots, and dipontic selection is only mildly occurring before adventitious shoot formation, 2) diplontic selection is rigorously occurring before shoot formation, and 3) diplontic selection is occurring after shoot formation and is causing the elimination of all but one cell type in each of the histogenic layers. The implications of these factors in a mutation breeding program are as follows: if certain cells are "chosen" to develop into adventitious shoots, and if diplontic selection plays a minor role, then a wide mutation spectrum, including semi-lethals, would be expected at relatively low irradiation doses; however, if dipontic selection plays a major role in determining which cells develop into adventitious shoots, then semi-lethals would be expected only at relatively high irradiation doses.

Edward P. Mikkelsen

Although there is no indisputable evidence for only one epidermal cell of the leaf petiole and its derivatives being involved in adventitious shoot formation, the fact remains that the majority of mutated adventitious shoots are non-chimeric. Therefore, irradiation of plant parts that give rise to adventitious shoots is still the best *in vivo* method of mutation breeding of vegetatively propagated crops.

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To Lynn, Tracy, and Mary

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INTRODUCTION

Cultivars of Rieger begonia, Begonia x hiemalis Fotsch, are triploid (Arends, 1970) and sterile. While the exact origin of the Rieger begonias is unknown, it is believed that they are the result of crosses between tetraploid B. x tuberhybrida (4x=56) with diploid B. socotrana (2x=28) (Doorenbos, 1973). Rieger begonias have 27 long chromosomes from B. x tuberhybrida and 14 short chromosomes from B. socotrana and hence, are 3x-1=41 (Arends, 1970). Because the Rieger begonias are sterile, new varieties cannot be produced by standard hybridization among themselves; either new crosses between B. x tuberhybrida and B. socotrana must be made, natural sports must be selected, or desirable mutations must be artificially induced. Since most varieties of Rieger begonias can regenerate adventitious shoots and roots from leaf-petiole cuttings, mutation breeding by subjecting leaf-petiole cuttings to mutagenic agents has been an effective means of producing new varieties (Doorenbos and Karper, 1975; Mikkelsen, Ryan, and Constantin, 1975).

As Broertjes (1968) has pointed out, the most effective use of artificial mutation induction is in propagation systems where an adventitious shoot originates entirely from

a single cell. In this regenerative process, and theoretically only under this condition, the majority of mutated adventitious shoots will be non-chimeric. If more than one cell is involved in the development of an adventitious shoot, and if a single cell of that group is mutated, then that portion of the shoot arising from that mutated cell will be genotypically different from the rest of the shoot--a chimeric condition. Of course, more than one cell in that group of cells giving rise to the adventitious shoot can be mutated; however, the probability of any two of them being mutated exactly the same is extremely low.

Many mutations resulting in chimeras are never detected. For example, if a mutation is a change in flower color, and if the mutated sector is a flower, then the mutation will be observed; but if that mutated sector is a leaf, then it will not be observed. If a mutated sector is detected, then that portion of the plant must be propagated asexually. If this new plant is still chimeric, the portion of the plant with the desired phenotype must again be asexually propagated. This process of selection and asexual propagation must be repeated until an entire plant of the desired phenotype is produced. Also, if a number of cells is involved in the formation of an adventitious shoot, there is the possibility of competition among these cells with respect to involvement in the formation of the shoot apical meristem. Cells incapable of dividing will not contribute to any growing

portion of the plant; whereas, those cells which are dividing relatively slowly could be displaced by faster dividing cells. This type of cell growth is termed diplontic selection. Because of these three disadvantages of chimerism (mutations which are not detected, the necessity of a few generations of asexual propagation to "true-up" the detected mutations, and diplontic selection), the efficiency of mutation breeding increases as the number of cells that are involved in the formation of an adventitious shoot at the time of mutation induction decreases. With fewer cells, the mutated sectors encompass a larger proportion of the plant and are more easily detected, and "trued-up". Also, there is less diplontic selection because fewer cells are involved.

Both Doorenbos and Karper (1975) and Mikkelsen et al. (1975) have produced mutations of B. x hiemalis Fotsch, by irradiation of leaf-petiole cuttings with x-rays, gamma rays or fast neutrons. Doorenbos and Karper (1975) and Mikkelsen (personal communication) have reported that a vast majority of the mutated shoots were not chimeric. From their results, it seemed logical that the adventitious shoots originated from single epidermal cells of the leaf petiole; however, there is no histological evidence reported to date to support or refute this assumption. Therefore, it was the purpose of this histological investigation of B. x hiemalis

Fotsch cv. 'Aphrodite Peach' to determine the origin of the adventitious shoots and roots, and especially to determine whether or not the shoots originated entirely from single epidermal cells.

LITERATURE REVIEW

The literature pertaining to histological studies of the origin of adventitious shoots and roots from leaf and leaf-petiole cuttings *in vivo* reveals that the process and location of organogenesis is different amongst the various species examined. The origin ranges from parenchymal and epidermal cells giving rise to shoots and parenchymal cells giving rise to roots, to epidermal cells giving rise to both roots and shoots. *Lilium* bulb scales (Walker, 1940) and *Peperomia* leaves (Harris and Hart, 1964) regenerate both shoots and roots endogenously. With *Sedum* leaf-petiole cuttings (Yarbrough, 1936), *Saintpaulia* leaf-petiole cuttings (Naylor and Johnson, 1937), and *Begonia rex* leaves (Hartsema, 1926), shoots develop from epidermal cells, and roots develop from endogenous, parenchymal cells. Likewise, with *Kalanchoe* (Broertjes and Leffring, 1972), *Streptocarpus* (Broertjes, 1968), and Achimenes (Broertjes, 1968), the shoots develop from "single" epidermal cells. With *Crassula* (McVeigh, 1938), the shoots and roots develop from cells of epidermal origin. A detailed description of the development of shoots and roots of *Lilium*, *Sedum*, *Saintpaulia*, and *Crassula* are given below.

In Lilium candidum and L. longiflorum, the shoots and roots of the detached bulb scale both originated from parenchymal cells (Walker, 1940). A few days after the bulb scale was removed from the bulb and planted, callus tissue formed over the cut surface. The cells at the cut edge had collapsed and the adjacent parenchymatous cells were embryonic with periclinal divisions. Later, divisions were along no particular plane. The actively dividing cells were distinguished by their small size, dense cytoplasm, and darkly stained nuclei. Rapid divisions of these parenchymal cells and the adjacent epidermal cells led to the development of the meristematic tissue of the bulblet primordia. A small bulblet appeared about two weeks after planting. After the development of a few leaf primordia, roots developed from callus near the traces of the bulb scale just below and lateral to the bulblet primordia. Root and shoot development continued independently until parenchymal cells between them became meristematic and differentiated into vascular tissue connecting the root and shoot. There were no vascular connections between primordia and bulb scale traces.

The development of adventitious shoots and roots in Sedum strahlii followed a different sequence of events (Yarbrough, 1936). The cells at the cut surface of the petiole collapsed within a few hours after being excised

from the plant. After approximately 30 to 48 hours callus arose from parenchymal cells of the petiole. About five days after excision, the callus had formed a large pad resulting from all the living parenchymal cells of the petiole having undergone divisions. At the same time, root primordia appeared within the interior of the callus. Shoot primordia appeared on the lateral surface of the callus within 10 to 12 days and were composed of both epidermal and subepidermal parenchymal cells of the callus tissue.

Saintpaulia ionantha represents a third mode of adventitious shoot and root formation (Naylor and Johnson, 1937). A few days after excision of the leaf from the plant, a callus tissue developed over the cut end of the petiole. Ten days to two weeks later, the petiole swelled unevenly around the wounded surface. Root initials derived from thin walled parenchymal cells between the vascular traces pushed out through the epidermis or down through the wound callus. There was no evidence that any of the roots originated directly from the wound callus. Redifferentiation of parenchymal cells between root primordia and petiole traces into vascular tissue served to connect the root primordia to the nearest petiole trace. Six to seven weeks after excision, shoot primordia began to form from the epidermis, one or two millimeters from the cut end. The first division of the epidermal cells was anticlinal. Further divisions were

randomly oriented. Again, redifferentiation of parenchymal cells into vascular tissue connected the shoot primordia with the nearest petiole trace. Each shoot was reportedly derived from a single epidermal cell, although adjacent epidermal and subepidermal cells of the petiole contributed to its final formation.

The development of adventitious shoots and roots in Crassula multicava represents another means of regeneration (McVeigh, 1938). Epidermal cells of the petiole began to divide within two days after excision from the plant. The first divisions were periclinal, later they were also anticlinal. The activity was not confined to a few cells; most of the cells around the entire petiole near the wounded surface divided. Groups of cells projected above the surrounding cells because of their more rapid divisions. New plants arose from these projected groups of cells. Initially roots were formed, and then vascular connections were made to the petiole traces by differentiation of parenchymal cells. Subsequently, the first pair of leaf primordia were formed. This represents a case where both roots and shoots arose from cells of epidermal origin.

MATERIALS AND METHODS

The cultivar of Rieger begonia chosen for this experiment was 'Aphrodite Peach', which is a product of mutation breeding of 'Aphrodite Rose' shoot tip cuttings. Aside from a different flower form and color, 'Peach' is capable of producing an average of about 60 adventitious shoots per leaf-petiole cutting under the optimal conditions of winter and about 20 under the suboptimal conditions of summer; whereas, 'Rose' produces about 10 adventitious shoots per petiole cutting in the winter and almost none in the summer (unpublished data).

Leaf-petiole cuttings at various stages of root and shoot development were obtained as needed from Mikkelsen's Inc., Ashtabula, Ohio, where they were grown under normal production methods as follows. Leaf-petiole cuttings were taken when the leaf petioles were about 1 to 1.25 cm long. The cuttings were planted in 5.5 cm. plastic pots containing a mixture of 45% peat, 45% perlite, and 10% soil, and the pH adjusted to 5.5. After being thoroughly "watered in", they were placed in the "rooting area" where the temperature was 22°C and the light intensity about 1200 ft-ca. They were kept moist by hand misting with a hose. After 4 weeks,

they were transferred to the "shooting area" where the temperature was maintained as close to 18°C as possible. The light intensity was increased to 6000-7000 ft-ca.

Upon receiving the developing cuttings they were rinsed in tap water to remove the soil. Any excess roots were cut off, the leaf and the upper part of the petioles were trimmed off, and the remaining pieces of petioles with the developing roots and shoots were dehydrated, stained and mounted using a modification of a technique described by Sass (1971). The basic procedure was as follows. The pieces were fixed in FAA for at least 24 hrs. Then they were dehydrated by soaking for 24 hrs. in each of six solutions of an n-butyl alcohol dehydration series, infiltrated, imbedded, and cast in "Paraplast'. Ten micron sections were microtomed, mounted on glass slides with Weaver's solution and dried on a hot plate at 55°C. The sections were then stained with safranin-fast green double staining. Usually, the slides were in safranin for 12 to 24 hrs. and in fast green for 2 to 5 mins. The slides were made permanent with 'Permount' and then were examined with a microscope. Photographs of selected areas were taken using fine grain 'Panatomic X' film.

RESULTS AND CONCLUSIONS

Microscopic observations made on serial sections of B. x hiemalis Fotsch cv. 'Aphrodite Peach' leaf-petiole cuttings at sequential times of development revealed the following sequence of events in the development of adventitious shoots and roots. At the time of excision, the parenchyma cells at the cut end of the petiole dehydrated and collapsed (Figure 1A). Within two weeks, the epidermal and subepidermal parenchymal cells at the petiole base had divided periclinally and anticlinally to form a small callus area (Figures 1B and 1C). This callus usually surrounded the entire portion of the petiole base; however, if the epidermis was not intact at some area of the petiole due to injury, callus formation by epidermal or subepidermal cells did not occur where the epidermis was injured (Figure 1D). Three or four weeks after excision, root primordia became evident as clusters or groups of cells below the surface of the callus divided rapidly, eventually becoming organized as root meristems (Figures 2A and 2B). The root primordia subsequently grew laterally through the callus and emerged at the surface of the petiole (Figure 2C). Roots were also initiated from internal parenchymal cells. These were

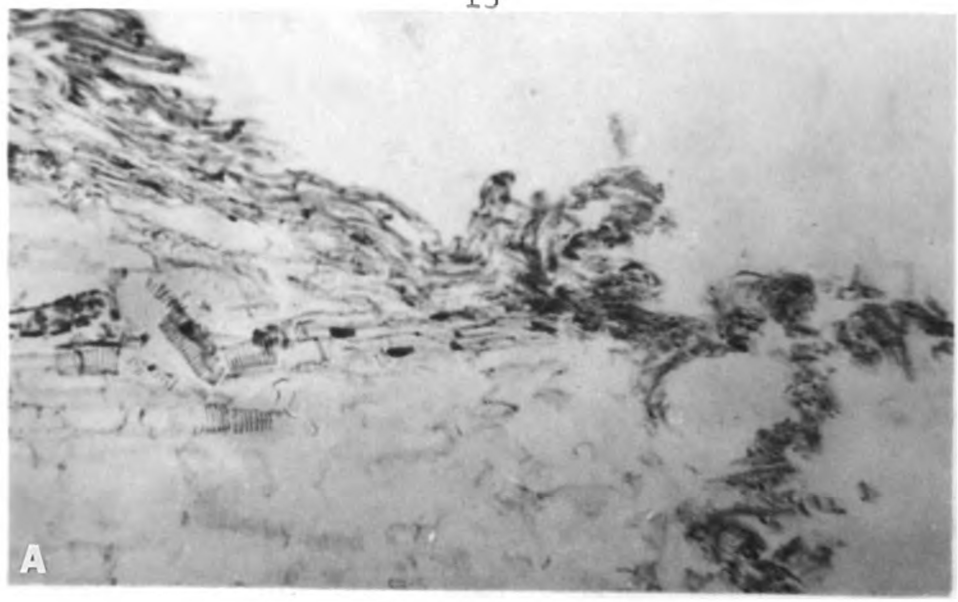
Figure 1. Initial stages of callus formation on Rieger begonia leaf-petiole cuttings.

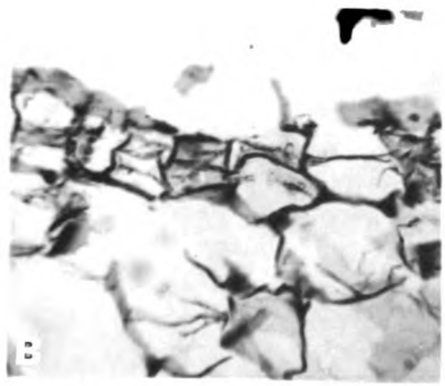
A. Longitudinal section (LS) of a petiole 2 days post-excision (PE) showing the collapsed cells at the cut end. 120X.

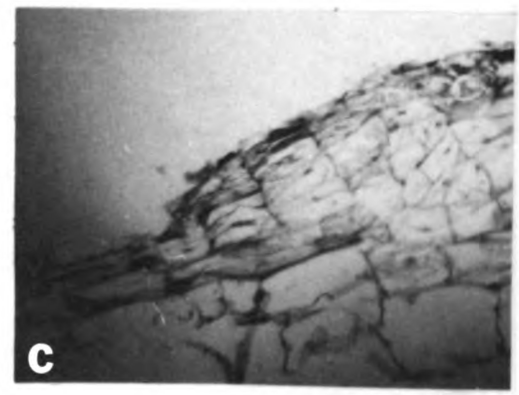
B. Cross section (CS) of a petiole 12 days PE showing the first periclinal divisions of the epidermal cells. 335X.

C. LS of a petiole 2 wks. PE showing periclinal and randomly oriented planes of division of epidermal and subepidermal cells. 30X.

D. External view at the cut end of a petiole 9 wks. PE showing the absence of callus and shoots where the epidermis was not intact. 12X.







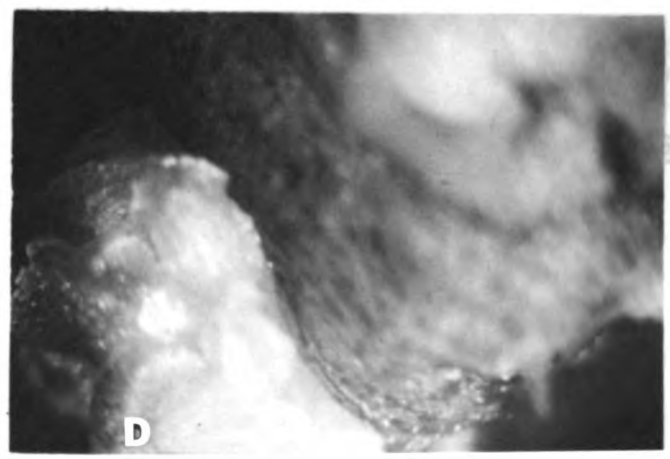


Figure 1

Figure 2. Root initiation and development. rp--root primordium.

A. LS of a root primordium 4 wks. PE. 335X.

B. LS of a more advanced root primordium 5 wks. PE. 335X.

C. LS of a root initial emerging at the callus surface 4 wks. PE. 120X.

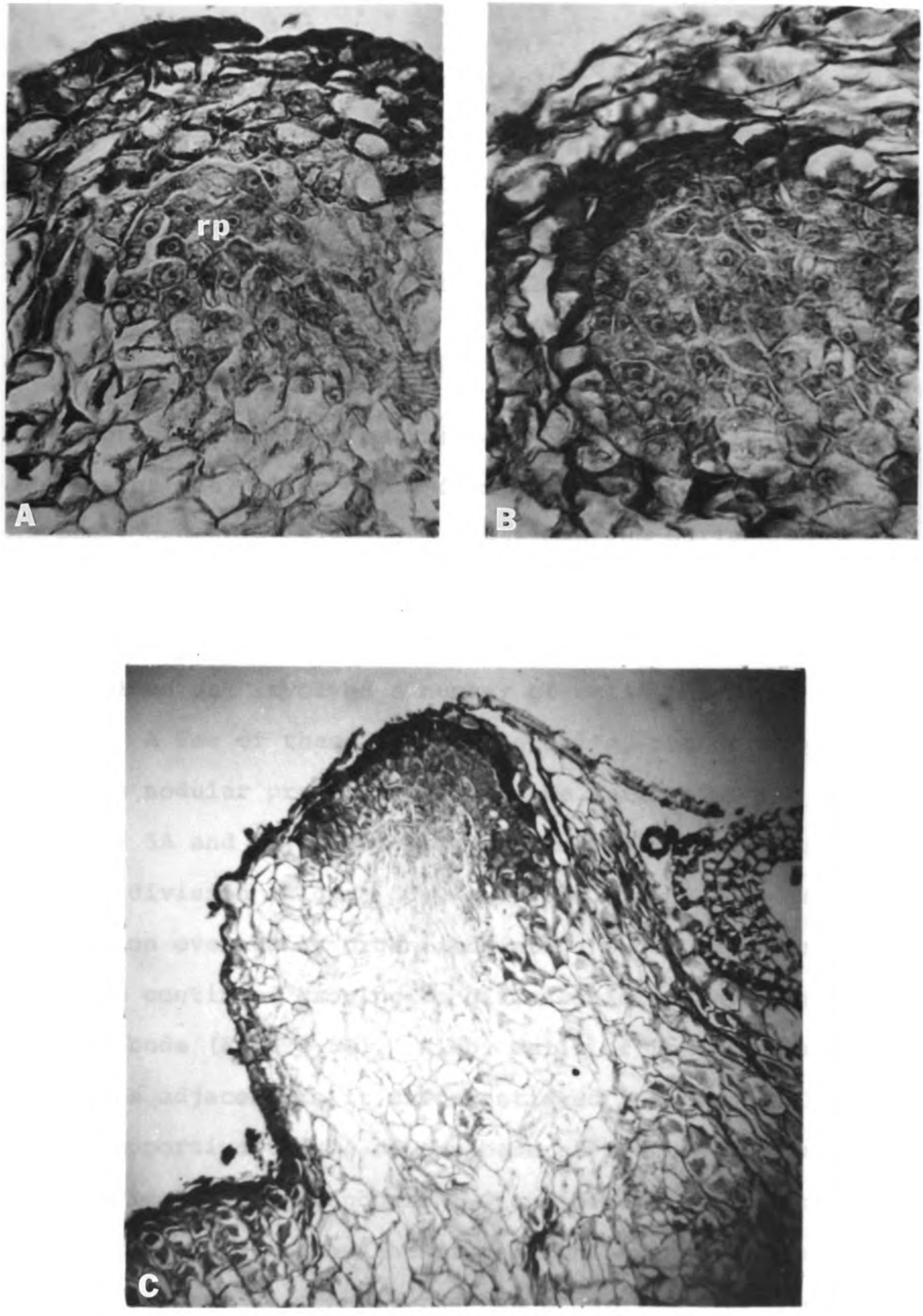


Figure 2

observed in the basal portion of petioles and were present even when the entire lower portion of the epidermis was injured and there was no epidermal callus (Figure 3A). Vascular strands between the roots and petiole traces were formed by redifferentiation of callus and parenchymal cells into vascular tissue (Figure 3B).

Shoot formation began 5 to 6 weeks after removal of the leaf-petiole cutting from the stock plant. Cells at the surface of the callus divided anticlinally forming a number of small daughter cells, and sometimes the callus cells directly below the surface also divided (Figure 4). This rapid division was not confined to a single cell and its derivatives but involved a number of cells and their derivatives. A few of these derivative cells, out of many, formed a nodular protrusion above the surface of the callus (Figures 5A and 5B). The size of the protrusion increased by cell division (Figure 5C), and some of the cells of the protrusion eventually organized into a shoot meristem. This meristem continued growing to differentiate leaves and lateral buds (Figure 5D). Also, cells of the protrusion and cells adjacent to it differentiated into bud scales and other supportive tissue of the base of the newly formed shoot (Figure 5E). Concurrent with the development of the protrusion, cells below the protrusion began to organize as vascular tissue to connect the new shoot vascular system with the leaf-petiole vascular system (Figure 5F).

Figure 3. Advanced root development. c--callus; p--parenchymal tissue; pt--petiole trace; ar--adventitious root.

A. External view 5 wks. PE showing a root initiated from a region lacking epidermis and callus. 15X.

B. LS of an adventitious root and CS of the leaf petiole showing vascular tissue connecting the adventitious root with a petiole trace. 32X.

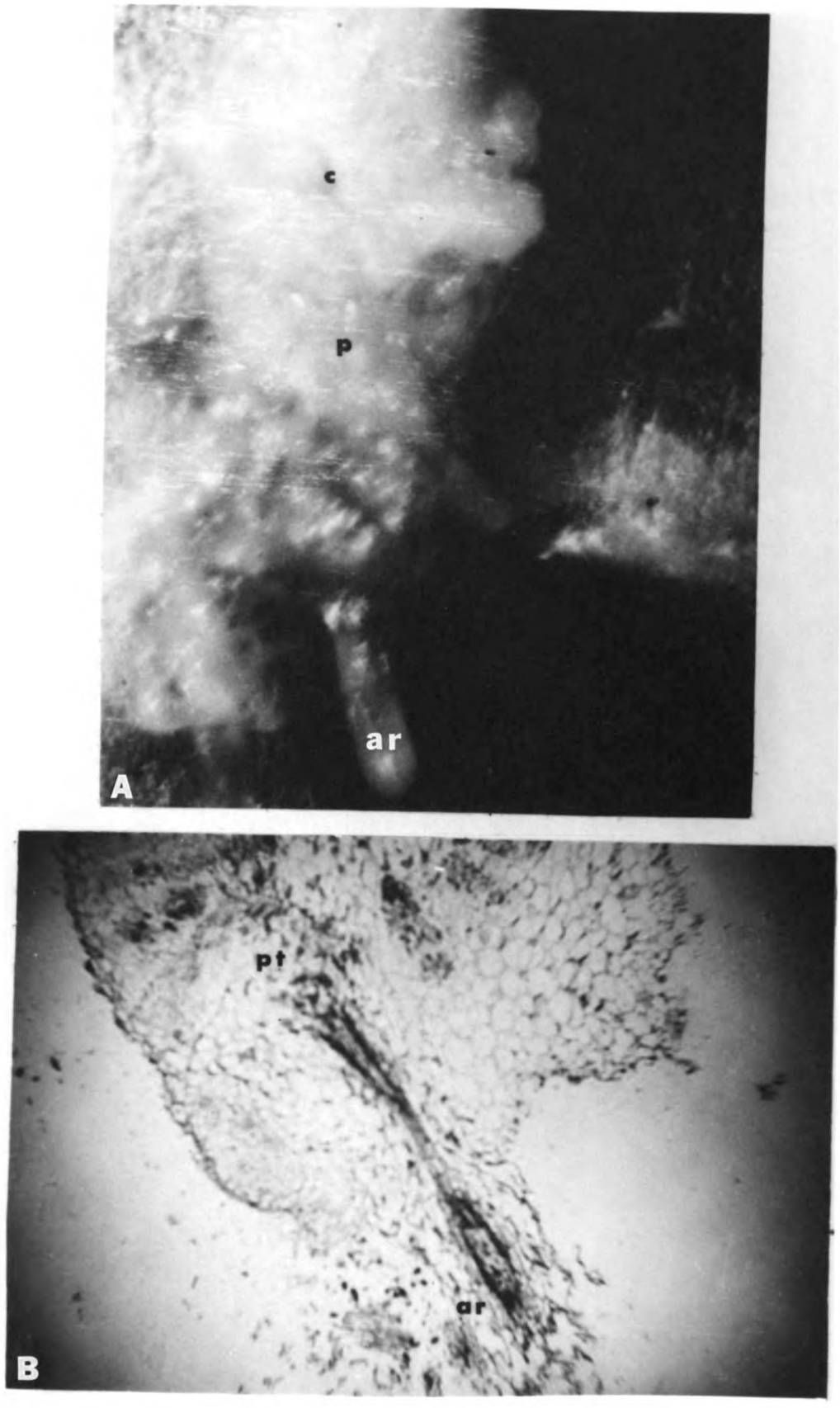


Figure 3

Figure 4. First stages of shoot formation.

A. CS of a petiole 5 wks. PE showing divisions of epidermal and subepidermal cells of the callus. 480X.

B. CS of another petiole 5 wks. PE, same as A. 480X.

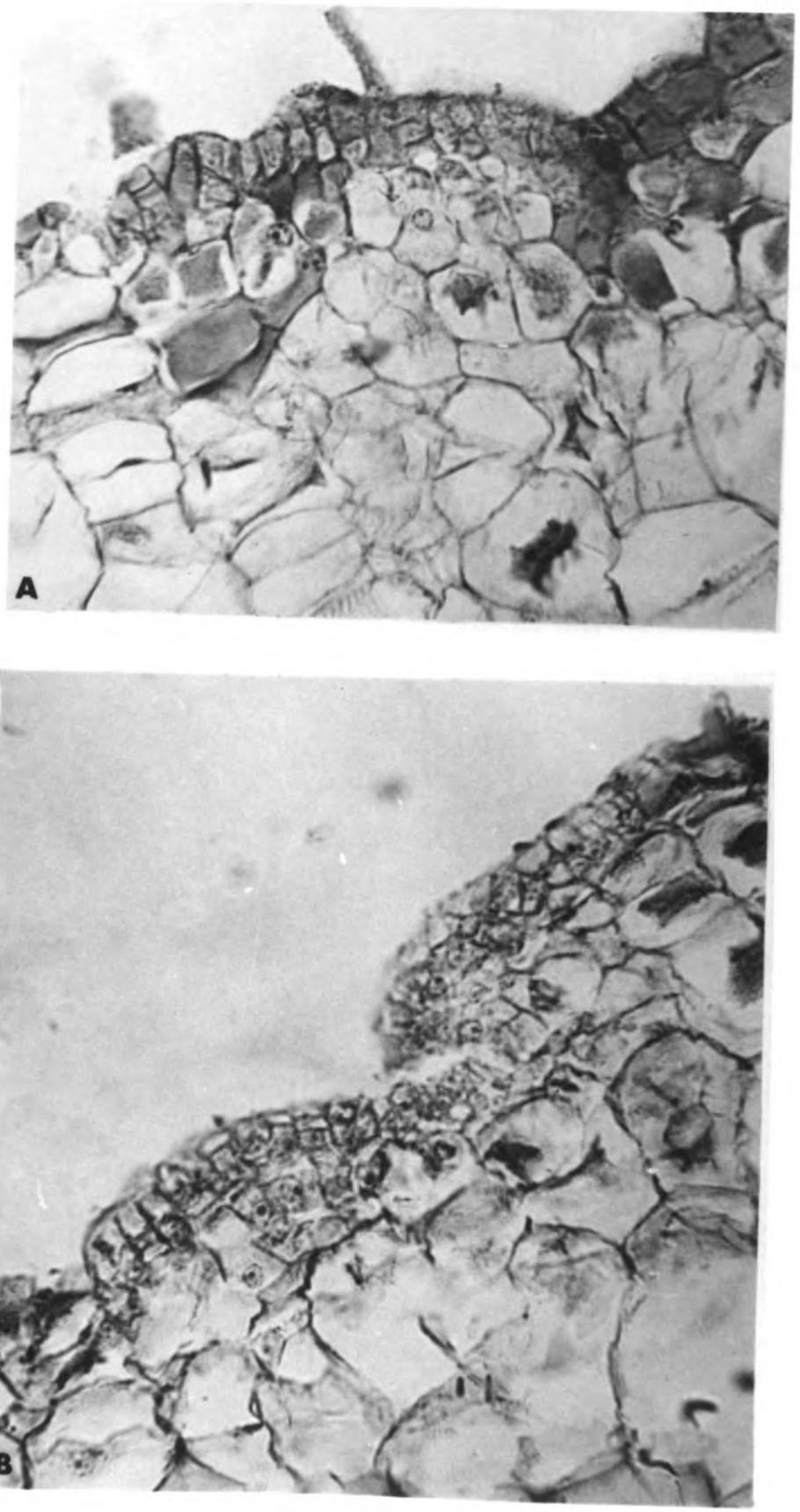


Figure 4

Figure 5. Advanced stages of shoot formation. l--leaf primordium.

A. LS of a slight protrusion 6 wks. PE showing anticlinal divisions. 335X.

B. LS of other protrusions above the callus 6 wks. PE. 480X.

C. LS of a more advanced protrusion 6 wks. PE. 335X.

D. Oblique section of an advanced initial 6 wks. PE showing a leaf primordium. The seemingly narrow connection to the callus is an artifact due to angle of the slice. 335X.

E. LS of a bud scale and a shoot primordium arising from the callus 6 wks. PE. 335X.

F. LS of a petiole 6 wks. PE showing the redifferentiation of callus cells below the surface to form vascular tissue. 335X.

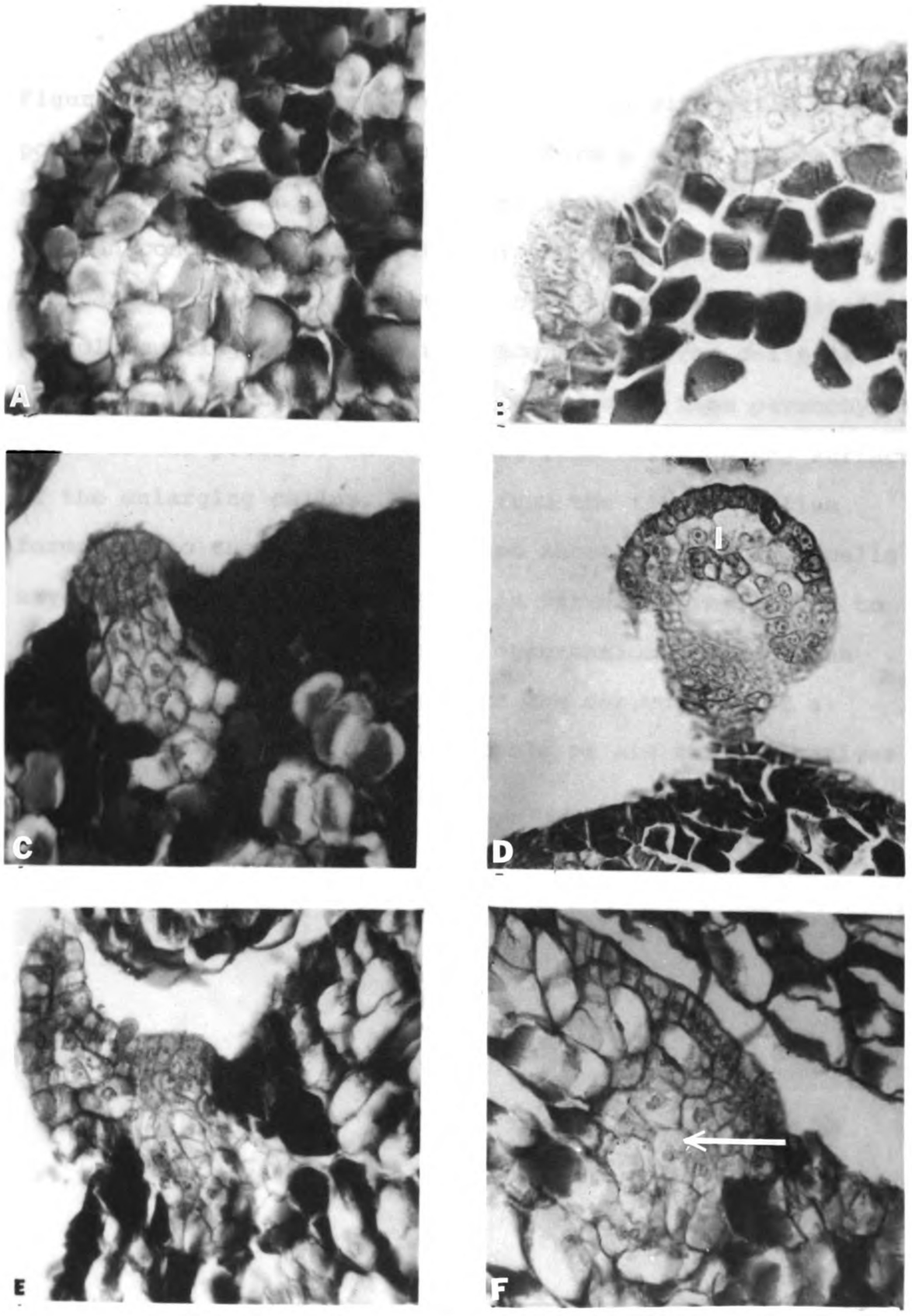


Figure 5

Figure 6A shows the formation of a callus with cells of a portion of the callus beginning to form a shoot initial. Figure 6B shows an advanced adventitious shoot.

In conclusion, the epidermal and subepidermal cells of the basal portion of the petiole of a Rieger begonia leaf-petiole cutting form a callus. Roots form from cells of the internal portion of the callus and also from parenchymal cells of the petiole. Shoots form from cells on the surface of the enlarging callus. Since from the time of callus formation to the time an organized shoot appears many cells have divided numerous times, it is virtually impossible to determine from the histological observations whether the cells of the entire new shoot are the derivatives of a single epidermal cell of the petiole or are the derivatives of a number of epidermal cells.

Figure 6. Summary view of shoot initiation and an advanced adventitious shoot. s--shoot initial.

A. LS of a petiole 6 wks. PE showing callus with the initiation of a protrusion to form a shoot. 120X.

B. LS of an advanced adventitious shoot 6 wks. PE. 120X.

Figure 6

DISCUSSION

Histological observations of leaf-petiole sections of 'Aphrodite Peach' suggest that adventitious shoots arise from a number of cells which may or may not be the derivatives of one epidermal cell of the petiole. Considering that 1) an epidermal cell divides numerous times in the formation of the callus, 2) that the cells of the callus surface divide several times before a protrusion develops, and 3) that only some of the cells of the protrusion eventually form the shoot apical meristem, it is possible by chance alone for the shoot apical meristem to be organized from cells, all of which are the derivatives of the same epidermal cell of the petiole. However, there appears to be no *a priori* reason why the shoot apical meristem could not be organized from cells which are the derivatives of several different epidermal cells.

The fact that the irradiation induced mutation work on leaf-petiole cuttings of *B.* x *hiemalis* Fotsch by Doorenbos and Karper (1975) and Mikkelsen *et al.* (1975; personal communication), have produced a low percentage of chimeric plants is evidence that the shoot apical meristem, at the time that the shoot emerges above the soil level, is

composed of an identical, or homogeneous, phenotype. However, it is not evidence that the entire adventitious shoot originated from a single epidermal cell of the petiole, nor is it evidence that the cells of the protrusion which organized into the shoot apical meristem were the derivatives of a single epidermal cell. The high percentage of non-chimeric plants only provides evidence that the shoot apical meristem is composed of a phenotypically homogeneous group of cells at the time that the shoot emerges above the soil line.

Naylor and Johnson (1937) originally reported that a single epidermal cell in Saintpaulia gives rise to the entire shoot except for the vascular strands which connect the vascular system of the shoot with that of the petiole. The most logical reason for the high percentage of non-chimeric plants which were obtained during radiation induced mutation breeding was that the entire plant came from one cell; therefore, the shoot apical meristem would obviously be genetically homogeneous (Sparrow, Sparrow, and Schairer, 1960; Broertjes, 1968). Actually, it seems that the published histological observations of both Naylor and Johnson (1937) and Broertjes (1968) do not indisputably support the hypothesis that the derivatives of only one epidermal cell are involved in the formation of the shoot apical meristem. The high percentage of non-chimeric mutants supports that

hypothesis, but does not prove it. As mentioned earlier, Walker (1940) has reported that a number of cells, both epidermal and parenchymal, are involved in the production of adventitious shoots in Lilium, but Broertjes (1972; personal communication) has stated that mutation breeding of lily bulb scales immediately after scaling produced mainly solid mutants as determined by flower color. Therefore, a high percentage of non-chimeric mutations is not sufficient evidence to show that adventitious shoot formation involves only a single cell. More recently Broertjes (personal communication) has stated that a number of epidermal cells are dividing at the time of adventitious shoot formation in Saintpaulia. Therefore, the fact that a number of cell types may be involved in the formation of an adventitious shoot in 'Aphrodite Peach' and concomitantly there is a high percentage of non-chimeric induced mutations is not an isolated occurrence. The question which arises is what processes are involved during adventitious shoot formation and subsequent growth of the shoot which usually insures a homogeneous group of cells in the shoot apical meristem at the time of shoot emergence above the soil level and subsequent flowering. There are a number of possible answers to this question, and one or all of them may be correct.

Although more than one epidermal cell of the petiole divides at the time of shoot formation, it seems likely that

one cell out of a group will divide first. As Broertjes (personal communication) states it:

> Many epidermal cells divide and form a number of meristems. In Saintpaulia, the number of epidermal cells per meristem is approx. 100-200. One cell, however, is the first one and this probably becomes the center of this group and grows out into a series of vegetative daughter cells. On top of this meristem one or a few cells then form the apex of the adventitious shoot and because of the restricted number of cells involved in it, the chance is very large that they are genetically identical, thereby forming a genetically homogeneous plantlet. The correct definition consequently is: the apex of an adventitious shoot ultimately originates from a single epidermal cell.

Broertjes (1972) further states that the cells which form the center of activity are somehow "chosen". Two explanations are apparent in his hypothesis. First, the cell whose derivatives will form the shoot apex are "chosen", and second, because of the number of cell divisions and of the restricted number of cells forming the apex, the homogeneity of the cells in the shoot apex is a matter of chance.

Another possible explanation for the high percentage of non-chimeric plants is that diplontic selection is taking place at the time of shoot formation. Cells which exhibit less physiological damage after irradiation treatment divide faster and contribute considerably more cells to the development of the callus. When the cells at the surface of the callus begin to divide to form the protrusion, the most vigorous cells divide faster and form the protrusion. Broertjes (1972) stated that diplontic selection is working

to a lesser degree before and including the time of the organization of the shoot apical meristem. The basis for his conclusion is that he has observed dwarf mutants even at low levels of irradiation when most cells probably were not mutated. The non-mutated cells were possibly more vigorous than the dwarf mutant cells; hence, the non-mutated ones should have successfully competed against the dwarf mutants if diplontic selection were taking place. However, there is no a priori reason to believe dwarfism is a semi-lethal characteristic resulting from cells which are physiologically less vigorous. If dwarfism were solely the result of less cell elongation, the cells could divide as rapidly or could even divide more rapidly than the non-mutated cells. The emergence of a chlorophyll deficient mutant or slower developing mutant would be more convincing evidence that diplontic selection was occurring to a lesser extent during the early stages of shoot development.

Diplontic selection is an accepted theory in the growth of a developing meristem; therefore, it is a possible explanation of how the shoot apical meristem could be composed of homogeneous cells at the time of emergence above the soil line. If a number of cell types were involved in the development of the organized meristem, then diplontic selection could conceivably result in elimination of all but one cell type in each of the histogenic layers. Sometimes,

mixtures of cell types in the layers of the apical meristem persist which ultimately result in variegation patterns of the leaves, shoots, or flowers. Such mutations have indeed been reported by Sparrow, Sparrow, and Schairer (1960) in their work on Saintpaulia. For some reason, they did not refer to these mutations as being chimeric.

Any or all of the above explanations may be the real reason why a shoot apical meristem emerging above ground is composed of a homogeneous group of cells when more than one adjacent epidermal cell of the petiole have divided so that by chance alone some chimeras would be expected. Cells could be "chosen" by some unknown factor, selected against only mildly before and at the time of meristem formation, and then if more than one cell type did occur in any of the histogenic layers of a meristem, all but one type in each layer would be eliminated by diplontic selection. As with most experimental observations, the combined information obtained from this histological study and from the irradiation mutation breeding projects of Doorenbos and Karper (1975) and Mikkelsen et al. (1975) are open to a number of possible explanations. What are the implications of these explanations for practical mutation breeding? If cells are indeed "chosen", and diplontic selection is less strict during the initial stages of development, then under relatively low irradiation doses a wide mutation spectrum,

including semi-lethals, would be expected. If diplontic selection is occurring during the early stages of shoot development as it does after shoot meristem formation, then a narrower mutation spectrum, excluding semi-lethals, would be expected. If the semi-lethals are desired, then they might be produced by subjecting the petioles to a high dose of irradiation so that most cells are damaged to the extent that they cannot divide, thus allowing the lesser damaged semi-lethal cells to develop into shoots. Regardless of the explanation for observing few chimeras, the fact is that few chimeras are produced by irradiating leaf-petiole cuttings; therefore, irradiation of plant parts that will give rise to adventitious shoots is a very efficient means of inducing desirable mutations in asexually propagated higher plants.

SUMMARY

Epidermal and subepidermal cells of the leaf petiole formed a callus at the basal portion of the petiole of Rieger begonia cv. 'Aphrodite Peach' leaf-petiole cuttings. Roots arose from cells of the internal portion of the callus and also from parenchymal cells of the petiole. Shoots formed from cells on the surface of the enlarging callus. Since from the time of callus formation to the time an organized shoot appeared many cells have divided, it is virtually impossible to determine from the histological observations made herein whether the cells of the entire new shoot are the derivatives of a single epidermal cell of the petiole or are the derivatives of a number of epidermal cells.

Adventitious shoots from irradiated leaf-petiole cuttings of B. x hiemalis Fotsch have been mainly non-chimeric. Several factors may be involved in ensuring non-chimerism when a number of cell types are dividing. First, the cell to give rise to the cells of the adventitious shoot may be "chosen". Second, diplontic selection may be occurring during callus and protrusion development. And third, diplontic selection may be occurring after the meristem has

organized and before the shoot emerges above ground. If diplontic selection is occurring to a lesser degree during the early stages of development because certain cells are "chosen", then a wide mutation spectrum including semi-lethals would be expected at low irradiation doses. If diplontic selection is occurring at the early stages of development, then semi-lethals, if desired, may be obtained only at high doses of irradiation.

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