AN ANATOMICAL STUDY OF THE ORIGIN AND DEVELOPMENT OF THE BULBLETS ON THE SCALE LEAVES OF LILY BULBS

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Brenda Frances Godden

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AN ANATOMICAL STUDY OF THE ORIGIN AND DEVELOPMENT OF THE BULBLETS ON THE SCALE LEAVES OF LILY BULBS

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BRENDA FRANCES GODDEN

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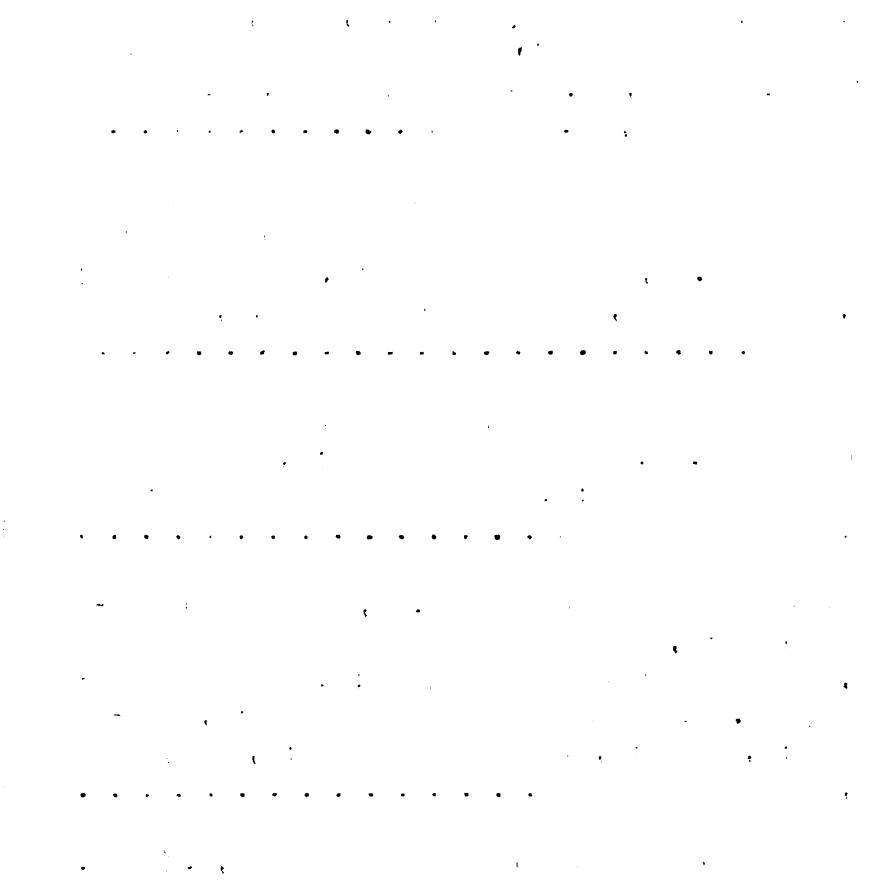

INTRODUCTION

Scale propagation in the genus Lilium has been an accepted procedure for both the commercial and amateur grower for more than half a century (Wallace 1879, Griffiths 1930, Emsweller 1957, Rockwell et al. 1961). Most rapid regeneration in the form of bulblets has occurred on scale leaves detached from the parent bulb following incubation of the scales in a moist medium at a temperature of 70 degrees F.

REVIEW OF LITERATURE

Priestley and Swingle (1929), in a comprehensive anatomical review of vegetative propagation, maintained that it was necessary to distinguish between the further development of a dormant, but already organized, meristem and the initiation of such a meristem as the result of developmental changes caused by mechanical isolation of a portion of the plant. They indicated that each species was distinctive in its behavior and required individual study before its performance as a self propagating unit could be determined.

Later anatomical investigations (Clamp 1934, McVeigh 1938, Naylor 1932, Naylor and Johnson 1937, Walker 1940, Yarbrough 1932) described the origin of regeneration within selected species and clarified this distinction between dormant or reassumed meristematic activity as was proposed by Priestly and Swingle.

Dormant meristems have been shown in Kalanchoe and Bryophyllum. Clamp (1934) showed residual primary meristems derived from the original apical meristem in Kalanchoe tubiflora. Similarly, entire dormant meristems are said to be present in Bryophyllum calycinum (Naylor 1932, Yarbrough 1932).

In contrast to this type of origin the mature cells in some other genera have been shown to reassume meristematic activity. A new individual arose exogenously by the divisions of a group of mature epidermal cells in Crassula multiclava (McVeigh 1938). In Saintpaulia ionantha the origin of the shoot was similar. It arose exogenously from epidermal cells whereas the root, in contrast, arose endogenously from parenchymatous tissue (Naylor and Johnson 1937).

In Lilium candidum and Lilium longiflorum the origin of regeneration was entirely endogenous with both root and shoot arising as a result of divisions of parenchyma (Walker 1940).

With the exception of Bryophyllum and Lilium, however, vascular connections were developed between the parent leaf and the new individual. In Lilium the vascular system of the young bulblet was indicated to be entirely independent of the parent bulb scale and at no time was any vascular connection observed (Walker 1940).

A phenomenum of Lilium is that regeneration only occurred after de-

tachment of the scale from the parent bulb. Hence regeneration might be associated with wound healing. Priestly and Woffenden (1922) formulated the following three point sequence of wound healing: "1) The wounded parenchyma surface becomes blocked by a deposit of suberin or cutin formed in the presence of air; 2) sap accumulates at the parenchymatous surface; 3) phellogen activity develops amidst this parenchyma and gives rise to callus tissue. In every case the essential antecedent to meristem formation is the blocking of the cut surface."

For the purpose of the present investigation it became necessary to make a clear distinction between callus tissue and periderm in relation to wound healing. Lauer and Krantz (1957) introduced a new interpretation following their study on wounding of Solanum tuberosum. They proposed that callus tissue was produced by an increase in cell number and that the resultant cells were capable of continued expansion and the production of buds. Periderm was suggested to be the result of re-differentiation of existing cells without division and without the capacity to produce buds. Further differences were: the relatively slow formation of callus when compared with the formation of wound periderm; callus formed only in non-dormant tubers, whereas periderm developed in both dormant and non-dormant tubers; and callus developed only after bud removal.

MATERIALS

The following three cultivars*: Olympic hybrids, Fiesta hybrids, and Bellingham hybrids, were selected because of their different, yet representative, external morphology of bulbs within the genus Lilium. The Olympic hybrids were selected forms of Lilium leucanthum centifolium. Fiesta hybrids were developed by a combination of Lilium dauricum seedling and Lilium davidii together with Lilium amabile and Lilium amabile luteum. Bellingham hybrids have resulted from hybridization of the native North American species (De Graff 1951).

*Supplied by the courtesy of Jan de Graff, Gresham, Oregon.

PROCEDURE

Eight outer scales were removed from bulbs of each of the three cultivars. The detached scales were dusted with a fungicide (Captan) to prevent basal scale rot and were laid horizontally in damp peat in wooden flats lined with polyethylene to retain the moisture. The bulb scales were incubated in darkness in moist peat at 70 degrees F for six weeks.

Three sample scales were collected at random at intervals of three days, examined for external features, cut into small portions and prepared for microscopic examination. The material was killed in a formalin-acetic acid - alcohol (5:5:90), dehydrated with ethyl alcohol, embedded in paraffin, sectioned 10 micra in thickness, and stained with safrannin and analin blue or with safrannin and fast green (Johansen: Plant Microtechnique). Sections were cut primarily in a direction parallel to the longitudinal axis and approximately two hundred and fifty preparations were mounted permanently in Canada Balsam.

RESULTS

EXTERNAL MORPHOLOGY

Bulbs:

The bulbs of the genus Lilium have a characteristic structure of spirally arranged, overlapping, fleshy scale-leaves arising upon a condensed disc like stem. Bulbs of Olympic and Fiesta hybrids were of similar appearance and were from 8 to 10 cms. in diameter. The Olympic bulb was a deep red color and the Fiesta bulb was normally white with some red pigment when exposed to light (a, b, Fig. 1). The Bellingham hybrids possessed two or more distinct bulbs arising from a rhizomatous stem base characteristic of the wild species from which they are derived (c, Fig. 1).

Scale leaves:

The outer scales of the Olympic hybrids averaged 6 cms. in length and 3 cms. in width. They had a sessile base and were elliptically concave toward the inside, tapering to an acute apex. The scales of Fiesta showed greater variation in shape, had a similar sessile base but an acuminulate tip. Parallel longitudinal venation was evident in some scales. Outer scales of the Bellingham hybrids were two-jointed, the distal section with a tapering shape comparable to the Olympic hybrid except for an average length of 3 to 4 cms. and width of 1.5 cms.

Bulblet development:

Bulblet development was found to be exactly similar in all three cultivars. The production of a proliferated callus over the wound surface was observed externally within 5 days. By 10 days distinct protuberances became evident on the callused surface and with leaf differentiation these protuberances became distinguishable as bulblets (Fig. 3). Throughout the incubation period the parent scale leaf remained firm and fleshy.

Bulblet development occurred predominantly, though not exclusively, on the adaxial surface (d, e, f, Fig. 1). Fiesta hybrids exhibited some capacity to produce bulblets on the margins of the scale leaf (f, Fig. 1). When veins were visible to the naked eye it was evident that bulblets were contiguous to the bases of these veins.

It was observed that new bulblets became visible over a period of six weeks so that, in the final collection, bulblets were at all stages of growth and development. Of the three cultivars, the Bellingham hybrids showed the greatest regularity in the time of development.

After six weeks the average number of bulblets on each scale of the different cultivars was: Olympic 1.9, Fiesta 3.0, and Bellingham 1.7.

INTERNAL MORPHOLOGY

Scale leaves:

The somewhat crescent shaped scales were surrounded by an irregular

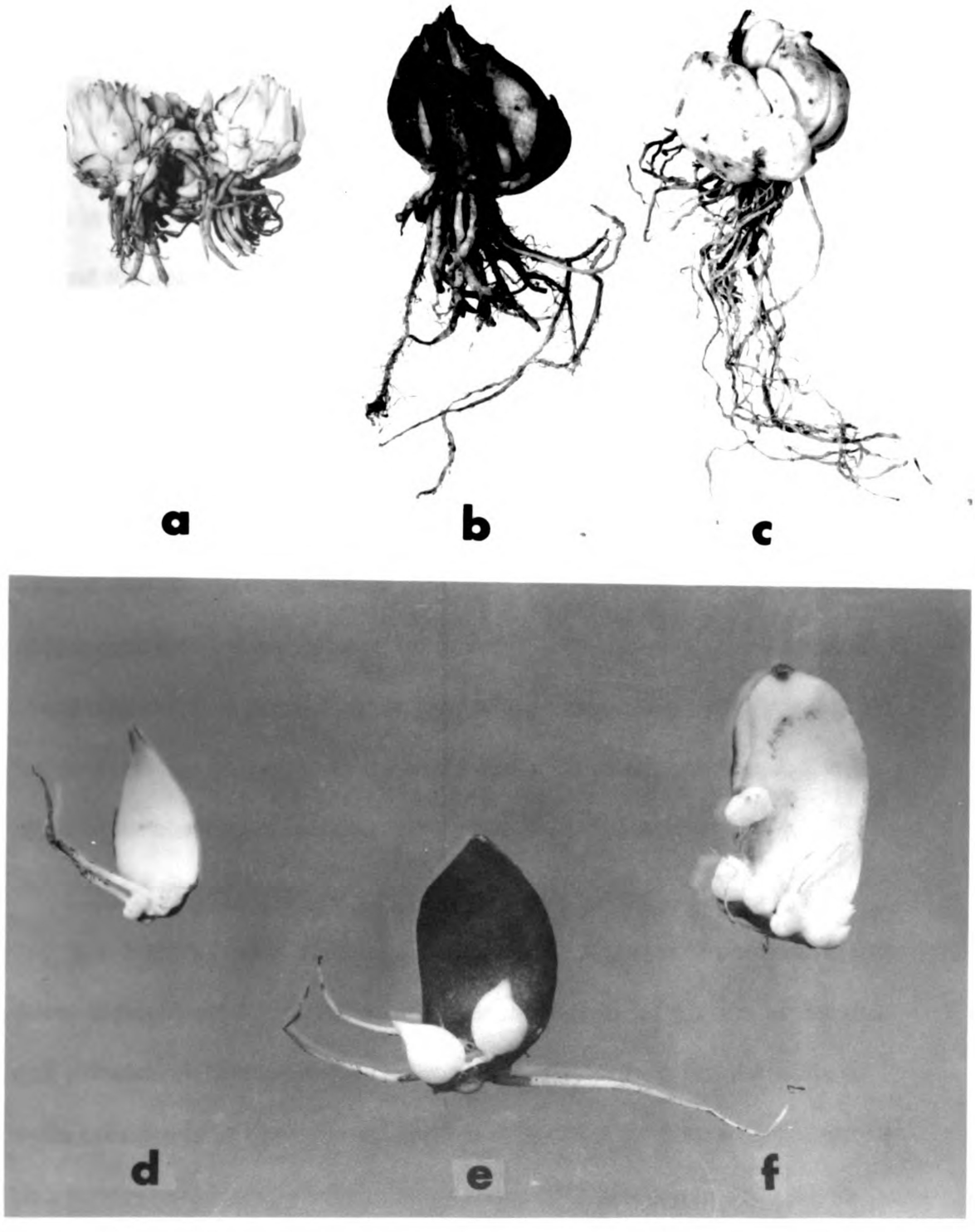

Fig. 1. a, b, and c, mature bulbs (x 1/2); d, e, and f, scale leaves and bulblets following six weeks of incubation (x 2/3).
a and d, cv. Bellingham; b and e, cv. Olympic; c and f, cv. Fiesta.

layer of epidermal cells. Undifferentiated parenchymatous mesophyll, heavily deposited with starch grains, filled the scale. Inconspicuous vascular bundles, frequently difficult to locate in the transverse view, were in no definite pattern. These bundles were small with xylem lying toward the adaxial, and phloem toward the abaxial surface.

Visible in longitudinal section in some scales examined immediately after detachment from the parent bulb was a distinctly separate zone of tissue adjacent to the wound surface (c, Fig. 2). The cells in this region were conspicuously rounded, were with heavier cell walls, and were without starch. In other scales this zone was absent and the cells were undifferentiated. Further study of several specially prepared sections confirmed the fact that the loose cells of this zone were merely a part of the stem tissue torn off with the scale and not a specialized separation layer of an abscission zone as might have been concluded.

Periderm and callus formation:

The wounded cells collapsed soon after detachment of the scale. The onset of periderm formation was indicated by an accumulation of suberin and globular material around the damaged cells and around the walls of cells contiguous to the wounded surface. From this surface cell destruction progressed distally with increased deposits of suberin and with vacuolation of the cells.

After three days nuclear divisions were exceedingly prolific in the

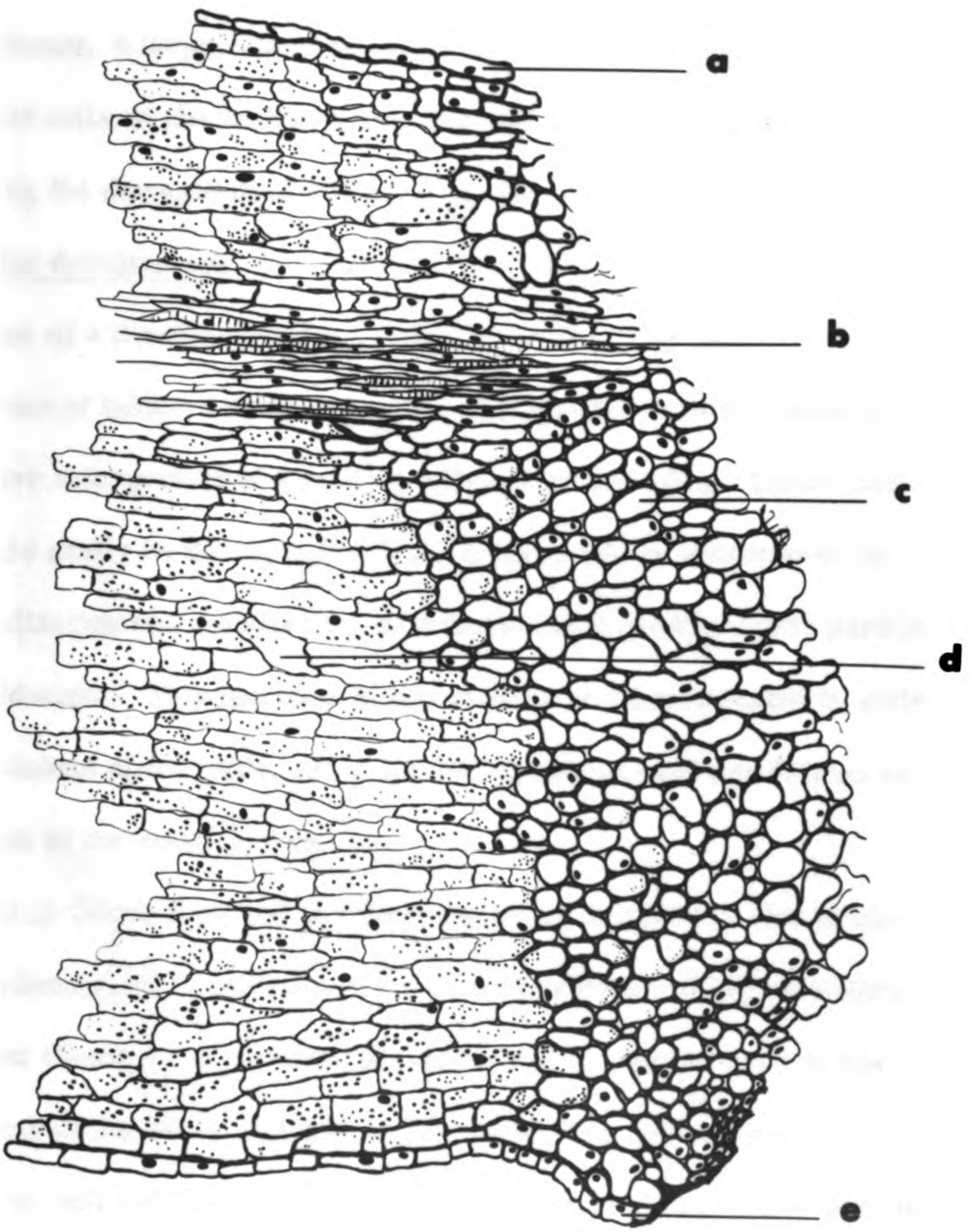

Fig. 2. Drawing of a longitudinal section of the base of a scale leaf immediately following detachment from the parent scale (x75).

a, adaxial epidermis; b, vascular tissue; c, stem tissue; d, mesophyll of leaf scale; e, abaxial surface.

parenchyma cells distal to the newly formed periderm. The earlier divisions were predominantly in a direction parallel to the wound surface. In this manner, a large volume of callus tissue was developed adjacent to the dead cells of the periderm crushing and subordinating this tissue and sealing the damaged area (2, 3, d, Fig. 4).

Bulblet development:

Stages of a developing bulblet are illustrated in Fig. 3.

Regions of bulblet initiation became distinguishable by the division and nuclear enlargement of a group of sub-epidermal cells. These divisions could easily be distinguished from those of callus development by their localization and the direction of division being predominantly parallel to the epidermis. This increase in cell number was accompanied by anticlinal divisions of the epidermal cells and a protuberance was formed on the surface of the scale (a, Fig. 4).

Later divisions occurred in all planes giving increase in size of the bulblet primordium. At this stage the first indications of differentiation of vascular tissue were observed occurring within existing cells of the parenchyma of the parent scale or in the cells of the callus tissue. A scalariform cell wall thickening characteristic of xylem elements appeared on those cells lying between the vascular strand of the parent scale and the developing primordium. There was some elongation of these cells (Fig. 5).

By a differential rate of cell division at the surface of the bulblet

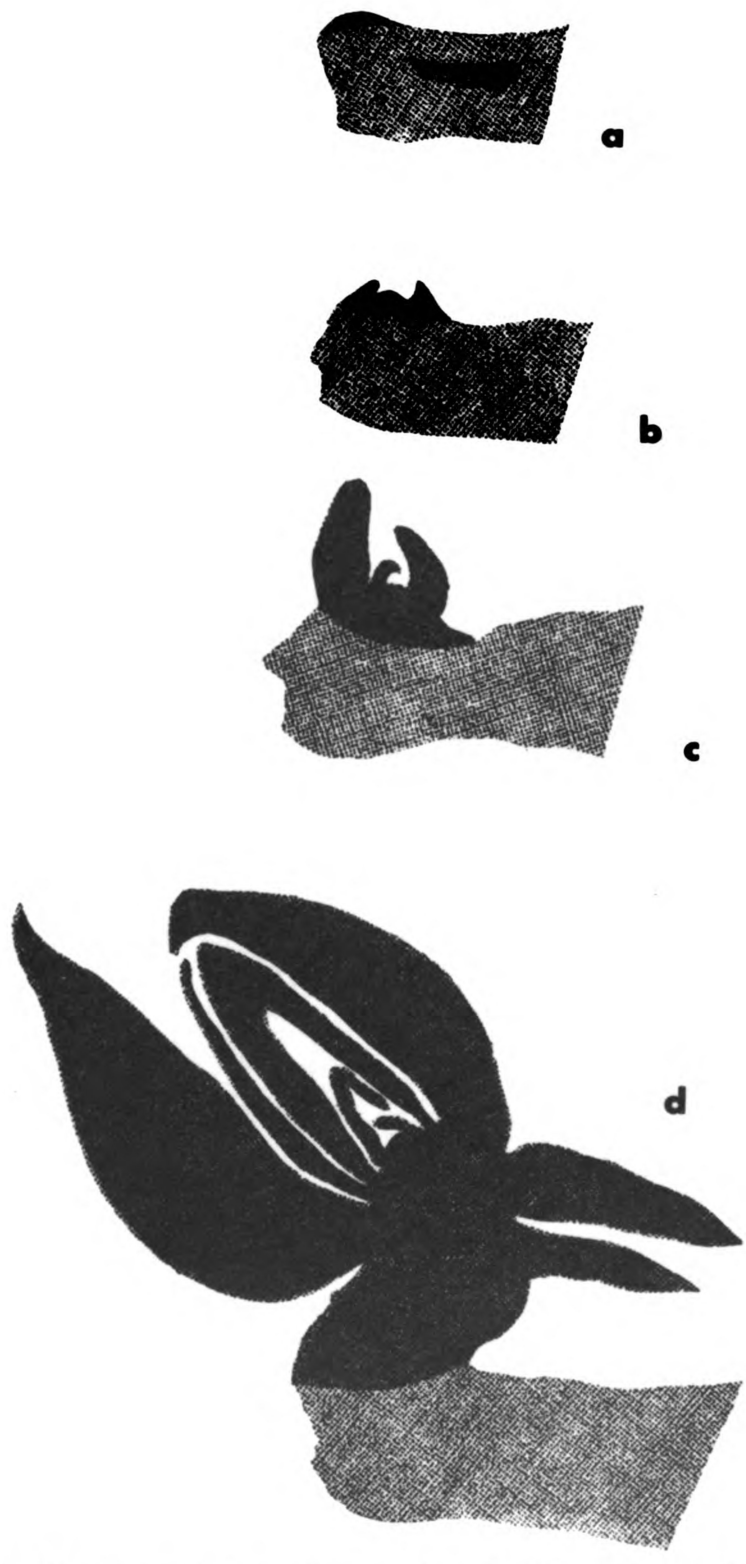

Fig. 3. Diagrammatic representation of stages of development of a bulblet. a, visible protuberance; b, formation of first leaf initials; c, later stage of leaf initials; d, developed bulblet.

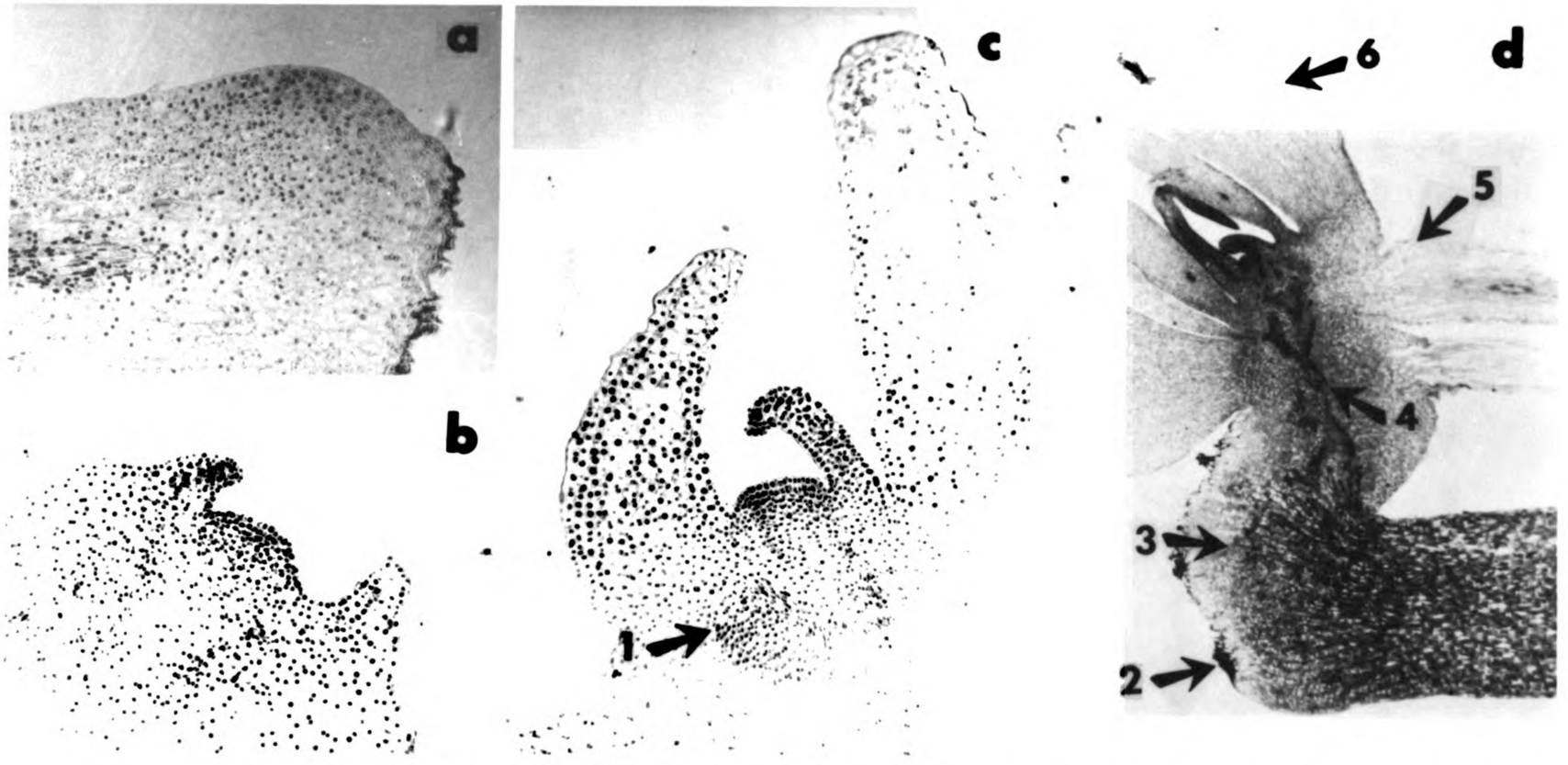

Fig. 4. Stages of development of a bulblet. a, visible protuberance (x45); b, formation of first leaf initials (x45); c, later stage of leaf initials (x45); d, developed bulblet (x13).
1, initiation of root primordium; 2, periderm; 3, callus; 4, vascular connection; 5, root; 6, outer bulblet scale.

primordium alternate leaf initials arose (b, Fig. 4). These increased rapidly in size to surround and enclose a central meristem portion which continued to give rise to successive leaf primordia.

The first indication of a root primordium was the formation of a densely stained group of meristematic cells within the bulbet protuberance below either the apical meristem or the leaf primordium (1, c, Fig. 4). A definite root apex developed and emergence was exactly similar to normal adventitious root development. At no time was rooting of the parent scale observed. Roots which externally appeared to have arisen from the bulb scale were shown to have their origin within the newly formed bulbet. Vascular tissue formed from the root meristem connected with that differentiated between the bulblet and the parent scale.

A bulblet after six weeks from the time of detachment of the scale possessed leaf scales and leaf initials and showed continuing development of leaf primordia (as illustrated in 6, d, Fig. 4). Vascular strands were forming in the larger scales. The central meristem had assumed the characteristics of a typical apical meristem exhibiting layers of both tunica and corpus. Two or three well developed roots were present (5, d, Fig. 4). Connecting vascular parenchyma between the parent scale and bulblet remained visible but further vascular differentiation appeared to have ceased (4, d, Fig. 4; Fig. 5).

Fig. 5. Enlargement of vascular connection of Figure d, 4. (x 70).

CONCLUSION

Wound healing at the broken surface of the detached scale followed the sequence proposed by Priestley and Woffenden (1922) of blocking of the cut surface and subsequent meristematic activity which gave rise to a callus tissue.

The present observations on Lilium have illustrated a distinction between callus and periderm tissue as was presented by Lauer and Krantz (1957). Their assumption that callus was an increase in cell number, whereas periderm was the re-differentiation of existing cells, has been substantiated and adopted. Lauer and Krantz' conclusion that callus formed only in non-dormant tubers and only after bud removal has not been verified.

The development of bulblets behind the wound surface was shown to be exactly similar in all three cultivars examined. It has been suggested therefore that the pattern of development described is consistent throughout the genus.

The bulblet arose on the leaf scale by simultaneous divisions of both epidermal and sub-epidermal cells and was not therefore entirely endogenous as has been stated by Walker (1940). This type of development of a new individual from reassumed meristematic activity in mature cells

was closely comparable, though not identical, to that shown by McVeigh (1938), Naylor and Johnson (1937) in Crassula and Saintpaulia. This type of origin in Lilium contrasted with that shown in Kalanchoe and Bryophyllum (Clamp 1934, Naylor 1932, Yarbrough 1932) in lacking residual meristems.

The dependence of the bulblet for food material from the parent scale during its early stages of initiation has been assumed. Vascular connections arose at the onset of bulblet development by the differentiation of parenchyma or callus cells lying between the vascular strands of the parent scale and the bulblet primordia. External observations have shown the tendency for bulblet development to occur at the base of veins. In spite of the fact that Walker (1940) had not observed vascular connections in Lilium longiflorum and Lilium candidum, they were present in Olympic, Fiesta and Bellingham hybrids.

Roots appeared as a subsequent development as the bulblet enlarged. In every case roots arose from the tissue of the bulblet and their origin could not be traced from parent tissue, as had been suggested in Saintpaulia (Naylor and Johnson 1937) and Lilium (Walker 1940).

SUMMARY

The external morphology of the bulbs of three cultivars (Olympic, Fiesta, and Bellingham hybrids), of the genus Lilium has been described. Scales detached from these bulbs were incubated in moist peat at 70 degrees F to promote bulblet development. Sample scales were taken at three-day intervals over a period of six weeks, and prepared for microscopic examination.

The origin of the bulblet has been shown to be derived by the simultaneous division of epidermal and sub-epidermal cells following periderm and callus formation on the wound surface. Prior to root development vascular connections developed between the bulblet primordium and the vascular tissue of the parent scale by the differentiation of existing parenchyma or of cells of the callus tissue.

The initiation of new bulblets occurred throughout the six week period of examination.

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