DEVELOPMENTAL VEGETATIVE MORPHOLOGY OF *GLYCINE MAX*

by

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INTRODUCTION

The increasing agronomic and industrial importance of the soybean, Glycine max L. has led to intensification of research in agronomy, genetics, physiology, and pathology. It has become evident that such studies would be aided by a greater knowledge of the structural development of the soybean plant. Morphological studies on soybean are few in number and consist of segments of structural detail. The earlier investigations did not stress the developmental pattern.

The immediate and specific objective of this present study is to investigate the developmental anatomy of the soybean plant from the dormant seed, through post-dormant events, germination, and subsequent seedling development up to floral initiation. The knowledge of the normal development would be particularly useful in studies that involve experimental treatment that may affect the normal course of development. The phase of the life-cycle studied in this paper is the period in which many current investigations are conducted.

REVIEW OF PERTINENT LITERATURE

Anatomical investigations reported in the literature that approach the study of a plant from a normal-developmental life cycle pattern are few.

Miller and Wetmore (20, 21, 22) described the developmental pattern of Phlox drummondii Hook. from embryonic stages to the mature condition, with emphasis on embryo orientation and vascularization of the developing plant. The developmental anatomy of Jatropha cordata (Orteg.) Muell. was described by Popham (27). The general status of organogeny, the histological pattern, and the condition of the histogens at seed dormancy were described. This was followed by an investigation of the post-dormant and young seedling stages up to 75 days, with the direction, place, and time of tissue differentiation in the root, hypocotyl, and stem. In the Leguminosae, an approach to the life-cycle pattern of investigation was reported in the papers by Yarbrough (35, 36, 37). The first paper reported the condition of the seed at dormancy and the other two investigations described the subsequent developing seedling stages to stem maturity.

Only a partial description of the dormant embryo of soybean occurs in the fragmentary literature on the subject.

The majority of the older anatomical studies of the dormant seed of the Leguminosae were concerned with the morphology of the outer seed coat and the hilum region. In these

studies the structure of the embryo was seldom reported in any detail, except for the cells of the cotyledons. Pammel (23) described the general seed characteristics of certain legumes. A more modern approach was utilized by Reeves (29, 30) in which the ontogeny and chemical components of the seed were studied.

Few investigators have described the interior cellular structure, or the organs of the embryo. Kondo (19) examined certain legumes, and Glycine was described as having two plumular leaves, a primary root system, and two cotyledons. Again, in this study, more emphasis was given to the cellular inclusions of the cotyledons and the gross external morphological features of the seed.

A recent and more detailed account of the root apex organization of soybean at dormancy was described by Sun (33). The apex was reported to be more complex than described by Bell (2), who reported the root tip as the "fourth angiospermous" type. Sun reported that the apex possesses two zones of initials, the stelar and the common initials. The distal portion of the latter he designated as the columella.

Bell (2) reported that prior to germination the epicotyl of the embryo possesses several foliage leaves. Kato, Sakaguchi, and Naito (18) described the presence of the first trifoliate leaf primordium at dormancy.

Relatively little literature describes the anatomical

changes of the next phase of plant development, reactivation. Picklum (26), described the tissue and cellular changes in the Zea embryo during germination and described imbibition, the rupturing of the pericarp, the subsequent protrusion of the coleorhiza, the time and position of the first post-dormant mitotic reactivation, and the initiation of the first post-embryonic leaves.

The structure of the shoot apex and leaf histogenesis have absorbed the attention of botanists for years. Literature review on the subject has been written by Foster (13), Popham (27), and Gifford (15). In conjunction with shoot apex studies of the Leguminosae, Boke (3), worked with phyllode formation in certain species of Acacia. Ball (1) described the shoot apex of Lupinus albus (L.) in an effort to establish the normal pattern of development of that part of the plant for purposes of further experimental studies.

The first shoot apex study reported for Glycine was presented in detail by Sun (32). He reported that the shoot apex is delimited into four zones; a two layered tunica, the central initiation zone, the peripheral and the rib meristem zone. Reviews concerning the theories and controversies of the cytohistological and topographical zones of cell initiation of the stem apex are discussed in the above mentioned papers by Foster, Gifford, and Popham.

The first leaf of Glycine was briefly described by Bell

(2) as a conical primordium that arises from the apical meristem.

The use of plastochronation as an approach to the study of the soybean stem apex and related leaf histogenesis was first reported by Sun (32). He observed that when a given leaf primordium had developed to a height of about 80-90 microns, the next primordium is initiated. Leaf initiation is indicated by anticlinal divisions in the tunica layer and periclinal divisions in the outer corpus.

According to Yarbrough (35), the cotyledons of Arachis hypogea L. enlarge during the course of early development and then become depleted of their food supply as protophloem and protoxylem differentiates. The cotyledonary axillary shoot is well developed, with one or two embryonic leaves. The condition of the cotyledonary axillaries of soybean at dormancy and later stages was not reported in the available literature.

An account of the developmental vegetative phase of a plant in the Leguminosae does not appear in the readily accessible literature. Certain aspects of vegetative morphology have been described, such as the vascularization study of Phaseolus vulgaris L. by Duott (8); the normal procambium and subsequent vascular differentiation study on Lupinus albus by Ball (1), and the developmental studies by Yarbrough on Arachis hypogea (35, 36, 37). A review of the

literature in this area was reported by Hansen (17), in his developmental study of Lotus corniculatus L.

Compton (7) studied the anatomy of the hypocotyl in certain legumes in relationship to possible phylogeny of the family.

The first ontogenetic study of the stem of soybean was described by Bell (2). He described the stem as an endarch collateral dictyostele; the annular, spiral, and pitted vessel segments; the primary phloem as consisting of sieve tubes and companion cells well separated by parenchyma cells; the pericycle immediately external to the primary phloem.

An ontogenetic investigation of a particular vascular bundle of soybean in a definite region in the stem, in relation to time of development from dormancy, has not been reported in the literature.

Esau (9) described the formation and structure of the phloem tissue in tobacco. In vascular studies of Linum, she described acropetal differentiation of phloem several plastochrones in advance of the xylem, and showed that xylem does not appear in isolated segments and which become continuous later. She also showed that in a procambium strand a sieve element differentiates before the first xylem element becomes evident (11).

Esau (12) described the development of the fibers at the outer periphery of the vascular bundle and showed that the

fibers are histologically part of the phloem.

Much of the early work on the morphology of legumes was concerned with the development of floral structures. The development of flower parts of Trifolium ochroleucum, Lathyrus sylvestris, and Lupinus varius was described by Payer (24). Frank (14) reported on the floral structures of Vicia cracca and Lupinus elegans. In conjunction with their studies of seed production, Coe and Martin (6) described the mature flower of Melilotus alba. Picklum (25) investigated the development of the inflorescence and flower of Trifolium protense. Guard (16) reported in soybean the first indication of a flower primordium is a knob-like structure in the axil of a bract. He did not describe floral transition, but dealt mainly with the order of emergence of the floral parts; sepals, petals, outer cycle of stamens, inner cycle of stamens and the pistil.

Borthwick and Parker (4) investigated the relationship between photoperiod and floral initiation in Biloxi soybeans. The study did not begin with the seedling grown under normal field conditions prior to treatment, however, the anatomical features of floral transition under experimental conditions were described in some detail.

The work of Kato, Sakaguchi, and Naito (18) began with floral transition and continued with the development of floral primordia, floral differentiation, megasporogenesis and

microsporogenesis, embryo-sac formation, fertilization and embryogeny.

MATERIALS AND METHODS

The soybean variety Hawkeye was used in this study. Seed of the 1956 crop was obtained through the courtesy of Dr. C. R. Weber of the Iowa State University of Science and Technology Experiment Station. The field material was from the 1957 plantings at the Agronomy farm. The planting date was May 28, 1957.

The seeds were germinated in plastic crispers on a substratum of screened sphagnum moss in the germinators of the Iowa State University Seed Testing Laboratory. Some seeds were planted in four-inch pots and germinated in soil in the greenhouse.

Collections of the germinating material were made at four-hour intervals up to 144 hours. The field material was collected on alternate days, at approximately the same time of the day, starting on the fifth of June, up to the time of first visible flowering.

The material was killed in Craf III and was dehydrated and infiltrated with wax using the ethyl alcohol-xylene series. Sections were cut at eleven microns. The stains used were iron-hematoxylin and safranin-fast green (31).

EXPERIMENTAL RESULTS AND OBSERVATIONS

The Dormant Embryo

The cotyledons

The thick fleshy cotyledon has a netted-veined vascular system (Figs. 1, 7). The epidermis consists of cuboidal cells that are approximately 16 microns in each dimension. Stomata are present on both surfaces. The mesophyll consists of two to three adaxial layers of palisade-like elongated cells, averaging 70 x 30 x 30 microns, and large cells which are loosely-arranged and have intercellular spaces (Figs. 1, 2). The cells contain globules of material of irregular size, shape, and undetermined chemical composition. An indentation is present on the abaxial surface of the cotyledons, and is prominent in certain varieties and barely perceptible in others. This pit is centrally located and above the large midvein of this organ (Figs. 1, 3). The large vascular bundles and the smaller veins have immature protoxylem, metaxylem initials, and mature protophloem (Fig. 4).

The plumule

The dormant plumule has two unifoliate leaves with conduplicate vernation. Mature protophloem and protoxylem initials are present but not abundant throughout the vascular system of the unifoliate leaves, and metaxylem initials are

present in some procambium strands (Fig. 6). Two stipules occur at the base of each unifoliate leaf. At the level of the stem apex, each stipule has one procambium strand (Fig. 8). The oblique meristem of the stem apex of the plumular axis has a uniseriate tunica, and a massive corpus in which the orientation of the cell walls indicate that periclinal and random divisions occur (Figs. 5, 9). Periclinal divisions had occurred in the outer corpus prior to dormancy, indicating that a leaf primordium had been initiated.

The radicle

The apical meristem of the dormant radicle consists of two zones; the stelar initials and the common initials and the other details of the root apex structure as described by Sun (33).

The cotyledonary axillary buds

The apical meristem is an oblique dome with a uniseriate tunica. Periclinal division of the outer corpus, adjacent to the tunica, indicate that a leaf primordium had been initiated prior to dormancy. The organization of the bud apex is the same as that of the main stem apex (Fig. 10).

Fig. 1. Cross section of cotyledons, showing the pit region, the large midvein, and the "spongy" mesophyll. (10x).

Fig. 2. Cross section of a portion of the adaxial surface of the cotyledon showing the cuboidal epidermal cells, the "palisade"-like cells. (100x).

Fig. 3. A section through the pit region of the cotyledon illustrating the normal epidermal cells, the plasmolyzed epidermal cells and the abnormal subadjacent cells. (200x).

Fig. 4. Tranverse section of a small vascular bundle in the cotyledon showing mature protophloem and immature protoxylem. (400x).

Epidermis	(E)
Abnormal epidermis	(EA)
Mesophyll	(M)
Pit	(P)
"Palisade" cells	(PA)
Protophloem	(PX)
Midvein	(V)

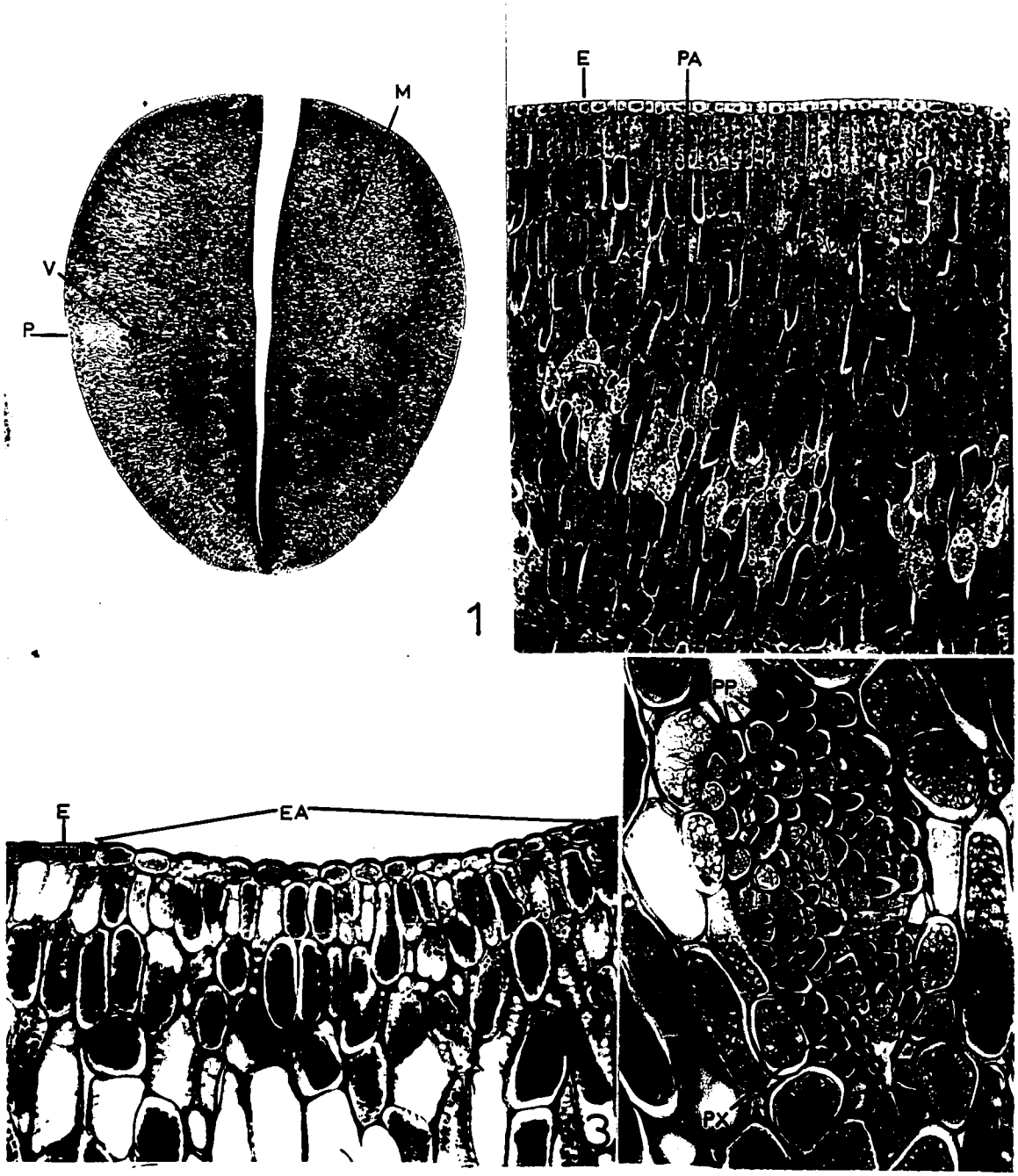
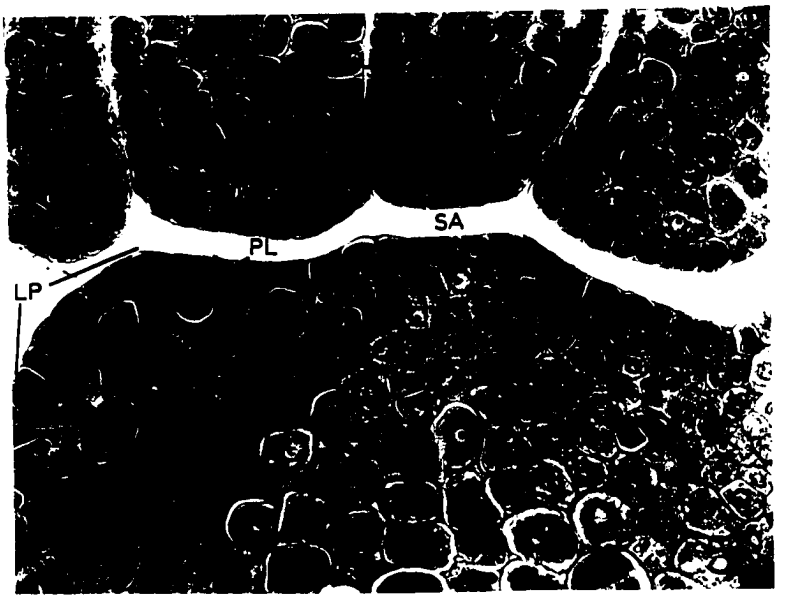

M
V
P
E
PA
1
PP
PX
E
EA
3

Fig. 5. Longitudinal section of stem apex region, showing the first trifoliate leaf primordium and periclinal divisions in the outer corpus. (400x)

Fig. 6. Cross section of a large vascular bundle in a unifoliate leaf. (400x)

Leaf primordium	(LP)
Periclinal divisions	(PL)
Protophloem	(PP)
Protoxylem	(PX)
Stem apex	(SA)
Unifoliate leaves	(U)


SA
PL
LP

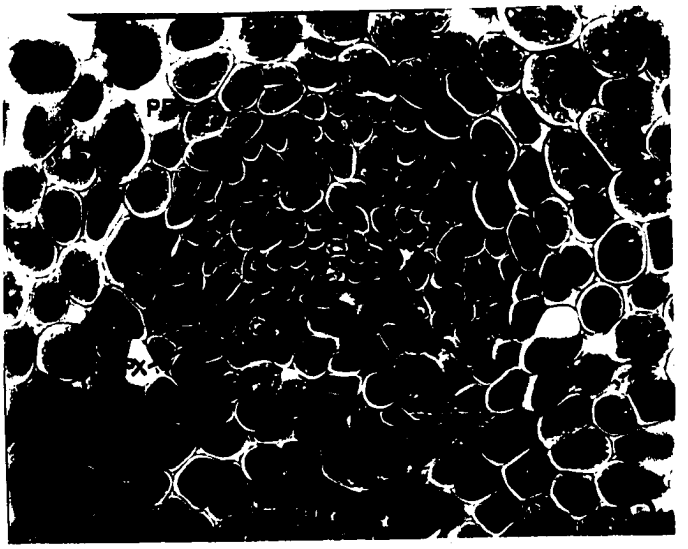

PF
X

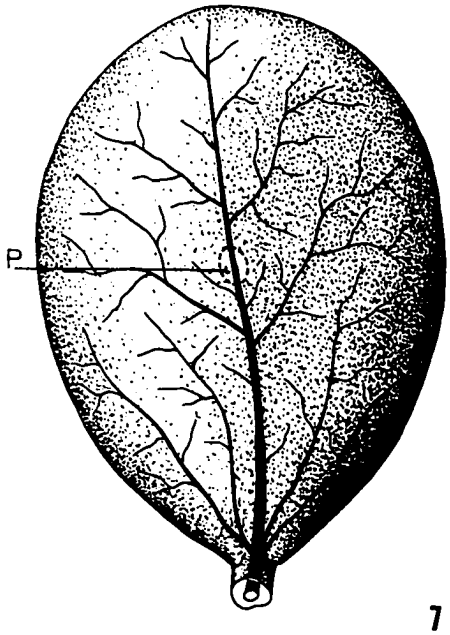
Fig. 7. A cleared dormant cotyledon, showing netted venation and pit on the lower surface. (4x)

Fig. 8. Cross section of the dormant plumular bud at the level of the stem apex. (75x)

Fig. 9. Detail of a portion of the stem apex region showing a procambial strand relationship with the first trifoliate leaf Primordium. (600x)

Fig. 10. Detail of the apex of the cotyledonary axillary bud. (600x)

Pit	(P)
Procambial cells	(PC)
Stipule	(S)
Stem apex	(SA)
Tunica	(T)
Base of unifoliate leaf	(U)



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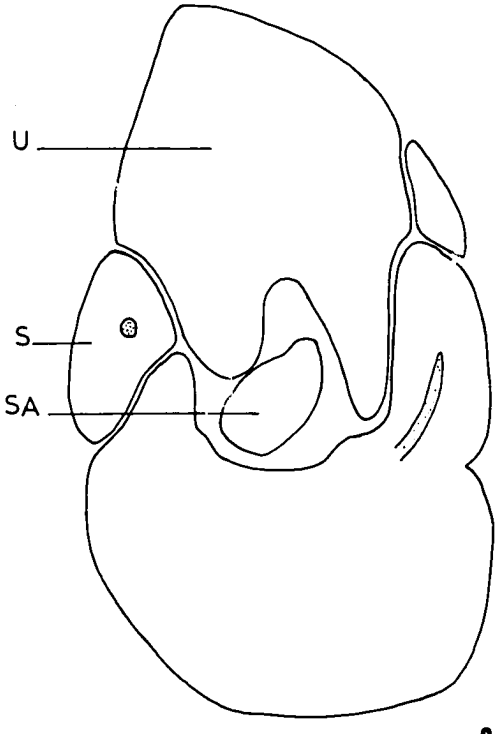

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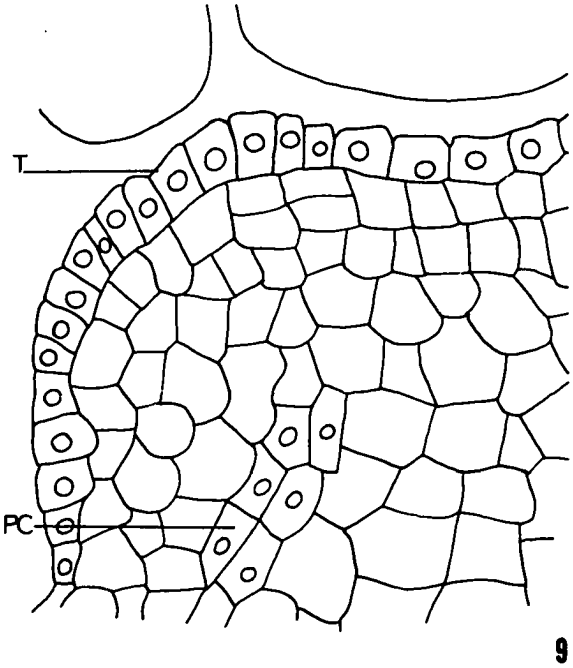

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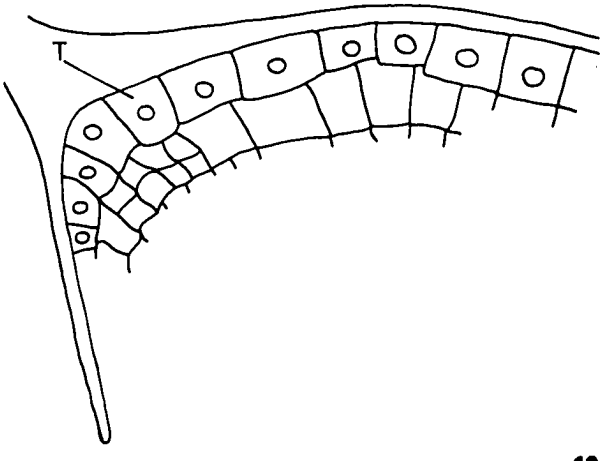

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Post-Dormant Reactivation

The time required for complete imbibition of the dry seed ranged from 18 hours in the germinator, to 24 hours in soil in the greenhouse. Reactivation was determined by the presence of mitotic figures in the organs of the embryo.

The radicle

Mitotic figures were first observed in the radicle 32 hours after the beginning of imbibition in the germinator. These figures were found 400 to 800 microns above the proximal side of the common initials in the cortex region. At 40 and 48 hours, mitotic figures were noted in the stelar region and in the apical generating zone, respectively (Figs. 11, 12).

The region that gives rise to the tissues of the radicle is composed of several stratified layers oriented at right angles to the axis. This is designated as the common initial region. Anticlinal divisions on the sides of the common initials contribute cells to the cortex. The distal cells of the common initials produce the central portion of the root cap by periclinal divisions. The peripheral portion of the root cap is produced by periclinal and oblique divisions from the outermost lateral derivatives of the common initials (Fig. 12).

The epidermis can be traced downward along the inner

Fig. 11. Longitudinal view of the radicle-plumular axis of the dormant embryo. (18x).

Fig. 12. Schematic drawing of the root apex region showing the two initial zones.

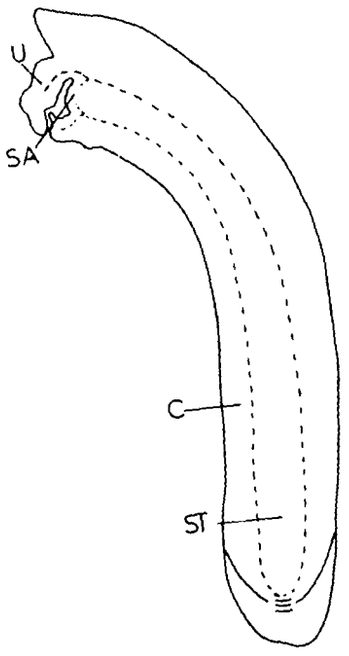
Figs. 13-15. Diagrams showing the loss of continuity of the protoderm in the apex region of the root. (600x)

Fig. 13. Protoderm cells and uppermost root cap cells, about 1600 microns from root tip.

Fig. 14. Protoderm and root cap cells, approximately 5800 microns from the root tip.

Fig. 15. Protoderm and root cap cells 300 microns from root tip, and the former display a loss of continuity.

Cortex	(C)
Common initials	(CO)
Calyptra	(CP)
Protoderm	(PD)
Stele	(ST)
Stelar initials	(SI)



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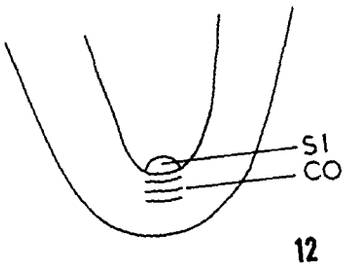

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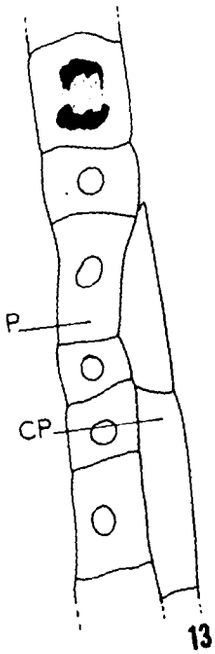

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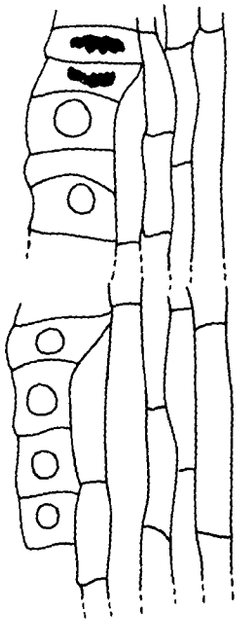

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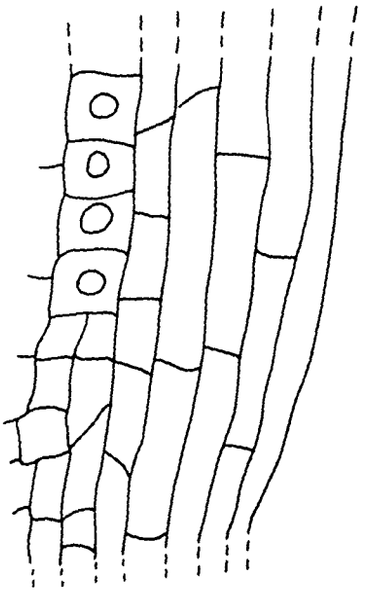

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limits of the root cap, about five cell layers deep, where the identity and continuity of the "protoderm" are no longer evident (Figs. 13, 14, 15).

The stelar initials form a hemispherical region at the proximal portion of the common initial region (Fig. 12).

The epicotyl

Mitotic reactivation was observed in the 36 hour collection in procambium strands within the corpus, about 50 and 100 microns from the stem apex. Periclinal divisions were present in the outer zone of the corpus. Divisions in the tunica occurred at 40 hours.

In the unifoliate leaves, mitotic figures were first found in the mesophyll at 36 hours. Epidermal hairs, which are present in the dormant condition, resumed enlargement at 36 hours. Mitosis in the epidermis of the first leaves was observed at 48 hours.

At 36 hours, mitotic figures were observed deep within the corpus of the apical meristem of the cotyledonary axillary bud. Cell division in tunica and outer corpus were resumed between 40 and 48 hours.

The cotyledons

No mitotic figures were observed in the mesophyll of the cotyledons. The normally cuboidal epidermal cells of

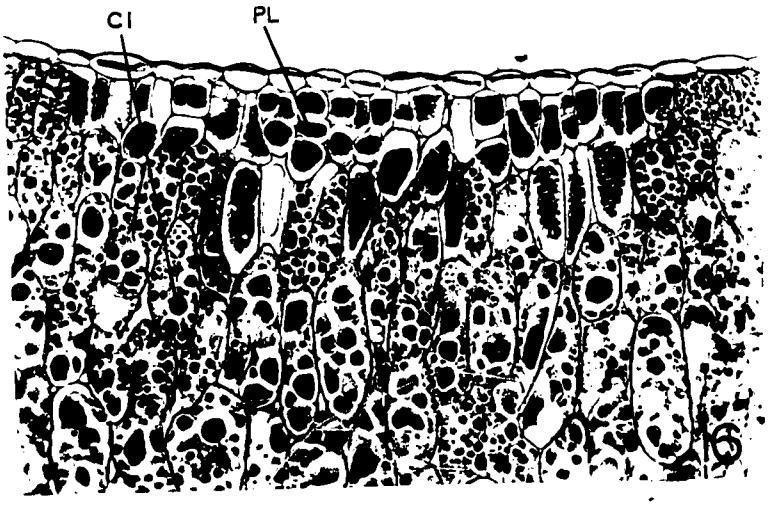
the cotyledon, in the region of the "pit", appeared to be in a state of hypertrophy at dormancy (Fig. 3). The cells directly below the epidermis, in this area, were also observed to exhibit some hypertrophy. The cellular contents of the epidermal and immediate subadjacent cells were collapsed, in comparison with the neighboring normal cells. After a period of 48 hours the cells associated with the depression, especially the epidermal cells, had undergone further plasmolysis. Periclinal divisions of some of the cells below the epidermis had occurred. The necrotic cells were deeply stained in prepared sections to a depth of about three cell layers (Fig. 16). This staining was more intense in the 7-day collection, and the hypertrophy was more pronounced. An increase of periclinal divisions of the subadjacent cells was evident. The remainder of the cells of the mesophyll had begun to display a degeneration (Fig. 17). By 14 days the necrosis had extended to a depth of six or eight cells. The cells below this area showed an increase in periclinal and random divisions (Fig. 18). Degeneration of the surrounding cells was prevalent, associated with external discoloration that indicates the beginning of senescence of the cotyledon.

Fig. 16. Section through the pit area of a cotyledon, two days after the beginning of germination. Cellular inclusions are prominent. (150x)

Fig. 17. Same section of the pit area, at seven days, showing the loss of cellular inclusions and the intense stain in the necrotic area. (75x)

Cellular inclusion (CI)

Periclinal divisions (PL)


CI
PL

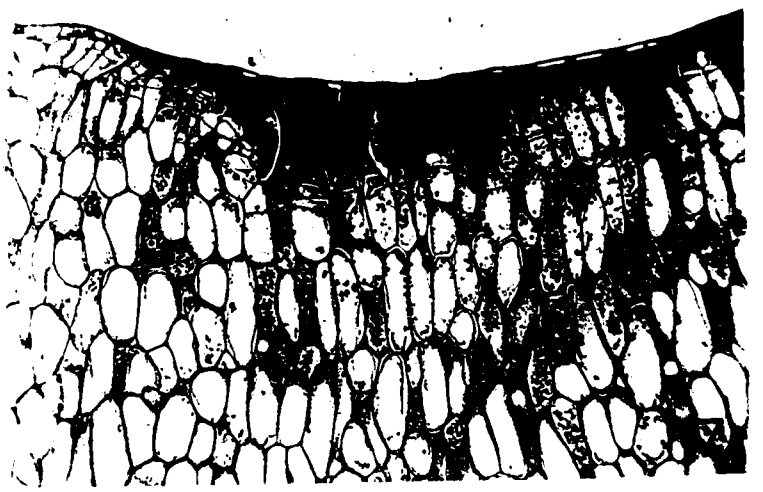
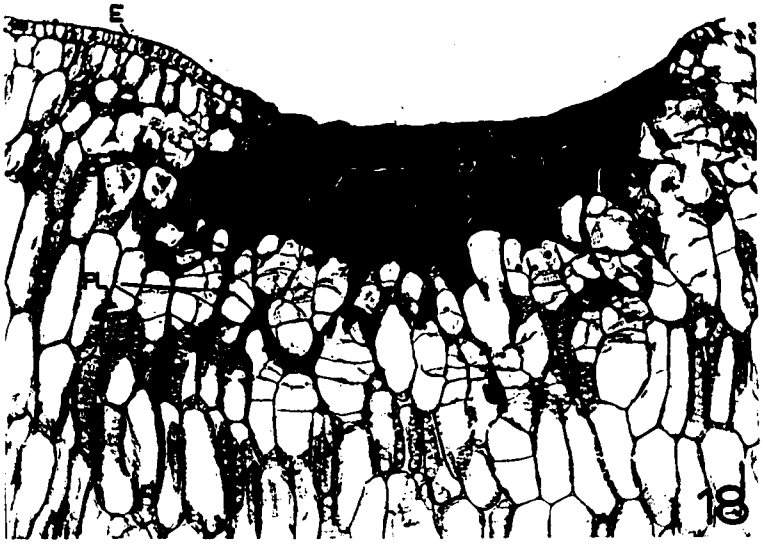


Fig. 18. The necrotic pit area at fourteen days after germination showing the extensive range of the abnormal cells and induced periclinal divisions. (100x).

Epidermis (E)

Periclinal division (PL)


E
18

Plastochronation from Dormancy to Floral Initiation and the Concurrent Cotyledonary Axillary Bud Development

The stem apex

The structure of the stem apex at dormancy has been described in previous pages (Figs. 5, 9). The development of the apex and related leaf formation during germination and emergence was studied. The term plastochrone in this study is defined as the time interval between the initiation of two successive leaves.

The width of the apex remains narrow, with some variability, until 72 hours, by which time it almost doubles in size (Table 1). At 84 hours a more prominent elongation of the internode between the unifoliate and the first trifoliate internode was observed (Fig. 22). Part of this general enlargement is associated with leaf formation, and at 84 hours the second leaf is initiated (Fig. 22). The first plastochrone was established, on the average, in an 80 hour period; however, a 60 hour interval was observed in some material (Figs. 19, 20, 21, 22). The formation of the third leaf, of the second plastochrone, extended over a period of two days, plus or minus a day, as compared with the first plastochrone of three and one half days (Table 2, Fig. 23). Leaves 6 and 7 were formed on 11 and 13 days, respectively, showing the two day plastochrone interval at later stages (Table 2,

Table 1. Measurements in microns of the developing shoot apex during the first plastochrone

Time in hours	Stipular base to the tip of the 1st leaf primordium	Apex to tip of 1st leaf primordium	Stipular base to stipular base
0	91-105	35-50	250-280
36	91-110	28-50	280-290
40	140-210	50-80	315
48	160-217	50-85	301-315
52	217	50-112	308-329
60	168-270	50-100	310-315
72	270-287	114-161	315-420
84	350-470	114-196	420-490

Table 2. The number of trifoliate leaves formed in relation to time in days after planting

Leaf number	1	2	3	4	5	6	7	8	9	10
Days	0	3.5	5.5	7	9	11	13	15	17	19

Figs. 19-22. Drawings of the stem tip region, showing the establishment of the first plastochrone.

Fig. 19. Stem apex at dormancy showing the primordium of the first trifoliate leaf. (70x).

Fig. 20. The first trifoliate leaf forty hours after the beginning of germination. (70x).

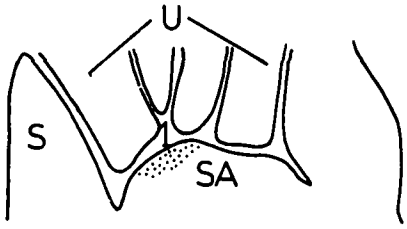
Fig. 21. The same region at fifty-two hours. (70x).

Fig. 22. The first trifoliate leaf is well formed and the initiation of the second trifoliate is evident.

Stipule (S)

Stem apex (SA)

Leaf number is indicated by the respective numerals.



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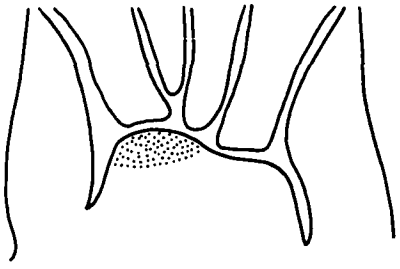

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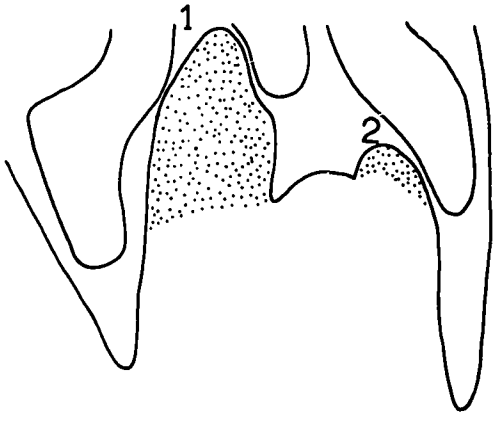

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Fig. 23. Outline of the epicotyl 132 hours after the beginning of germination, showing the initiation of the third trifoliate leaf. (20x)

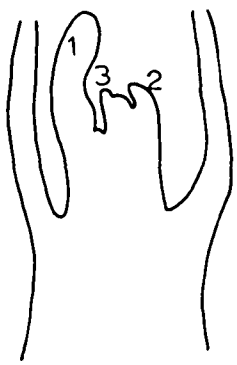
Fig. 24. A floral primordium showing the beginning of sepal formation. (240x)

Fig. 25. The shoot apex eleven days after planting.

Fig. 26. The young shoot thirteen days after planting.

Sepal primordia (SE)

Leaf number is indicated by the respective numerals in the drawings.



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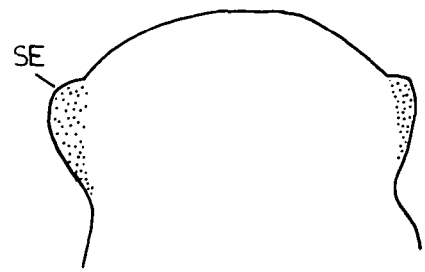

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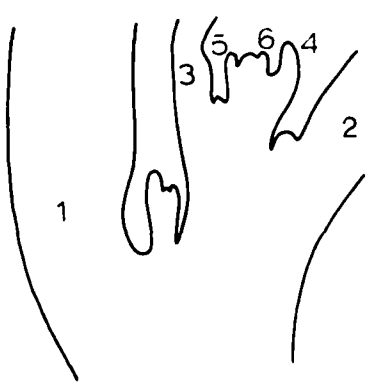

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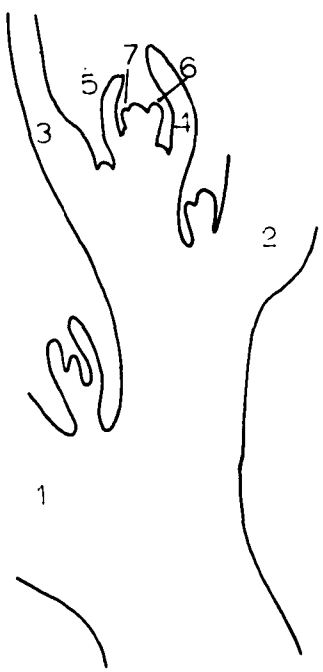

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Figs. 25, 26).

Floral initiation

Floral initiation was observed at the 10 to 12 leaf stage, or 20 to 24 days (Figs. 24, 27). The apex broadens at this time and shows two lateral sepal primordia. The first evidence of floral initiation is in the bud in the axil of the first trifoliate leaf. Twenty-five days after the beginning of germination, the primordia of all floral members are evident in the axillary shoot of the first trifoliate leaf (Fig. 28).

The cotyledonary axillary

The height of the dormant cotyledonary axillary bud ranges from 20 to 50 microns, and its morphological status was described in preceding pages (Fig. 10). The first major change after dormancy was observed at 96 hours, at which time the first leaf had enlarged and initiation of the second leaf had occurred (Fig. 29). By the seventh day the cotyledonary axillary shoot has two to three leaves and a height of approximately 400 microns (Fig. 30). Four days after the flowering is visible on the main stem, the buds in the axils of the cotyledons attain maximum size and structural development. At this time the axis of the buds is approximately 1200 microns in length, has five and possibly six leaves, and a

Fig. 27. Longitudinal section of portion of a stem 21 days after planting. The first and second trifoliate leaves are not shown. (48x)

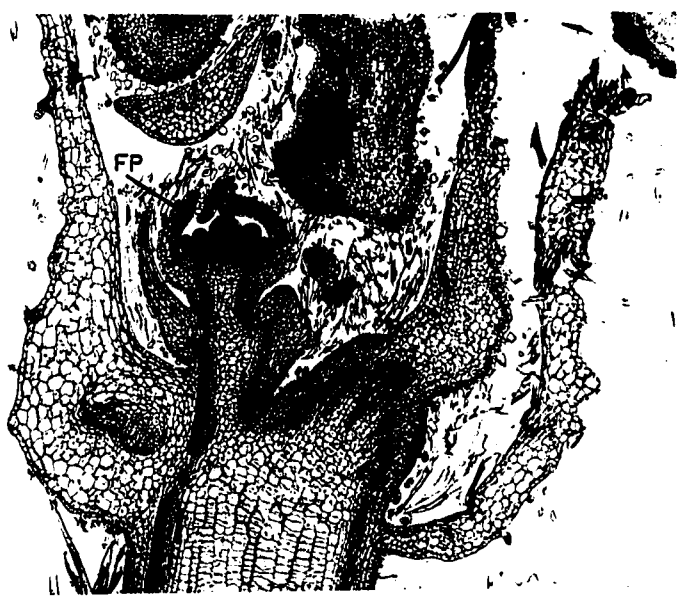
Fig. 28. Section through a floral primordium in the axil of the first trifoliate leaf. (48x)

Floral primordium (FP)

Stem apex (SA)

The leaf number is indicated by the respective numerals.


SA


FP

Figs. 29-31. Outlines of the cotyledonary shoot from four days after planting up to senscence.

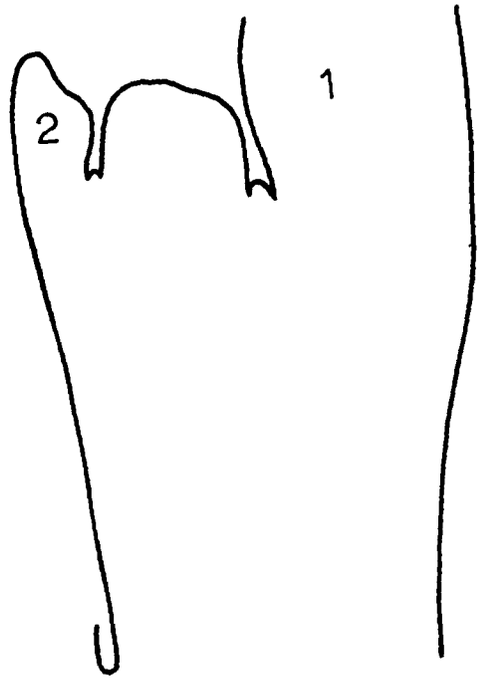
Fig. 29. The cotyledonary axillary shoot four days after planting.

Fig. 30. The cotyledonary axillary shoot seven days after planting.

Fig. 31. A cotyledonary axillary bud at the maximum structural development, 35 days after planting.

Leaf number is indicated by the respective numerals.

29



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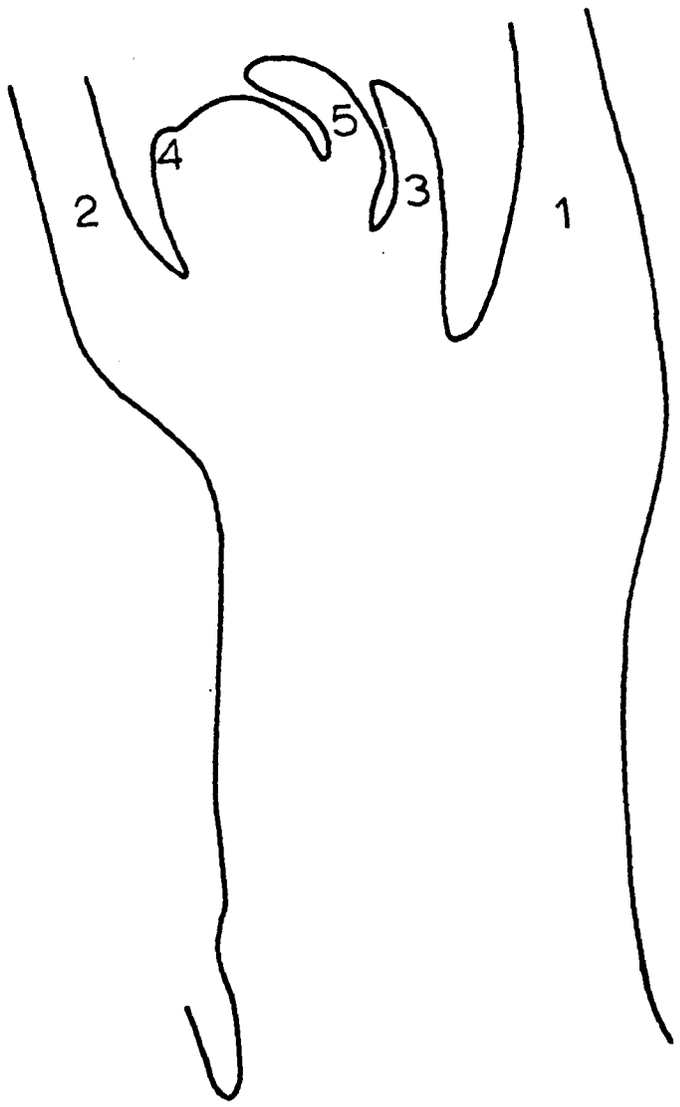

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vegetative apex (Fig. 31). These cotyledonary axillaries are capable of reactivation and extensive growth and fruiting.

Histogenesis of the Vascular Bundle

Longitudinal sections of dormant stem apices indicate that procambial cells occur approximately five to six cells below the uniseriate tunica, in association with the initiation of a leaf primordium. The cells between the outer corpus layer and the procambium strand undergo both periclinal and anticlinal divisions. The periclinal divisions of the outer corpus, designated T-2 by Sun (32), illustrate the correlation between leaf initiation and the acropetal development of procambial strands (Figs. 5, 9).

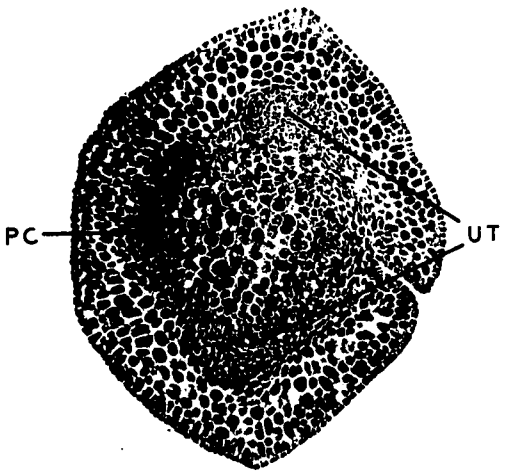
Serial transverse sections at dormancy section show densely stained procambial cells and vacuolated ground meristem cells about 60 to 100 microns below the tip of the leaf primordium. The procambium of the stem is a continuous ring at this level (Fig. 32). The strand to the unifoliate leaf is more advanced in differentiation than the smaller, densely-stainable cells of the procambial ring of the stem. This trace has mature protophloem, immature protoxylem and in some instances a indication of metaxylem initials (Fig. 6).

Strands in the 28 and 32 hour collections of the epicotyl contain spiral and annular protoxylem elements. These mature vessels were observed in the unifoliate and cotyledonary

Fig. 32. Transection of the shoot approximately 100 microns below the tip of the leaf primordium. The procambial ring is delineated by the vacuolation of the pith region, the abaxial sides of the bases of the unifoliate leaves and the future cortical region. (80x).

Fig. 33. Transection of a vascular bundle in the first internode region, 36 hours after the beginning of germination. The radial seriation, which had occurred prior to dormancy, is evident. Mature protoxlyem and protophloem are established at this time of development. (400x)

Cortex	(C)
Procambial ring	(PC)
Protophloem	(PP)
Protoxylem	(PX)
Radial seriation	(RS)
Unifoliate trace	(UT)



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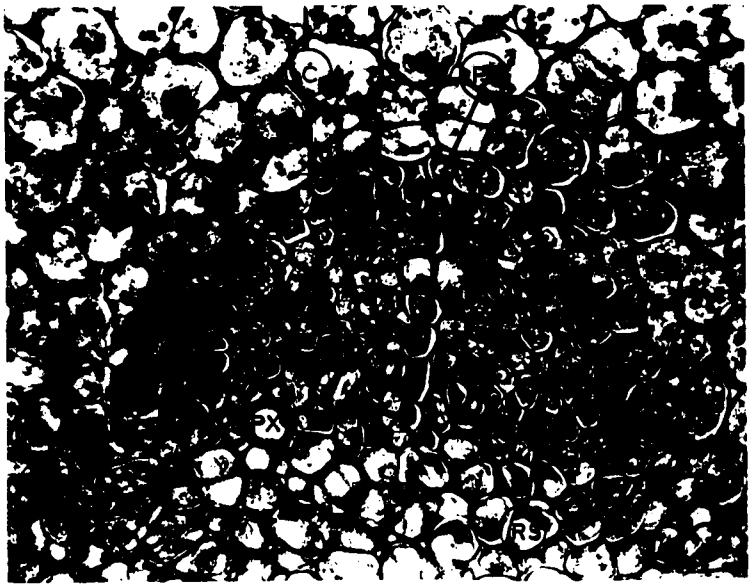


traces, approximately 350 and 800 microns below the stem apex, respectively.

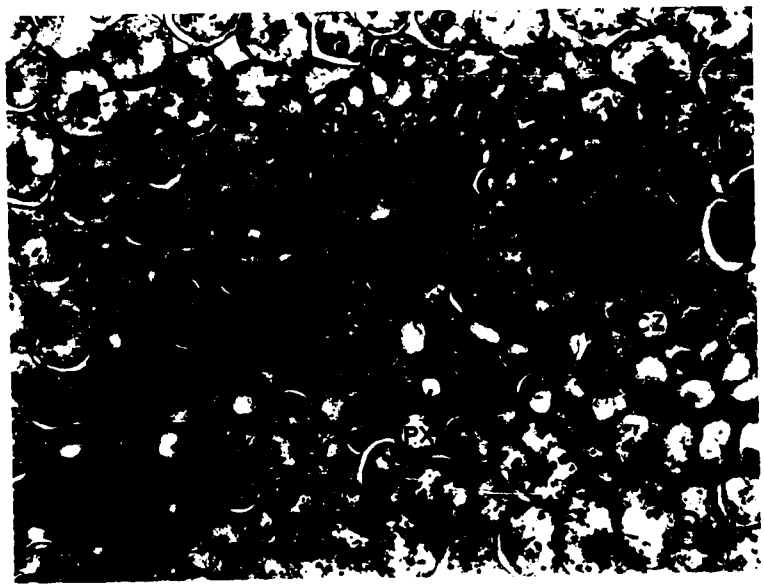
Cross sections about 100 microns below the apex of the 36 hour stem material have mature protophloem and protoxylem in the traces of the unifoliate leaves. Along with this development that occurred since dormancy, the cells of the strands have undergone elongation, and mitotic figures occur in the protophloem region. One or two distinctive layers of active procambium cells outwardly adjacent to the protophloem are evident (Fig. 33).

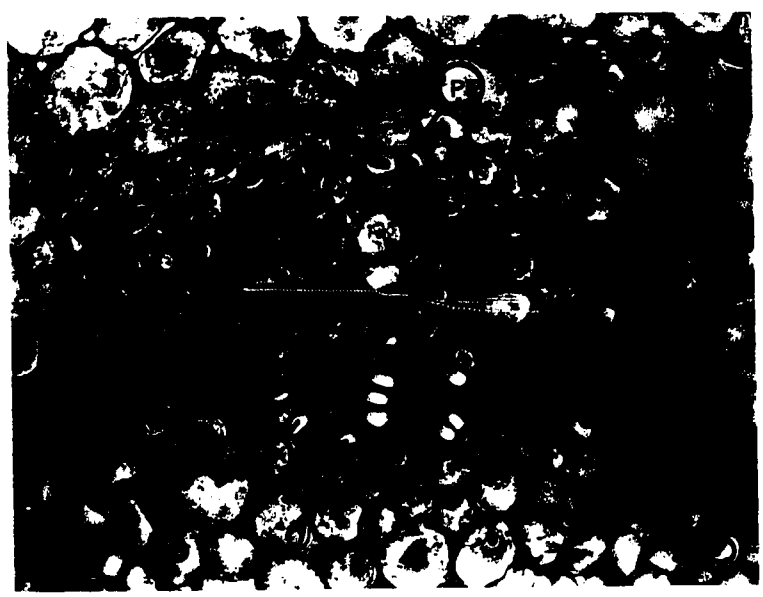
After 48 hours, greater mitotic activity occurs in the smaller cells of the procambial ring of the stem and also in the cells of the trace of the unifoliate leaf. The divisions in the smaller cells are generally in random planes, however an increase in the number of tangential divisions is evident. A radially seriated zone of cells is evident in the bundle that had been established prior to dormancy. This zone will be tentatively designated as the cambiform zone, which differs from procambium in that the cells of the cambiform zone divide predominantly in tangential planes, as opposed to random divisions displayed by procambial cells. Reactivation occurs in this zone at this stage of development (Fig. 34). The number of protophloem elements increases from one to three by 36 hours, to four to eight by 48 hours. One mature protoxylem vessel was found at 36 hours as compared with two at 48 hours.

Fig. 34. Vascular bundle 48 hours after the beginning of germination, at a higher level than the previous figure of the same bundle (33). The cambiform zone is evident. (400x).

Fig. 35. The vascular bundle at 60 hours after germination shows an increase in mature protophloem and protoxylem elements. Metaxylem initials are evident. (400x)

Cambiform zone	(CZ)
Metaxylem initials	(MI)
Protophloem	(PP)
Protoxylem	(PX)




P

The cell boundary between the protophloem elements and the possible future endodermis is no longer evident. Some protophloem elements are found directly adjacent to the endodermal cells, indicating that the fibers are formed within the region of the protophloem.

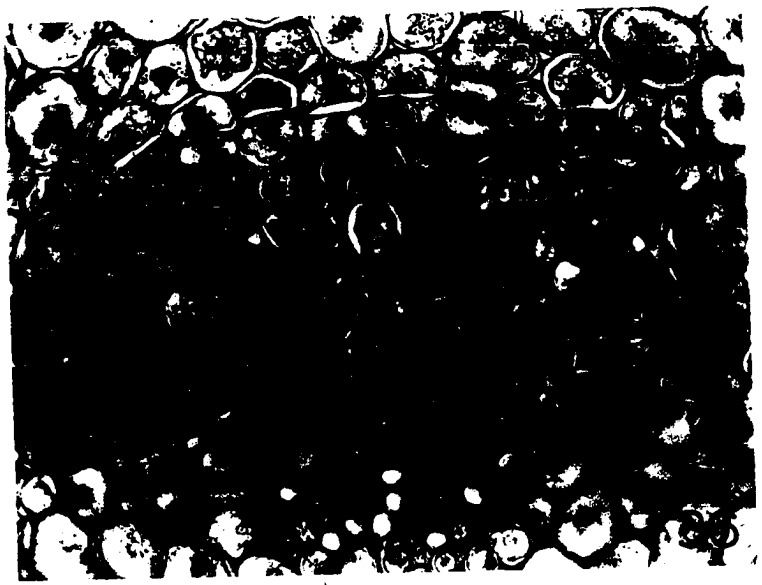
By 60 and 72 hours an increase in the number of matured protophloem elements is evident. Companion cells are not found in the protophloem. Metaphloem initials are present directly inward and contiguous with the protophloem. The number of mature, radially arranged spiral protoxylem elements increases. The metaxylem initials, which are rectangular in cross sectional outline undergo slight enlargement, loss of nuclei, and increased vacuolation, but thickening of the wall was not observed at this time of development. Anticlinal division of the cambiform derivatives occurs in the bundle. Periclinal and anticlinal divisions occur in the cortex (Figs. 35, 36).

The vascular bundle leading to the unifoliate leaf is still immature at 5 and 6 days, as mitosis of procambial cells is evident; however, mature metaphloem elements with companion cells are present. Most of the cambium derivatives form secondary xylem vessels, fibers, tracheids and xylem parenchyma as reported by Bell (2). A vessel type, which he did not report, with highly oblique end walls is present. During differentiation of metaxylem and secondary xylem the first-

Fig. 36. The protophloem elements are interspersed throughout the fiber mother cells. The position of metaphloem is established by 72 hours. (400x)

Fig. 37. Shows the position of metaphloem initials, the increased fascicular cambial activity and the initiation of interfascicular activity. (200x)

Fascicular cambium	(FC)
Fiber mother cells	(FM)
Interfascicular cambial activity	(IA)
Metaphloem initials	(MTI)
Metaphloem mother cells	(MTM)
Protophloem region	(PPR)



formed protoxylem undergoes disintegration, and the beginning of obliteration of the first-formed protophloem is evident at this time. The obliteration process is due partly to random division and enlargement of the fiber mother cells and subsequent maturation of these cells into fibers that are dispersed throughout and around the outermost protophloem elements. Obliteration is just being initiated at this stage and only a few protophloem elements are concerned. The initiation of interfascicular cambial activity is evident (Figs. 37, 38).

Nine day material shows a definite starch sheath, formed from the inner cell layer of the cortex immediately exterior to the protophloem. The ultimate number of fibers in a bundle has been established by this stage. They are generally angular in outline, without intercellular spaces and do not have secondary wall thickenings at this time (Fig. 39).

By 20 days the bundle displays considerable activity of the fascicular and interfascicular cambium. Directly below the interfascicular region and its immediate derivatives, a zone of sclerified parenchyma is evident. There are layers of secondary phloem and xylem formed in the larger bundles and few secondary elements associated with the smaller bundles of the developing vascular tissue. There is a layer of thin-walled parenchyma cells between the phloem fibers and the metaphloem. The fiber cell walls now display secondary thickening (Fig. 40, 41).

Fig. 38. The beginning of obliteration of the outermost protophloem elements is evident. Metaxylem initials are evident. (200x).

Fig. 39. Shows a starch sheath directly outside of the phloem fiber region. (200x)

Fibers	(F)
Fascicular cambium	(FC)
Metaxylem initials	(MI)
Metaxylem	(MX)
Obliteration	(O)
Secondary xylem	(SS)
Starch sheath	(S)


38

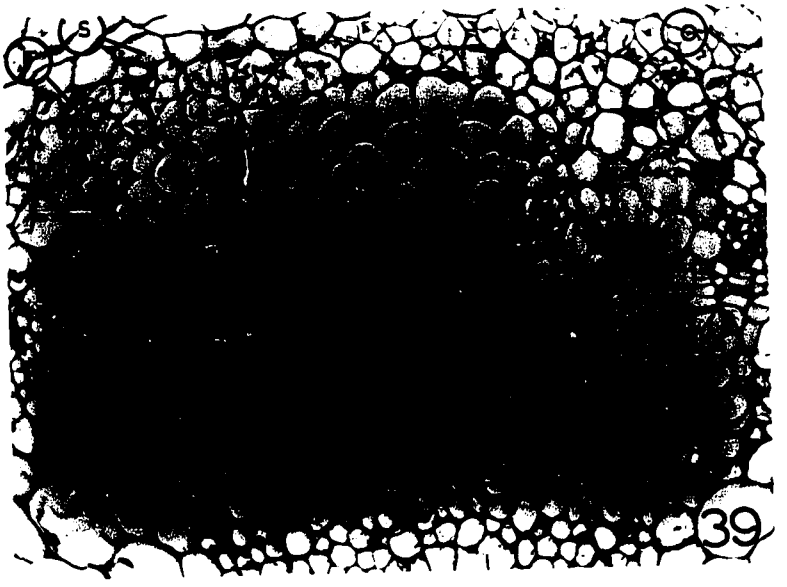
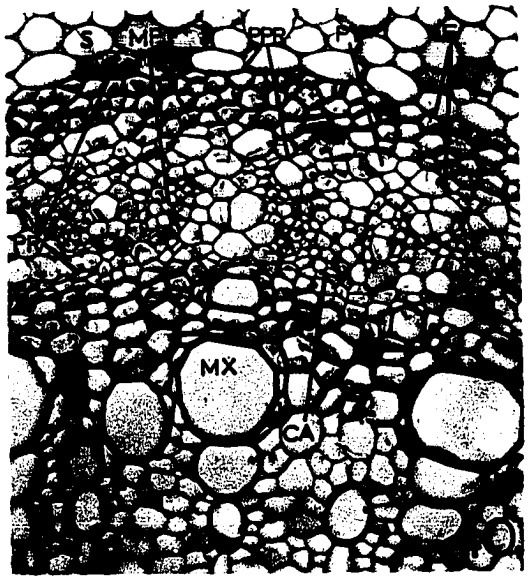
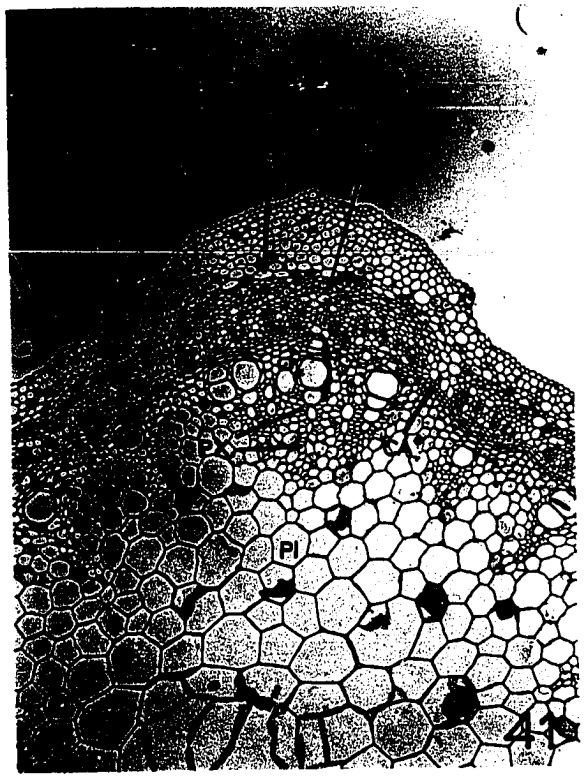

S
39

Fig. 40. A transection in the first internode region at 20 days showing cambial activity, metaxylem, and early secondary xylem. (170x)

Fig. 41. Transection of a portion of a mature stem. (48x)

Cortex	(C)
Cambium	(CA)
Epidermis	(E)
Fibers	(F)
Metaphloem	(MP)
Metaxylem	(MX)
Pith	(PI)
Protophloem region	(PPR)
Primary phloem	(PR)
Phloem parenchyma	(PY)


S
MF
PP
P
MX
CA


PI

DISCUSSION

Most of the earlier investigations on soybean dealt mainly with the seed characteristics and stored food materials, and did not describe the cellular detail and organogeny of the dormant embryo.

A small, centrally situated pit is present on the lower surface of the cotyledons. This anatomical feature of the dormant cotyledons indicates a structural weakness which develops during embryogeny. At dormancy an unknown bacterium may occur in the pit region, which may be the place of entrance for the bacteria. In view of this, an investigation of the post dormant reactivation period of inoculated and non-treated seeds should be made.

The vascular development and morphological status of the unifoliate leaves, cotyledons, and stem apex prior to dormancy, at dormancy, and during the early stages of germination could well be extended to other legumes. Such studies would permit concise interpretations of modifications induced by irradiation, chemical treatment and genetical studies. A case in point is Carlson's (5) use of the normal-abnormal approach in the study of the effects of maleic hydrazide on the meristems of three plants.

A detailed study of the root apex was omitted in this study, owing to the fact that it was investigated by Bell (2)

and Sun (33). Sun's use of the work "columella" for the distal portion of the common initials may be open to question, as the transversely layered cell region of the common initials appears to be a uniform meristem. In this paper the classical terms "common initials", "diffuse generating zone" or "open zone" are still applicable.

The general pattern for reactivation in soybean is somewhat similar to that reported for Zea by Picklum (26). Mitotic figures are first observed 32 hours after germination in the cortex of the root, some distance away from the apical generating zone. Figures occur subsequently deep within the corpus of the stem apex and of the cotyledonary axillary bud, and still later in the apices. The last structures which display mitotic figures are the tunica of the stem apex and the protoderm.

The histogenesis and leaf development was described by Sun (32). In the present study the narrow stem apex was found to widen during the course of leaf formation. At approximately 80 hours the second leaf is initiated. This is the first plastochrone. The third leaf is initiated about 132 hours after the beginning of germination and after these initial stages the subsequent leaf initiations occurred at two-day intervals. Sun (32) reported that when a given leaf primordium attains an approximate height of 80 to 90 microns, the next leaf was initiated. Sun's report is in general

agreement with this present study; however, he did not describe the early and later stages of plastochronation.

The cotyledonary axillary develops two to three leaves during the early stages of germination and then growth slackens until 20 or 25 days, when the bud is essentially inactive. These buds do have the anatomical potentiality to develop into mature fruiting shoots if the main axis is decapitated during the early growth period. Weber (34) reported a 10% loss in yield from decapitated plants.

The development of the vascular bundle between the cotyledonary and unifoliate node was investigated. At 48 hours a tangential orientation of the procambial cells is evident. This fascicular cambial activity produces radially seriate metaxylem and unstratified metaphloem. The interfascicular cambium is initiated approximately five or six days after germination and by 20 days a complete cambium is formed. Esau (12) stated:

> If some procambium remains in a meristematic state after the completion of primary growth, it becomes the cambium of the secondary body. This cambium is called fascicular, since it originates within the bundles or larger segments of the primary vascular system. (12, p. 381)

In soybean, both the procambium and fascicular cambium seem to be the same meristem, at different, overlapping developmental stages. In view of the precocity with which the fascicular cambium is initiated in soybean, the terms, primary

and secondary plant body are convenient, but should carry certain reservations. For instance, the radial seriation of the so-called metaxylem in soybean, in radial continuity with the presumed secondary xylem and with fascicular cambium require further study.

The derivatives of the bundle cap consisting of so-called "pericyclic" fibers have been re-examined in soybean. The first protophloem elements were found abutting the inner cortical cell layer, and there is no distinct tissue between the cortex and phloem that could be ontogenetically termed pericycle. The cells that differentiate into fibers are ontogenetically part of the primary phloem, therefore they should be designated as phloem fibers. This agrees with Esau (12) in her report on the development of the primary phloem fiber in Linum perene L.

SUMMARY

An investigation of the developmental anatomy of Glycine max, (L.) Merr., Var., Hawkeye, was made, beginning with the status of organogeny and tissue organization of the dormant seed, through the post-dormant and emerging seedling, and subsequent development to floral initiation.

The dormant cotyledons have netted venation. The large, main vascular bundles and the smaller veins have immature protoxylem, metaxylem initials, and mature protophloem. The mesophyll consists of palisade and spongy parenchyma. A centrally located pit occurs on the abaxial surface of the cotyledons. The epidermal and subadjacent cells of the pit area are slightly plasmolyzed and hypertrophied at dormancy.

Mature protophloem and protoxylem are present in the vascular system of the dormant unifoliate leaves. Metaxylem initials are evident in some strands. The oblique stem apex consists of a uniseriate tunica and a massive corpus. The primordium of a trifoliate leaf had been initiated prior to dormancy, as evidenced by periclinal divisions of the outer corpus.

The apical meristem of the dormant radicle consists of two zones, the stelar initials and the diffuse common initials.

The organization of the dormant cotyledonary axillary bud is the same as that of the dormant main stem apex.

In the radicle, mitotic reactivation may occur at 32 hours after the beginning of germination, in the cortex region, approximately 400 to 800 microns above the apical generating zone. In the apical generating zone mitotic figures occur in the 40 and 48 hour collection.

In the shoot, mitotic reactivation occurs in the procambium strands within the corpus at 36 hours, approximately 50 to 100 microns below the stem apex. Reactivation in the unifoliate leaves occurs at 36 and 48 hours in the mesophyll and epidermis, respectively. Mitotic figures are present in the corpus of the cotyledonary axillaries in the 36 hour collection, and in the tunica at 40 to 48 hours.

Increasing necrosis of the cells in the pit region of the cotyledon is evident in the 2, 7, and 14 day collections. Periclinal divisions occur in the subadjacent cells. By the fourteenth day, degeneration of the mesophyll cells of the cotyledons near the necrotic zone is prevalent, along with external discoloration.

The dormant stem apex is narrow, and almost doubles in width by 72 hours. The first plastochron occurs approximately 80 hours after the beginning of germination, the second by about 130 hours, forming the second and third leaf, respectively. Each subsequent leaf is initiated at two-day intervals. At the 10 to 12 leaf stage, or 20 to 24 days after germination, floral initiation is microscopically evident in

the axillary shoot of the first trifoliate leaf. The primorida of all the floral member are present in this same position on the stem at 25 days.

The height of the cotyledonary axillary buds increases from 20 and 50 microns at dormancy to approximately 1200 microns at the time of flowering on the main axis, then increase in height ceases.

At dormancy, the vascular system of the stem consists of a procambium ring, with an enlarged strand to each unifoliate leaf. Thirty six to 48 hours after germination, mitotic activity increases in both the smaller cells of the procambial ring and the procambium of the unifoliate trace, and there is an increase in the number of mature protophloem and protoxylem elements. Divisions in the procambium are at first in random planes, but a tendency to tangetially oriented divisions is evident by 48 hours. Radiately seriated metaxylem, and unstratified metaphloem are differentiated. The radially seriated arrangement of the metaxylem is related to the very precocious cambium-like activity in the bundle. This active zone may be designated fascicular cambium, even at this early stage. Interfascicular cambial activity is initiated in the internode above the cotyledons approximately five to six days after germination, and the complete cambium is operating by 20 days.

A definite starch sheath is derived from the innermost

cortical layer, and is evident by nine days. The ultimate number of fibers had formed in the vascular bundle at this stage. The sclerenchyma cells that had been known for many years as "bundle cap" of pericyclic derivation, are formed within the region of the protophloem and are ontogenetically phloem fibers.

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