American Journal of Botany 20: 309-327 (1933)

STRUCTURE AND GERMINATION OF TOBACCO SEED AND THE DEVELOPMENTAL ANATOMY OF THE SEEDLING PLANT

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(Received for publication July 28, 1932)

INTRODUCTION

The genus *Nicotiana* (Solanaceae) is composed of some 50 or more species, according to Garner (1927), and possibly as many as 70 species, according to Setchell (1921) and East (1928). For purposes of classification the genus was divided into four sections by Don (1838): Tabacum, Rustica, Petunioides, and Polidiclia. These were accepted by succeeding authors, including Comes (1899), but East (1912) and Setchell (1912) both agree that the one species and its variety in the section Polidiclia belong in Petunioides. Later, East (1928) concludes that the Petunioides section is not justified from the genetic evidence. He states: "There are a number of genetic centers which may possibly be made the basis for various generic subdivisions when our information is more complete." Chromosome numbers in the genus are diverse, the haploid numbers of some 33 different species thus far determined being 8 or 9, 10, 11, 12, 16, and 24 (East, 1928; Gaiser, 1930), appearing in no way related to the present grouping into sections.

It is generally conceded that the genus is of New World origin, Central America and northwestern South America being centers of distribution, according to DeCandolle (1884), Vavilov (1926, 1931), and East (1928), though *N. suaveolens* Lehm. is reported from Australia and three other species which may be merely varieties of the latter are listed from the region of the Malay peninsula (East, 1928). Some species are herbaceous annuals, others perennials, and some even shrubby or subarborescent (four such species are distinguished by Goodspeed, 1932, including *N. Wigandioides* Koch.), or arborescent (*N. glauca* Graham). Certain species produce showy garden flowers, while still others are valued for their leaves. Among the latter is *Nicotiana tabacum* L., our common tobacco of commerce, sharing its importance to some extent with *N. rustica* L. (Bailey, 1916).

While there is considerable diversity of opinion as to who it was that first took tobacco or tobacco seed to Europe, there does seem to be a general agreement that it took place in the first half of the sixteenth century. Since its introduction into Europe four centuries ago it has developed into one of the most important plants of commerce, statistics for the year 1930 indicating a production of over one and a half billion pounds (*International Yearbook*

of Agriculture, p. 210, 211), Soviet Russia and several smaller countries not included. The increase in its culture has been marked in the past decade. Diseases take a heavy toll in some regions.

An ideal study in the developmental anatomy of a seed plant should perhaps include four periods in gross development: The *first* is concerned with the embryo plant—i.e., its development from the zygote to the mature embryo in the seed. This particular stage is determinate, in that it has a definite beginning and end. The *second* period is concerned with the enlargement of these first-formed parts, ending in the usual category of the seedling plant. The *third* period consists, at first, of enlargement which takes place as growth is accelerated (in tobacco it includes the stages in which the first ten to fifteen leaves develop), and this grades insensibly into the condition in which are developed all vegetative structures typical of the adult plant in size and character. This period of vegetative development may extend over a longer time than any of the others and is often marked by considerable vegetative plasticity, due to extremes of soil moisture, light, or other conditions. It is a period during which, in the growing plant, "continued embryology holds sway with its successive origination of new organs" (Bower, p. 315). The *fourth* is a period which occurs after a certain physiological balance has become established within the plant, resulting in the development of parts leading to and including flowering and fruiting.

The earlier stages in the embryogeny of tobacco have been worked out by Souèges (1920), to illustrate the origin of the dermatogen, plerome, and periblem, and the study here presented is confined to the development of the seedling plant and the beginning of the period of rapid enlargement which follows, chiefly as regards the hypocotyledonary axis.

The results that follow, though little more than confirmatory of many preceding investigations on seedling anatomy, attempt to give more detailed information on the developmental side of what has become one of our most important economic plants. The present paper represents the first of a series of studies on the structure and development of *Nicotiana tabacum* L.

MATERIALS AND METHODS

The greater part of the seeds and seedlings of tobacco (*Nicotiana tabacum*) used in this experiment were of the Cash variety, although Havana Seed and Cuban Shade varieties were used as additional material. Less extensive observations were made on *N. Langsdorffii* Weinm., *N. glutinosa* L., and *N. rustica*, each species representing a different chromosome group. In germinating the seeds no particular attention was paid to temperature, though Haberlandt reports an optimum of 27° C., with the minimum and maximum at 15° and 31°–33° C., respectively. No seed treatment nor special precaution was taken with regard to light, but it seems well to touch on these points because of their relation to germination.

Goodspeed (1913) has shown that treatment of seed from 10 to 12

minutes with 80 per cent H_2SO_4 and washing for a short period markedly increases the percentage of germination for several *Nicotiana* species. While the necessity of light for germination is a problem on which there are many diverse data and subsequent conclusions, Honing (1916, 1926, 1930) finds that such varieties as Cuban Shade, Connecticut Broadleaf, and many of the varieties grown in the Carolinas and Virginia show only a small percentage of germination in the absence of light as compared with its presence (0–10 per cent), while Havana Seed in darkness shows 41–50 per cent as good germination in darkness as in light. *N. rustica* under similar circumstances shows 71–80 per cent as good germination. All figures were based on observations at the end of seven days. Goodspeed (1919) reports that "there is no doubt that the seed of five representative types of *Nicotiana tabacum* and of five varieties of *N. rustica* will germinate readily in darkness." Numerous other papers deal with the subject.

The material taken for microscopic study was fixed in formal-acetic-alcohol, imbedded by the paraffin method, sectioned at 10–12 microns, and stained with safranin, being counterstained with Delafield's haematoxylin or fast green.

In the studies on seeds as well as seedlings, serial sections were cut both longitudinally and transversely.

THE SEED

The seeds of tobacco are very small; egg-shaped though somewhat flattened, and with a prominent raphe along one side, ending in the projecting hilum at the small end of the seed (fig. 1, *A*). They have a finely reticulated surface and are dark brown in color. Though the seeds vary in size according to variety, they average, according to Kondo (1921), 0.75 mm. long, 0.53 mm. broad, and 0.47 mm. thick. a thousand weighing 0.08 gram.

The protective seed coat (not illustrated in detail) includes a prominent epidermis. Both layers of the double inner wall are cutinized and the inner layer is slightly lignified. The outer walls are thin, of cellulose with some pectic material, and are lightly cutinized. The fact that the thin outer walls bend inward at maturity accounts for the reticulated appearance of the seed. The subepidermal layers, usually three in number, are composed of thin-walled parenchyma cells which are crushed at maturity. It is necessary to study an immature seed to get the seed coat in full detail, as pointed out by Grintescu (1915) for *N. rustica*. A single layer of nucellar tissue persists just inside the layers of parenchyma, between the latter and the endosperm. Inward from this are some three to five layers of rather thick-walled endosperm cells (fig. 1, *K* and *L*), largely isodiametric, rich in aleurone and oil droplets and densely protoplasmic.

A longitudinal section through the seed (fig. 1, *L*), passing through the plane of symmetry of the straight or slightly curved embryo, shows the latter to be about 0.7 mm. long and surrounded by endosperm. The cotyledons

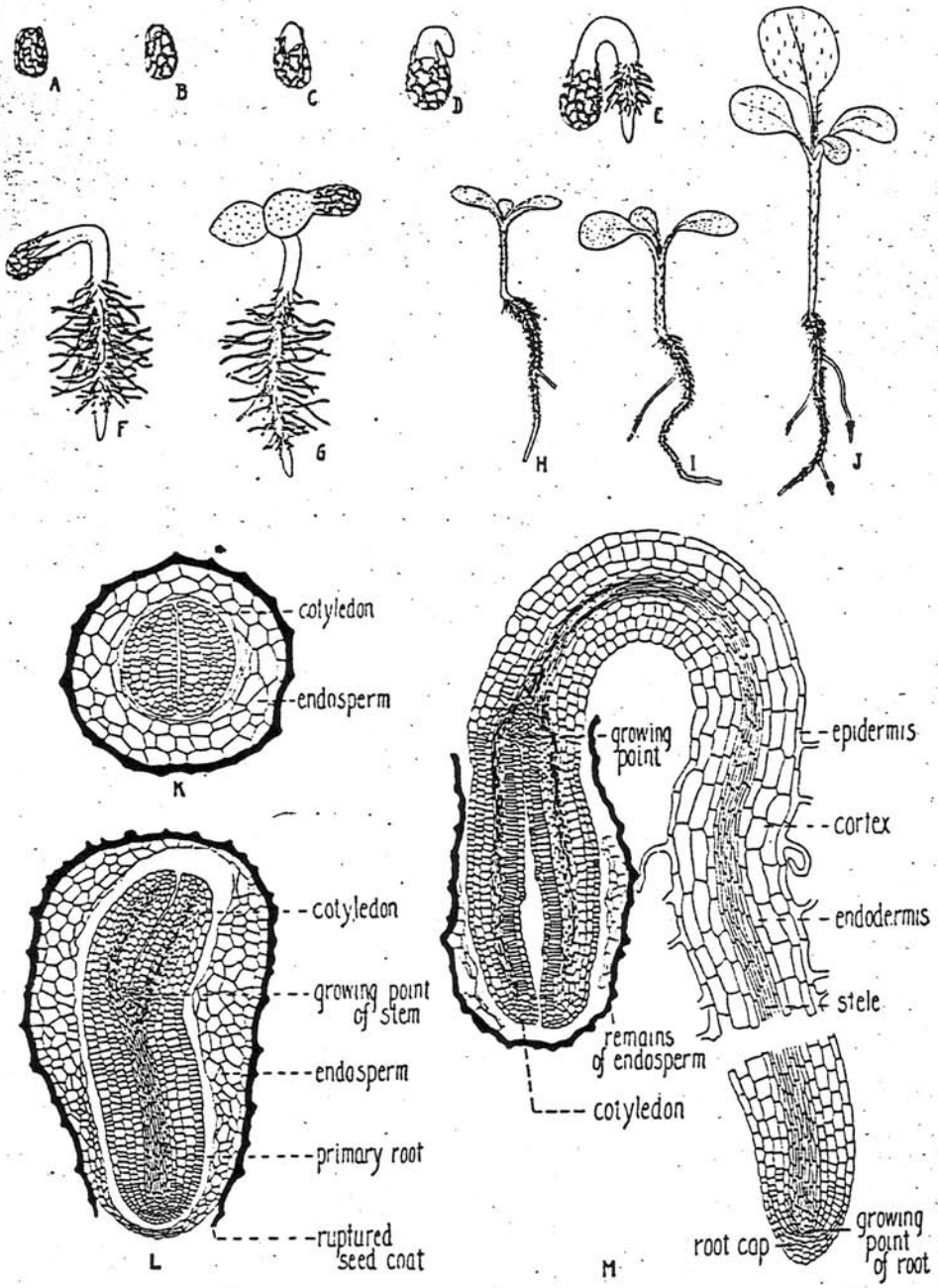

Fig. 1, *A–M*. *A*, dormant seed (×8). *B–D*, stages in germination at the end of six days (×8). *E–G*, same, at the end of nine days (×8). *H–J*, seedling stages at the end of sixteen to eighteen days (×2). *K*, transverse section through dormant seed at the cotyledonary level (×70): *L*, longitudinal-median section through seed just starting to germinate, showing ruptured seed coats (×50); see *B* above. *M*, longitudinal-median section through seedling at the end of nine days (×50).

are six layers of cells in thickness, including upper and lower epidermis (fig. 1, *K*). Their provascular midrib is well defined, as is the central strand of the hypocotyl, the latter being oriented toward the small end of the seed. In the hypocotyl there are four layers of cells outside the stele, including the epidermis (fig. 1, *L* and *M*). It is of interest to note that the mesophyll of the cotyledons is of the same number of cell layers in thickness as the cortex plus the epidermis of the hypocotyl. The embryonic cotyledons are some seventeen upper epidermal cells long, other layers having about the same

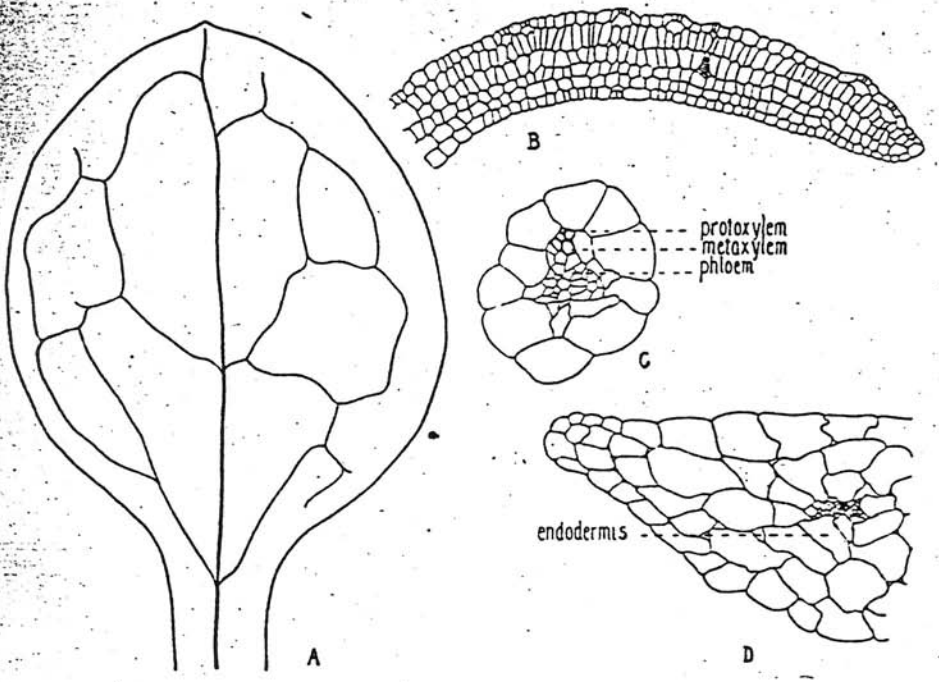

Fig. 2. *A–D*. *A*, diagram of mature cotyledon showing entire vascular system (× 20). *B*, longitudinal section of cotyledon of a seedling nine days old (× 70); see fig. 1, *G*. *C*, transverse section through midvein of mature cotyledon, midway between base and tip (× 150). *D*, transverse section through mature portion of cotyledonary petiole (× 120).

number, and eighteen upper epidermal cells wide (fig. 1, *K* and *L*). Behrens (1892) reports "two procambial strands corresponding to the bundles of the cotyledons" in the hypocotyl. This seems to be the condition only at the upper end as they diverge into the cotyledons. The growing point of the stem, though only slightly developed, is markedly conical in shape.

GERMINATION

Germination is irregular in most cases, as Behrens (1892) pointed out. The first germinations usually take place six to eight days after planting.

The seed coat breaks under the pressure of the developing primary root, near the micropylar end of the seed (fig. 1, *B* and *L*). The hypocotyledonary axis continues elongation and in 10–12 days the primary root is several millimeters in length, has developed copious root hairs, and has started to function (fig. 1, *C–F*). The upper hypocotyl is slightly hairy. During the same period the cotyledons have remained within the seed coats (fig. 1, *M*), usually withdrawing about the twelfth day, though the old seed coats often adhere to one of them for a few days longer (fig. 1, *G*). By the fifteenth day the first leaf above the cotyledons is visible, followed by the second on the seventeenth or eighteenth day (fig. 1, *H–J*). Growth accelerates rapidly from this time on. These stages in germination are discussed below from the point of view of internal development.

ONTOGENY OF THE PRIMARY ROOT

The growing point of the root is developed at the time of differentiation of the embryo, the lower end of the hypocotyledonary axis possessing definite though immature root structure.

The primary root is diarch (fig. 3, *A*-1), the protoxylem and metaxylem being practically indistinguishable from each other. The primary phloem groups consist of two small masses of parenchyma, one on either side of the xylem arm. The pericycle is clearly distinguishable as the outer layer of the stele and gives rise to a few lateral roots after a period of fourteen to sixteen days (fig. 1, *H*). The Casparian strip identifies the endodermis, the other two layers of the cortex being composed of considerably enlarged parenchyma cells. The epidermis is thin-walled and gives rise to copious root hairs. The number of cell layers of the cortex corresponds to the number present in the embryo, there being no increase in cell number in transverse section as the cortex enlarges.

ONTOGENY OF THE HYPOCOTYL

The rapid increase in the length of the hypocotyl is due both to cell division and enlargement, *all new cell walls of the primary body being laid down perpendicular to the axis*. The number of cells in a transverse section of the hypocotyledonary axis of the seedling before secondary thickening would, therefore, be the same as the number present in the embryo.

Serial sections up through the axis of a seedling ten days old disclose the internal conditions at this early period of development (fig. 3, *A*-1 to *A*-13). It is clear at this stage of development that most of the anatomical root-to-stem change takes place in the region immediately below the cotyledons, from the level shown in figure 3, *A*-6, to approximately the cotyledonary level (fig. 3, *A*-13), a distance of about 0.5 mm. This portion of the hypocotyl is still elongating, and the cotyledonary traces of annular and spiral elements are clearly discernible (fig. 3, *C*). The protoxylem of the trace to the first leaf distal to the cotyledon has differentiated (fig. 3, *A*-12), though the leaf has

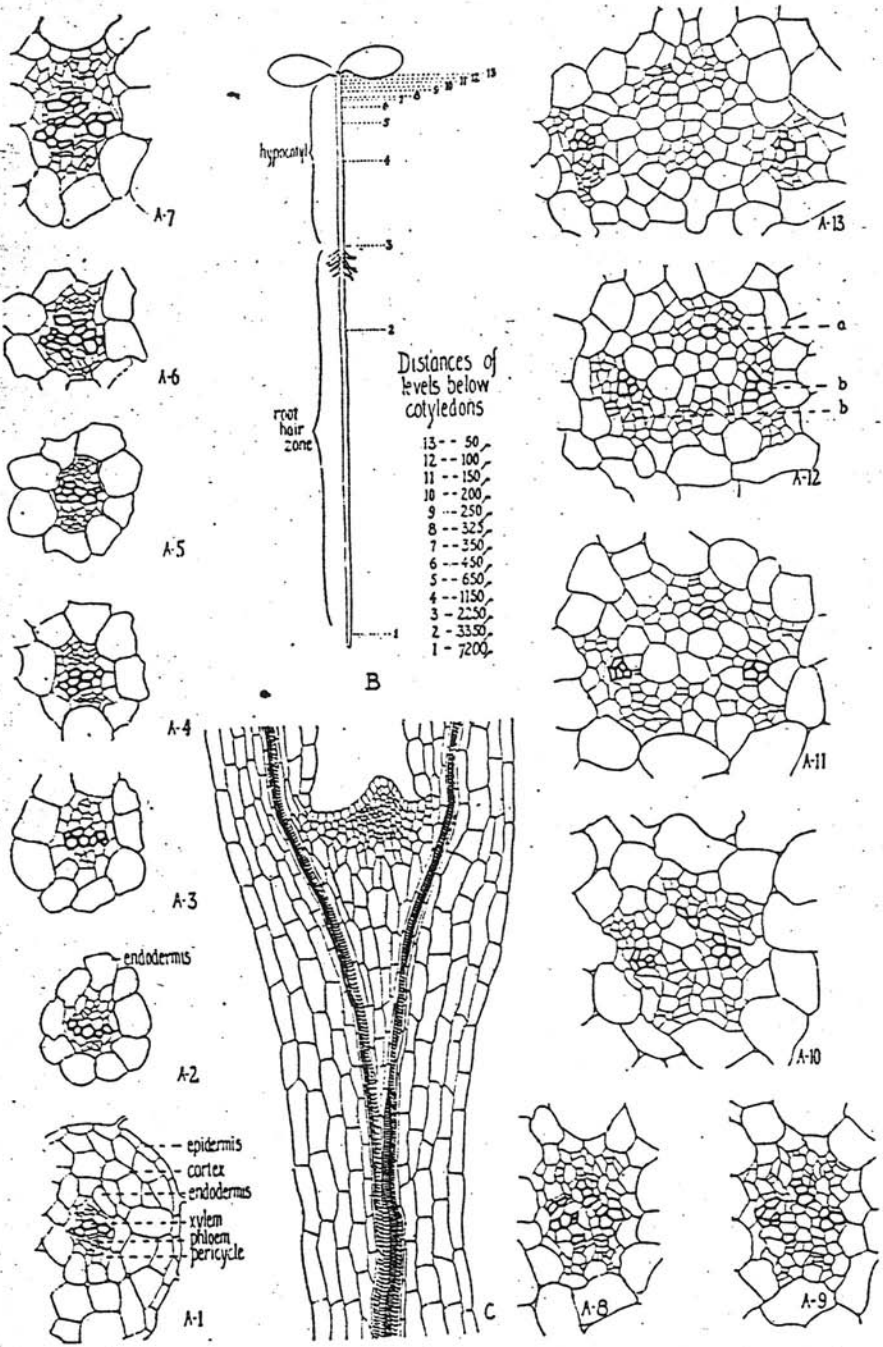

Fig. 3, *A*-1 to *A*-13, *B*, *C*. All figures from seedlings ten days old. *A*-1, transverse section through portion of root at the level indicated in *B*-1 (× 150). *A*-2 to *A*-13, transverse sections of stele and endodermis at successive levels indicated in *B*-2 to *B*-13 (× 150). (*A*-12. *a*, xylem of trace to first leaf distal to cotyledon. *b*, xylem of cotyledonary traces.) *B*, sketch of seedling (× 1½) to show levels at which transverse sections *A*-1 to *A*-13 were taken. *C*, longitudinal-median section through hypocotyl showing transition region, divergence of cotyledonary traces, and growing point of stem.

not yet developed. The only clearly differentiated cells in the stele at this level are the xylem cells of these three traces, though the endodermis is easily identified (fig. 3, *A*-10 to *A*-12). The origin of the pith is evident at level *A*-8, 325 microns below the cotyledons, where the diarch root xylem becomes divided into several groups. The two more prominent groups become the xylem of the cotyledonary traces, the two less prominent ones ultimately supplying the first and second leaves distal to the cotyledons. At the level at which the pith is first evident, each phloem group from the root bifurcates, so that the xylem of each cotyledonary trace is central and is flanked on either side by a phloem group. The phloem on one side of one cotyledonary trace forks again, one of these strands accompanying the xylem destined for the first leaf distal to the cotyledons (fig. 3, *A*-10; fig. 1, *H*), the other remaining with the cotyledonary trace. At the time these phloem changes are taking place, the protoxylem becomes reoriented so that the xylem of each cotyledonary trace is endarch.

The axis of a seedling three to four weeks old is the first to show clearly the relationship between internal and external phloem. At this period of its development the seedling has two to three leaves visible above the cotyledons (fig. 1, *J*). Secondary thickening has just begun. The change from the exarch to the endarch condition of the primary xylem, though difficult to make out because the seedling of tobacco is greatly reduced, takes place in a seedling of this age about 2 mm. below the cotyledonary level and is completed within a distance of approximately 1 mm. The fact that transition takes place less than 0.5 mm. below the cotyledonary level in the younger material indicates that the region immediately below the cotyledons has undergone elongation. With regard to the establishment of the internal phloem, it is evident for the first time in material of this age that strands of external phloem diverge inward approximately 1.4 mm. below the level at which the cotyledons are attached to the axis.

A hypocotyl four to six weeks old, cut in serial transverse sections, shows the relationship of external and internal phloem in its final ramifications (fig. 4, *B*; fig. 5, *A–D*). The secondary xylem is considerably increased in amount. In some material the first two phloem strands enter the pith 180 degrees from each other, being laid down gradually inward through the cotyledonary gaps, the internal condition in each case being established within a perpendicular distance of 60 microns. When the first two strands of internal phloem are oriented in this manner at a level about 1.4 mm. below the cotyledons, they are usually followed by two more strands which become established internally at a level approximately 0.7 mm. below the cotyledons. These also "invade" the cotyledonary gap (opposite side of the cotyledonary trace from the previous "invasion"), and are 180 degrees from each other. Other internal phloem strands may have differentiated in material of this age, in which case they usually connect with the external phloem through the gaps already mentioned or through vascular rays, often immediately

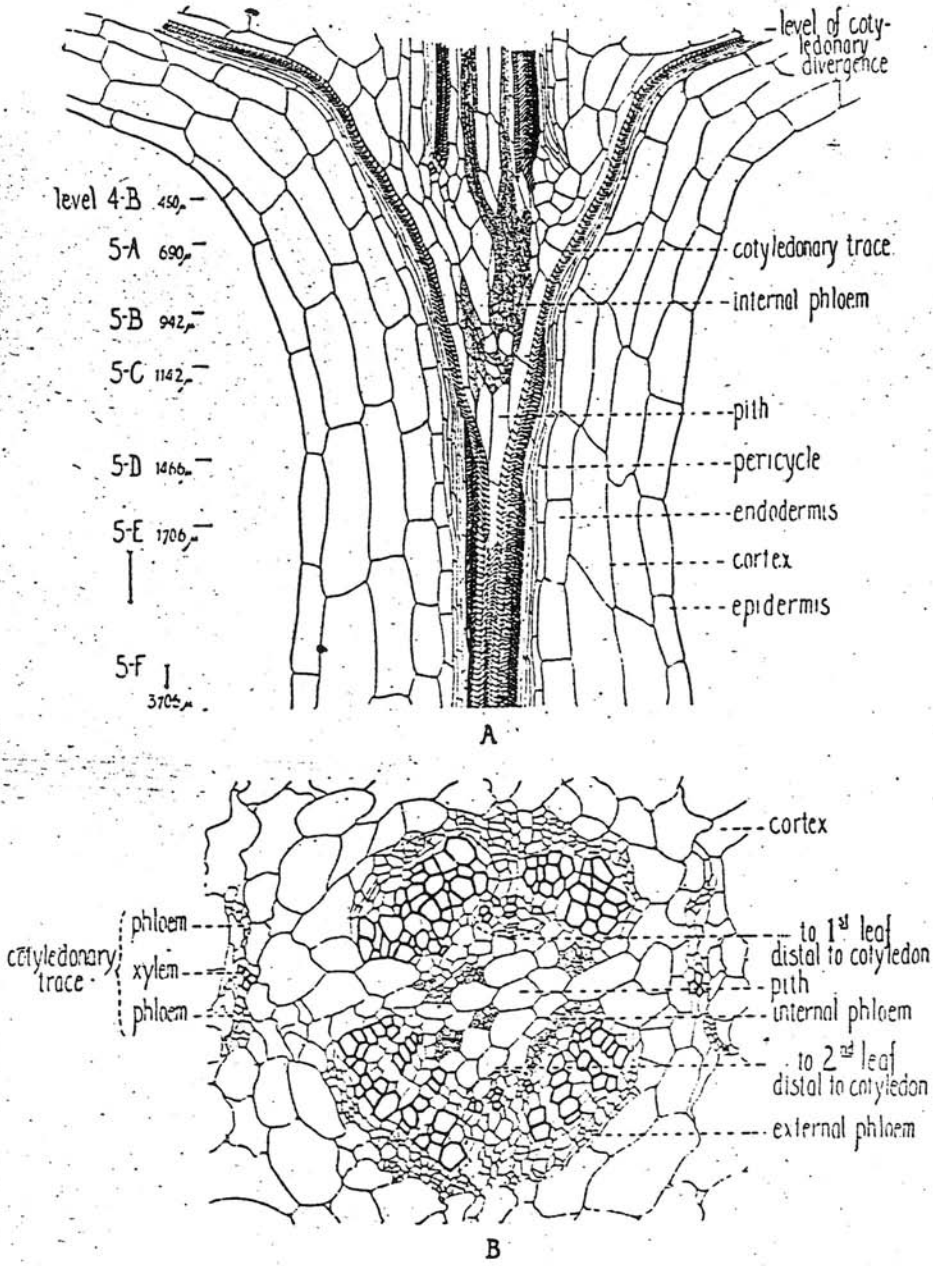

Fig. 4, *A* and *B*. Both figures taken from plants six weeks old. *A*, longitudinal-median section through upper hypocotyl showing orientation of internal phloem, divergence of traces to the cotyledons and the lower end of the first internode (× 50). Levels 4, *B*, and 5, *A–E*, indicate levels at which transverse sections of same age were taken, and refer to figure 4, *B*, and figure 5, *A–E*. Distances below the level of cotyledonary divergence are indicated at each level. *B*, transverse section through portion of the hypocotyl at the level indicated above as 4, *B* (× 140), approximately 450 microns below actual cotyledonary divergence.

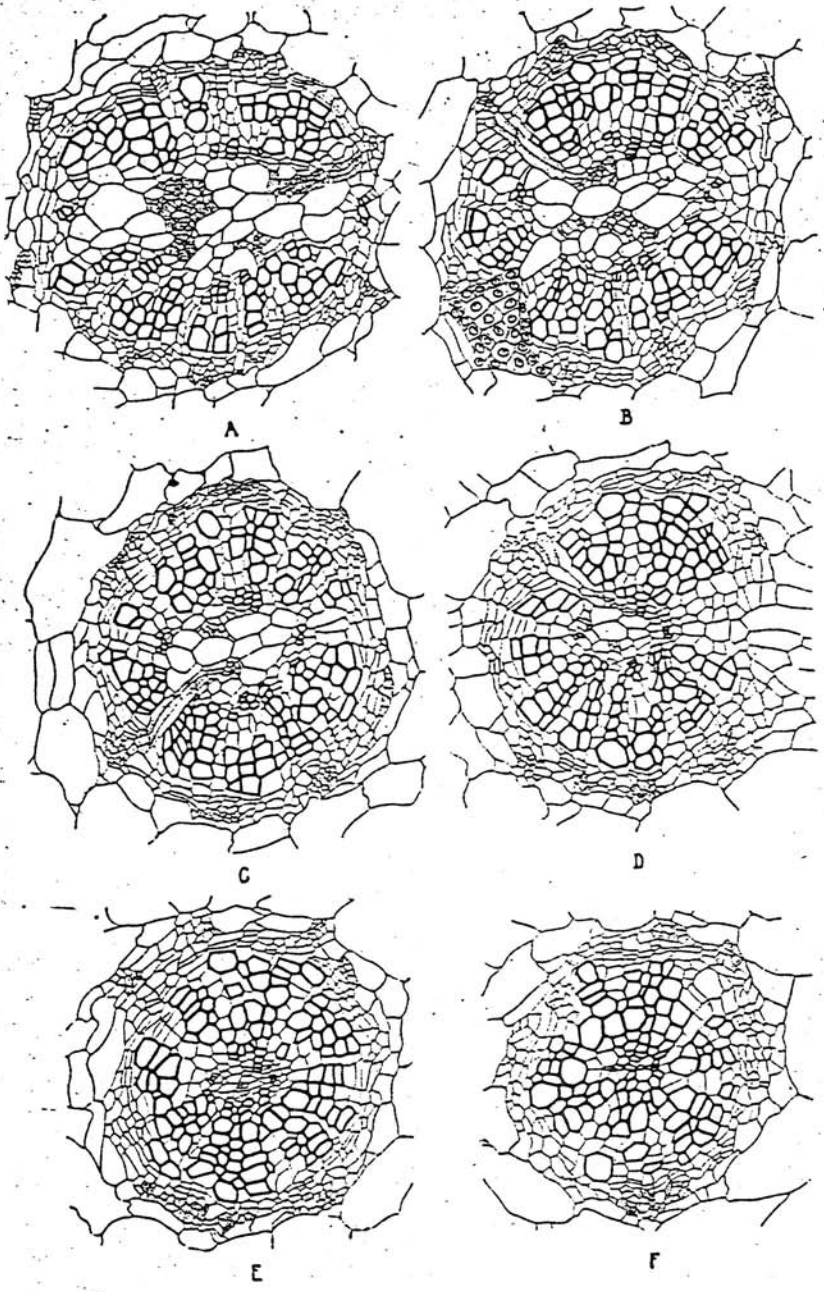

FIG. 5.

beside the trace to the first leaf distal to the cotyledons, well below the level of the leaf gap.

Apparently there is no definite pattern for the establishment of the internal phloem, for it occurs in diverse ways. A common scheme of accomplishment is illustrated in figure 5, *A–D*. In this case, three of the internal phloem groups "enter" the pith from one side of the axis, while the fourth enters from the opposite side. A fifth internal phloem strand, very small in size, ends blindly in the pith (fig. 5, *D* and *E*). As previously indicated, each phloem strand which differentiates inward from the external phloem accomplishes the change within a distance of 60 microns. As further secondary thickening takes place, the connection between internal and external phloem groups is by means of phloem parenchyma laid down by cambium. The internal phloem anastomoses to form something of a phloem plate just below the cotyledonary level (fig. 4, *A*). From four to seven groups of internal phloem usually appear in transverse section at this level.

The root system of the mature tobacco plant is largely adventitious, and such roots are being initiated by the pericycle throughout the hypocotyl of a seedling four to six weeks old (fig. 5, *B*).

The number of epidermal and cortical cells present in a transverse section of the hypocotyl at this stage of development is essentially the same as in the corresponding structure of the embryo, but they are considerably enlarged. There is only a small amount of external phloem present, primary and secondary, and this appears to be largely parenchyma. The secondary xylem elements are scalariform or reticulate in their markings.

ONTOGENY OF THE COTYLEDONS

All new cell walls in the cotyledons (except in provascular regions) are likewise laid down perpendicular to the plane of the upper and lower epidermis. The number of cell layers in a mature cotyledon is, therefore, the same as in an embryonic cotyledon.

Fig. 5, *A–F*. All figures are from serial transverse sections through the hypocotyl of a seedling six weeks old (× 140). *A*, 240 microns below transverse section shown in fig. 4, *B*, showing internal phloem connected through cotyledonary gap with the external phloem. *B*, 252 microns below *A*. The connecting phloem shown in *A* has joined the external strand. Another internal phloem group is shown connecting with the external phloem. At the lower left is the primordium of an adventitious root. *C*, 200 microns below *B*, showing connecting phloem strand. In the late primary condition this would have appeared as taking place through the cotyledonary gap. The laying down of secondary xylem has made the connecting phloem actually secondary, through a vascular ray. *D*, 324 microns below *C*, showing the last remaining internal group connecting with the external phloem. *E*, 240 microns below *D*, showing a vestige of internal phloem which ends blindly a little lower down. The xylem of the cotyledonary traces is closing in toward the center and the pith is much reduced. *F*, 2 millimeters below *E*, showing pith no longer present. The diarch primary xylem is characteristically root-like, though buried in secondary xylem.

The upper epidermal cells are the first to divide in the cotyledons, and stomata of the upper epidermal layer are well differentiated long before the cotyledons leave the old seed coat. The palisade cells assume their characteristic appearance early, division having started almost simultaneously with the upper epidermis. Growth is predominantly basipetal until the seed coat is shed, expansion then taking place rather evenly throughout the lamina. The petiole continues to elongate for some time.

The cotyledons at the time of emergence average forty-eight upper epidermal cells in length, with other cell layers about as follows: lower epidermis, forty-two; palisade, fifty-six; spongy parenchyma, thirty-seven. Since seventeen cells is the average for each layer in the cotyledons of the dormant embryo, it is obvious that the palisade undergoes the greatest number of divisions, followed by the upper and lower epidermis, and lastly the spongy parenchyma. The latter through meristematic activity is responsible for all vascular bundles of the cotyledon except the midrib, which is already differentiated in the dormant embryo.

At the end of ten to twelve days the primary xylem is mature in the cotyledons as well as in the hypocotyl. In the midvein it consists of annular and spiral (occasionally scalariform) elements, and is distinctly endarch in its development from petiole to tip of cotyledon. Only slender elongate parenchyma cells have been observed in the phloem of the cotyledons.

The emergence of the cotyledons from the seed coat is due to their predominantly basipetal growth. At this time the cells of the upper epidermis increase rapidly in size, thus opening the upper cotyledonary surfaces to the sunlight. The lower epidermal cells are somewhat slow to divide and differentiate, for as long as the cotyledons remain in the seed the lower epidermis acts as an absorptive layer, taking in food from the endosperm (Behrens, 1892).

The mature cotyledons of the Cash variety at the end of their period of expansion are some forty epidermal cells wide and seventy cells long (not including the short petiole), indicating almost twice the number of cell divisions in length as in width. Even in length the number of cells is only four times the number present in the embryo, which indicates a considerable cell enlargement. The greater number of these divisions take place before the cotyledons emerge from the seed coats, as indicated above. The average area of the cotyledons is 14.4 sq. mm., while the average total length of veins per cotyledon is 27.3 mm.

In the petiole of the mature cotyledon (fig. 2, *D*), the endodermis is clearly differentiated as the bundle sheath and may be distinguished by its Casparian strip. The outer epidermal wall and cuticle of the cotyledon are rather thin, offering little mechanical protection against the invasion of parasitic organisms.

DISCUSSION

Earlier mention has been made of the fact that this work is largely confirmatory of the results of previous studies of seedling plants. Its principal contribution is to give more detailed information on the developmental anatomy of tobacco, one of our most important economic plants.

As to the weight of tobacco seeds, Kondo (1921) reports them as averaging 0.08 g. per thousand, but Berthold (1931) shows that seeds are heavier if some of the flowers are removed from the inflorescence and relatively few capsules are allowed to develop. Seed weight per 1000 in such a case runs as high as 0.0859 g., as against a minimum of 0.0723 g. without trimming. Berthold has also shown that seeds in the center of the inflorescence are heavier.

With regard to the structure of the seed, the findings here are in almost complete agreement with those of de Toni and Paoletti (1891), working on *N. tabacum*, and with those of Grintescu, working on *N. rustica*, while the descriptions and figures of Harz (1885, p. 1020, 1021) and Behrens (1892) are inadequate.

The relatively rapid acceleration in the growth of the seedlings, once started, was noted and measured by Behrens (1892). He found the dry weight of the plant at the end of 20 days to be 38 times its seed weight, indicating a reasonably rapid development for so minute an embryo and small amount of stored food.

From an anatomical point of view, the principal works which have included mention of *Nicotiana* and related genera have been those connected with studies of the origin or merely of the presence of internal phloem and the root-stem transition. Several early studies go into the origin and presence of internal phloem in solanaceous plants; but Gérard (1881), working with *Datura*, was the first to determine that *branches of external phloem were laid down inward to become internal phloem*. Scott (1891) finds evidence in *Ipomoea versicolor* Meissn. which confirms Gérard. The internal phloem of the hypocotyl joins the external phloem of the root, having differentiated outward between the converging protoxylem groups of the cotyledonary trace.

Herail (1885), from a study of developing petioles of tobacco leaves, among other things, concluded that the internal phloem had its origin in pith parenchyma, was not connected with the external phloem, and should be called, therefore, "medullary phloem." He made the point that the internal phloem always has the same structure as the external phloem. Flot (1893), on the other hand, distinguishes the perimedullary zone (the part which borders the woody portion of the bundle on the inside) in which the internal phloem arises, as having its origin in the procambium. He uses tobacco in support of this contention (fig. 10, pl. 4; fig. 15, pl. 6).

Lamounette (1890), in one of the more comprehensive works on internal phloem, reported on tobacco as well as numerous species from genera of

various families with internal phloem. He, with Herail, held that internal phloem is derived from parenchymatous cells adjacent to the procambial ring, and not from the procambium. Because of their interpretation that the internal phloem is of a different origin and is not, therefore, a part of the vascular bundle, they object to the term "bicollateral" proposed earlier by de Bary (1877). Lamounette lists 21 families with internal phloem, and states (p. 214) that he has never observed immediate relations between the internal and external groups. (The fact that one internal phloem strand in tobacco did not join the external phloem is evidence that such a union does not necessarily have to take place, and in some plants may not, as reported by Lee, 1912, p. 737, for *Schizanthus pinnatus*.) According to Lamounette, plants with internal phloem may be placed in two categories: (1) those having internal phloem at the lower end of the hypocotyl, in the immediate neighborhood of the root; (2) those in which the internal phloem appears at the summit of the hypocotyl, at the level of divergence of the cotyledons. He further concludes that the internal phloem of the hypocotyl is independent of the phloem of the root and external phloem of the bundles of the hypocotyledonary axis. Its formation in the hypocotyl is always after the formation of external phloem and woody elements to which it is adjoined. In this last observation he is in accord with Scott and Brebner (1891), reporting on *Chironia peduncularis* (p. 277), and other authors, as well as the observations on tobacco reported here. Lamounette also mentions that in those forms in which the internal phloem first appears in the lower portion of the hypocotyl it is usually present in the cotyledons; and conversely, when it first appears at the summit of the hypocotyl, it is usually absent from the cotyledons. He interprets these types as grading insensibly one into another in the different families which possess internal phloem. He has further made two artificial classes: (1) a group in which the hypocotyl shows internal phloem at the time of greening of the cotyledons, such as *Cucurbita maxima* and *Solanum nigrum;* (2) those in which the hypocotyl is deprived of internal phloem at this stage, as *Ipomoea leucantha, Oenothera biennis,* and *Fuchsia corymbiflora,* indicating later relative maturity of the tissue in question. In the stem the internal phloem may differentiate at the same time that other vascular elements appear (Cucurbitaceae), or much later (Basellaceae).

From a phylogenetic point of view, Lamounette concluded that the internal phloem must have formed primitively in the stem and secondarily in certain axial hypocotyls, its development being an abnormal formation due to the division of pith cells.

Except for the conclusion that internal phloem arises in the pith and, therefore, ultimately from the ground meristem, the observations recorded here are in complete agreement with those of Lamounette. There is little doubt that for the most part in tobacco internal phloem strands are merely inwardly differentiating branches of external phloem, which would mean, classically, at least, that they originate in the provascular meristem rather than

from the ground meristem, as Lamounette holds. It is becoming increasingly clear, however, that we shall have to revise still further our rather traditionally fixed notions concerning the origin of tissues. The internal phloem in the tobacco seedling appears only at the top of the hypocotyl, which would make it fall into the second of Lamounette's earlier categories. This would likewise call for its being absent in the cotyledons, and it is absent. It would also fall into the second of his artificial classes with regard to time of appearance, for it does not appear until several days after the cotyledons first become green. As pointed out above, it does not differentiate until long after the first-formed xylem and external phloem are mature.

Scott and Brebner (p. 267) describe the transition in the hypocotyl of *Browallia viscosa* H. B. and Kth., which appears to resemble closely the condition in tobacco, as follows:

> On tracing the hypocotyl downwards to the taproot, the changes which we find in the position of the tissues are as follows: the pith gradually thins out; the two lateral bundles disappear, becoming confluent with those of the cotyledons. The primary xylem groups of each cotyledonary pair approach each other and ultimately unite, turning their protoxylem outwards. In the transitional region the strands of internal phloem successively pass out between the converging xylem bundles and one by one reach the strands of the external phloem, with which they fuse. The external phloem strands concentrate themselves on the two sides of the vascular cylinder, between the two centripetal xylem groups, which now represent the cotyledonary pairs. Finally these two groups themselves unite at the center of the root, forming the diarch xylem plate, and at this point the last of the internal phloem strands passes out and joins the normal phloem.

In the only work found on the anatomy of the young tobacco plant, de Toni and Paoletti (1891) discuss the origin of tissues in the root of *Nicotiana* and give a brief account of the structure of root, stem, leaf, fruit, and seed. They agree with Lamounette and Herail with regard to the origin of internal phloem. Lee (1912), however, in a study of seedlings representative of numerous families and genera, finds the internal phloem continuous with the external, except as noted above. In the case of *Nicotiana alata* Link and Otto, he reports that internal phloem is not present, though probably because the seedlings examined were not sufficiently mature.

Artschwager (1918) in studies on *Solanum tuberosum* found strands of external phloem becoming oriented as internal phloem in the hypocotyl, comparable to the findings of Gérard, Scott, Scott and Brebner, and others. Thiel (1931), working with *Solanum melongena,* showed the root-stem transition beginning low in the hypocotyl, the final transition taking place in the cotyledons. His figures show an inward differentiation of external phloem strands until they are finally clearly oriented as internal phloem. At the same time this is taking place, metaxylem (diarch root) is "breaking up" in the hypocotyl into several groups which become reoriented.

Kennedy and Crafts (1931), reporting on the transition region of the older material of root and rhizome of wild morning-glory (*Convolvulus*

arvensis), note that the transition occurs just below the surface of the ground, the xylem being dissected into four or five divisions, and the "internal phloem of the stem divides and passes out on each side of the wedge-shaped xylem segments, ultimately joining the outer phloem of the root." While these results are contrary to the findings of several earlier authors working on younger material in the same family, they agree closely with the observations of Scott and Brebner and others, and with observations on tobacco reported here. It has been shown in the latter that the inward differentiation of branches from the external phloem may take place through cotyledonary gaps, or later through vascular rays. As secondary thickening takes place, the connecting phloem strands are necessarily secondary, being laid down by the cambium along with ray parenchyma. This is in agreement with Scott and Brebner (p. 269) when they suggest that the phloem connecting internal and external groups must come from the cambium as secondary thickening takes place.

Lee (p. 745) states: "Broadly speaking, in the smaller seedlings the transition region is short, and the rearrangements are concluded in the upper part of the hypocotyl; while in the larger seedlings the region of transition is very extended." With regard to primary xylem and size of seedlings, Hill and deFraine (1913, p. 269) conclude that diarchy is characteristic of smaller ones, as is a single median strand at the base of the cotyledonary petiole (p. 268). These generalizations apply rather well to the tobacco seedling.

SUMMARY

1. In the seed the inner walls of the epidermis are heavily thickened, while the outer walls are thin with a light cuticle. The outer walls bend inward at maturity, giving the seed its reticulated appearance. There are three subepidermal layers of thin-walled parenchyma outside a single persisting layer of nucellar tissue. The endosperm consists of three to five layers of thick-walled cells, rich in aleurone and oil droplets.

2. Increase in the length of the hypocotyl is due both to cell division and cell enlargement. No new cells are added in a radial direction; so the number of cells in a transverse section of the seedling hypocotyl before secondary thickening is the same as the number present in the dormant embryo.

3. The pith in the mature seedling extends approximately 1.5 mm. below the level of cotyledonary divergence. All structures of the tobacco seedling are greatly reduced; hence xylem transition from the exarch to the endarch condition is difficult to make out. It takes place about 2 mm. below the cotyledons, on the average, being completed within a perpendicular distance of approximately 1 mm.

4. The internal phloem does not differentiate until several days after all other primary tissues. The external phloem gives rise to four main internal phloem strands, the first differentiating inward through a cotyledonary gap about 1.4 mm. below the level of cotyledonary divergence. This is followed

at successively higher levels by three more strands, until the internal phloem is established at a level 0.7 mm. below the cotyledons. Occasionally there are additional strands. While these inwardly differentiating strands do not follow any exact pattern, it is common to find strands "entering" the cotyledonary gaps, one on either side of each cotyledonary trace. As secondary thickening takes place, the connection between internal and external phloem groups is through phloem parenchyma laid down by the cambium along with the ray parenchyma in the vascular rays.

5. Increase in the size of the cotyledons is likewise by cell division and enlargement, all new cells being laid down in a plane parallel to the upper and lower epidermis (except in the development of vascular bundles), so that the mature cotyledons are the same number of cell layers in thickness as the cotyledons of the dormant embryo. Growth of the cotyledons is at first basipetal, expansion of the blade later taking place equally throughout. The Casparian strip identifies the endodermis as the bundle sheath, well up into the cotyledonary petiole. The midvein is clearly endarch collateral throughout its length, with the phloem more or less in two groups through the petiole. The lower epidermis acts as an absorbing layer to take food material from the endosperm and is thus slightly behind the upper epidermis in development.

6. The cuticle throughout the seedling is thin and should offer little mechanical resistance to the attacks of disease-producing organisms.

The present investigation was carried on while I held a National Research Council Fellowship in the Biological Sciences. I am indebted to Professor Edmund W. Sinnott for helpful suggestions during the course of this and subsequent investigations on tobacco. The Department of Botany at Columbia University most courteously provided laboratory and greenhouse facilities. To O. E. Street, E. G. Moss, and F. A. Wolf, I owe my thanks for seeds and various other materials. Dr. Sophia Eckerson has been kind enough to check my interpretation of the microchemistry of the seed coat.

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