III. ORIGIN AND DEVELOPMENT OF AXILLARY BUD IN TRIGONELLA FOENUM-GRAECUM L.

Trigonella foenum-graecum is a herbaceous plant belonging to the family Papilionaceae. It grows to a height of 2-3 feet. The life span of the plant does not exceed four months. A fully grown plant has branches up to third or fourth order. The leaves are trifoliate compound and alternate having 1/3 phyllotaxy (Fig. 1). The first leaf produced after the emergence of the two cotyledons is simple (Fig. 1). The two free lateral stipules of an earlier leaf protect the younger leaves and apex. Flowers are axillary and solitary.

Shoot apex

Quick succession of leaf initiation and early
differentiation of bud meristem are the two significant features of the morphogenetic activity of the dome-shaped shoot apex. Four intergrading phases of the plastochron, the early pre-leaf initiation, the late pre-leaf initiation, the leaf initiation and the post-leaf initiation phases are recognized.

The shoot apex at the early stage of pre-leaf initiation phase shows stratification on the flank of the peripheral meristem which is more or less opposite to L1 leaf (Fig. 2). The late stage of the pre-leaf initiation phase of the shoot apex shows stratification up to 4 or 5 layers in the peripheral zone which is more or less opposite to L1 leaf (Fig. 3). Increase in the area of the shoot apex and stratification of its cells at the time of leaf initiation are commonly reported (Gifford, 1950; Sussex, 1955; Shan, 1960). But in *Garrya elliptica* (Reeve, 1942), 'there is a distinct stratified appearance in the corpus following emergence of the most recently initiated primordium'. Before leaf initiation the
stratification is less. However, Gifford (1954) associates increased stratification with the maximal area phase in *Garrya elliptica* (Reeve, 1942). In *Trigonella* the diameter/height range is from 107 μ/69 μ to 118 μ/66 μ in the pre-leaf initiation phase.

A further increase in the size of the shoot apex is found at the leaf initiation phase (Fig. 4). The diameter/height range is from 121 μ/67 μ to 127 μ/61 μ. The stratification is more pronounced. In this phase the leaf initiation is identified by periclinal divisions in a sector of T2 (Fig. 4). But, as in *Drimys winteri* var. *chilensis* (Gifford, 1951a), periclinal divisions in T2 and corpus occur simultaneously during leaf initiation (Fig. 4). Further anticlinal divisions in tunica and divisions in various planes in the corpus build up the leaf primordium.

The shoot apex at the post-leaf initiation phase has a basal diameter/height range of 90 μ/31 μ to 102 μ/42 μ, when measured at the level of the
first visible node. Eventhough the two layered tunica divides predominantly by anticlinal divisions, there was a solitary case of a periclinal division in T1. T2 shows periclinal division in connection with leaf initiation only. The corpus divides in various planes. A poorly defined central meristem is distinguished. Comparatively its cells have greater cytonuclear ratio and less avidity for staining (Fig. 5). Usually this zone consists of T1, T2 at the summit and a few subjacent corpus cells. The densely stained peripheral meristem is not well stratified. The rib meristem is of vacuolated cells.

After the initiation of a leaf primordium, the shoot apex above it is raised up prior to the initiation of the next leaf primordium. Following the initiation or emergence of a leaf primordium in *Salix laevigata* (Reeve, 1948) there may be periclinal divisions in the cells of upper layers of stratified corpus or even in a centrally located cell of the otherwise distinct T2 layer. In *Drimys lanceolata* (Gifford, 1950), periclinal divisions occur in T2 of the central zone after the initiation of leaf.
primordium. In *Ephedra altissima* (Paolillo and Gifford, 1961) the central meristem zone is raised up by elongation of its lower derivatives during a plastochron. The details of this phase of the morphogenetic activity of the shoot apex during the plastochron, as studied in *Ephedra*, are not available in the literature for many angiosperms. In this regard, the observations on *T. foenum-graecum* need some elaboration.

Figure 6 shows a periclinaly divided cell in the third layer of the central meristem. Subjacent cells are elongated. The orientation and thickness of walls suggest that this complex of six cells is derived from a common cell. Figure 7 illustrates some corpus cells dividing periclinally. They are in the peripheral flank where the next leaf is likely to be initiated. In figure 7, one of the central meristem cells can be seen dividing anticlinally. These features shown in figures 6 and 7 suggest that, as reported for *Ephedra*, the elevation of the central meristem after the initiation of a leaf is due to periclinal divisions.
in the cells of the central meristem followed by elongation of the lower derivatives. The anticlinal divisions in the cells of the central meristem contribute to the growth of the peripheral meristem. But in *T. foenum-graecum*, after the initiation of a leaf, all cells of the peripheral meristem other than those which have already taken part in leaf initiation, are raised up by periclinal divisions and elongation of lower derivatives as shown in figure 7.

**Origin of the bud**

The inception of the bud meristem is observed in the apical meristem adaxial to either the first (Fig. 8) or the second leaf primordium (Fig. 9). It is a densely stained zone. The early bud meristem becomes a 'detached meristem' as in *Syringa vulgaris* (Garrison, 1949a) and *Hibiscus cannabinus* (Kundu and Rao, 1955). The peripheral meristem cells adaxial to the bud meristem vacuolate and appear less stained. This makes the bud meristem appear to be
detached from the peripheral meristem of the apex (Fig. 55). Wardlaw (1943a) originally used the term 'detached meristem' in the case of a fern where a single apical cell gives rise to lower derivatives and a portion of which gets 'detached' by vacuolation of surrounding cells and forms the early bud meristem. With regard to angiosperms the term is referred to a portion of the peripheral meristem of the shoot apex, which is differentiated as the earliest bud meristem. In Dianthera americana the bud meristem is described as originating from residual meristem at the axil of the second leaf, and it is specially mentioned that the bud has not originated from the flank meristem of the main shoot apex (Sterling, 1949). But in the photomicrograph (Plate II, Fig. 17) the bud meristem is visible as a part of the peripheral meristem near the axil of the second leaf. Garrison (1949a) interprets the early bud of Syringa vulgaris found in connection with the second or third leaf as a detached meristem. In Drimys winteri axillary bud activity is first perceptible in connection with the
fourth or fifth leaf primordium, and the bud is considered as a 'detached meristem' (Gifford, 1951b). The buds of *Juglans cinera* arise from the residual meristem derived from the shoot apex (Garrison, 1955).

In *Cayratia carnosa* the early bud meristem is visible simultaneously with the leaf initiation (Shah, 1960).

In *T. foenum-graecum* the early bud meristem adaxial to the leaf which is initiated by periclinal divisions in T2 is three or four layers deep from the periphery (Fig. 8). Occasionally the leaf initiation occurs in the third layer from the periphery and the bud meristem adaxial to it is four or five layers deep.

The cells of the young bud meristem, especially cells of T2 and corpus appear deeply stained. Probably the differential stainability indicates varying morphogenetic potentialities. These cells may show compact alignment (Fig. 9). Figures 9 and 10 show that the bud trace procambium and the trace procambium of the axillant leaf are different. The early bud meristem has only one bud trace. The second bud trace
develops later in the development.

**Further development of the bud**

The bud meristem increases in volume by more anticlinal divisions in tunica and periclinal divisions in corpus. The anticlinal divisions are more along the peripheral side of the bud meristem (Figs. 11, 12, 55, 56). In the initial stages the third and fourth corpus layers of the bud divide periclinally, and corpus cells further below undergo divisions in various planes (Figs. 11, 12).

During further development, this type of irregular divisions are not found as most of the corpus cells divide anticlinally and periclinally (Figs. 13, 14, 56, 57). This histogenic activity results in the protrusion of the bud meristem (Figs. 13, 14, 55-58).
A zone of the cambium-like cells is observed on the adaxial side of the bud meristem (Figs. 11, 12, 56). This zone is not found in the earliest stage of the bud meristem (Figs. 8, 9). It is developed by frequent anticlinal and periclinal divisions of ground meristem cells formed between the bud meristem and apical meristem. The cells of the cambium-like zone do not take part in the early development of the bud as they do in *Michelia fuscata* (Tucker, 1963).

By the time the bud assumes a prominent convexity, it occupies an axillary position (Figs. 15, 57, 58). The corpus cells at the base of the bud vacuolate leaving the apex of the bud as a eumeristematic hood (Fig. 15). In the further stage of development, a less stained central meristem, compact and deep staining peripheral meristem, vacuolated rib meristem and pith cells below it are differentiated (Fig. 16). Two procambial strands are visible (Fig. 16). The zone of cambium-like cells gradually loses its identity. Its cells differentiate into cortical...
parenchyma, procambium and pith cells of the bud (Figs. 16, 59).

The prophyll initiation is identified by periclinal divisions in T2 or outermost corpus layer of a well developed bud (Figs. 17, 18). Only one prophyll is formed, which is at right angles to the axillant leaf (Figs. 17, 18, 60-63). The prophyll is similar to the other leaves of the bud. The differentiation of procambial strands for the prophyll and the next leaf is precocious (Fig. 16). The first leaf of the bud is also initiated by periclinal divisions in the sector of T2 (Fig. 19).

**Floral bud**

The flowers of *Trigonella* are solitary and axillary. The apex is crowded with floral buds (Figs. 20, 21). The initiation of the flower bud is indicated by a prominent hump (Fig. 22, F1). This is in contrast with the early meristem of the vegetative bud where there is no bulging (Figs. 3, 8, 9). In *Garrya elliptica* the axillary floret
primordium until the emergence of its prophyll is not having histological distinctions from the axillary foliage bud in the early stages (Reeve, 1943). In *Trigonella* the difference between vegetative and floral buds is more pronounced in the subsequent stages (Figs. 23, 24). Figure 23 shows a floral bud at the second node where T1 and T2 show regular anticlinal divisions, whereas corpus cells have mostly divided by horizontal and vertical divisions so as to make the bud more or less flat. The bud at this stage shows a meristematic cap surrounding the vacuolated region. When the corpus cells divide by various planes, the appearance of the bud shows a marked difference (Fig. 24). At this stage the floral whorls are initiated, and procambial strands are blocked out. The buds shown in figures 22-24 are related respectively to L1, L2 and L3 leaves down from the shoot apex. Their structural prominence will be more striking when compared with the vegetative buds related to L1, L2 and L3 leaves (Figs. 8, 11, 12). In *Trigonella* sometimes the lower bud, VB, remains small and vegetative, while the buds at higher nodes are prominent and floral (Figs. 20, 21).
Early vascularization of the main apex

Transverse sections of a number of shoot apices have been examined. The following description of main apex and bud typifies the general observations. The various levels expressed in \( \mu \) are calculated by counting the serial sections from one stage to the other.

A transection slightly below the extreme tip of the shoot apex shows a light stained central meristem, CM, and dense and deep staining peripheral meristem, PM (Fig. 25). Rib meristem, RM, and peripheral meristem, PM, are observed still below. At a depth of 28 \( \mu \), the cortical part of the ground meristem, GM; procambium, PC; and residual meristem, RE, are distinct (Figs. 27, 64). All the three, i.e., the procambium, the residual meristem and the ground meristem are differentiated from the peripheral meristem. The highly vacuolated pith cells, P, are derived from rib meristem (Figs. 24, 64-66). The procambial cells have narrow lumen and deep staining nucleus and cytoplasm. The residual meristem cells
are comparatively bigger and lightly stained. The cells of the ground meristem are more vacuolated than the cells of the procambium and the residual meristem.

The second leaf, L2, shows its median vascular strand, MT, and one of the two lateral strands, LT, at this level (Figs. 27, 66, 68). The first leaf, L1, with its median strand, MT, is also seen (Fig. 27). The vacuolation of cells on the abaxial and adaxial sides of a leaf primordium makes the procambial cells in between very distinct. Similarly, in the axis region, the vacuolated cells in the cortex and pith block out procambium and residual meristem in between (Figs. 64-72).

The three vascular strands of L1 leaf become associated with the axial cylinder of procambium and residual meristem at lower levels (Figs. 28-30, 64-66). At 147 µ level, the second leaf, L2 is associated with its two stipules on either side (Figs. 30, 66-68). Five procambial strands, one median leaf trace, MT, two laterals, LT, and two
stipule traces, ST, are present (Figs. 30, 68). At 163 μ level, the stipule trace procambium, ST, shows its connection with the lateral trace, LT (Figs. 31, 67, 68). Approximately at a depth of 177 μ, the median leaf trace, MT, and one of the lateral traces, LT, are seen with their respective leaf gaps, whereas the other lateral trace has become a part of the axial vasculature (Fig. 32). The node is trilacunar.

The procambial strands are differentiated acropetally. The presence of residual meristem as well as procambium in the shoot apex shows that the peripheral meristem differentiates into residual meristem and procambium.

Vascularization of the bud during prophyll initiation

In the longitudinal section, a single procambial bud trace is observed in the earliest stage of the bud (Fig. 9). It is differentiated from the peripheral meristem of the main shoot apex as the early bud meristem originates from the apical meristem. The procambial strands present in a
developing bud are in continuation with the original bud traces which in their early stages were near the base of the bud (Fig. 16). The part of the procambial strand, pc, has its origin in the differentiated cells of the bud meristem, and its distinction from the earlier formed part of the bud trace is recognized by the disappearing identity of cambium-like cells (Fig. 16). The distinction between the early bud trace lying at the base of the bud meristem and its extension into the growing bud as a result of differentiation of bud tissue is difficult to make out in a well developed bud. For the convenience of general description, therefore, the definition of branch trace by Esau (1960) is very useful. The branch trace is defined as "a vascular bundle in the main stem extending between its connection with the vascular tissue of the branch and that with another vascular unit in the main stem". The procambial traces of the prophyll and early leaves have precocious development in the bud. The various stages of procambialization in the bud at successive nodes of the axis are illustrated in figures 33-45.
In a transection the bud meristem appears as a bright stained zone at the second node (Figs. 33, 67-69). It is closely situated to the axial vasculature. At about 21 μ below the earlier described stage; the edges of the axial vasculature, separated by the ground meristem of the median leaf gap, appear dense and deep stained, suggesting the positions of the two bud traces (Figs. 34, 70, 71). Further down, the median and the lateral leaf traces become a part of the axial vasculature (Fig. 72).

The bud at the third node shows the precocious procambial strands for the single prophyll and the first leaf (Figs. 35-39, 73-81). The course of the bud traces is described from the base of the bud upward. The two bud traces, BT, originate from the axial vasculature, AV (Figs. 35, 73, 74). They develop acropetally into the bud by differentiation of the bud meristem. The procambial strands and the residual meristem differentiated in the bud together constitute the provascular system of the bud, which appears as two arcs at this level (Fig. 36). In each arc there are three procambial strands, the
branches of the bud trace strand (Fig. 36). As there is only one prophyll, the procambium on the 'prophyll sector' of the bud is more prominent (Figs. 35, 36, 73-75). The gradual differentiation and organization of three strands from a single bud trace are traceable in figures 73-75. A similar histogenic development for the opposite bud trace is observed at higher level of the bud. The prophyll and leaf trace procambia are seen at this stage (Figs. 36, 76).

Above 21 μ, the provascular system of the bud appears as a ring consisting of the precocious procambial traces of prophyll and leaf, and the residual meristem (Figs. 37, 78). At about 35 μ level, the rib meristem, rm; peripheral meristem, pm, and the procambial strands, mp and mt, of the bud are observed (Fig. 38). About 56 μ nearer the bud apex, the less stained central meristem and the bright stained peripheral meristem are seen (Figs. 39, 78-81).

Similar development of provascular system of the bud at the fourth node is illustrated in figures 40-45.
The prophyll initiation occurs at the fourth or fifth node. The bud traces, BT, take their origin from the axial vasculature, and at 14 μ level each of them organizes into three strands (Figs. 46, 47). These procambial strands and the residual meristem cells differentiated from bud apex constitute the provascular system of the bud. The provascular system appears semilunar at about 35 μ level (Fig. 48). Still above, it appears as a complete ring in a transverse section (Fig. 49). Ten to fifteen procambial strands are present. Of the 11 procambial strands (Fig. 49), 5, 8 and 11 are prophyll traces; while 4, 3 and 10 are the traces of the next leaf as can be observed at higher level (Fig. 50). After the departure of the prophyll traces, the vascular system of the bud shows only three leaf traces; the rest of the provascular system is of residual meristem (Fig. 51). Almost near the bud apex, the median and the lateral strands are distinct in the peripheral meristem (Fig. 52).

Vascular interrelationships

The young internode of T. foenum-graecum
possesses 10-15 primary vascular strands. A series of nodes and internodes with 11 primary vascular strands shows complicated interrelationships (Figs. 53, 54). The node is trilocular. The lateral traces join with the median and enter the petiole. Again it divides into three, and each goes to each of the three leaflets. Before a lateral trace fuses with the median, it gives out a branch to the stipule.

The bud traces are related to leaf traces, but not to those of the axillant leaf.

Figure 54 is the schematic representation of figure 53 to show the interrelationship of bud traces in consecutive nodes and internodes. For convenience and clarity, the relationships of median and lateral leaf traces and bud traces are separately dealt with.

Eleven primary vascular strands are numbered and the course of each is shown (Fig. 54). Vascular strands 2, 5 and 7 are leaf traces for the fourth node, N4. The lateral traces, 2 and 7 branch towards
the stipules (projections, s) before joining with the median leaf trace, 5. The lateral leaf trace, R2, at the third node, N3, is related to 6e and P of lower internode. The strand 6e is related to 6c of N4. The lateral leaf trace, R1, of the first node, N1, can also be traced to a branch, 6e, which is likewise related to the bud trace.

The median leaf trace, 6d, at N1 and the bud trace, 6c, at N4 are branches of a common strand, 6a, found below the fourth node. At every node, the fusion and branching of the two strands from the internode below mainly contribute to keep the equal number of primary vascular strands in the internode above. The strands 6 and 8 at N4, 10a and 10b at N3, and 10f and 10d at N2 are examples.

Thus a median leaf trace is linked with a trace complex which is two nodes below (vascular strand 6d in figure 54). The lateral leaf traces have links with vascular trace complexes in the nodes immediately below (10e), or, two or three nodes farther below (R1 and 3) (Fig. 54). A leaf
gap is formed by the departure of each leaf trace;
but the bud trace does not show a similar gap.