IX. ORIGIN AND DEVELOPMENT OF AXILLARY AND ACCESSORY BUDS IN MENTHA SPICATA L.

Mentha spicata L. is a herbaceous member of the family Labiatae. It is a plant of medicinal importance, and is cultivated in many parts of Gujarat.

The leaves are simple, opposite-decussate, exstipulate, ovate with dentate margin and acute tip (Fig. 1). They are richly aromatic. There are two buds, one axillary and the other accessory, one below the other. The development of the accessory bud is inhibited apparently due to the presence of the axillary bud.

Shoot apex

During the various stages of the plastochron
the shoot apex shows various forms, ranging from flat to dome shaped. The main plastochronic phases are, the pre-leaf initiation, the leaf initiation and the post-leaf initiation phase. The criteria used to distinguish these phases are the shape of the shoot apex, cytohistological zones, the stratification of the apical meristem and the ratio between the height of the youngest leaf primordium \((l_1)\) and the width of the shoot apex \((sa)\), whenever possible.

In general the shoot apex shows two tunica layers, enclosing a mass of corpus. The cells of tunica divide predominantly by anticlinal divisions. The corpus cells undergo divisions in different planes. The central meristem, peripheral meristem and rib meristem are distinct only in certain phases.

In the early stages of pre-leaf initiation phase the central meristem is not distinct (Fig. 2). The shoot apex is more or less flat. The emergence of the youngest leaf primordium, \(l_1\), is prominent and it is adaxially inclined towards the flat shoot.
apex. The corpus is poorly stratified. The $l_1/sa$ ratio ranges from $0.1-0.2$.

In the late pre-leaf initiation phase the shoot apex is slightly convex and exhibits distinct zones (Figs. 3, 4, 58). The cytohistological zones are present. The stratification in the peripheral meristem is four or five layers deep.

The main features of the advanced stage of the pre-leaf initiation phase are the prominent semispherical shape of the shoot apex, increased stratification and the presence of cytohistological zones (Fig. 4). The $l_1/sa$ ratio is $0.4-0.5$. The increase in stratification and dimensions of the shoot apex indicate the approaching stage of leaf initiation.

The leaf initiation phase is identified by periclinal divisions in T2 (Figs. 5, 59). The other features are the increased volume of the shoot apex and the presence of cytohistological zonation.
In the post-leaf initiation phase the leaf buttress is formed by periclinal divisions in T2 and the subjacent corpus cells (Figs. 6, 60). When the leaf buttress grows to a young primordium, it becomes adaxially inclined to the flattened surface of the shoot apex, and at this stage the shoot apex is considered to be at early pre-leaf initiation phase. There are no distinct zones in the shoot apex in post-leaf initiation phase. The stratification of the apical meristem is poor.

Origin of axillary bud

The earliest bud meristem in Mentha is identified by a set of cells which have undergone more anticlinal divisions in the peripheral meristem near the second leaf primordium (Figs. 7, 8, 61). Such localised anticlinal divisions may be more prominent in T1 and T2 (Fig. 7), or both tunica and corpus cells may show characteristic alignment due to these divisions (Fig. 8). Two layers of tunica and one or two layers of corpus constitute the early bud meristem which is
eumeristematic. Gradually the peripheral meristem cells on the adaxial side of the early bud parenchymatize and detach it from the main apex. Few parenchyma cells surrounding the bud undergo anticlinal and periclinal divisions and form a zone of cambium-like cells (Figs. 8, 9). A non-median section of the early bud meristem shows the bud trace in the form of residual meristem between the axillant leaf trace and the bud (Fig. 10).

Further development of the axillary bud

Tunica layers do not take an active part in the development of the bud. The tunica expands by anticlinal divisions keeping pace with the enlarging mass of the corpus. The corpus cells in the third and fourth layers divide in oblique, anticlinal and periclinal planes (Figs. 11, 12, 63). This histologic activity results in the protrusion of the bud (Figs. 11-13, 63). The anticlinal divisions result in the radial increase of the bud. All the cells of the bud meristem up to this stage are eumeristematic (Figs. 11-14). The bud is in
axillary position at this stage (Fig. 14). When the bud has well emerged by the activity of the corpus cells and by the expansion of the tunica, a few cells on the side of the axillant leaf are seen vacuolated. These cells constitute the early accessory bud meristem (Fig. 15). During this stage the cells at the base of the axillary bud also appear vacuolated (Fig. 16). In the next stage the bud apex simulates the main apex in having two tunica layers enclosing a corpus. The periclinal divisions in T2 cells in the sector of the apex indicate the prophyll initiation (Fig. 17). At this stage there is no distinct central meristem. There are two prophylls and their initiation is simultaneous in the two opposite sectors (Figs. 17, 64). A distinct central meristem is absent at this stage (Fig. 17). But during further development of the prophyll a light stained central meristem is differentiated (Fig. 18). The transverse zone of cells comprising of pith, procambium and cortical region is distinct by their comparative narrow size (Figs. 18, 65, CZ). The
size and shape of these cells show that they are derived from the cambium-like zone which now gradually loses its identity. Its derivatives produced at the centre of the bud differentiates as pith cells and contribute in raising the bud. Some of its derivatives differentiate into procambium and outer ground meristem tissue. After the emergence of the prophyll primordium the bud apex appears flat (Figs. 19, 64, 65). The procambium of the prophylls differentiates acropetally (Fig. 19). The anticlinal divisions in tunica and the divisions in various planes in the corpus of the bud apex make it convex (Fig. 20). The characteristic cytohistological zonation is evident (Fig. 20). During the initiation of leaves in the bud periclinal divisions occur in T2 (Figs. 21-23). The leaf trace procambium differentiates up to the site of leaf initiation (Figs. 22, 66). At this stage it appears that the bud shows the structural and physiological attributes of the main apex.
Accessory bud

The accessory bud of *M. spicata* originates from the axillary bud meristem. After the emergence of the axillary bud, but before any cytohistological zonation is evident, a few cells of the axillary bud on the side of the axillant leaf are distinct (Fig. 24). They are comparatively less stained. This group of cells is the earliest visible accessory bud meristem. Two tunica layers and two rows of corpus cells constitute the earliest accessory bud. Later it is separated from the axillary bud by parenchymatization of cells adaxial to it. At this stage the accessory bud appears eumeristematic (Fig. 25). The development of the accessory bud is very slow. The accessory bud meristem shows a few anticlinal divisions when the initiation of the first pair of leaves occurs in the associated axillary bud (Fig. 22). The bud gradually protrudes out due to periclinal divisions in its corpus (Figs. 26-29). The anticlinal divisions are more frequent in tunica layers (Figs. 28, 29).

Further development of the accessory bud is
inhibited apparently due to the presence of the axillary bud.

**Vascularization of the main apex**

The transverse section of the main apex at 8 μ level from the tip has a peripheral meristem, PM, surrounding the rib meristem, RM (Figs. 30, 67). At a level of 24 μ the procambial strands, PC, the residual meristem, RE, and outer ground meristem, GM, are visible (Fig. 31). The procambial cells are narrow and their nuclei stain brightly. The residual meristem cells are denser and more staining than the cells of the ground meristem. The outer ground meristem, the procambium and the residual meristem are differentiated from the peripheral meristem of the apex. At 48 μ level the vascular system of the first leaf L1 is seen in figure 31. At a lower level, it resolves into two leaf trace strands (Fig. 32). The two procambial strands become associated with axial vasculature, AV, at 64 μ level (Fig. 33). Thus every leaf trace is formed by the fusion of two strands (Figs. 32, 33, ...
68). The node is unilacunar. The vascular system of a leaf primordium becomes distinct when the cells at its abaxial and adaxial sides appear vacuolated.

**Vascularization of the bud during prophylly initiation**

The earliest bud meristem has residual meristem connection with the axillant leaf trace procambium (Fig. 10). The vascular relationship between the bud and the axis at the second and third nodes is shown in figures 34-37. The bud with respect to second leaf L2 is eumeristematic at 88 \( \mu \) below the main apex (Fig. 34). At 136 \( \mu \) level from the main apex, the bud meristem is close to the trace procambium of the subtending leaf, L2 (Fig. 35).

The bud in connection with the third leaf shows the vascular connection with the axillant leaf trace (Fig. 36). At 160 \( \mu \) below the main apex, the bud appears eumeristematic (Fig. 37).

The course of the vasculature in the bud
related to fourth leaf, L4, is described from the base of the bud upward. The branching of the bud trace complex, BC, from the axillant leaf trace is considered to be at 0 \( \mu \) level (Figs. 38, 69). The continuous differentiation of the procambial traces in the bud is by procambialization of a few cells of the peripheral meristem and residual meristem of the bud. The procambium already formed along with the residual meristem constitutes the provascular or primary vascular system of the bud. The pattern of procambial distribution foreshadows the pattern of primary vascular system of the branch. The provascular system of the bud at 24 \( \mu \) level appears as two arcs (Figs. 39, 70). Each procambial strand which is a continuation of the bud trace bifurcates during its acropetal course. At 24 \( \mu \) level each arc of provascular system and its procambial strands are more distinct at 48 \( \mu \) level (Figs. 40, 71). Of the two procambial strands one is accessory bud trace, AT, and the other, axillary bud trace, BT, (Fig. 40). The provascular system appears, along with the bright stained cells of the accessory bud, in the
form of a loop at 72 μ level (Figs. 41, 72). Near the bud apex at 104 μ level the residual meristem and procambium form more or less a discontinuous rectangular provascular system (Figs. 42, 73). Each prophyll trace is formed by fusion of two procambial strands (Figs. 42, 73, 74). The apex of the bud is eumeristematic (Fig. 43).

Vascularization of the bud with prophylls

Figures 44-52 illustrate the primary vascular organization of the axillary bud in Mentha. The bud trace complexes, BC, are related to the axillant leaf trace (Fig. 44). At this level only a single strand of procambium is present on each side. Four branches of this procambial strand appear differentiated at higher level of the bud (Fig. 45). These branches differentiate partly from the peripheral and partly from the residual meristem of the bud. The procambial differentiation is acropetal. Thus at 72 μ level the provascular system of the bud is in the form of two arcs, each consisting of residual meristem and four procambial
strands (Fig. 45). The pattern of differentiation of the residual meristem from the bud apex and the development of the procambial strands at 96 μ level makes the provascular system to appear as a loop in transverse view opening towards the axillant leaf trace (Fig. 46). The part of the accessory bud meristem can be seen at this stage.

Of the four procambial strands, on each side of the provascular system, one each differentiates as a trace of the accessory bud (Fig. 47). At 136 μ more branches of the procambial strands are differentiated. The provascular system consists of a ring with twelve procambial strands and residual meristem (Fig. 48). These procambial strands are for prophylls and leaves of the bud. As the prophyll trace procambia and the procambia for the first few leaves of the bud differentiate quicker than others, at 144 μ level there are only eight strands (Fig. 49).

The procambial strands, 6 and 7 (Fig. 49) at a higher level join to form the prophyll trace
complex '6-7' (Fig. 50). Likewise strands 2 and 3 form the complex '2-3' for the other prophyll (Figs. 49, 50). The remaining four procambial strands are for the first pair of bud leaves (Figs. 50, 51). The bud apex at 168 μ level is eumeristematic.

Vascular interrelationships

The vasculature of the axis, and its relation with leaf and bud are illustrated in figures 53-57. The common pattern of vascular interrelationship is shown in figure 53. The other four figures illustrate its variations.

Twelve primary vascular strands are generally observed in a young internode. Six are shown in figure 53 and are numbered 1-6, below the fourth node, N4. The other six strands, 1', 2', 3', 4', 5' and 6' are not represented in the figure. At the fourth node, N4, the vascular strands 3 and 4 join to form the leaf trace of the fourth leaf, L4 (Fig. 53). Similarly 3' and 4' fuse to form the leaf trace on the opposite side at the fourth
node, N4, which is not shown in figure 53. The vascular strands 2 and 5 bifurcate into 2a, 2b and 5a, 5b in the node. The vascular strands 2b and 5a traverse up to the second node, N2, and form the leaf trace for the second leaf, L2. Meanwhile, the vascular strands 2a and 5b traverse up to the third node, N3, and which divide in the same fashion as the strands 2 and 5 in the fourth node, N4. Each of the pair of strands 1 and 1', and 6 and 6', form the leaf trace for the leaves at N3. Thus a pattern of vascular anastomosis is established in successive nodes and internodes.

The two bud trace complexes, BC, arise on either side of the trace of the subtending leaf (Fig. 53). The accessory bud trace, AT, arises from the bud trace complex, BC.

Four variations are found to this common pattern of the vasculature.

In figure 54 there are slender vascular
strands, $X_1$, associated with the usual two strands, 3 and 4 in the formation of the leaf trace at the fourth node, N4. Similarly in the formation of L2 trace, two slender strands, $X_2$, are also associated (Fig. 54). $X_2$ strands arise as a branch of the strand 5a at the level of the fourth node. Sometimes two $X_2$ strands are formed in a slightly different manner (Fig. 55). The vascular strand 5 gives rise to 5X, 5a and 5b at the level of the fourth node (Fig. 55). The vascular strand, 5X, thus formed bifurcates at the level of N3; one of the resultant branches joins with another strand 2X formed as a branch of the vascular strand, 2b (Fig. 55). Thus, the two vascular strands $X_2$ of the L2 leaf in figure 55 are different in origin from the $X_2$ strands of L2 trace in figure 54.

Similarly the three smaller strands, $X_1$ contributing in the formation of L4 leaf traces in figures 54 and 55 appear similar. But it is possible that their relation with the older strands may be different as can be observed in figures 56 and 57.
In figure 56, the strand 5 gives rise to 5X, 5a and 5b at the fourth node. The vascular strand, 5X thus formed, trifurcates at the level of the third node. These three form the $X_1$ strands taking part in the formation of L2 leaf trace.

In figure 57, the division of the vascular strand 5 results in the formation of 5X, 5a and 5b. The strand 5X bifurcates at the level of N3. The strand 2b gives rise to a branch 2X at N3 level. The strand 2X traverses parallel to the two strands formed by 5X. These three strands are contributing to the formation of L2 leaf trace (Fig. 57).