STUDIES ON THE DEVELOPMENT OF POLYAD GRAINS OF *CALLIANDRA HAEMATOCEPHLA* HASSK. WITH ELECTRON MICROSCOPIC TECHNIQUE

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Abstract: Development of polyad grain of *Calliandra haematocephala* Hassk. was studied. Electron microscopy technique preparation was adapted for this study. That is, pre-fix in glutaraldehyde, post-fix in osmium tetraoxide, dehydrate in acetone, embed in plastics, cut with ultramicrotone, thick section (1 μ thick) stained with methylene blue-azure II, examine under light microscope. Different developmental stages, namely, undifferentiated, one-, two-, four-, five-, six-, and eight-celled stages were revealed. Each nuclear division is followed immediately by cytokinensis which is common in Monocotyledons and also found in this species. Sporopollenin starts adding on the surface when 4-celled stage is reached (wall formation). Although cell plate has already separated two daughter cells away but sporopolleninous wall had not appeared at that time. Longitudinally, there can be several octads formed from each locule, however, horizontally there is only one expected at the same level. In other words, there was only one pollen mother cell existing at the initiation stage. Accompanying events, such as tapetal layer (development and disintegration), and reinforcement of endothecial cells are also reported.

I. INTRODUCTION

Polyad (octad) pollen of *Calliandra haematocephala* Hassk. (see Fig. 1) has a very unusual morphology. First of all, octad grains are not common. Tetrads are a fairly common grains throughout the angiosperms, there are approximately 50 families of angiosperms in which tetrads or dyads can be found in their members. Polyads (more than 8), on the other hand, are relatively rare. Only these families, Asclepiadaceae, Orchidaceae, Annonaceae, Hippocrataceae, and Leguminosae have them. In Asclepiadaceae and Orchidaceae, pollen grains from the same locule of the anther stay together and form so called pollinia and massulae. In Annonaceae, octads found are very different from what we see in *Calliandra haematocephala* on the basis of their arrangement. Those in Annonaceae have their two tetrads, either in tetrahedral or decussate form, sticking together and forming octads (Walker 1971).

Polyadg grains of tetrad, octad, hexadecad, 32–, 64–celled can be found throughout most of the Mimosaceae members. While octads can only be expected in some species of *Calliandra*. (Sorsa, 1969). Usually, polyads are arranged more or less in a 3-dimensional configuration in this family. This can be demonstrated by those polyads of 16–, 32, and 64–celled. As we can see in these genera, such as; *Parkia*, *Acacia*, *Anadenanthera*, and *Adenanthera* etc..

Octads of 2-dimensional arrangement of *Calliandra haematocephala* is of particular interest. It is the only genus in this family that has this unique kind of arrangement.

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It is the purpose of this report, to trace their developmental history. Questions like, what kind of orientation of the daughter cells after cell form division is accomplished will hopefully be answered in this paper.

II. MATERIALS AND METHODS

Fresh (head) flowers of different sizes, ranging from 4 mm in diameter—the youngest one, to 6 mm, 10 mm, 15 mm, ..., to fully mature head—about 7 cm in diameter (after anthers and filaments are stretched out) were picked from *Calliandra haematocephala* tree. In other words, size interval of flower buds were as small as possible. Hopefully, consecutive development of polyads can be traced without skipping any stage. Anthers of these heads were then excised under a dissecting microscope. In most of the young heads, anthers were so little that only 60 magnification is possible for the manipulation. Then these anthers of same size as a group were fixed in glutaraldehyde, rinsed with sodium cacodylate buffer, post-fixed with osmium tetra-oxide, rinsed with sodium cacodylate buffer again. Materials were then dehydrated through acetone series, embedded in plastics, trimmed for cutting. LKB ultramicrotone was used, thick sections (1 μ thick) were then picked up by steel loop, stained with methylene blue-azure II, and were ready to be observed under the microscope. For detailed informations about fixation, embedding, sectioning, dehydration, and staining, see Dawes 1971.

III. RESULTS AND DISCUSSIONS

In general, the development of polyads of *Calliandra haematocephala* can be divided into following stages, namely;

1. Isodiametric: Being just emerged from the floral structure, sporogenous cells are isodiametric, more or less homogeneous, and the differentiation apparently has not started yet. (see Fig. 2)
2. One-celled stage: Pollen mother cell being differentiated from the sporogenous cell pool, gets more nutrition and grows bigger than the surrounding cells (see Figs. 3, 4, and 5). From sections examined, pollen mother cells are at 4th the layer counting from periphery. First layer is epidermis. It undergoes anticlinal divisions in order to meet the increment of the anther. It might stay intact or be broken into pieces later when the anther dehisces. The layer next to epidermis, will become the endothecium as we will see later. This layer of cells will reinforce its surface and mechanically support the anther. Next layer is the tapetal layer, cells will divide periclinally and form 2 or 3 layers. They will literally dissolve their contents from innermost layer going centrifugally, and supply the nutrition for the growth of polyad. Tapetal cells are usually characterized by densely staining and distinct nuclei.
3. Two-celled stage: Pollen mother cell divides (Fig. 6), and forms two daughter cells (see Figs. 7, 8). Cytokinensis follows right after the nuclear division, which is common among the monocots (Esau, 1965). Cells of tapetal layer divide, three tapetal layers are formed.
4. Four-celled stage: Two daughter cells of the first division grow bigger (Figs. 9, 10, 11, 12) as a result of absorbing nutrition from dissolving tapetal cells (innermost tapetal layer, see Figs. 9, 10). And divide into four new cells arranged in a rhomboidal shape (see Figs. 13, 14, 15, 16). Also innermost tapetal layer has already disintegrated and endothecial layer starts reinforcing.

5. Five-celled stage: one of the tetrad divides and form pentad. In Figure 17, the dissolving of tapetal cells and the absorption from pentad can clearly be demonstrated from the right part of the photograph.
6. Another cell next to the divided one eventually divides and hexad is formed. It is still possible although it has not been seen from sections that two of the tetrad can divide at the same time and form hexad.
7. Eight-celled stage: Eventually, two of the hexad which did not divide at the tetrad stage divide and form octad. (see Fig. 36, front view, and also Figs. 25–35)

Stomium that locates between two locules, when pollen mature will split and form a longitudinal slit, this will give octads a way to escape. This can be seen in Figure 35.

IV. CONCLUSION

The whole series of developmental stages can be concluded in Plate VII. Diagrams depict the developmental history. Periphery is not covering here.

a. Isodiametric stage—sporogenous cells are more or less isodiametric, cells have not differentiated yet (see Fig. 2).
b. One-celled stage—pollen mother cell stands out, peripheral sterile part relatively small. Tapetal cells are densely stained and have very distinct nuclei (see Figs. 3, 4, and 5).
c. Cell grows larger, starts dividing (see Fig. 6).
d. Two-celled stage—two daughter cells formed as the result from the division, cytokinensis follows right after nuclear division, tapetal cells divide and form 3 layers (see Figs. 7, 8).
e. After being nourished from dissolving tapetal contents (see figs. 9, 10), newly formed cells grow larger (see figs. 11, 12), and finally divide (not seen in section).
f. Four-celled stage—four cells arranged in rhomboidal shape, formed as a result from second division. Cells of inner tapetal layer have already disintegrated. Endothecial cells start reinforcing (see Figs. 13, 14, 15, and 16). Sporopollenin starts being built on the surface of the tetrads.
g. Five-celled stage—one of the tetrad grows relatively bigger than the rest three and starts dividing (not seen in section), and forms five-celled mass (see Fig. 17).
h. Six-celled stage—the one next to the divided one also divides (not seen in section), and forms six-celled (hexad) mass. Tapetal layers (rest two) also disintegrate (see Figs. 18, 19, 20, 21, 22, 23, and 24) or this process might happen the other way around.
i. Two of the tetrad divide at the same time (not seen in section). This gives rise to hexad.
j. Eight-celled stage—The rest two of the hexad divide, form octad. (see Fig. 36, front view)

There is one single vascular bundle sits in the middle of the four locules as seen in cross section (Fig. 27). Dehiscence of anther forms a longitudinal slit. The opening—stomium locates between two pollen locules (Fig. 35). Endothecial layer is thick enough as a mechanical support. These all can be seen when the anther (and polyad) becomes mature.

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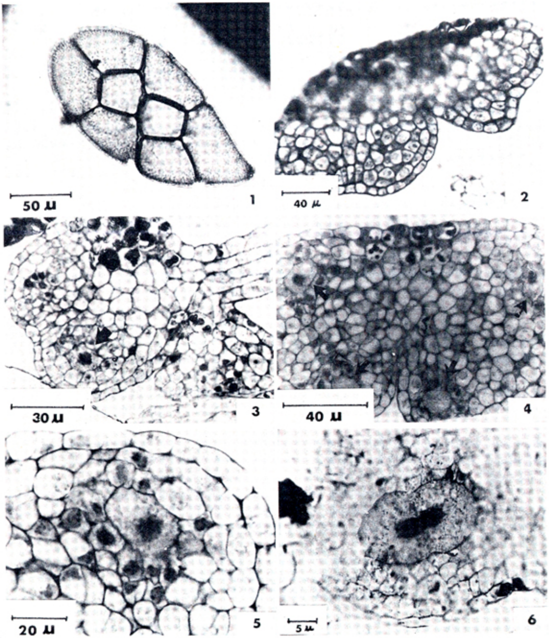
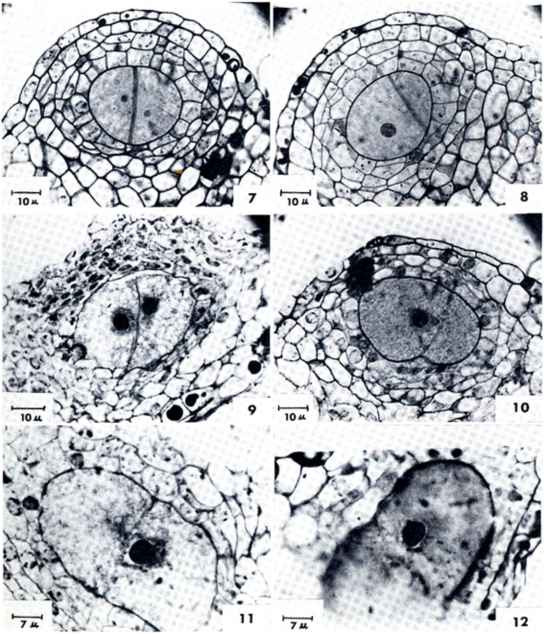
PLATE I



Explanation of Plate I

Fig. 1. Octad pollen grain of *Calliandra haematocephala* Hassk. front view.
2. Cross section of anther at early stage, cells more or less isodiametric, differentiation has not yet been taking place.
3. Longitudinal section of anther and filament, pollen mother cell has already shown some substantially differences (arrow pointed)
4. Cross section, showing four locules of anther at four corners (arrows pointed).
5. Cross section, showing one of the pollen mother cells.
6. Cross section, showing cell division of pollen mother cell,

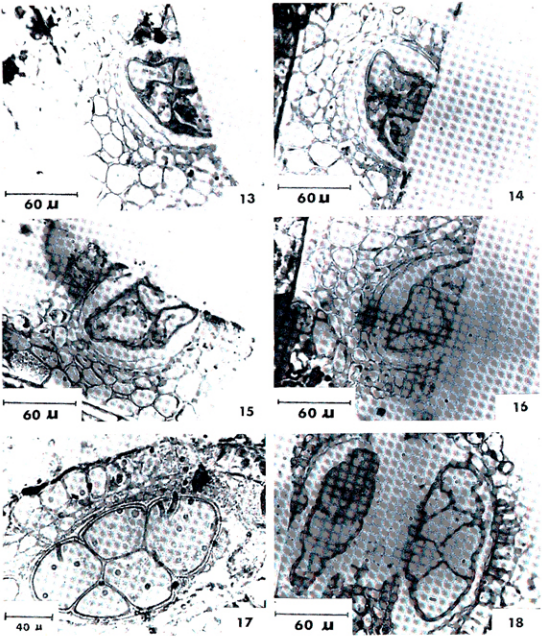
PLATE II



Explanation of Plate II

Fig. 7-12. Cross section, showing two daughter cells formed and growing bigger. Figs. 8, 10, 11, 12, one of the nuclei is not seen in these figures. Tapetal cells undergo periclinal divisions, three layers are formed as seen in figures 7, 8.

PLATE III



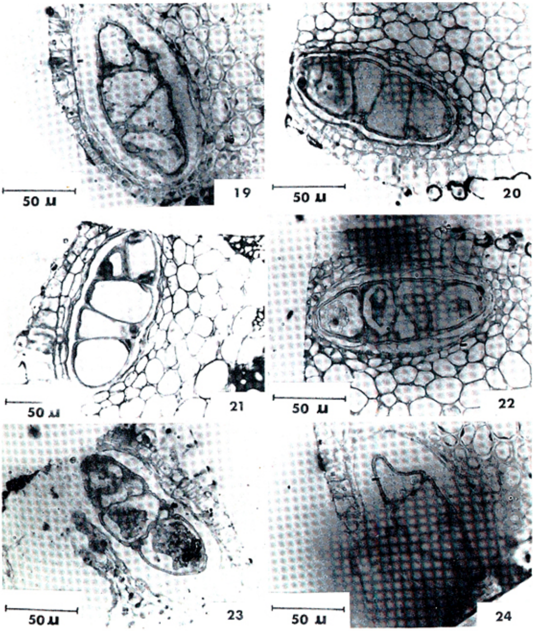
Explanation of Plate III

Fig. 13-16. Cross section, front view of tetrad, tapetal layer starts disintegrating (only the innermost one) endothecial reinforcement can also be seen.

17. Cross section, front view of pentad.

18. Cross section of anther, showing hexad stage, right one, front view, left one, side view.

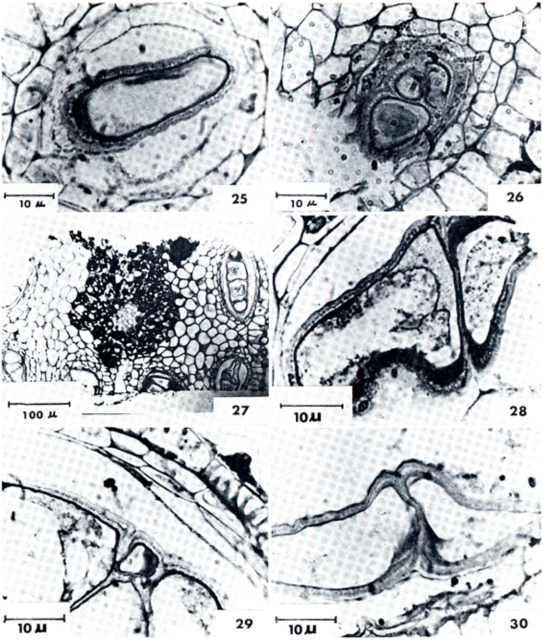
PLATE IV



Explanation of Plate IV

Fig. 19-24. Side view of hexads.

PLATE V



Explanation of Plate V

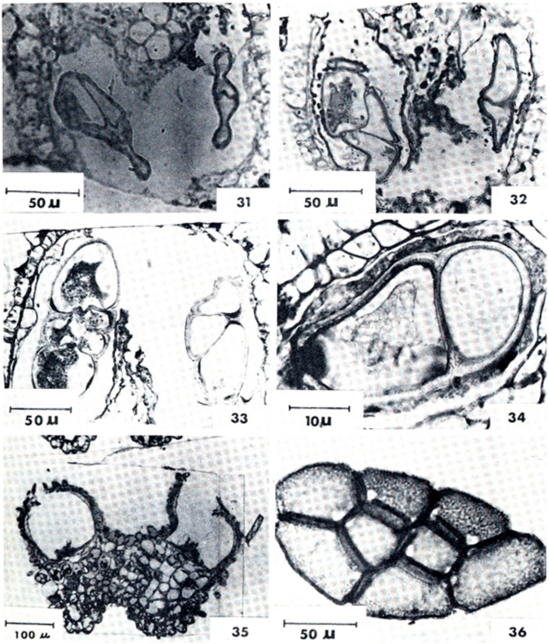
Fig. 25. Octad, cutting plane through lowermost one of the octads, showing tectate pollen wall

26. Octad, cutting is through lower part.

27. Cross section of anther at eight-celled stage.

28–30. Sections of octad cut from different angles, tectate pollen wall can be detected.

PLATE VI



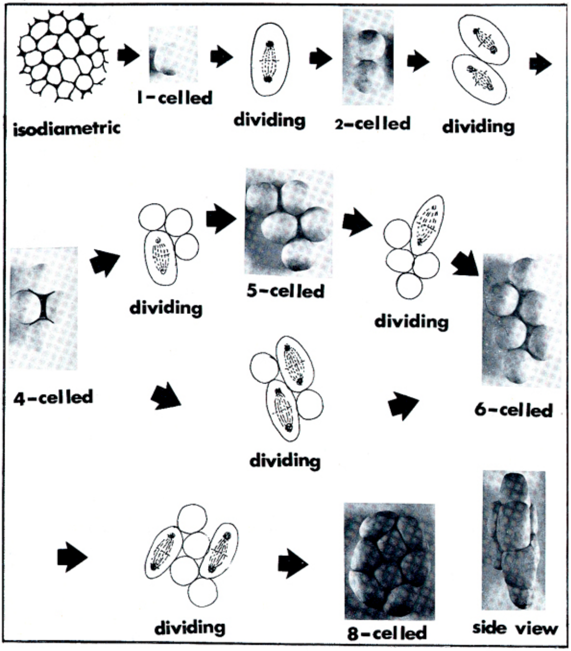
Explanation of Plate VI

Fig. 31-34. Octads within anther cutting planes are from different angles.

35. Cross section of anther after octads are all shed.

36. External morphology of mature octad, front view.

PLATE VII



Explanation of Plate VII

Schematic diagrams, showing the possible development of an octad.