2. Materials and Methods

WHEN THE BRANCHES AND LEAVES ARE BUSY IN PREPARATION OF THE FOOD, THE FLOWERS ARE ACTIVE IN MAKING OF THE FRUIT.
* CYAMOPSIS TETRAGONOLOBA (L.) Taub.
The samples of fruits in their different stages of development for the present study were collected from various parts of Gujarat, Tamilnadu and Kerala. The following Fabaceous members, either growing as wild or cultivated were selected for their ovary and pericarp studies:

1. Abrus precatorius L.
2. Cyamopsis tetragonoloba (L.) Taub.
3. Cajanus cajan (L.) Millsp.
4. Crotalaria striata DC.
5. C. verrucosa Linn.
6. C. pallida DC.
7. Clitoria ternatea L.
8. Centrosema pubescens Benth.
9. Glycine wightii (Grah. ex W. & A.)
10. Sesbania sesban (L.) Merr.
12. Vigna mungo L.
13. V. wightii Benth.
14. Atylosia scarabaeoids L.
15. Canavalia gladiata (Jacq.) DC.
17. Erythrina indica L.
18. Flemingia macrophylla (Willd.) Merr.
19. Indigofera linnaei L.
20. Mucuna prurita Hook. (L.f)
23. Trigonella foenum-graecum L.

Before processing for sectioning, length and breadth of ovaries from the buds as well as from opened flowers; of fruits at various developmental stages; of mature and dry fruits were measured. Then to facilitate the study
they were cut into three equal parts called basal, middle and terminal and their cut pieces were also fixed in Formalin-Acetic acid-Alcohol (FAA) and aspirated to remove air. The fixed materials of ovaries and basal, middle and terminal parts of different stages of fruits were cut into suitable size and washed 2-3 times in 60% ethanol. They were then dehydrated in tertiary butyl alcohol, infiltrated and embedded in tissue prep (56.5°) as described in Jensen (1962). Transections and longitudinal sections at about 7-9 um thickness were cut on Spencer-820 Rotary microtome. In addition to this, free hand sections of fresh as well as fixed materials were also used for histochemical tests for their tissues and contents.

Routine sections were stained in Safranin O and Fast green FCF (Berlyn and Miksche, 1976); Tannic acid-Ferric chloride, Safranin O and Fast green FCF (Foster, 1934), Toludine blue O in citrate buffer (O'Brien et al., 1964), Safranin O and Hematoxylin (Delafields) (Johansen, 1940), Toludine blue O and Basic Fuschin combination (Pizzalato, 1980).
Epidermal peels or paraoblique sections were taken with the help of sharp razor blades, needle and fine forceps. Sometimes epidermal peels were obtained by treating desired pieces of the material with 10% cupric sulphate and conc. hydrochloric acid (Mohan Ram and Nayyar, 1974). The peels were stained with Delafield's haematoxylin, then counterstained with safranin and mounted in 20% glycerine jelly. For studying the fiber and sclereids, epicarp and endocarp, of mature or dry fruits of various taxa were macerated in concentrated nitric acid and potassium chlorate.

Crystals and thick walled cells were observed with polarization and phase contrast microscopes. For observation, slides were deparafinized and unstained before mounting in DPX.

For fluorescence microscopic studies fresh hand sections and deparafinized sections were used to localize various metabolites. The following stains were used. (a) Aniline blue (0.05% in 0.067 KH PO buffer) for starch grains (Currier, 1951), (b) Fluorochrome or Dansyl chloride for basic proteins (Ringertz, 1968),
For histochemical tests, microtome and fresh hand sections were used. The following chemical combinations were used to localize various metabolites. (a) Ferric chloride and sodium carbonate for tannins (Johansen, 1940); (b) Mercuric bromo phenol blue for proteins (Mazia et al., 1953), (c) Periodic acid - Schiff's reaction for polysaccharides (Jensen, 1962), (d) Iodine potassium iodide for starch (Johansen, 1940), (e) Sudan black B for lipids (Bancroft, 1974), (f) Phloroglucinol-HCL for lignin (Jensen, 1962), (g) Hydrogen peroxide and silver nitrate for calcium oxalate crystals (Pizzolato, 1964), (h) Coomassie brilliant blue for proteins, (i) I$_2$KI-H$_2$SO$_4$ reaction for cellulose (Johansen, 1940).

Mature fruit walls both from fresh and fixed (FAA) materials were used to study the outer and inner surface sculptures. The pericarps were cut into 5 mm pieces and dehydrated through graded acetone series. After dehydration, materials were mounted on specimen stubs using 'fevicol' adhesive and kept for air drying. The
air-dried samples were immediately coated with a thin conducting film of Gold-palladium using SEM coating unit Nanotech Thin Film Ltd. (England). Samples were observed with the Cambridge stereoscan S-10 microscope (England) at 'ATIRA' Ahmedabad.

For light microscopic observations, Olympus and Nikon research microscopes (Japan) were used. Phase contrast microscopic observations and photomicrographs were taken with the help of AO spencer phase contrast microscope (USA). Zeiss Axiomat (Germany) is used for polarized observation and photomicrography. Black and white and colour photographs for bright field were taken with Carl-zeiss photomicroscope I (Germany). The photographs of the entire fruit materials in the laboratory and in field were taken with Minolta Camera XG1 (Japan). Line drawings were made with the help of side tube Camera lucida (Carl Zeiss-Jena - Germany) and xerox copies were obtained from Modi xerox 1025 series (India).