Studies of the Ovule and Seed Development of Guar

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Introduction

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) appears to have great potential as a field crop because of its production of mannogalactan gum. The gum is produced in the seed and is located in the tissue surrounding the embryo. In legumes, the endosperm is commonly digested and absorbed during embryo development so that the mature seed consists only of a seed coat surrounding an embryo. The persistence of a food-storage tissue in the guar seed is of botanical interest as well as of commercial significance. Therefore, the nature of the development of this food-storage tissue has been investigated.

The problem being studied is the morphological development of the guar ovule and seed. Primary concern will be given to endosperm development. Certain aspects of the development of the female gametophyte late embryo stages, and associated parts of the seed are included to show their relationship to endosperm growth.

Methods and Materials

The plant material used in this study was *Cyamopsis tetragonoloba* cv. 'Texsel' obtained from the 1962 Guar Research Field Plot at the Purdue University Agronomy Farm.

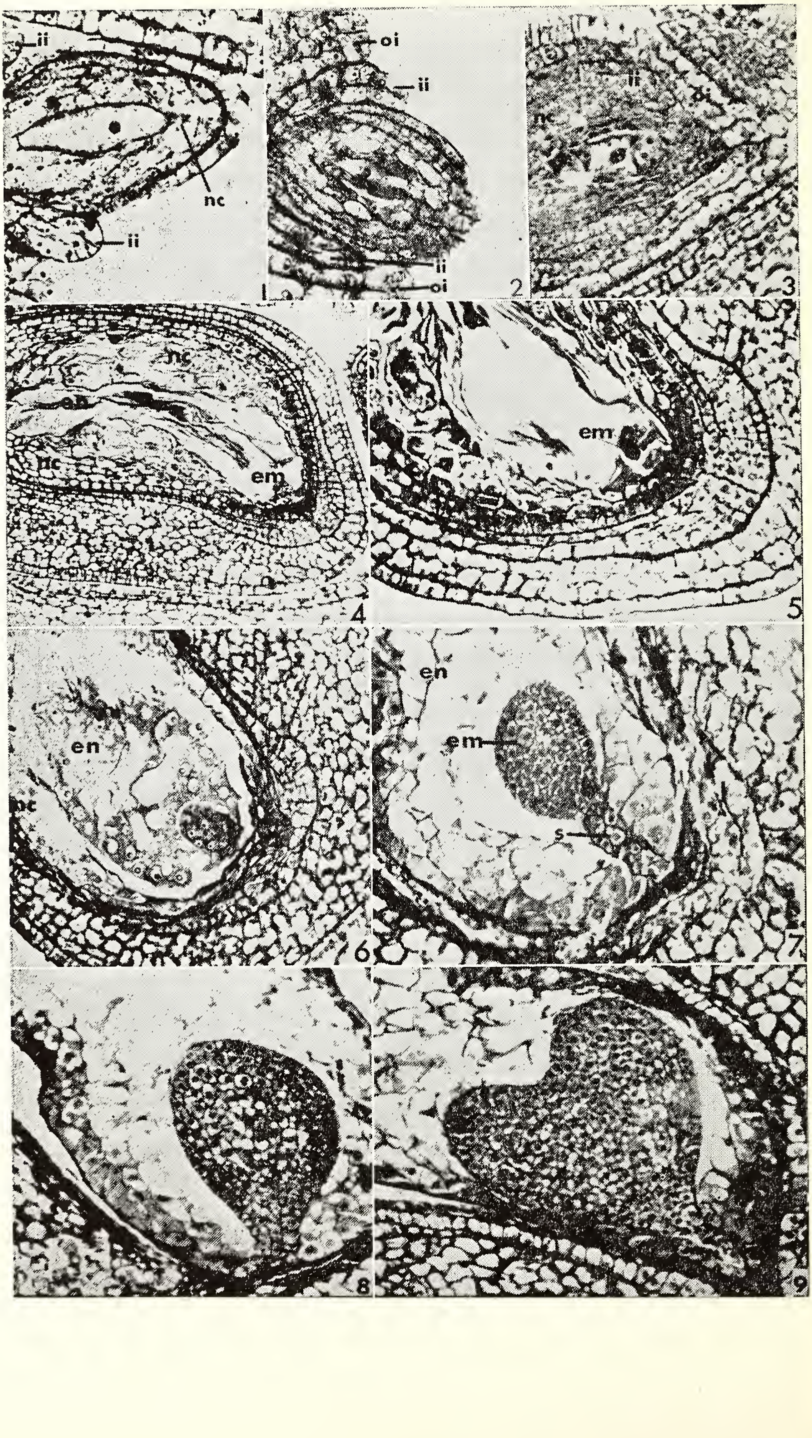
In preparation for sectioning the fresh material was killed and fixed in Randolph's Modified Navishin (Craf) solution (4). After killing and fixation, the material was dehydrated in an ethyl-alcohol to xylol series followed by infiltration with paraffin.

The material was sectioned on a rotary microtome at a thickness of 6-8 micron and stained with a combination safranin and fast green series. Microphotographs were taken on a Zeiss photomicroscope.

Results

Early stages of guar ovule development are found in the young flower bud. The crassinucellate ovule consists of nucellular tissue surrounded by two integuments, supported on a funicular stalk. The embryo sac develops within the ovule, and after fertilization the growing embryo and endosperm eventually fill the guar seed. The nucellus is broken down as the endosperm develops, and by the time the seed matures the nucellar tissue is no longer present. The endosperm persists in the mature seed and the outer integument is modified to form parts of the testa.

INTEGUMENTS: Two integuments are initiated as evaginations at the base of the young ovule and these eventually grow around the nucellus (Fig. 1). Each integument consists of two cell layers that divide anticlinally. The integuments away from the funiculus grow at a more rapid rate than the two integuments nearest the stalk (Fig. 2).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The inner and outer integuments on either side also grow at different paces. Initially the growth of the inner integument appears more pronounced, but as development proceeds the outer one passes it. By the time the female gametophyte has matured (Fig. 3), the edges of the outer integument have met above the nucellar apex, and the edges inner integument have not yet reached this point. About the time of fertilization however, the inner integument forms an envelope around the nucellus, and the meeting of the integuments at the nucellar apex forms the micropyle.

After fertilization the ends of the integuments become closely appressed above the nucellar apex. The outer integument thickens and differentiates (Fig. 4). The inner integument appears to remain only two cell layers thick and does not undergo further differentiation. As development proceeds the two cell layers of the inner integument around the embryo sac becomes less and less distinguishable, except at the micropylar end where the two cell layers can be distinguished through heart shaped embryo stages (Fig. 21).

In the mature seed (Figs. 24, 25) the inner integument has been broken down or resorbed, and it appears that only scattered and fragmented cells remain. The outer integument has been modified as the seed matured to form parts of the seed coat or testa. A mesophyll layer (Fig. 25), which lies near the endosperm, has been formed from the inner part of the outer integument. Hour-glass cells compose the intermediate layer between the mesophyll and the palisade cells. These cells are columnar, like the outer palisade cells, but they are much larger and sometimes appear slightly waisted in the middle. The inner ends appear more irregular in shape than do the outer ends. The palisade cells are slender, tightly packed columnar cells. Their outer ends are covered with a cuticle (Figs. 24, 25).

EMBRYO SAC FORMATION: Within the ovule a single, functional megaspore (Fig. 1) survives and eventually grows into the embryo sac. This functional megaspore undergoes mitotic divisions to form a monosporic, 8-nucleate female gametophyte structure (Fig. 3). Two synergids lie on either side of the egg cell at the micropylar end of the embryo sac. Three antipodal nuclei occur at the chalazal end, and two

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Figures on ovule and embryo development, 1-9: 1. Early stage of the young guar ovule found within the flower bud. A massive nucellus (nc) surrounds the surviving functional megaspore. Inner (ii) and outer (oi) integuments have evaginated from the base of the ovule. X430; 2. A young guar ovule showing the growth of the integuments around the nucellus. X380; 3. The 8-nucleate female gametophyte of guar. A massive nucellus (nc) surrounds the embryo sac. The outer integuments (oi) have reached the nucellar apex. The inner integuments (ii) have not yet reached the apex. X370; 4. Early globular embryo (em) soon after fertilization. Free nuclear endosperm (en) is spread through the embryo sac. Nucellar tissue is still prominent. X90; 5. Early globular embryo (em) showing free endosperm nuclei. X320; 6. Globular embryo surrounded by free nuclear endosperm (en). X250; 7. Late globular embryo (em) with suspensor (s). Early cell wall formation in the embryo. X300; 8. Late globular embryo just before cotyledonary regions become prominent and cellular endosperm. X250; 9. Heart shaped embryo. Cotyledonary regions are clearly distinguishable. X225.

polar nuclei are located in the center of the sac. The mature ovule at this time is in a campylotropous position.

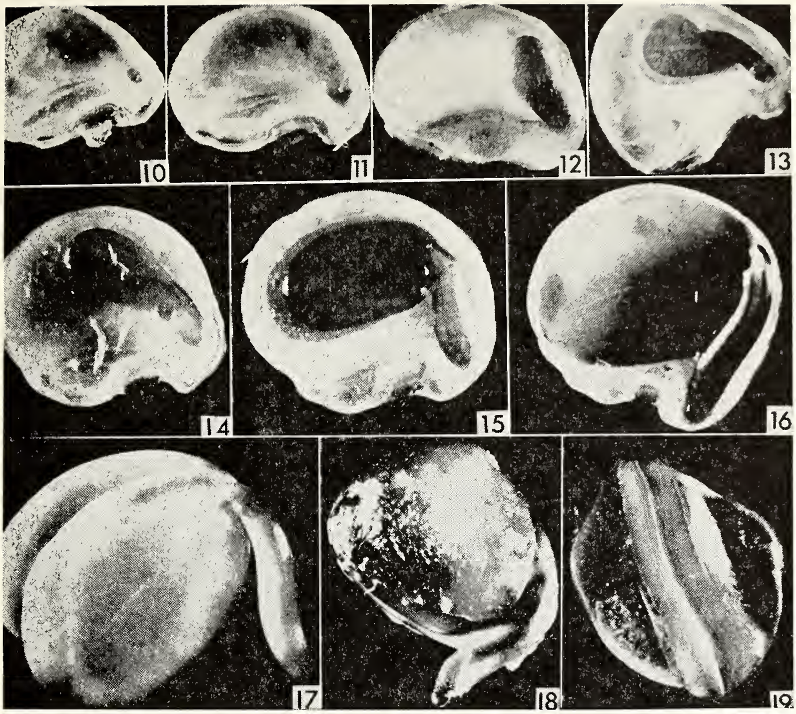
THE NUCELLUS: As the embryo sac develops it is surrounded by a massive nucellus whose several cell layers separate the sac from the nucellar epidermis (Figs 1, 2, 3). As the endosperm tissue expands it does so at the expense of the nucellus. A sequential comparison of all stages from soon after fertilization (Fig. 4) to the time of maturity (Figs. 23, 24, 25) shows that the nucellus is ultimately broken down or resorbed. Right after fertilization, when the endosperm is still all free nuclear, most of the nucellus is still present (Fig. 4). By the heart shaped embryo stage most of it has been broken down (Fig. 21), and by later stages (Fig. 22) only a very few, if any, nucellar cells remain.

THE EMBRYO: Very early divisions of the embryo have been thoroughly worked out by Anataswamy Rau (2) and therefore have not been a major concern of this study. According to Anataswamy Rau the zygote divides transversely to form a basal cell and a terminal cell. The basal cell subsequently forms the suspensor supporting the embryo, and the terminal cell develops into the embryo proper.

Later events in the development of the embryo in this study can be described in sequential stages according to embryo shape. The terminal cell gives rise to a globular stage by a series of anticlinal and periclinal divisions (Figs. 4, 5, 6). As divisions proceed the embryo becomes less globular; height and breadth are approximately equal, but it is somewhat flattened in the third dimension (Figs. 7, 8). Divisions in the suspensor do not keep pace with those of the embryo proper (Fig. 7), resulting in fewer but larger cells. The hypophyseal part of the embryo, which gives rise to the extremity of the root and root cap, is initiated at the very base of the embryo, just above the area of attachment with the suspensor (Fig. 7). Further divisions in the procotyledonary region (Fig. 8) produce the heart shaped stage (Fig. 9), where the cotyledonary parts become clearly distinguishable.

After the heart stage the cotyledons undergo longitudinal growth (Figs. 10, 11) until a 'torpedo' stage is formed (Fig. 12). At this stage the hypocotyl appears lengthened and is almost as long as the cotyledons that have flattened against each other. As further growth occurs the cotyledons turn back within the ovule (Figs. 13, 14). Later the cotyledons and hypocotyl lie almost at right angles to each other (Fig. 15), and at seed maturity the embryo (Figs. 16, 17) has almost completely filled the seed. The cotyledons and hypocotyl lie very close to the edge of the testa (Fig. 16). Chlorophyll was observed in the embryo from the later heart shaped stages to the time of maturity.

THE ENDOSPERM: A nuclear type of endosperm is initiated at the time of fertilization by the fusing of a sperm nucleus and the two polar nuclei. The 3n nucleus undergoes a series of mitoses shortly after fertilization. At first free endosperm nuclei become distributed somewhat evenly through the embryo sac (Fig. 4). A short time later the free nuclear endosperm appears more concentrated around the embryo (Fig. 6). The embryo at this time is in a globular stage.



Figures on embryo and see development, 10-19: 10. Heart shaped embryo within immature seed. X21; 11. Late heart shaped embryo. X19; 12. "Torpedo" stage embryo. X17; 13 and 14. Later embryo stages. The cotyledons have begun to turn back within the ovule or the immature seeds. X16; 15. Late embryo, just before maturity. X14; 16. The embryo within the mature seed. X13; 17. The mature embryo that has been removed from the seed before drying has occurred. X10; 18. The mature embryo surrounded by endosperm. X13; 19. Cross section of the mature seed before drying has occurred. X14.

Cytokinesis is initiated in the endosperm at the micropylar end of the embryo sac near the embryo (Fig. 7). This cell formation extends toward the chalaza until most of the endosperm is cellular. However apart from this main body of cellular endosperm many free endosperm nuclei can be seen at the chalazal end (Figs. 20, 21) until the embryo has reached the heart shaped stage (Fig. 21). After this time the number of free endosperm nuclei evidently gradually diminish and degenerate. A free nuclear tubular portion is often visible at the tip of the main body of endosperm (Fig. 20).

The outer layer of the main endosperm body is composed of small cells, while the cells of the interior are larger and have thinner walls. The cells of the outer layer are more dense and stain darker at all stages (Figs. 7-9, 20-25).

THE MATURE SEED: In the mature seed the nucellus has been broken down or resorbed and the endosperm completely surrounds most of the embryo (Figs. 18, 19, 23) and the outer border of the endosperm lies next to the testa (Figs. 24, 25) that developed from modifications of the outer integument.

At maturity, just before the seed begins to dry, the endosperm appears almost transparent (Figs. 18, 19) and it comprises the bulk of the seed. Upon drying the endosperm becomes more opaque.

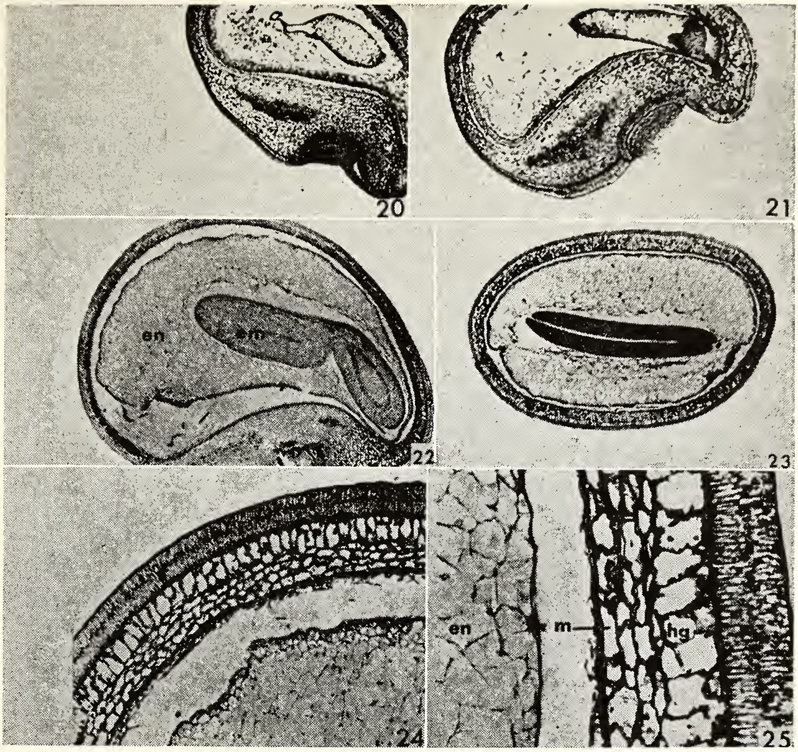
Discussion

Anataswamy Rau (2) has carefully diagramed the successive cell divisions in the embryo development of guar from the two-celled proembryo stage through the early globular stages. The epiphyseal, cotyledonary, hypocotyledonary, and hypophyseal regions were pointed out in the stages he presented. The evidence presented here (Figs. 4, 5, 6) on early embryo development agree with several stages diagramed by Anataswamy Rau (2).

Johansen (5) has outlined the details of the Trifolium Variation, a basic type of embryo development. It appears that Anataswamy Rau has assigned *Cyamopsis psoraloides* to the Trifolium Variation largely on the basis that the early divisions of this embryo conform to those of *Trifolium minus*. The study presented here was not mainly concerned with the successive divisions of the early embryo, but it does show evidence that the suspensor of this embryo conforms to the Trifolium Variation by having a suspensor whose cells differ from those of the body of the embryo in form, number, and plane of division (Fig. 7). Therefore, on the basis of the work of Anataswamy Rau and evidence presented in this study, the early embryo development of guar appears to largely conform to the Trifolium Variation as outlined by Johansen.

Anataswamy Rau (1) presented an analysis of the early endosperm development in guar and several related species. The results on early endosperm formation, and ontogeny for guar presented here largely support the analysis done by Anataswamy Rau on this species. Anataswamy Rau mentioned the presence of a free nuclear tubular portion at the tip of the main body of endosperm, or in the chalazal portion, during early stages. He did not give reference to any comparable stage of embryo development when this tubular portion was present. However, in this study a free nuclear portion at the tip of the main body of endosperm (Fig. 20) was observed only during late globular-early heart shaped stages of the embryo. Possibly it was also present at earlier stages; but due to the delicate structure of this tubular portion, it might have been destroyed in sectioning.

Corner (3) stated that two microscopic characters distinguish the leguminous testa: 1) the external palisade, developed from the outer epidermis of the outer integument, and 2) the hour-glass cells, developed from the outer integument. According to Corner any testa with these two microscopic features is apparently leguminous. A magnified section of guar testa from the side of the seed shows these two characteristics and identify the testa as being leguminous (Figs. 24, 25). Corner further stated that hour-glass cells having inner ends that are very irregular are often characteristic of Papilionaceae. This characteristic



Figures on embryo and seed development, 20-25: 20. Longitudinal section through the immature seed at a late globular embryo stage showing a portion of cellular endosperm and a free nuclear tubular portion at the tip of the main body of endosperm. X35; 21. Longitudinal section of the immature seed at a heart shaped embryo stage showing the cellular endosperm body surrounded by free nuclear endosperm. X30; 22. Longitudinal section of the immature seed at a later embryo stage (em) showing the cellular endosperm (en) which surrounds the embryo. X35; 23. Cross section of mature seed showing cotyledons, endosperm, and testa. X30; 24. Section of the mature seed showing a portion of the endosperm and testa. X90; 25. Enlarged section of mature seed showing endosperm (en) and a portion of testa from the side of the seed. Testa structure: mesophyll layer (m), hour-glass cells (hg), and palisade cells (p). X180.

also appears in the hour-glass cells of guar (Fig. 25), and helps to place it in this sub-family.

Summary

Some morphological aspects of the developing ovule and guar seed are described. Emphasis is placed on endosperm development. The endosperm is important as a source of mannogalactan gum, and the fact that the endosperm persists in the mature guar seed is an unusual situation for a leguminous species.

The guar ovule is found within the flower bud and consists of a massive nucellus surrounded by two integuments. Within the ovule a

monosporic, 8-nucleate female gametophyte is formed. After fertilization the nucellus is gradually broken down or resorbed as the endosperm develops, and at seed maturity the nucellar tissue is not present. The inner integument remains only two cell layers thick, and it is evidently broken down or resorbed before the seed matures. The outer integument becomes complicated to form various parts of the testa. The cell structures of this guar testa from the side of the seed compare favorably to the testa cell structure of other species of Papilionaceae.

Early stages of embryo development were found to be similar to early stages of the basic Trifolium Variation, and descriptions of Anataswamy Rau (1). Later stages of embryo development are described according to embryo shape. Throughout most of its development the embryo is almost completely surrounded by endosperm.

A nuclear type endosperm is initiated at fertilization. During early stages many free endosperm nuclei are distributed through the embryo sac. A short time later the free endosperm nuclei are more concentrated in the area around the embryo. Cytokinesis is initiated during globular stages and progresses from the micropylar to the chalazal end of the embryo sac. Free endosperm nuclei persist in the chalazal end even after cytokinesis is initiated. A free nuclear tubular portion was observed at the tip of the main body of endosperm during late globular-early heart shaped embryo stages. At seed maturity the endosperm surrounds the embryo and it comprises the bulk of the seed.

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