Aspects of the Reproductive Biology of *Zieria prostrata* (Rutaceae)

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Abstract

Zieria prostrata has tetrasporangiate anthers with a five layered wall, the innermost layer being the secretory tapetum with binucleate cells. The processes of microsporogenesis and microgametogenesis is normal with no evidence of either meiotic abnormalities or pollen sterility. The mature pollen grains are two-celled and tricolpate. There are four ovules in each ovary. They are anacampylotropous, bitegmic and crassinucellar. There may be several archesporial cells but usually one grows into a megaspore mother cell. Meiosis is normal and gives rise to a tetrad of megaspores. The development of the female gametophyte follows the monosporic Polygonum type. The endosperm is of the Nuclear type. The embryo development appears to be of the Onagrad type. Although all the ovules appear to form seeds, nearly half become infected with insect larvae which eat through the endosperm and embryo. The seeds are exotestal, monoembryonate and ex-arillate. Seed germination under laboratory conditions is extremely low. This cannot be explained in terms of seed coat anatomy. The fruit wall shows sub epidermal oil glands.

Introduction

Zieria prostrata is an endangered species of the ROTAP category 2E, restricted to three headlands in the vicinity of Coffs Harbour. It has horticultural potential because of the beautiful flowers and prostrate habit and is relatively easy to propagate from stem cuttings. Griffith (1991) studied the distribution of the species, assessed its conservation status and suggested recovery objectives. Virtually nothing is known of the reproductive biology of the species which may help in effective management aimed at conserving the species in the wild. This study has been undertaken at the request of National Parks and Wildlife Service of New South Wales with the financial assistance from the Australian Flora Foundation.

Material and Methods

Flower buds, flowers and fruits of various stages of development were collected by Mr Hans Wissman during successive field trips between August 1993 and February 1995 from a small population of about six plants at Emerald Beach, North of Coffs Harbour. For the microscopic examination of the histological stages in reproductive development, these were fixed on spot in a mixture of 70% ethanol (90cc), glacial acetic acid (5cc) and formalin (5cc). After two days in the fixative, the materials were repeatedly washed in 70% ethanol and were gradually dehydrated and embedded in paraplast. Sections were cut at a thickness of 12 to 16 microns, dewaxed, stained in safranin and fast green, and mounted in euparol on microscopic slides following conventional procedures (see Prakash

1986). Camera lucida drawings were made of suitable stages in embryological development

For seed germination experiments in Armidale, conducted between 19 January and 3 February 1994, 300 seeds were collected from mature fruits. Before sowing in garden soil at about 250C, one-third of these were rubbed on sand paper, one-third were aerated, and the remaining one-third were both rubbed with sandpaper and then subjected to aeration.

Observations

Floral morphology

The plants form dense prostrate mats with rooting wherever the stems touch the ground in which the identity of individuals is hard to determine. The flowers (Fig. 1A) are bisexual, regular, complete, and borne in small inflorescences of 3-7 flowers in Spring. There are four petals which are white at maturity but tinged with pink in bud with prominent stellate hairs (Fig. I B) on the outside. Alternating with petals are four free stamens. The nectaries are prominent. The gynoecium is tetracarpellary, syncarpous and tetralocular. There are two seeds in each locule.

Anther development

The anthers are tetrasporangiate (Fig. 2A). The anther wall consists of five layers of cells (Fig. 2B): the epidermis, endothecium, two middle layers, and the tapetum. The tapetum is of the glandular type with binucleate cells (Fig. 2E) that are most prominent at the time of meiosis. The microspore mother cells are surrounded by callose (Fig. 2C). Meiotic divisions (Fig. 2D,E) are normal and result in tetrahedral microspore tetrads which separate into individual microspores (Fig. 2F) on dissolution of the callosic wall of the spore mother cell. The mature pollen grains are tricolpate and bicelled (Fig. 2G). The endothecium starts to become prominent after meiosis and develops fibrous thickenings at pollen maturity.

Ovule development

The ovules are bitegmic, anacampylotropous, and crassinucellar (Fig. 3 F). Initially the outer integument is three-layered whereas the inner is twolayered (Fig. 3B). The number of archesporial cells varies from one to many (Fig. 3A). Parietal cells are cut off (Fig. 3B) and these, together with divisions in nucellar epidermis (Fig. 3C), constitute a multilayered tissue between the micropyle and the embryo sac. The development of the female gametophyte (Fig. 3C-E) follows the monosporic Polygonum type. The mature female gametophyte (Fig. M has an egg apparatus of three cells, and two polar nuclei; the antipodals, being ephemeral, are absent at maturity. There is large amount of starch present in the embryo sac. The polar nuclei fuse before fertilisation (Fig. 3F).

Seed development

The zygote is situated at the micropylar end of the embryo sac (Fig. 4A). It gives rise to a proembryo following the Onagrad type of development (Fig. 4B). In a young seed, the seed coat (Fig. 5A) shows 3 or 4 cell layers in the testa (derived from the outer integument) and 2 or 3 layers in the tegmen (derived from the inner integument). The mature seeds are small, rounded, black, monoembryonate, ex-arillate and exotestal with thickened, closely set, radially elongated but scarcely thickened columnar cells in the testa derived from the outer and inner epidermal layers enclosing in between rounded parenchyma cells with intercellular spaces. Tannins abound - particularly in the outer two layers. The cells of the tegmen also enlarge but remain highly vacuolate and develop an irregular outline - suggestive of their imminent collapse. The seeds, in their anatomy, resemble those of Ruta and other members of the subfamily Rutoideae (Prakash and Lim 1995).

Fruit wall

The fruit wall (Fig. 6A) consists of about 10 layers of thin-walled parenchymatous tissue bounded by a prominent outer epidermis and a two layered zone of somewhat smaller cells on the inner side. Oil glands occur beneath the outer epidermis.

Seed Germination

Of the three treatments, only those that were rubbed with sand paper and subsequently aerated for 24 hours showed any signs of germination. Of the 100 seeds only 3 germinated after 3 weeks. There was no further germination beyond this period The radicle emerges first as a minute white knob but eventually elongates. One of the seeds showed the emergence of three seedlings although the histological examination did not show any signs of polyembryony. Of a sample of twenty seeds floated on water, only four sank straight away sank to the bottom, and another five sank 12 hours later. The remaining kept floating and on examination showed insect larvae that evidently ate away the endosperm and the embryo.

Discussion and Conclusions

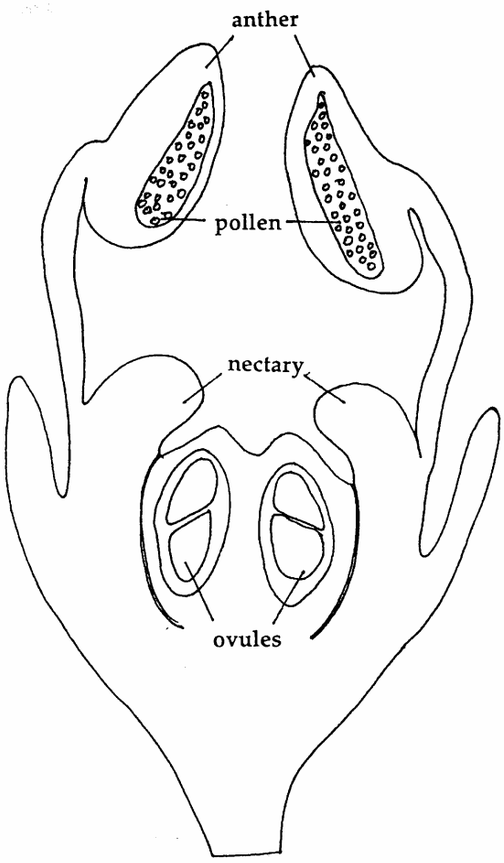
The investigation showed that reproductive development in the material of *Zieria prostrata* under study was normal and broadly comparable with that in other rutaceous plants (Prakash and Lim 1995). There was no sign of either sterility in the anther or the ovule. The pollen grains are normal looking and appear capable of germination. On the other hand, nearly half of the seeds produced were predated by larvae of some unknown insect. Seed germination was difficult under experimental conditions but the thickness of seed coat does not appear to be the cause.

References

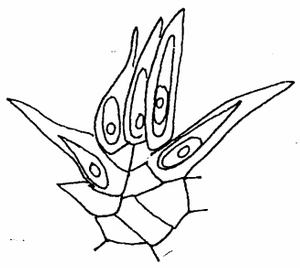
Griffith, S.J. 1991. Species recovery plan for *Zieria prostrata.* Australian National Parks and Wildlife Service Endangered Species Program, Project No. 155.

Prakash, N. 1986. Plant Microtechnique. University of New England, Armidale.

Prakash, N. and Lim, A.L. 1995. Reproduction in Rutaceae - a review. *In K.* G. Mukherjee (ed.) Professor N. N. Bhandari Commemoration Volume of Phytomorphology, International Society of Plant Morphologists, Delhi. pp. 1-50.



A. L.S. FLOWER BUD



B. STELLATE HAIR FROM PETAL BUD

FIG.1

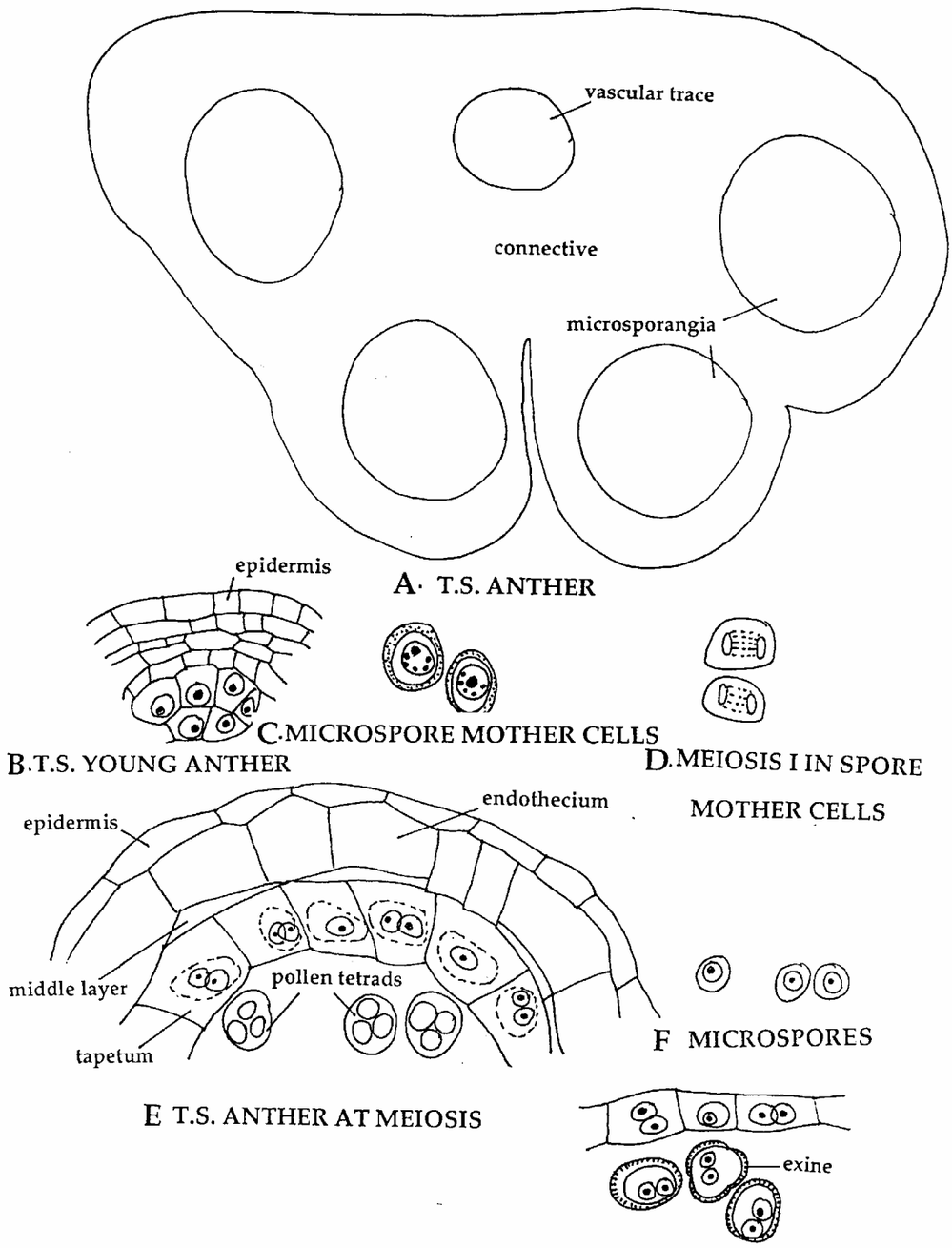
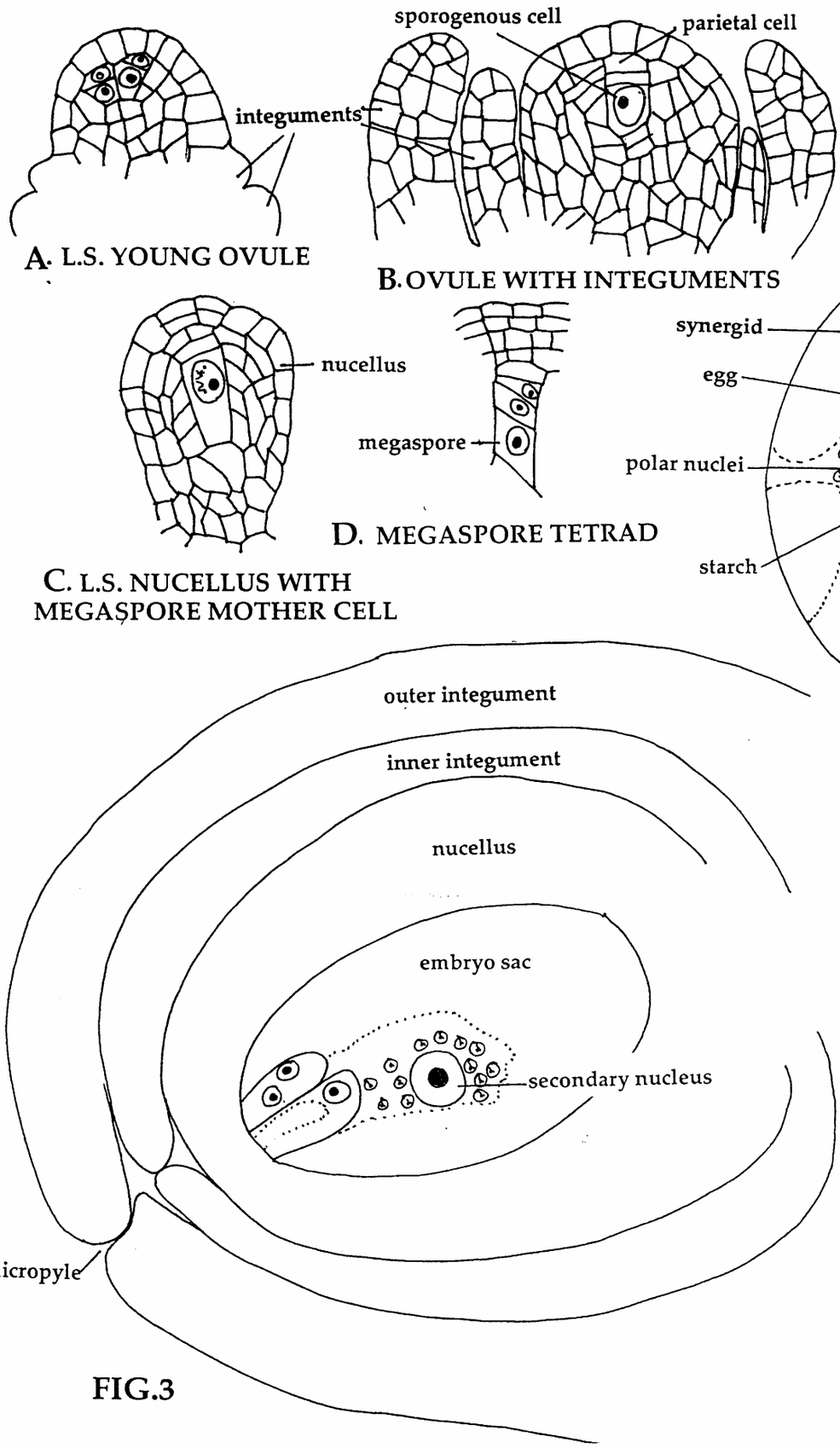
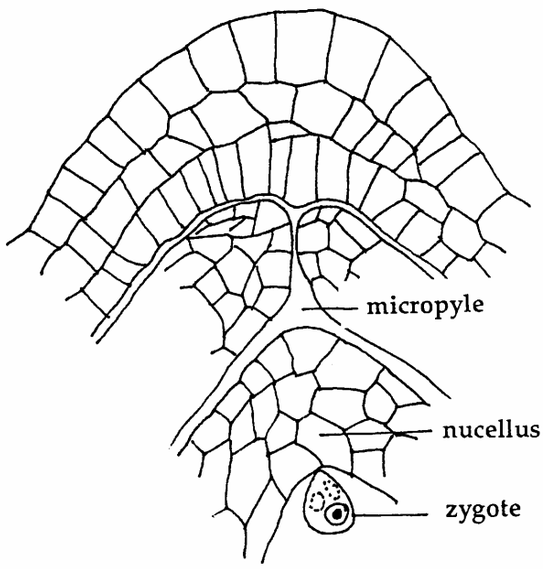
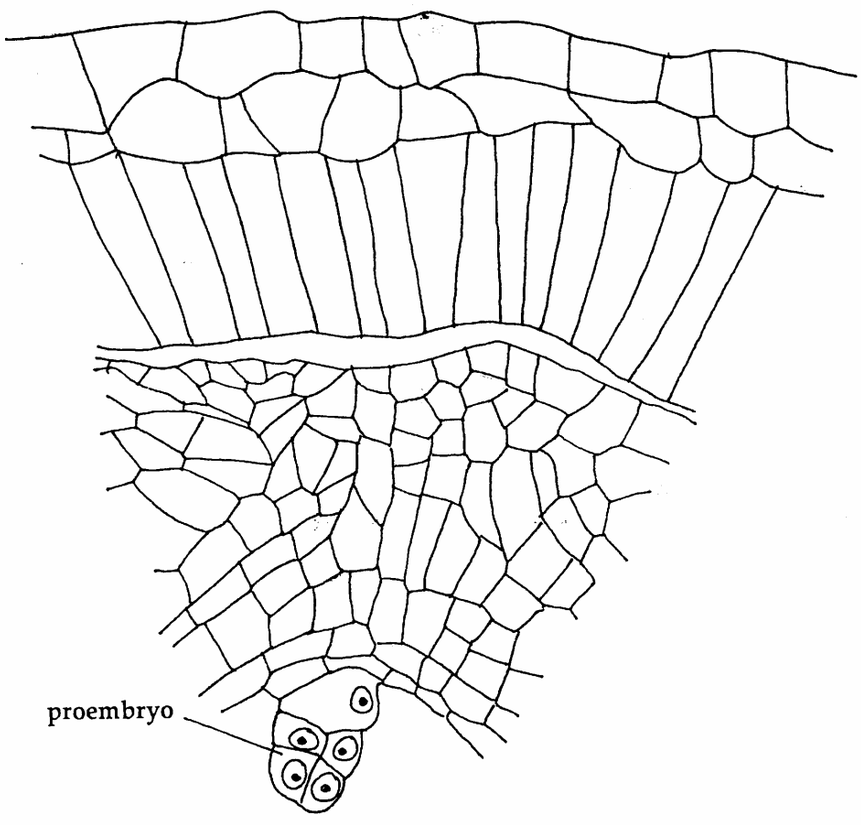

vascular trace
connective
microsporangia
epidermis
A· T.S. ANTHER
C.MICROSPORE MOTHER CELLS
B.T.S. YOUNG ANTHER
D.MEIOSIS I IN SPORE
MOTHER CELLS
endothecium
epidermis
middle layer
pollen tetrads
tapetum
F MICROSPORES
E T.S. ANTHER AT MEIOSIS
exine
G. TAPETUM AND MATURE POLLEN

FIG.2


integuments
sporogenous cell
parietal cell
A. L.S. YOUNG OVULE
B. OVULE WITH INTEGUMENTS
nucellus
megaspore
D. MEGASPORE TETRAD
C. L.S. NUCELLUS WITH MEGASPORE MOTHER CELL
synergid
egg
polar nuclei
starch
E. ORGANISED EMBRYO SAC
outer integument
inner integument
nucellus
embryo sac
secondary nucleus
micropyle
FIG.3
F. OVULE WITH MATURE EMBRYO SAC

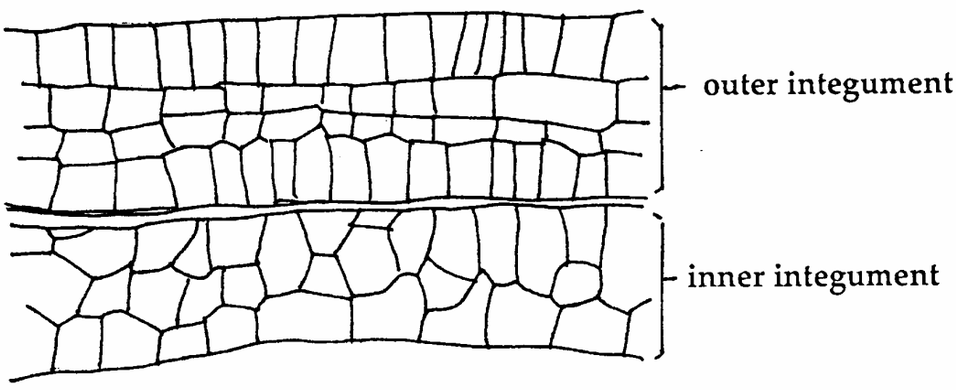


A. L.S. MICROPYLAR PART, YOUNG SEED

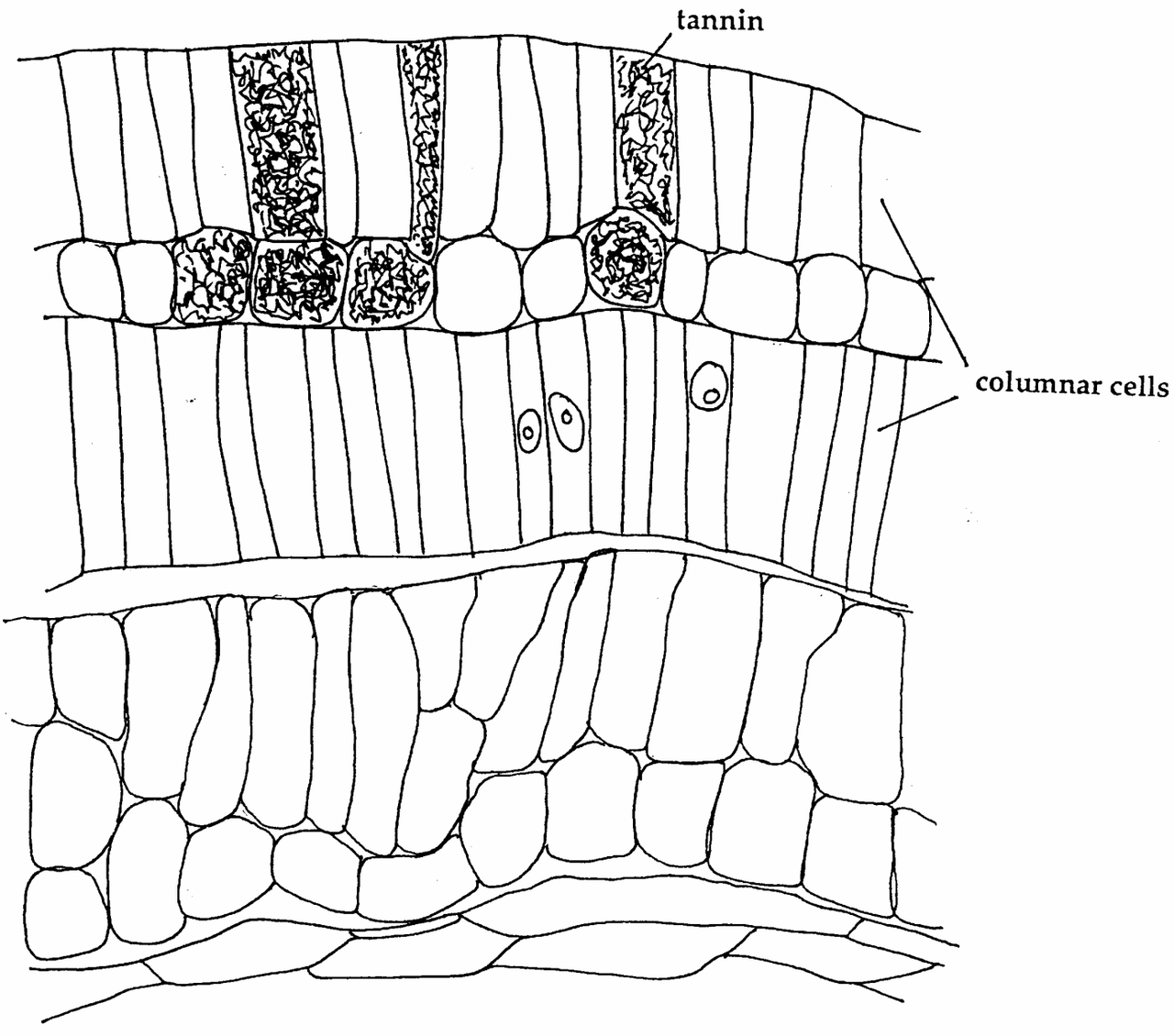


B. L.S. MICROPYLAR PART, OLDER SEED

FIG.4

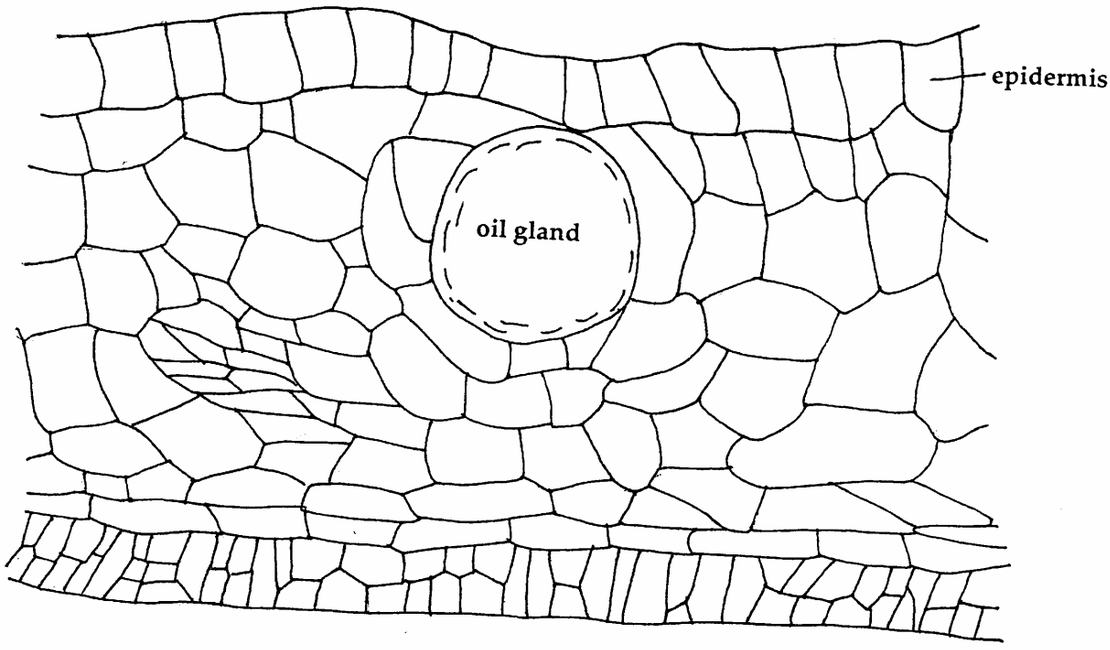


A. T.S. YOUNG SEED COAT



B.T.S. SEED COAT, RIPE SEED

FIG.5



A T.S. FRUIT WALL

FIG.6