Indian Journal of Natural Rubber Research, 8(1) : 8-12,1995

DEVELOPMENTAL ANATOMY OF GERMINATING SEED OF *HEVEA*

D. Premakumari and P. Sobhana

Premakumari, D. and Sobhana, P. (1995). Developmental anatomy of germinating seed of *Hevea*. *Indian Journal of Natural Rubber Research*, 8(1): 8 - 12.

Developmental aspects of germinating embryo of *Hevea* were studied with emphasis to the growth changes at the pre-emergence stage. The shoot pole of the mature embryo is a dome-shaped meristem consisting of a unilayered protoderm, a hypodermal region, the procambium and ground meristem. Prolaticifer initials are distributed in all the three zones below the protoderm and in the cotyledons. The root pole is blunt and undifferentiated.

Vascular differentiation is basipetal in the shoot axis starting with laticifer differentiation followed by sieve tube and xylem formation in the order. Primary laticifers are articulated and anastomosing. Root differentiation is irregular. Lateral root development advances before the initiation of tap root development.

Key words : *Hevea*, Embryo, Germination, Laticifer, Seed.

D. Premakumari (for correspondence) and P. Sobhana, Rubber Research Institute of India, Kottayam - 686 009, Kerala, India.

INTRODUCTION

Hevea fruit is a regma which dehisces, when mature, along the septae. The testa of a mature seed is highly mottled and of varied colour from chestnut brown to darker- shades. It is hard and impermeable except at the micropyle. The hylar region is depressed and covered with two thin layers of cuticle. The tegma is many layered, papery and light and is tightly placed in between the testa and the endosperm. The embryo is oriented inside the endosperm tissue with root pole facing the micropyle. There are two veined cotyledons situated dorsiventrally inside the endosperm. The cotyledons remain close together and crumbled, inside the seed.

Germination of *Hevea* seed has been dealt with in detail by researchers (Calvert, 1887 and Gomez, 1982) and little controversy exists regarding the general aspects. Information on the sequence of developmental changes is insufficient and controversy exists on the origin of laticifers. This work was taken up to study the developmental changes of the zygotic embryo of *Hevea* during germination with special emphasis to the growth changes at the pre-emergent stage .

MATERIALS AND METHODS

Fruits of *Hevea brasiliensis* were collected at the yellow-brown pericarp stage when the seeds are fully mature (Premakumari, 1975) and the seeds were collected by mechanically breaking the pods. Seeds of a single clone, RRII 105, were used for this study. Immediately after collection the seeds were put for germination in petridishes filled with moist river sand and the moisture level was retained by sprin-

kling water every day. Embryos, along with the cotyledons, were dissected out from five seeds each on the day of collection and two days, five days and seven days after sowing and fixed in formalin acetic alcohol. Paraffin blocks of the fixed materials were prepared. Serial microtome sections were taken at 1= 0 µm thickness and safranin-fast green staining was done following the standard procedures (Johansen, 1940).

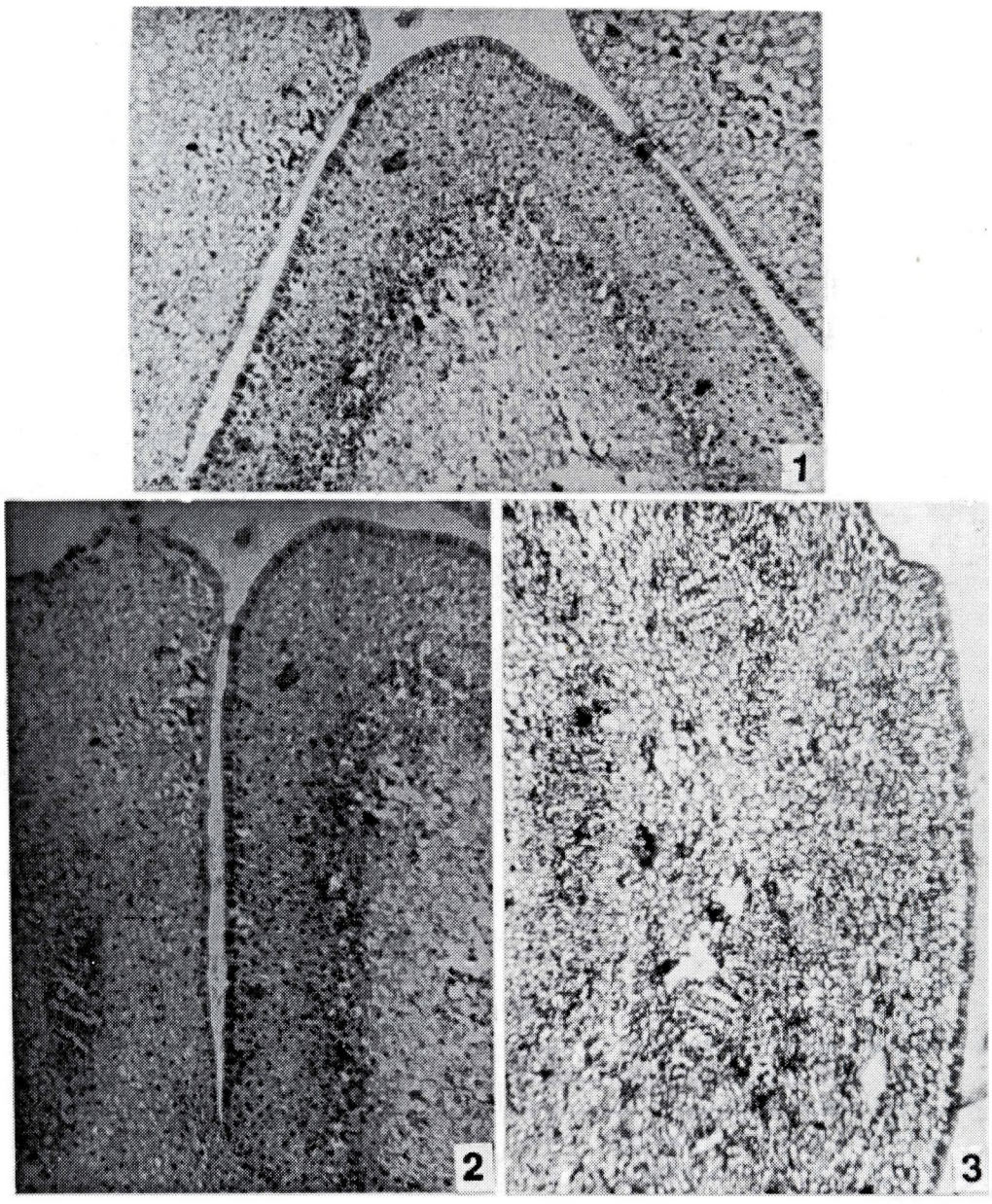
RESULTS AND DISCUSSION

Longitudinal sections through the axis of the embryo along with the cotyledons in the mature seed, before imbibition of water, reveal the structure of a mature embryo. The shoot pole of the embryo (Figure 1) is represented by a mass of meristem which is dome-shaped and has four differentiating regions. The outermost is a one layered protoderm which later undergoes anticlinal division to produce the primary dermal tissue . Panikkar (1974) observed a two-layered tunica for the embryo of *Hevea* seedlings while Gomez (1982) is in agreement with the present observation. The hypodermal region consists of ten rows of cells. These are nearly rectangular in shape, thin walled and contain bigger nuclei and dense cytoplasm. The cells divide anticlinally and periclinally to produce the cortex of the developing shoot. Below the cortical zone the procambial zone is well differentiated. At this zone the cells are elongated as a result of periclinal division and are oriented parallel to the axis of the embryo. The innermost ground meristem is characterised by larger isodiametric cells with thin walls, dense cytoplasm and starch content. According to Gomez (1982) the promeristem contributes to the protoderm, cortical region and the procambium and the origin of pith is from the ground meristem.

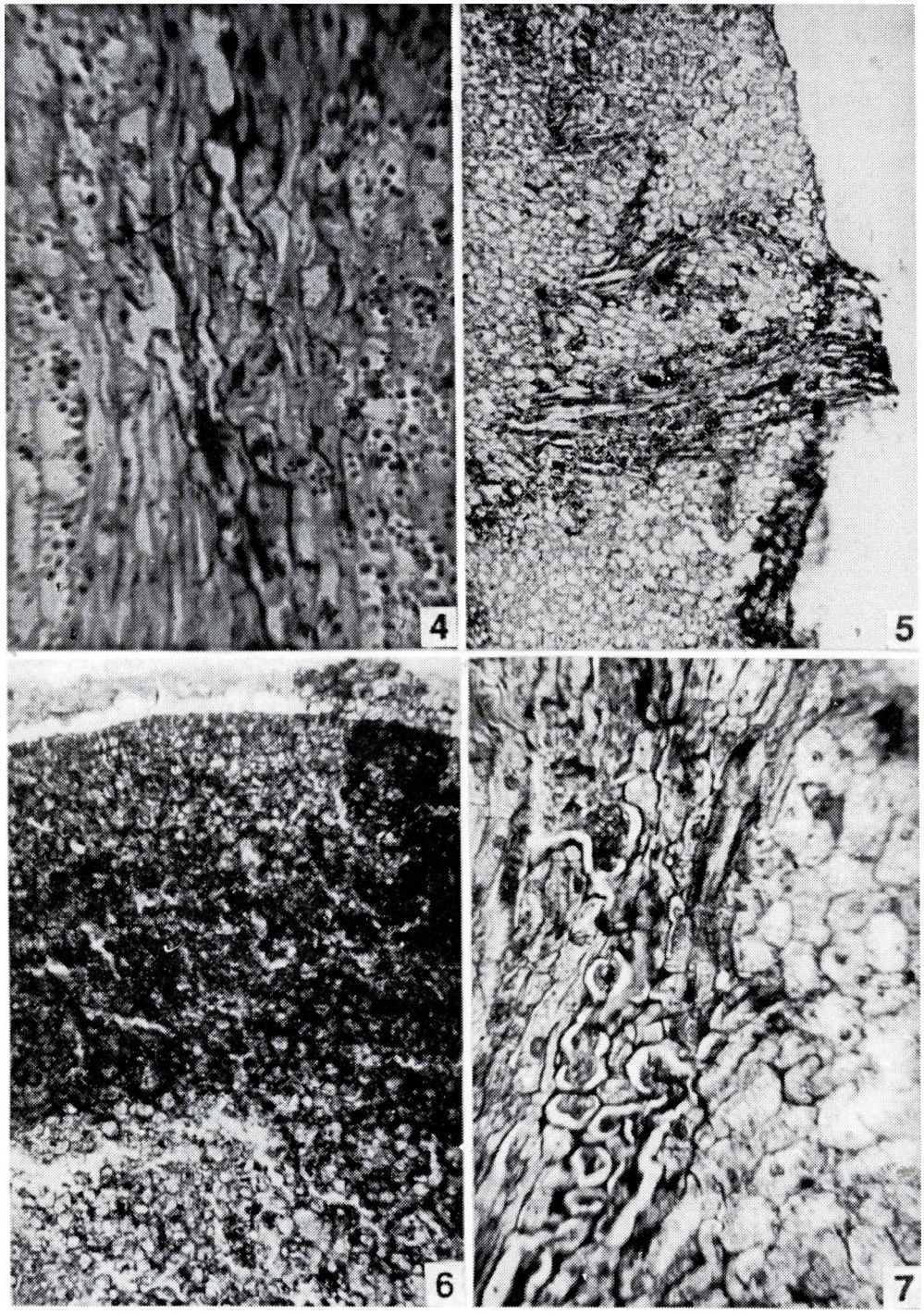
Prolaticifer initials are distributed in all the three zones of the apical meristem and also in the cotyledons. This is in agreement with Calvert (1987) who identified three systems of laticifers in the stem of *Hevea*, namely hypodermal, principal and medullary . The prolaticifers are long, irregular in shape and anastomosing. According to Gomez (1982) no medullary and hypodermal systems were observed in the modern definitions of these regions and the principal system, as observed in the procambial region, belongs to the phloem proper.

At the root pole of the embryo differentiation of the zones is not pronounced. The root pole end is blunt and no active meristem for the primary root formation is present at the embryonic stage while lateral root meristems are present. Presence of prolaticifers was not observed at the root pole region at this stage. There is no dormancy period for *Hevea* seed. Even before fruit dehiscence, on availability of sufficient moisture after maturity, the seeds may germinate inside the pod. On water imbibition, the cotyledonary cells are activated. The first developmental changes of the germinating seed are initiated in the axis of the embryo at the junction of the cotyledons and the axis. In the longitudinal section of the embryo with the cotyledons, two days after sowing (Figure 2) the apical meristem is more elongated and activated, especially at the junction of the axis and the cotyledons. Vascular bundles are present in the cotyledons and the procambial region of the shoot pole is active. Growth proceeds to the two poles. Rapid growth and elongation of the hypocotyl region takes place as a consequence of which the tip of the root pole of the embryo pushes the operculum to the side and emerges through the micropyle on the seventh day.

Before the opening of the micropyle, growth changes occur in the different regions. The lateral root primordia resume



Figs. 1. Longitudinal section of the embryo, through the axis with cotyledons in mature seed before imbibition of water. x84; 2. Longitudinal section of the embryo two days after sowing. x84; 3. Cross section of the root pole showing vascular supply to the lateral roots. x84.



Figs. 4. Longitudinal section of the hypocotyl region just below the shoot/root axis. x300; 5. Cross - section of the root pole at root emergence stage, showing longitudinal view of the vascular supply to the lateral root x84. anastomosing laticifers are abundant; 6. Cross section of the root pole below the lateral root divergence at the same stage. x84; 7. Longitudinal view of the vascular supply to the leaf primordium. x300, anastomosing laticifer initials with coenocytic nuclei are viewed.

activity in synchrony with the elongaton of the hypocotyl region. A part of the procambium and cortical region contribute to the formation of the lateral roots. As the root pole tip of the hypocotyl just emerges, lateral root rapidly elongate from about 500 µm above the radicular tip. Vascular differentiation at this part is complete and vascular supply to the lateral roots have also taken place (Figure 3). Immediately after the resumption of the lateral root meristems, the apical shoot meristem initiates leaf primordia. By the time the root pole end emerged, the incipient shoot system had already developed five leaf primordia.

By this time vascular differentiation had also been advanced. The pattern of vascular differentiation is basipetal in the shoot axis as reported earlier (Gomez, 1982) while at the root pole it is irregular. The irregularity of vascular differntiation in germinating seed of *Hevea* is indicated by Gomez (1982) though the pattern has not been described. Five days after sowing elongation growth is more rapid just below the junction of the shoot/root axis (Figure 4) but vascular differentiation has started . Laticifer initials are formed first followed by sieve tube initials while primary xylem has not yet formed. At this stage vascular differentiation is more pronounced at the region where lateral root initials are produced. The vascular cylinder, pith and cortex are differentiated. Vascular supply to the lateral roots is clear in the cross section of the root axis at this region seven days after sowing. Anastomosing laticifer initials are abundant along with the vascular tissue (Figure 5) in the axis. At the same stage vascular differentiation had advanced less below the point of lateral root divergence (Figure 6). Developing primary laticifers were very abundant as seen in the longitudinal section at the proximal part of the leaf primordium of the germinating seed seven days after sowing (Figure 7). These are formed by the fusion of laticifer initials and are thus articulated. Anastomosing takes place at the points of contact. Earlier reports (Bobilioff, 1923; Panikkar, 1974; Gomez, 1982) are in agreement with the present observation while it is contradictory to the report (Zhao Xiqian, I987) that the primary laticifers of *Hevea* are non articulated. However, the occurrence of prolaticifers at the embryonic stage and their development prior to the development of other tissues indicate some probable role of this tissue in growth.

ACKNOWLEDGEMENT

The authors are grateful to Dr. M.R.Sethuraj, Director of Research and Dr. C.K. Saraswathyamma, Deputy Director, Botany Division, Rubber Research Institute of India for providing facility for this study and to Dr . A . O . N . Panikkar, Director of Training, Rubber Board for valuable comments.

REFERENCES

Bobilioff, W. (1923). Anatomy and physiology of *Hevea brasiliensis* . Zurich Institute Orell Fussli.

Calvert, A. (1987). The laticiferous tissue in the stem of *Hevea brasiliensis. Annals of Botany,* 1: 75.

Chin, H.F., Aziz, M., Aug, B.B. and Hamsa, S. (1981). The effect of moisture and temperature on the ultrastructure and viability of seeds of *Hevea brasiliensis. Seed Science and Technology,* 9 : 411-22.

Gomez, J.B. (1982). Anatomy of *Hevea* and its influence on latex production. Malaysian Rubber Research and Development Board, Kuala Lumpur.

Johansen, D . E . (1940). Plant microtechnique . Ed . 1. New York, McGraw Hill.

Panikkar, A.O.N. (1974). *Anatomical studies in Hevea brasiliensis.* Ph.D. Thesis, Birla Institute of Technology and Science, Pilani, India.

Premakumari, D. (1975). Viability of rubber seeds from green pods. *Rubber Board Bulletin,* 12(1): 24-25.

Zhao Xiuqian (1987). The significance of the structure of laticifer with relation to the exudation of latex in *Hevea brasiliensis. Journal of Natural Rubber Research,* 2 : 94-98.