

Morphological and histochemical changes in the egg and zygote of *Lagerstroemia speciosa*. I. Cell size, vacuole and insoluble polysaccharides*

P RAGHAVAN and V J PHILIP**

Department of Botany, University of Calicut 673 635, Kerala, India

** P.O. Box 43844, Nairobi, Kenya

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Abstract. In *Lagerstroemia speciosa*, the decrease in size of the egg and its micropylar vacuole immediately after fertilization is followed by a progressive and marked expansion of the cell. The PAS-positive cell wall material in egg is confined to the micropylar half. Soon after fertilization, but before completion of decrease in size of the zygote, its cell wall grows in thickness. A complete wall is not formed around the zygote. The bulk of the insoluble polysaccharides in the cytoplasm is localized at the chalazal pole of the egg and zygote. Following fertilization, the size and number of starch granules in the egg cytoplasm significantly increased followed by a decrease and again an increase during zygote development. The morphological changes in the egg following fertilization are probably related to the osmolarity of the cell and of the vacuole which would account for the change in cell size.

Keywords. *Lagerstroemia speciosa* ; variation in vacuolar size ; cell wall ; insoluble polysaccharides.

1. Introduction

The zygote is the fundamental structural and functional unit which constitutes a new beginning and affords opportunities for investigating growth, development, differentiation, assumption of form and functional activities. Ultrastructural changes have been described in the fertilized egg of a few plants such as cotton (Jensen 1968), *Capsella bursa-pastoris* (Schulz and Jensen 1968), *Zea mays* (Diboll 1968), *Petunia hybrida* (van Went 1970b), flax (D'Alascio-Deschamps 1972), barley (Norstog 1972) and *Quercus gambelii* (Mogensen 1972 ; Singh and Mogensen 1975). But little is known about the metabolic changes accompanying the formation of the zygote. The present study deals with the structural and certain histochemical changes that result on fertilization of the egg of *Lagerstroemia speciosa* and its subsequent development up to the first division of the zygote.

This paper is dedicated to late Professor B. G. L. Swamy.

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2. Material and methods

Ovules and seeds of *Lagerstroemia speciosa* (Linn.) Pers. at different stages in development were collected at weekly interval from areas in and around Calicut University campus, fixed instantaneously in the field in formalin-acetic-alcohol or Carnoy's fluid. Conventional method of dehydration through tertiary butyl alcohol series was employed and serial microtome sections (10–15 μm) were prepared. Insoluble polysaccharides were demonstrated by PAS-reaction (Jensen 1962). Tissue oxidation was carried out in 0.5% periodic acid in distilled water for 20–30 min. Response to the stain was the same by sections fixed by either fixatives. Treatment of tissues fixed in Carnoy's fluid with 1% aqueous mercuric chloride solution gave a negative reaction with Schiff's reagent. Confirmatory test carried out with potassium iodide–iodine stain indicates that the materials stained with PAS is constituted of starch grains and that these occur in plastids. Cellular, nuclear and vacuolar areas were calculated from camera lucida drawings of the stained preparations.

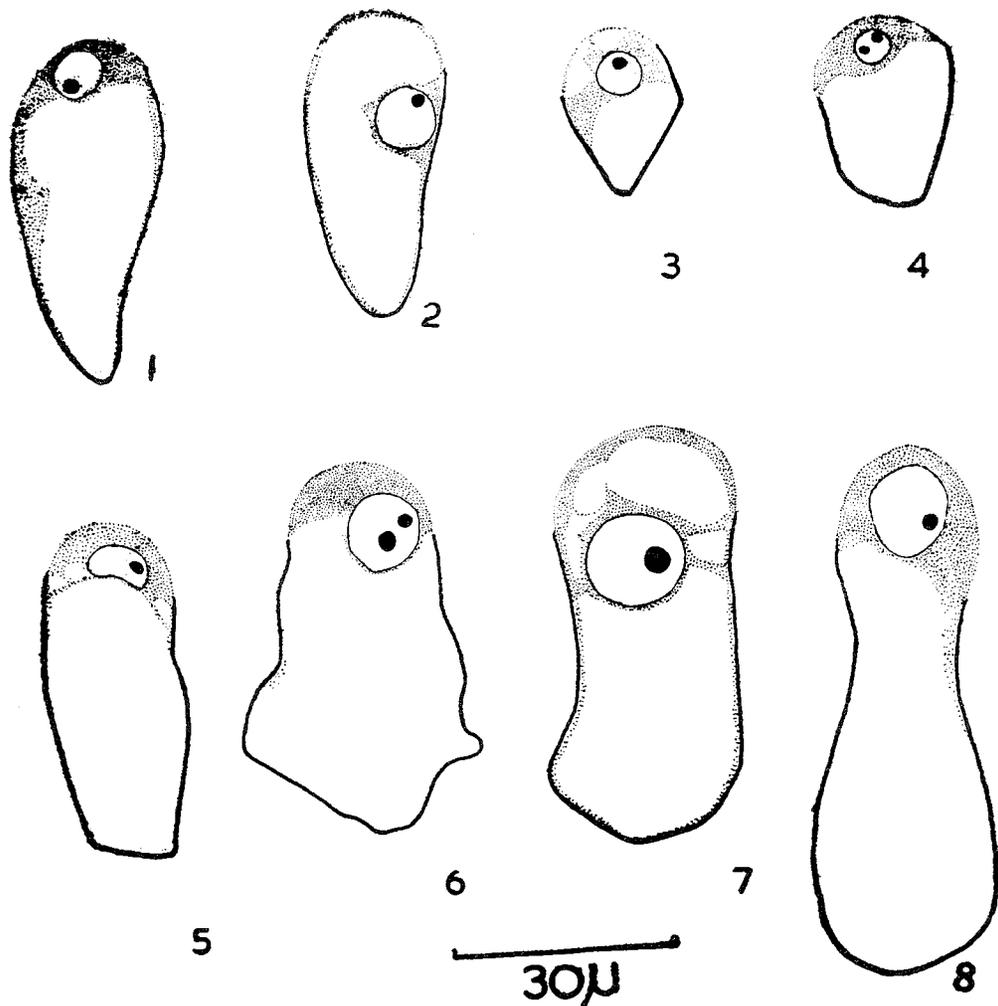
3. Results and discussion

3.1. Change in size of the cell and its vacuole

The pear-shaped egg cell with its broad chalazal and tapering micropylar poles ranges from 457 μm to 632 μm in length. It contains a thin layer of cytoplasm surrounding a large vacuole which reaches almost the cell wall at the micropylar pole. The cytoplasm is concentrated in the chalazal half. The nucleus is confined to either the chalazal pole (figure 1) or to a lateral position in the chalazal half (figure 2).

Immediately after fertilization the cell shrinks to about 240 μm and the vacuole also decreases (figure 3). This decrease in size is followed by a progressive and marked expansion of the cell (figures 4–8), so that the maximal size attained is about five-fold the initial size of the [zygote and about two and a half-fold the size of egg cell (figure 8). Furthermore, a progressive flattening of the micropylar region of the zygote occurs. In few preparations the nucleus was seen displaced towards the centre of the cell due to the appearance of two smaller vacuoles at the chalazal pole (figure 3). Subsequently these two apical vacuoles expanded disproportionately to the remainder of the cell and displaced the nucleus to a more central position (figure 7). In 75% of the zygotes examined, only the micropylar vacuole was present which enlarged retaining the nucleus at the chalazal pole itself.

The observed size changes in the egg cell and zygote of *Lagerstroemia* cannot be accounted for merely by biological variation among the preparations examined. In cotton also a prominent shrinkage of the zygote occurs (Jensen 1964, 1968), but not in *Capsella* (Schulz and Jensen 1968) and barley (Norstog 1972). Cell enlargement and cell elongation are considered as general features of the zygote preparing for division. Assuming that cell shrinkage is due to loss of water, the process is reversible because water from the exterior can be abstracted by the maturing zygote. The underlying osmotic changes in the zygote and the



Figures 1-8. *Lagerstroemia speciosa*—changes in size of egg, zygote and their vacuole. All are longisections; micropylar pole towards bottom of page. 1. Egg cell; the major part of cytoplasm is confined to chalazal pole. 2. Egg cell showing lateral disposition of nucleus and cytoplasm at chalazal half. 3. Zygote; note decrease in cell volume, and formation of two smaller vacuoles at the chalazal pole. 4-8. Zygote at later stages in development. Note the progressive increase in size and the flattening of micropylar region.

surrounding milieu result from metabolic changes at these two sites. One of these changes is the reversible soluble carbohydrate/polysaccharide transformation in zygote to be discussed in § 3.3.

3.2. Cell wall

Both in the egg and in the zygote the extent of cell wall present is variable. Electron microscopic studies have shown that in mature eggs of cotton (Jensen

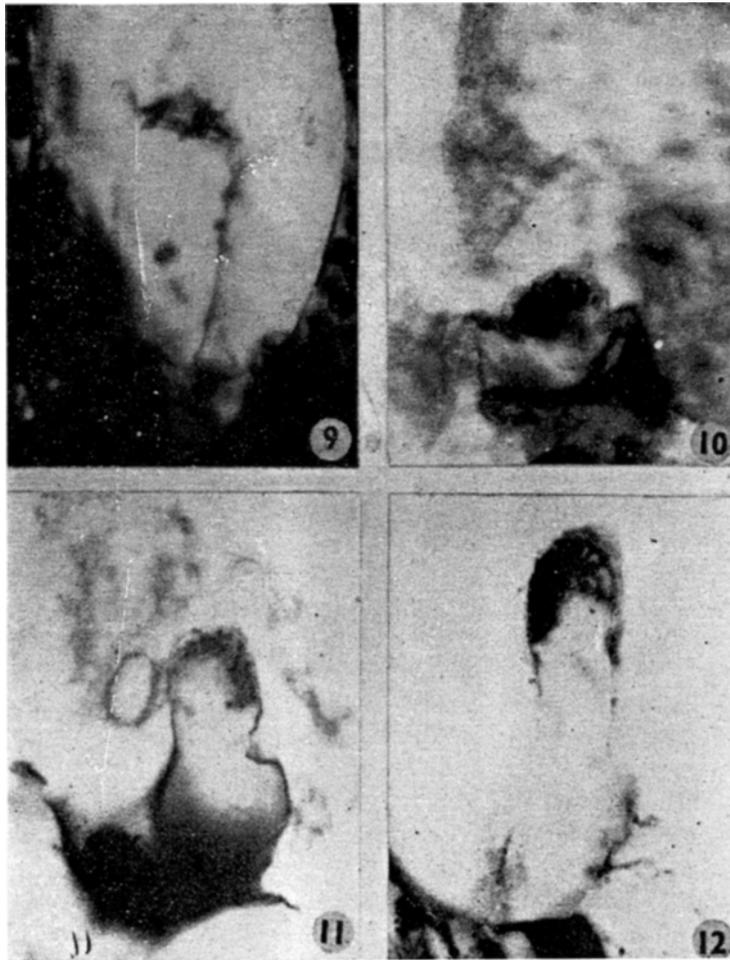
1964, 1965), *Torenia fournieri* (van der Pluijm 1964), maize (Diboll and Larson 1966) and *Petunia hybrida* (van Went 1970a, b) the cell wall extends to only half way up the micropylar pole. However, in the egg cell of *Capsella bursa-pastoris* the wall extends almost over the entire cell; at the chalazal pole the structure is honey-combed with large gaps (Schulz and Jensen 1968). Thus, in all the species investigated the egg cell shows regions of the plasma membrane at the chalazal pole in direct contact with the embryosac, a feature which possibly enables the egg to derive nutrition directly from the central cell. In general, in angiosperms the micropylar region of the zygote is anchored to the wall of the embryosac, and during development wall formation extends over the open chalazal region and envelops the zygote all round. In *Capsella bursa-pastoris* simultaneously with the deposition of the wall material in the gaps in the chalazal region, the wall in the micropylar portion of the zygote becomes thickened (Schulz and Jensen 1968). In barley the wall of the zygote is thicker at the micropylar region than elsewhere (Norstog 1972).

In *Lagerstroemia speciosa* a positive periodic acid-Schiff reaction was obtained both in the cell periphery and in the cytoplasm of the developing embryo. But in the egg the reaction was confined to the micropylar half, resembling in this respect, cotton, maize, *Torenia* and *Petunia*. But, unlike in cotton, in *Lagerstroemia* a complete cell wall is not formed around the young zygote. In the egg the reaction product is visible as a fine film extending to 60% of the perimeter of the cell from the micropylar pole (figure 9). Soon after fertilization, but before completion of decrease in size of the zygote, the cell wall grows further in length covering 70% of the cell perimeter measuring $56\ \mu\text{m}$ (figure 10). Subsequently, however, the addition of wall material does not appreciably increase the thickness, and the percentage of wall material to the perimeter of zygote remains unchanged (figure 11). In a nearly mature zygote the wall extends to $112\ \mu\text{m}$ from the micropylar pole covering 80% of the cell perimeter (figure 12), and its thickness is slightly less than that in the two earlier stages but more than that in the egg.

Street and Öpik (1970) have pointed out that during cell expansion (elongation), the cell wall does not thin out, but there occurs a proportionate increase in cell wall synthesis. It is not clear how a cell would respond to a decrease in cell size. Because in both the egg and the zygote of *Lagerstroemia speciosa* the cell wall is present only at the micropylar half, a decrease in size of the young zygote will not presumably impose as much strain as it would have been if the zygote had an entire cell wall.

3.3. Insoluble polysaccharides (starch granules)

Histochemical staining with periodic acid-Schiff reagent showed, besides cell wall, numerous granules of insoluble polysaccharides in the cytoplasm of both egg and zygote, especially localized in their chalazal pole. In all stages of development beginning from egg, the starch granules are heterogeneous in size and varied in number. The egg cell contains about 50 tiny starch granules (figure 9). Following fertilization, there is a significant increase in their size and number. In the young zygote, which showed a decrease in size, the number of starch granules increased to 90-100 (figure 10). As the zygote enlarged, but before attaining



Figures 9-12. *Lagerstroemia speciosa*—median longisections of egg and zygote after treatment with periodic acid-Schiff reagent. 9. Egg with PAS-positive wall covering 60% of its perimeter from the micropylar pole. Note the size and number of starch granules at the chalazal pole. 10. Zygote as seen immediately after formation; note further growth of wall in thickness, decrease in cell size, and number of starch granules. 11. Zygote at a later stage, showing decrease in size and number of starch granules but with no appreciable change in wall material deposition. 12. Nearly mature zygote with the wall covering 80% of the cell perimeter. $\times 850$.

maturity, the number of granules decreased to about 60 ; their size also decreased (figure 11). In the nearly mature zygote, a second increase in number to about 100 granules can be noticed (figure 12), accompanied by an increase in size.

In cotton the egg cell contains one or two small starch granules per plastid and there is no specific association of the plastids with other organelles in the cell. Following fertilization, when cell size is decreasing, the plastids accumulate along with mitochondria, around the nucleus (Jensen 1968). At this stage, starch begins to accumulate in the plastids. The close association of the nucleus, plastids and ribosomes presumably facilitates the ready elaboration of the biosynthetic system (s) and the transfer of the enzyme concerned to the granule or the cytoplasm, as the case may be. During maturation of the zygote, when additional wall material is being formed so as to complete the wall around the entire cell, the number of plastids remains unaltered, but their size increases. Also, the number of starch grains per plastid increases so that the plastids become filled with starch.

The occurrence of starch grains in the egg cell of *Lagerstroemia speciosa*, the increase in their number soon after fertilization and their presence in large amounts in the mature zygote, are in accord with the findings in cotton (Jensen 1968), but the decrease in number and size of starch granules which occurs in the intermediate stage is distinctive of *Lagerstroemia*. The increase in number and/or size of the starch granules following fertilization is accompanied by the decrease in the vacuolar volume. Similarly, a decrease in the number and size of starch granules is sometimes associated with increased vacuolar size, as when the early zygote reaches the intermediate stage of development. Such a formation of starch granules at the expense of soluble sugars stored in the vacuole would reduce the osmotic pressure and result in a diminution of cell and vacuolar size. This would account for the morphological changes, namely, a marked decrease in size, on fertilization of the egg. The reverse process, namely starch degradation and transfer of soluble sugars to the vacuoles, would increase the osmolarity of the cell and vacuole. This would account for the increase in size of the early zygote and its vacuole. Contrary to expectation, the second increase in number and size of starch granules, which occurs in the mature zygote is associated with an actual increase both in cell and vacuolar size.

A significant point is that cell wall synthesis in the zygote occurs at the micropylar half, even though the protoplasm is concentrated at the chalazal half. Evidently, the polarity of the cell does not extend to the metabolic activity of the cell relative to wall synthesis.

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