

Relationship between development of endosperm transfer cells and grain mass in maize

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Abstract

The most basal endosperm cells of maize (*Zea mays* L.) began differentiating into transfer cells in 10 days after pollination (DAP). The thickening and ingrowths forming in the transfer cell wall were slow during 10 and 15 DAP. There were many vesicles, silky and string ball objects in cytoplasm, and the number of mitochondria and rough endoplasmic reticulum increased. After 15 DAP, the wall thickening and ingrowths forming in the transfer cells sped up. By 20 DAP, the transfer cell zone had developed, there appeared 65 - 70 rows of cells in width and 3 - 4 layers of cell in depth, the obvious cell wall ingrowths presented strong positive reaction with periodic acid Schiff's reagent. After 20 DAP, no significant change appeared in the shape and structure of the transfer cells, and the transfer cells entered function stage. In the mature kernels (53 DAP), the most basal transfer cells were filled with ingrowths, however, dense cytoplasm was also found in these cells. The nuclei had quite irregular shapes in these cells. Some transfer cells contained black grains and crystals. A black layer formed in the pericarp tissue adjacent to the transfer cell zone. Full development of endosperm transfer cells was important for reduction of kernel abortion and increase of kernel mass.

Additional key words: cytoplasm, endoplasmic reticulum, ingrowth, mitochondria, nuclei, vesicles, *Zea mays*.

Introduction

Transfer cells in plant seeds have been described (*e.g.* Hu *et al.* 1983, Gao *et al.* 1992, Wang *et al.* 1992). Through endosperm transfer cell zone in maize the mother nutrition enter into endosperm and embryo. Its development and length of function stage are important for development of embryo and nutrient accumulation in seeds. The form of endosperm transfer cells at 23 DAP in maize has been described by

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Davis (1990), but there has been no report concerning their development. This report deals with the relationship between development of endosperm transfer cells and grain mass.

Materials and methods

Plants of hybrid maize (*Zea mays* L.) cv. Ye Dan No. 13 were grown in University field. Female spike segments with middle site cut off were obtained 5, 10, 15, 20, 25, 30, 35, 45, 53 DAP, some of them were fixed in acetic acid:ethanol (1:3), dehydrated, and embedded in paraffin as usual. The others were fixed firstly in 2.5 % glutaraldehyde and then in 2 % osmium tetroxide, rinsed in the same buffer and dehydrated in an ethanol series, and embedded in *Epoxide resin 812*. Serial sections 2.5 μm thick were cut using glass knives and were stained with periodic acid-Schiff's reagent (PAS) and counterstained with toluidine blue O (TBO). Ultrathin sections were cut using *Nova-V* ultramicrotome (LKB, Bromma, Sweden) and poststained with uranyl acetate and lead citrate and observed in a microscope *JEM-1200EX* (JEOL Ltd., Tokyo, Japan).

Results and discussion

A lot of endosperm cells formed at 5 DAP. At 10 DAP, the cytoplasm of endosperm cells with large nuclei and many recognizable organelles was quite dense. Both outer and radial wall of the most basal cell layer began to thicken, but no wall ingrowths were observed. On the other hand both cytoplasm and walls had getting more and more thinner from base to inner layers of endosperm (Fig. 2). At the 20 DAP, there were about 3 - 4 layers of endosperm transfer cells which extended in depth. The wall ingrowths containing numerous PAS-positive materials began to form and became more and more short from base to inner layers (Fig. 3). Endosperm transfer cells distributed as a zone inside pericarp, and the wall ingrowths at the edge of zone just adjacent to aleurone were shorter (Fig. 1). The transfer cell zone contains about 65 - 70 rows of cells from the close to the distant side of embryo in the longitudinal direction. There were some black material accumulating in ovary tissue adjacent to the transfer cell zone, and this tissue dried to form the black layer of kernel when the seeds matured.

At 10 DAP, the walls adjacent to placenta and the base of radial walls began thickening and a few wall ingrowths occurred in the most basal cell layer of endosperm, but their radial walls in the top, inner walls and other endosperm cell walls above basal layer were still thin. There were obvious wavy curve in the middle and top radial walls (Figs. 4, 9). In the 3 - 4 layers of cell bordering pericarp, the nuclei with clear nucleolus and nuclear envelope appeared prominent and regular. Numerous plastids congregated around the nuclei. There were abundant mitochondria, rough endoplasmic reticulum (RER), ribosome and vacuoles of various size in cytoplasm. Some vacuoles contained wadding black material (Figs. 4, 9). But there

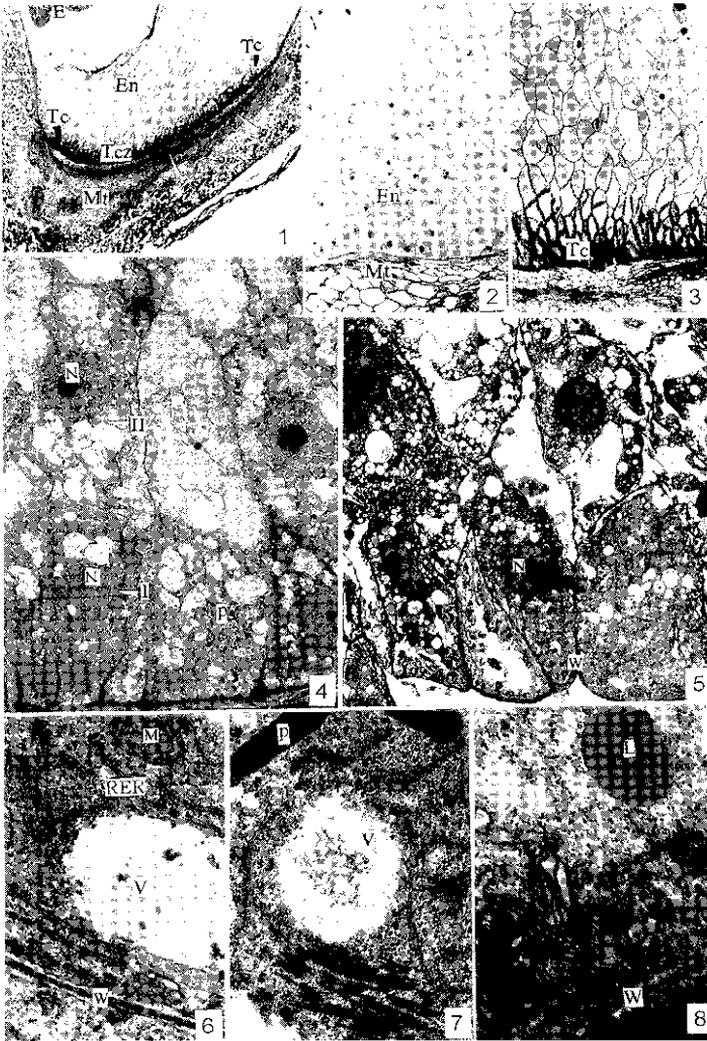


Fig. 1. The longitudinal section of kernel at 25 DAP showing shape and location of transfer cell zone ($\times 100$).

Fig. 2. The most basal endosperm cells at 10 DAP; cells began just differentiating ($\times 250$).

Fig. 3. The transfer cells had developed at 20 DAP ($\times 200$).

Fig. 4. The I and II cell layers of endosperm basal part at 10 DAP ($\times 100$).

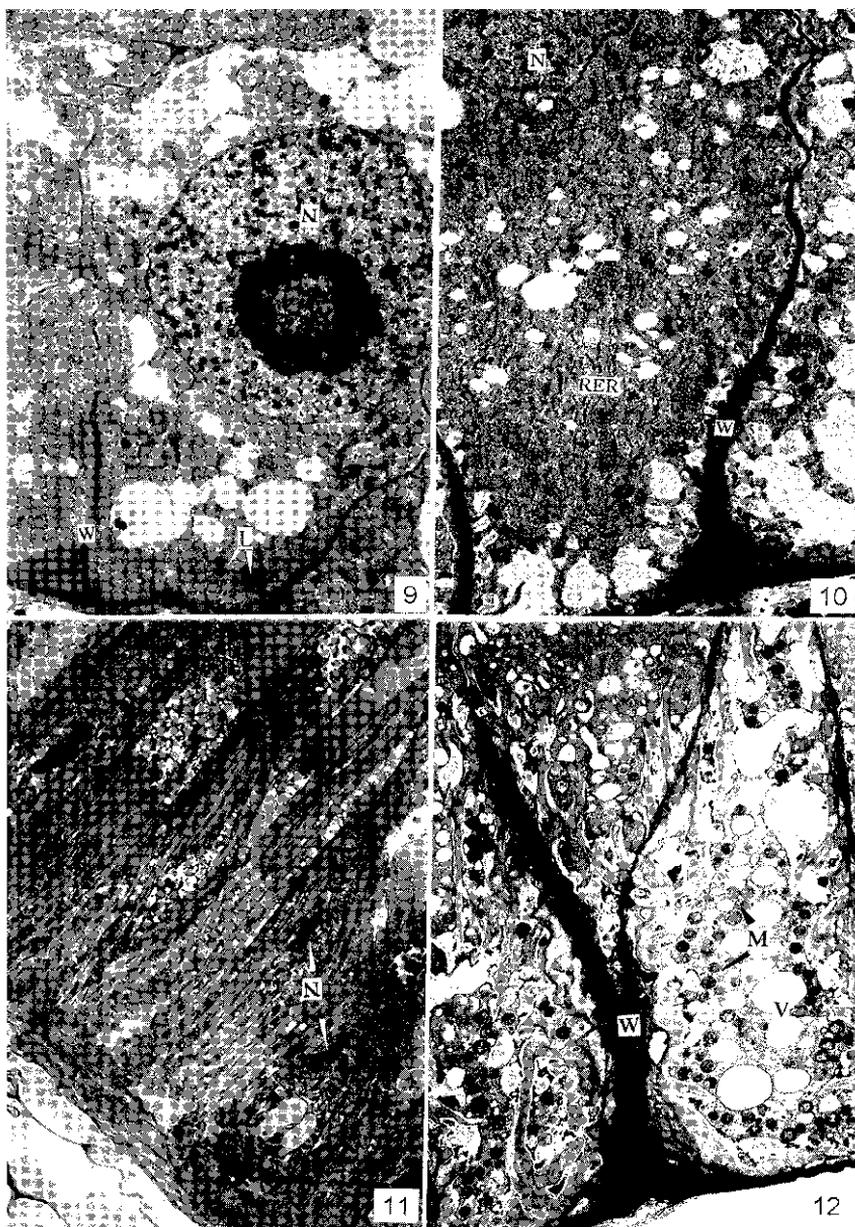
Fig. 5. The endosperm basal cells, showing differences among cells and nuclei at 15 DAP ($\times 1\ 000$).

Fig. 6. Many vesicle with membrane in endosperm basal cells at 15 DAP ($\times 12\ 000$).

Fig. 7. Microfibrils in cytoplasm ($\times 15\ 000$).

Fig. 8. String shape objects on cell wall ($\times 20\ 000$).

(Bl - black layer, E - embryo, En - endosperm, L - lipid body, M - mitochondrion, Mt - maternal tissue, N - nucleus, P - plastid, RER - rough endoplasmic reticulum, Tc - transfer cell, Tcz - transfer cell zone, V - vacuole, W - cell wall).



Figs. 9 - 12. The morphological character and structure of the most basal endosperm transfer cells.

Fig. 9. 10 DAP, nucleus, small lipid bodies and cell walls ($\times 4\ 000$).

Fig. 10. 15 DAP ($\times 5\ 000$).

Fig. 11. 20 DAP ($\times 12\ 000$).

Fig. 12. 20 DAP, cell wall ingrowths, vacuoles and mitochondria in cytoplasm ($\times 3\ 000$).

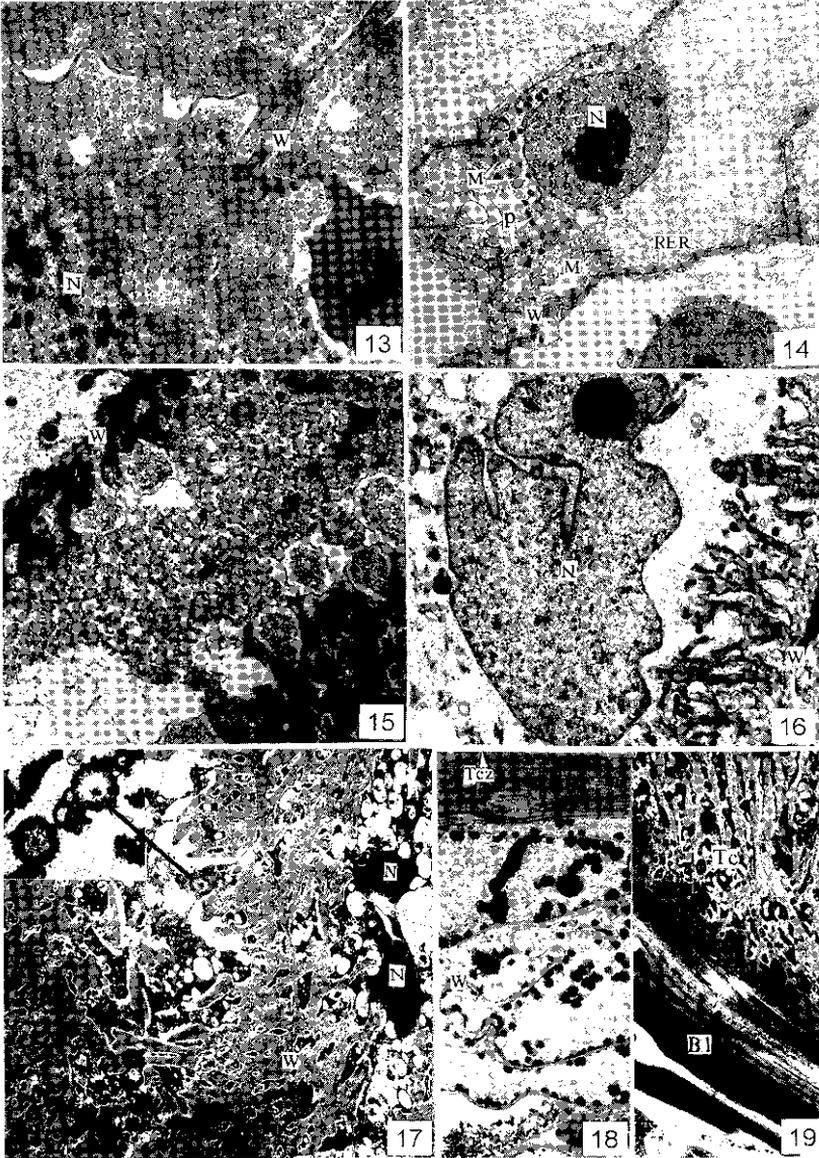


Fig. 13. The most basal transfer cell, showing projected tumor on cell wall ($\times 8\ 000$).

Fig. 14. Development of the third and fourth transfer cells; thin cell at 10 DAP ($\times 2\ 000$).

Fig. 15. Cell wall ingrowths and vesicles with membrane at their ends at 20 DAP ($\times 3\ 000$).

Fig. 16. Nucleus of changed shape ($\times 3\ 000$).

Fig. 17. The most basal transfer cells in mature kernel; cell wall ingrowths, nucleus and crystals ($\times 3\ 000$); inserted fig. on the left upper showing enlarged crystals ($\times 12\ 000$).

Fig. 18. Maternal tissue under transfer cell zone; folded cell walls and black grains in cells ($\times 2\ 500$).

Fig. 19. Black layer in the basal part of mature kernel ($\times 2\ 000$).

were more RER, small vacuoles (Fig. 1) and a few lipid bodies (Fig. 9) in the most basal layer of cells.

After 10 DAP, the wall ingrowths of endosperm transfer cells developed slowly. Up to 15 DAP, the region of occurring wall ingrowths failed to extend to the top in the most basal layer (Figs. 5, 10), but more changes occurred within these cells. Some of them were dense and contained more mitochondria and RER, but other contained many vacuoles (Figs. 5, 10). The form of nuclei became irregular and some nuclei passed through the wall (Fig. 5). Therefore, the cytoplasmic movement could be considered a way of transferring materials among cells. Plenty of coated vesicles (Fig. 6), microfibrils (Fig. 7) and objects of string ball shape (Fig. 8) occurred in the cytoplasm near the wall. At some position the wall produced a tumour-like ingrowths (Fig. 13). These structures might be precursors of wall ingrowths. After 15 DAP, both thickening and ingrowths forming in transfer cell walls undergo rapidly. The transfer cell zone that spread 3 - 4 layers in radial direction formed roughly at 20 DAP. In longitudinal section, wall ingrowths were longest in the most basal layer and more and more short from the base to top. The nuclei became long, the nuclear envelop getting clearless (Fig. 11), while the cytoplasm were still dense and numerous mitochondria and black objects could be observed (Fig. 12).

In the third and fourth (from the base) layers of endosperm, both development and structure of wall ingrowths were different from the first and the second layers. Before the wall ingrowths formed, their walls were very thin, the cytoplasm was also relatively thin with little ribosomes but abundant mitochondria and RER on two sides of the wall (Fig. 14). As development proceeds, wall ingrowths often extended towards a cell in which this extensions appeared branched and numerous coated vesicles were connected to the end of them (Fig. 15). In these cells, the cytoplasm was slightly dense with much black material and abundant mitochondria that were located in the open space among wall ingrowths. The form of the nuclei appeared various (Figs. 15, 16). These wall ingrowths were not very long but appeared in good order. In addition, in both the 5th and 6th layers, wall ingrowths occurred only in few cells.

After 20 DAP, the transfer cells had no evident change in form except the walls continued to thicken slowly and ingrowths had slight extensions. In the mature caryopsis of maize, the transfer cells at the most basal layer were almost completely filled with wall ingrowths. The cytoplasm with numerous vacuoles and crystal matter was dense (Fig. 17) and some black material was observed in both cytoplasm and vacuoles. The nuclei changed into irregular shape (Fig. 17). Under the transfer cell zone there were a few cell layers of ovule as the development of caryopsis, their cytoplasm were disappeared and the walls were thick and tortuous. Plenty of black grains accumulated within the cells (Fig. 18) forming a black layer when the caryopsis was matured (Fig. 19).

After 20 DAP, the development of transfer cells finished and they were fully functioning. The rapid development of endosperm transfer cells in maize finished at the early stage of grain development, when the grains just had begun to milking. The well development wall ingrowths of transfer cells enlarged the transport channel of symplasm and reduced the resistance to transport (Lou 1980) to embryo and

endosperm from the mother bodies. Kernel volume and mass increased rapidly (Table 1). During 20 - 25 DAP, the transfer cells function was best, and the grain mass increased in the most speed. By the mature stage of kernel, the cytoplasm of endosperm starch cells was disappeared, but the transfer cells were still alive and functioning, this pattern perhaps was one of reasons that the kernel mass still increased.

Table 1. Grain development in maize. The volume of grain was measured according to Hinage drainage method (Qi 1980).

DAP	Volume [mm ³ grain ⁻¹]	Volume increase [mm ³ grain ⁻¹ d ⁻¹]	Dry mass [mg grain ⁻¹]	Mass increase [mg grain ⁻¹ d ⁻¹]
5	42.3		3.67	
10	84.9	8.5	7.29	0.72
15	168.9	16.8	42.37	7.02
20	237.8	13.8	94.06	10.34
25	291.1	10.7	145.41	10.26
35	378.2	8.7	254.72	10.93
45	435.0	5.7	342.00	8.73
53	447.3	1.6	364.64	2.83

The form and function of endosperm transfer cells have been reported in a number of plants. But only in maize (Davis *et al.* 1990) and *Coix lacryma-jobi* (Gao *et al.* 1994) such numerous well developed wall ingrowths could be observed. Previous studies on *Vigna senensis* (Hu *et al.* 1983) revealed that the number of endosperm transfer cell and ingrowths were less and their function stage were also shorter than in maize. In maize, their function stage lasted 30 d, and they accumulated unsolvable compounds from beginning to end. The dry mass of grain still increased during the late stage of maize grain development (Table 1, also Gao *et al.* 1992), even in cut ear the grain mass can increase. So it is very important to promote well development of the endosperm transfer cells and delay formation of black layer which could reduce grain abortion and promote the formation of big and plump grains.

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