

Ultrastructure of the Suspensor Cells in the Natural Tetraploid *Trifolium pratense* L. (Fabaceae)

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Abstract : This paper reports observations of the development and cytology of this embryonic organ in the natural tetraploid *Trifolium pratense* L. during embryogenesis. In the natural tetraploid *Trifolium pratense* (var. Elçi) the division of the zygote occurs in the transverse or oblique direction, resulting in the formation of two unequal cells. The basal cell is a larger than the terminal cell. The smaller terminal cell divides further and give rise to the embryo proper. The larger basal cell develops into vacuolated suspensor. In proembryo stage, the terminal cell nucleus becomes elongated, and occupies a large part of the cell. The basal cell nucleus which contains several electron-dense regions. The growth rate of the suspensor cells is very rapid during earlier stages of embryogenesis. In the early globular embryo stage, the suspensor consists of four or six cells and contains mitochondria, ER, ribosomes, lipid droplets, small protein bodies and vacuoles. In the late globular embryo stage, the suspensor cell expanded like massive structure. During embryogenesis, the suspensor cells are usually filled with small vacuoles are less electron-dense, and fewer ribosomes than the adjoining embryo cells. The suspensor undergoes at the cotyledone stage embryo and is not present in the mature seeds.

Key words : *Trifolium pratense*, suspensor cells, embryo development, ultrastructure.

Doğal Tetraploid *Trifolium pratense* L.' de Suspensor Hücrelerinin Ultrastrüktürü

Özet : Bu makalede, doğal tetraploid *Trifolium pratense* L. 'nin embriyogenez sırasında embriyonik organın sitolojisi ve gelişimi üzerine olan gözlemleri kapsar. Doğal tetraploid *Trifolium pratense* (Elçi çayırüçgülü)'de zigotun ilk bölünmesi enine veya eğiktir ve eşit olmayan iki hücre oluşur. Bazal hücre terminal hücreden büyüktür. Daha küçük olan terminal hücre bölünerek embriyoyu oluşturur. Büyük bazal hücre ise vakuollü suspensoru meydana getirir. Proembriyo evresinde terminal hücrenin çekirdeği uzar ve hücrede daha büyük bir alanı işgal eder. Bazal hücre çekirdeğinde elektronca yoğun bölgeler mevcuttur. Bu bitkide suspensor hücreleri embriyogenezin erken evrelerinde oldukça hızlı gelişir. Embriyo gelişiminin erken küresel evresinde 4-8 hücrelidir. Suspensor hücrelerinin sitoplazmasında küçük vakuoller, lipid ve protein cisimcikleri ile ribozom, mitokondri ve ER bulunduğu gözlenmiştir. Embriyo gelişiminin küresel evresinde suspensor hücrelerinin sayısı artar. Embriyogenez süresince suspensor hücreleri küçük vakuollü ve elektronca az yoğundur ve embriyo hücrelerine oranla daha az ribozom içerirler. Embriyo gelişiminin kotiledon şekilli evresinde dejenere olan suspensor hücreleri olgun tohumlarda gözlenmemiştir.

Anahtar kelimeler: *Trifolium pratense*, suspensor hücreleri, embriyo gelişimi, ince yapı.

1. Introduction

The suspensor is an embryonic organ and is essential to embryo development [1]. In most flowering plants, after fertilization the first division of the zygote divides into one terminal and one basal cell. The smaller densely cytoplasmic terminal cell divides

further and give rise to embryo proper. The larger basal cell develops into a highly vacuolated suspensor [2].

Angiosperms suspensors vary widely in size and morphology from a single cell to a massive structure composed of hundreds of cells [3-5]. A few suspensors produce elaborate outgrowths (haustoria) that invade surrounding endosperm and maternal tissues. In most cases, the suspensor functions early in embryogenesis and enters a process of degeneration during later stage of development.

Ultrastructural [2, 6, 1], Cytochemical [7, 6, 8], and biochemical studies [9-13] with a variety of angiosperms have shown the suspensor can serve as a conduit for nutrient flow and may provide unique metabolites for the growth of the embryo proper [4, 12, 13, 2, 1, 14, 15, 16]. Suspensor cells may also be polytene, polyploid, and multinucleate [17, 18]. In most of plants, multiplication of nuclear DNA content and polytenization of chromosomes are often associated with differentiation of the suspensor.

Some of the most unusual suspensors have been identified among the Fabaceae [1]. In *Phaseolus*, the suspensor develops quickly to form a large and wide column, other suspensors in Fabaceae consist of just one or two cells [5].

This paper reports observations of the development and cytology of this embryonic organ in the natural tetraploid *Trifolium pratense* L. during embryogenesis.

2. Materials and Methods

Plants of the natural tetraploid *T. pratense* L. variety E2 which was adapted to be a plant having chromosomes ($2n = 4x = 28$) by counting chromosomes at root tips were grown under natural conditions (Fig. 1). The mentioned plant was collected from 'Tortum' region in Erzurum (Türkiye) by Elçi [19]. For light and electron microscopic observations developing ovules were excised from ovaries under binocular-microscopy at three developmental stages according to ovary length:

Developmental stages of examples	ovary lengths of examples (μm)
Developmental stage 1	between 750 μm and 950 μm
Developmental stages 2	between 950 and 1150 μm
Developmental stage 3	between 1150 and 1350 μm

Approximately 255 ovules were examined. Ovules were fixed 96 % in ethanol: acetic acid (3:1) for 12 h. Dehydration was carried out in an ethanol-xylol series, and the materials were embedded in paraffin. For electron microscopic examinations, ovules were fixed in 3% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2) for 3 h at room temperature. Materials were postfixed in 1% osmium tetroxide for 3 h at room temperature. Then the samples were dehydrated in an ethanol series, transferred to 100 % propylene oxide and embedded in Epon 812 [20]. Ultrathin sections were stained with uranyl acetate and lead citrate. Ultrastructural observations were made with a Jeol CXII transmission electron microscope (TEM) at 80 Kv.

3. Results

In the natural tetraploid *Trifolium pratense* L. three main stages in development of the suspensor can be distinguished;

Stage 1. Differentiation of the basal cell (proembryo- ovary lengths of examples between 750 μm and 950 μm).

Stage 2. Full development and function (late globular embryo proper- ovary lengths of examples between 950 and 1150 μm).

Stage 3. Senescence of the suspensor (late heart embryo proper-ovary lengths of examples between 1150 and 1350 μm).

Stage 1. Two-celled embryo: The first division of the zygote occurs in the transverse or oblique direction, resulting in the formation of two unequal cells (Figure 1A). The basal cell is a larger than the terminal cell (Figure 1B). The terminal cell will contribute to the formation of the embryo proper, and the basal cell will form the suspensor. The terminal cell nucleus becomes elongated, and occupies a large part of the cell. The nucleus has an electron dense nucleolus which contains several small vacuoles. At the micropylar end of the basal suspensor cell, extensive wall ingrowths appeared along the cytoplasmic face of the cell wall. Short strands of rough ER and mitochondria are observed adjacent to these ingrowth. Mitochondria are numerous but they contain relatively few short cristae and electron-translucent areas (Figure 1C). The proembryo cell wall is very thin and has no visible middle lamellae.

Stage 2. Late globular embryo : When the embryo proper reach the early globular stage of development the basal cell undergoes further differentiation, and consists of four or six cells (Figure 2A). Plasmodesmata occur in the common walls between the suspensor cells and embryo cells. There are several electron-dense inclusions in the space between the plasmalemma and the cell wall.

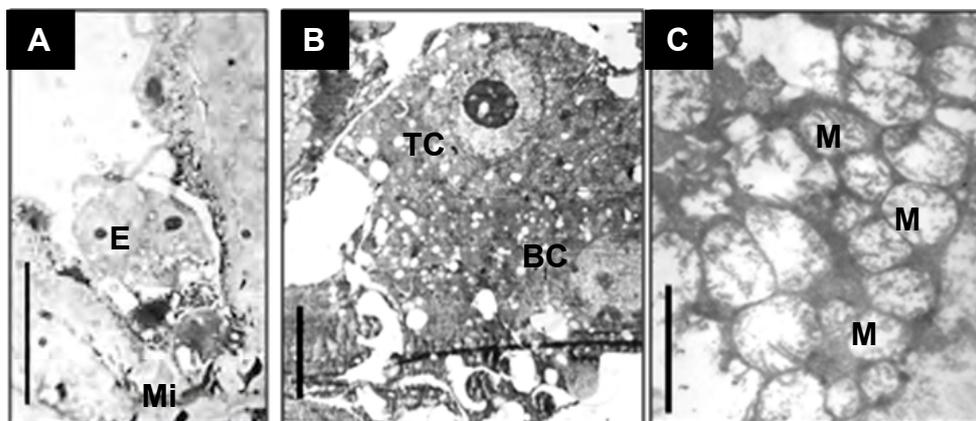


Figure 1. The proembryo stage of *T. pratense* L. **A.** Paraffin section of the ovule showing the two-celled proembryo (*Ep*). Micropyle (*Mi*). Bar = 50 μm . **B.** Electronmicrograph of the proembryo showing terminal (*TC*) and basal (*BC*) cells. Bar = 1 μm . **C.** Electronmicrograph of the basal cell cytoplasm contains numerous mitochondria (*M*). Bar = 10 μm .

The cytoplasm of the suspensor cells contains mitochondria, ER, ribosomes, lipid bodies, small protein bodies, and vacuoles (Figure 2B).

Well-developed wall ingrowths are present along the walls of the suspensor (Figure 2C) at the micropylar end. The cytoplasm contains several organelles. Rough ER is poorly developed and giving a swollen appearance of various degree. Some of the vacuoles contain electron-dense deposits. Mitochondria are numerous and contain few short cristae. A few dictyosomes and small protein bodies are seen in the cytoplasm. Numerous lipid bodies are present and they are large and electron-dense (Figure 2D),

In some ovules the suspensor shows different kinds of shapes, and size during embryogenesis (Figure 3A-C), but the cells are vacuolated and stain less intensely for protein and nucleic acids than adjacent cells of the embryo proper.

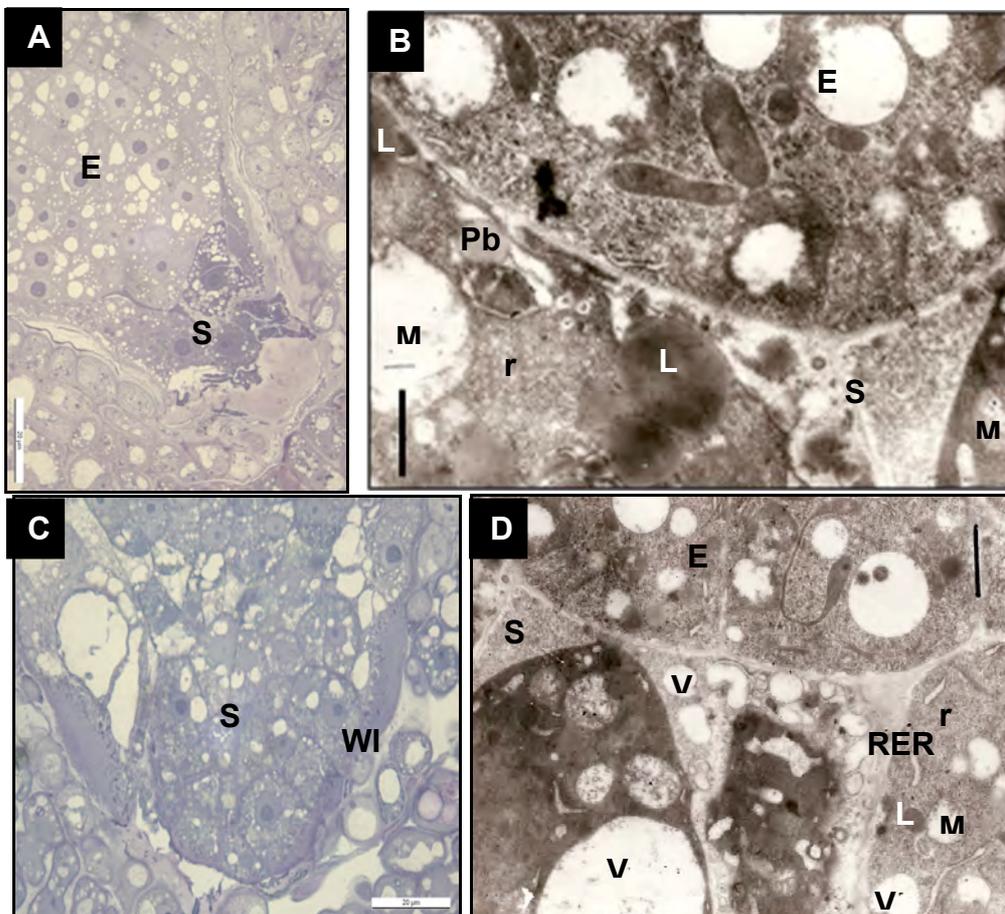


Figure 2. The globular embryo stage of *T. pratense* L. **A.** Light micrograph of an early globular embryo (*Ep*) and suspensor (*S*). Bar = 20 μ m. **B.** Electron micrographs of sections from the suspensor cytoplasm showing several organelles. *M*: mitochondria, *L*: lipid bodies, *r*: ribosomes, *Pb*: protein body. Bar = 20 μ m. **C.** Late globular embryo stage of a semithin section showing the wall ingrowths (*WI*). Bar = 20 μ m. **D.** Electronmicrograph of the late globular embryo stage showing the cytoplasm of the suspensor cells. The cytoplasm contains numerous small or large vacuoles (*v*), mitochondria (*M*), rough ER (*RER*), lipid (*L*), and ribosomes (*r*). Bar = 3 μ m.

Late globular embryo stage, the suspensor cells continued to enlarge and most small vacuoles appeared within the cell, and well-developed wall ingrowths were present along the walls of the embryo sac near the integumentary tapetum (Figure 4A-B). Before the cotyledons are initiated, a cuticle forms over the surface of the globular embryo, but this cuticle is absent around the suspensor. In this stage the endosperm develops rapidly and embryo cells have dense cytoplasm, abundant organelles [21].

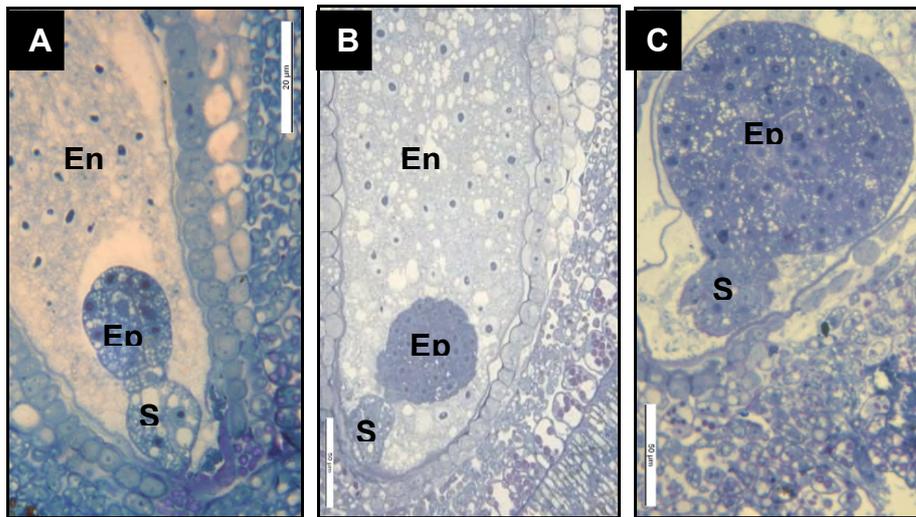


Figure 3. Light micrographs of semithin sections showing different shapes and size the suspensor. **A.** Early globular embryo stage. Bar = 20 µm. **B.** Mid globular embryo stage. Bar = 50 µm. and **C.** Late globular embryo stage. Bar = 50 µm. *Ep*; embryo proper, *En*; endosperm, *S*; suspensor.

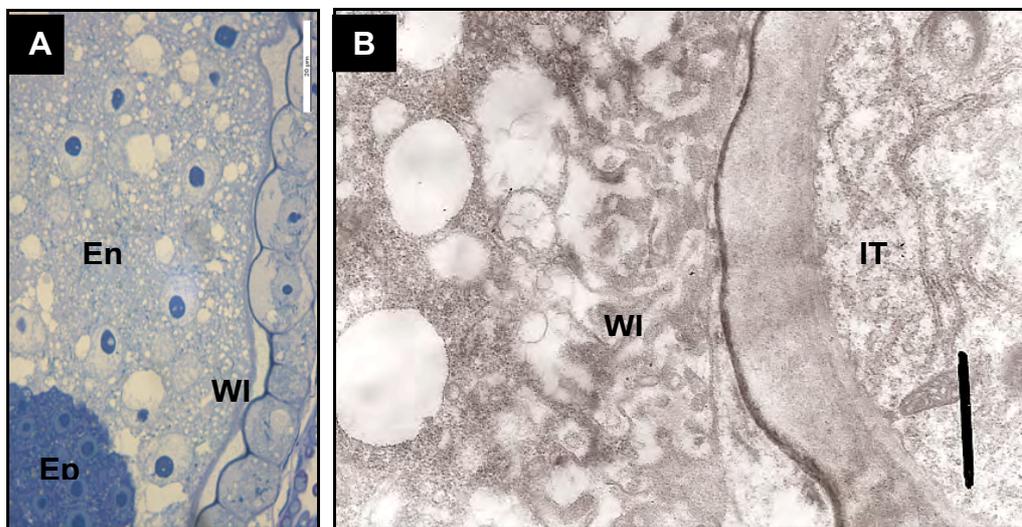


Figure 4A. Light micrograph of a semithin section showing the late globular embryo stage (*Ep*). *En*: endosperm. Bar = 20 µm. **B.** Electronmicrograph enlargement of the cytoplasm showing well-developed wall ingrowths (*WI*) are present along the walls of the embryo sac near the integumentary tapetum (*IT*) cells. Bar = 2 µm.

Stage 3. Late heart embryo : At this stage, the suspensor cells are highly vacuolate and protruded beyond the inner integument. It continued to grow toward the micropyle, but it never grew beyond the outer integument of the seed coat. During the heart-stage of embryogenesis the suspensor becomes large, and less electron dense (Figure 5A-B). Vesicle-like structures appear in the nuclei of suspensor cells. The organelles were not clearly defined. The suspensor of *T. pratense* L. degenerates at this stage, and is not present in the cotyledonary embryo stage (Figure 5C).

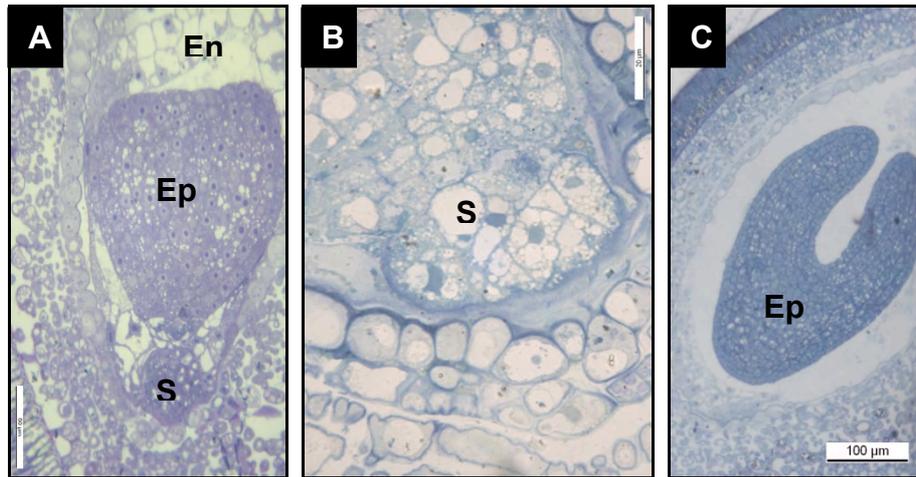


Figure 5A. Light micrograph of a semithin section showing the heart-stage embryo (*Ep*). *En*: endosperm, *S*: suspensor. Bar = 20 µm. **B.** Detail of the degenerated suspensor cells are highly vacuolate and electron translucent areas. Bar = 20 µm. **C.** Light micrograph of a semithin section showing the cotyledonary embryo stage (*Ep*). Note the suspensor is not present. Bar = 100 µm.

4. Discussion

The suspensor in flowering plant is indispensable organ for the early growth of the embryo proper [1]. In many plant species the embryo suspensor is a fast growing and short-lived organ.

The present study illustrates certain cytological changes in the suspensor cells of *T. pratense* L. during embryogenesis. The growth rate of the suspensor is often very rapid during earlier stages of embryogenesis which becomes a massive structure that reaches its maximal size at the late globular embryo stage of development and then degenerates during subsequent late heart stage of embryo proper.

In *Phaseolus*, the suspensor is more active than the embryo proper in RNA and protein synthesis during early stage of development [22, 9, 23].

Ultrastructurally, the suspensor basal cell in *T. pratense* L. wall ingrowths have been observed in cells presumed to have a role in absorption and translocation of metabolites from maternal tissues to the embryo proper. Mitochondria found near these ingrowths may play a role in energy dependent transport of nutrients.

The occurrence of wall labyrinths and protuberances in the suspensor has been described in numerous reports, [24, 6, 8, 16] Gunning and Pate, [25] stated that the projections multiply the absorptive surface of the plasma membrane in transfer cells. The suspensor of *T. pratense* L. contains structural modifications to facilitate transport, but the cells are less stained for protein and nucleic acids than that of the embryo proper.

The development and cytology of the suspensor in this plant concluded that the basal cell is an active transfer cell absorbing nutrients from the maternal tissues, metabolizing and transporting them through the suspensor cells to the embryo proper in early stage of embryogenesis, later degenerates at the late heart stage of embryogenesis and is not present in the mature embryo. [2, 26-29].

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