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MORPHO-ANATOMIC TRANSFORMATION OF THE SWEET SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH SUBSP. *BICOLOR*) EMBRYO, IN THE FIRST DAYS OF GERMINATION

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ABSTRACT
The sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis has a complex structure. The value of caryopsis consists not only in insuring the species reproduction, but has other usage, one of this is as malt. In the African countries, similar to rise, the sweet sorghum represents a source of starch, proteins, and is being used as food for people and animals. Sweet sorghum is also used to produce ethanol, currently utilize as biofuel in many countries.
The embryo's endosperm incorporates it's nutritive reserves, and is providing the energy necessary for the plantlet to grow. Though the sweet sorghum endosperm was cytological and biochemical studied, one can observe that the study of embryos morphology and the dynamic produced at this level in the first 48 hours were neglected, as the aspects related to it's fast growing characteristic. We suggested that such a plant material may serve as experimental model in many studies of plant physiology.
Keywords: *Sorghum,* embryo, germination, coleoptile, umbilical cord.

INTRODUCTION
The sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*), is a monocotyledonous plant, a highly appreciated gramineous plant in the geographic regions where the wheat culture is not developing well (Africa, South America, Central America). But, in the last decades, the culture of sweet sorghum has spread into Europe, including Romania, being used as raw material in the biotechnological industry, mostly in producing biofuels, respectively ethanol. In many African states the germinated sweet sorghum is used as malt, due to its endosperm which incorporates especially β and α amylases (*Botes* et al., *1967a; 1967b*).

In the scientific literature, we identified three articles (Rooney and Miller, 1982; *Glennie* et al., 1983; *Sautier* and *O'Deye*, 1989), in which the structure of sweet sorghum caryopsis is described. None of the authors collectives mentioned above is presenting exactly and in detail the morpho-anatomic aspects of the sweet sorghum fruit and seed. This lead us to examine at optical and electron microscope the caryopsis structure, as much as in our plant physiology experiments we wanted to use this material to examine the influence of light, at different wave lengths and intensities, on the sorghum plantlets growth.

The examination of sweet sorghum caryopsis was made in different viewing angles, even to the scanning electron microscopy (SEM), in the early stages of germination, also at seed pericarp, testa or endosperm level, but these studies did not referred to embryo structure (Adams et al., 1975; *Glennie* et al., 1983; *McDonough and Rooney*, 1990). This fact determined us to perform mainly, a microscopic level study of the sweet sorghum caryopsis in germination, focusing on the examination of the embryo's structure.

MATERIALS AND METHODS
In our experiments we used sweet sorghum caryopsis (*Sorghum bicolor* (L.) Moench subsp. *bicolor*), obtained from the Lovrin Agricultural Research and Development Station, in Timiş county.

The germination of sweet sorghum caryopsis was done in the dark, on Whatman filter paper, humidified with bi-distilled water. The paper was placed in a plastic recipient of 30cm long, 15cm wide and 10cm high. At 12, 24, 36 and 48 hours from the time of germination start, we took samples for fixation and we examine afterward the sections prepared through them, in optic, scanning and transmission electron microscopy (FEI Tecnai 12). Fixation of the plant material was made in steps: prefixation by glutaraldehyde 2,7%, prepared in phosphate tampon 0,1M, pH 7.4, lasting for 60-90 min. at 4^{O}C; followed by washing the fixation substance in phosphate tampon 0,15M, pH 7.4 (4 consecutive baths of 60 minutes each, the 4^{th} over night, all being performed at 4^{O}C); afterward, postfixation phase of the samples for 75-90 minutes, in osmic acid 2% solution (OsO_4), prepared in 0,15M phosphate tampon, 7,4 pH, operation also executed at 4^{O}C (*Kay*, 1967 and *Ploaie* et al., 1979). Following, sectioned materials bathing was performed in 2 baths, in 1,5M phosphate tampon, for 15 minutes each. Afterward, the samples were dehydrated in multiple successive acetone baths, administrated in different, increasing concentrations, of 30%, 50%, 70%, 80%, 90%, 100%. After samples infiltration with 812 epoxide resin, the resin blocks were modeled and successively the

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ultramicrotome LKB Leica sectioning was performed. From these sections semi fine samples were realized, 300nm thick; the sections staining was made with ETS (Epoxy tissue stain). A double contrasting of the sections was performed, by applying a samples treatment with uranyl acetate solution (for 13 minutes), afterward with led citrate solution (for 6 minutes), thickness of ultra fine samples was of 60nm (*Weakley,* 1981 and *Hayat*, 2000). The sections were examined in optical microscopy, fitted with image capturing equipment.

In the case of samples examined in scanning electron microscopy, FEI Quanta 250, they were initially fixed in glutaraldehyde 2,7% and mounted on microscope holder, which was previously cooled to a temperature of 3° C (*Goldstein*, 2003).

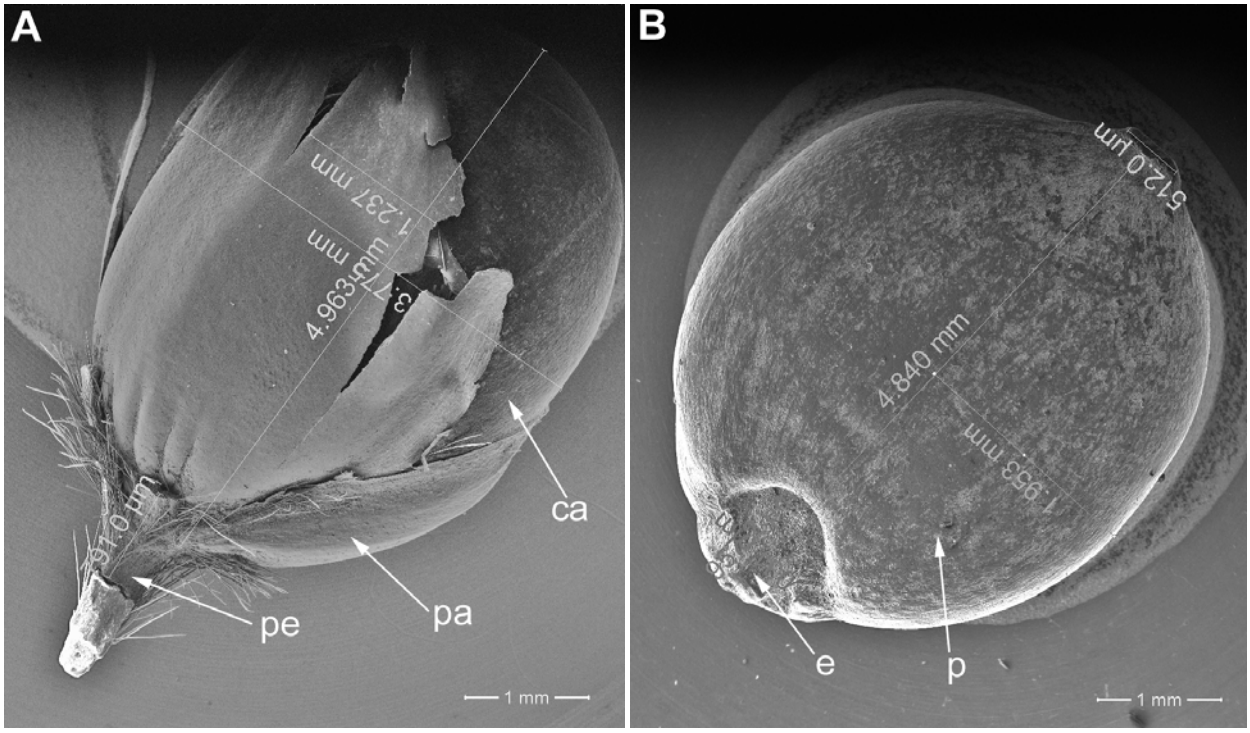
Most representative sweet sorghum images at caryopsis level, in different germination phases, including from within the embryo and endosperm, were captured and presented in this paper, figures 1 - 9.

RESULTS AND DISCUSSIONS

The germination of sweet sorghum embryo and plantlet growth are quite fast. This fact, we believe, is explained by endogenous reserves of the endosperm, easy to use by the embryo in the growth process. The sorghum endosperm contains mainly proteins, but also starch. By enzymatic hydrolysis the energy needed by the embryos cells is provided, both in the mitosis processes and the growth process. In the sorghum caryopsis (Fig. 1A) the embryonary zone (Fig. 1B-D) is located right in the vicinity of the former floral peduncle (Fig. 1A). In the non-germinated caryopsis, the embryo occupies a small part of the caryopsis volume. In figure 2 we present a schematic longitudinal section, imagined through non-germinated caryopsis of sweet sorghum, schematics elaborated by *Sautier* and *O'Deye*, (1989), and modified by us.

After the first germination hours, the embryo grows in volume (Fig. 1 C), the scutellum gaining a continuously bigger cell mass, thus, at 12 hours after caryopsis germination start it is of about the size of the embryo. The embryonary axis is linked through an "umbilical cord", a tissular "bridge" which – upon our knowledge – don`t have a consecrated term in the scientific literature, none the less the role of this formation is, mostly, essential in providing the embryo with nutritive products, through the scutellum.

At 12 hours after the caryopsis were put to germinate (Fig. 1C) one may observe that radicles, still inside the seed, is detaching of the coleorhiza, and the scutellum parenchyma (Fig. 1D) contains in its cells small corpuscular formations of a nature undefined in the scientific literature. In the same time, we observe (Fig. 1D) that the coleorhiza, has already breakthrough the pericarp. In exchange, the coleoptile (Fig. 1D), has not yet breakthrough the caryopsis pericarp.



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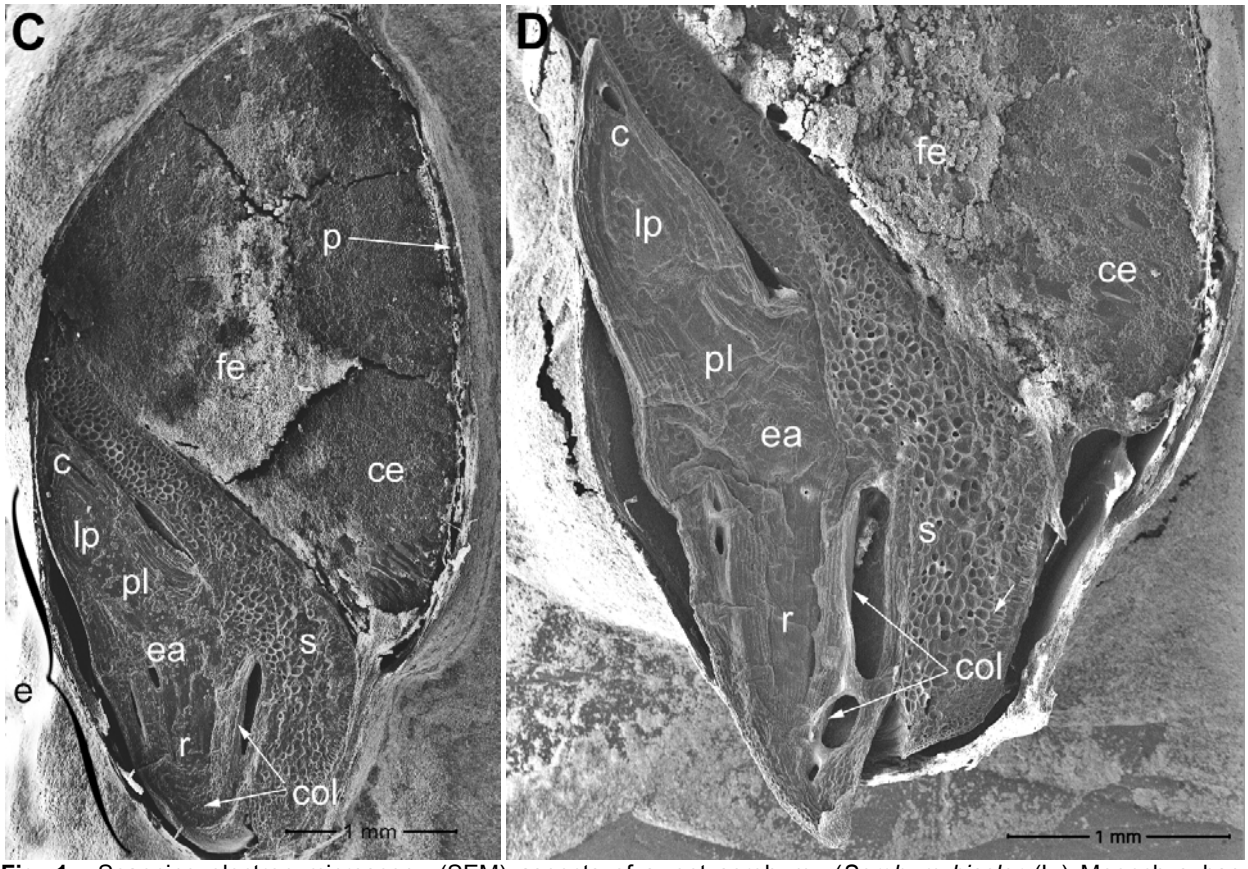


Fig. 1. Scanning electron microscopy (SEM) aspects of sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis, where: A – appearance of the undecorticated sorghum caryopsis; B - decorticated sorghum cariopsis; C - longitudinal section thru sorghum cariopsis, at 12*h* from germination; D - longitudinal section thru sorghum caryopsis, at 24*h* from germination; *c* - coleoptile; *ca* - caryopsis; *ce* - corneaus endosperm; *col* - coleorhiza; *e* - embryo; *ea* - embryonal axis; *fe* - floury endosperm; *lp*- leaf primordia; *p* - pericarp; *pa* - palea; *pe* - peduncle; *pl* - plumule; *r* - radicel; *s* - scutellum.

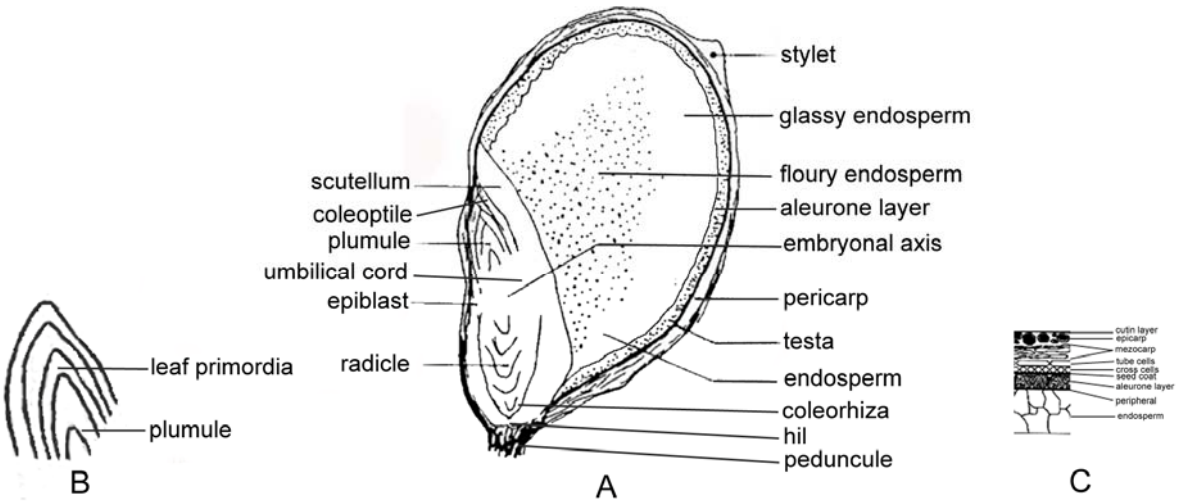


Fig. 2. Schematic representation of sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis (after *Sautier* şi *O'Deye*, 1989, modified by us): A - caryopsis; B - coleoptile; C - pericarp

In the sorghum caryopsis sketch, conceived by *Sautier* and *O'Deye* (1989) were not nominated a series of morpho-anatomical elements, as: aleurone layer, the embryonary axis, the coleoptile, coleorhiza and the connecting zone among embryo and scutellum, formation which we called the "umbilical zone", which we did not find explained in any scientific paper.

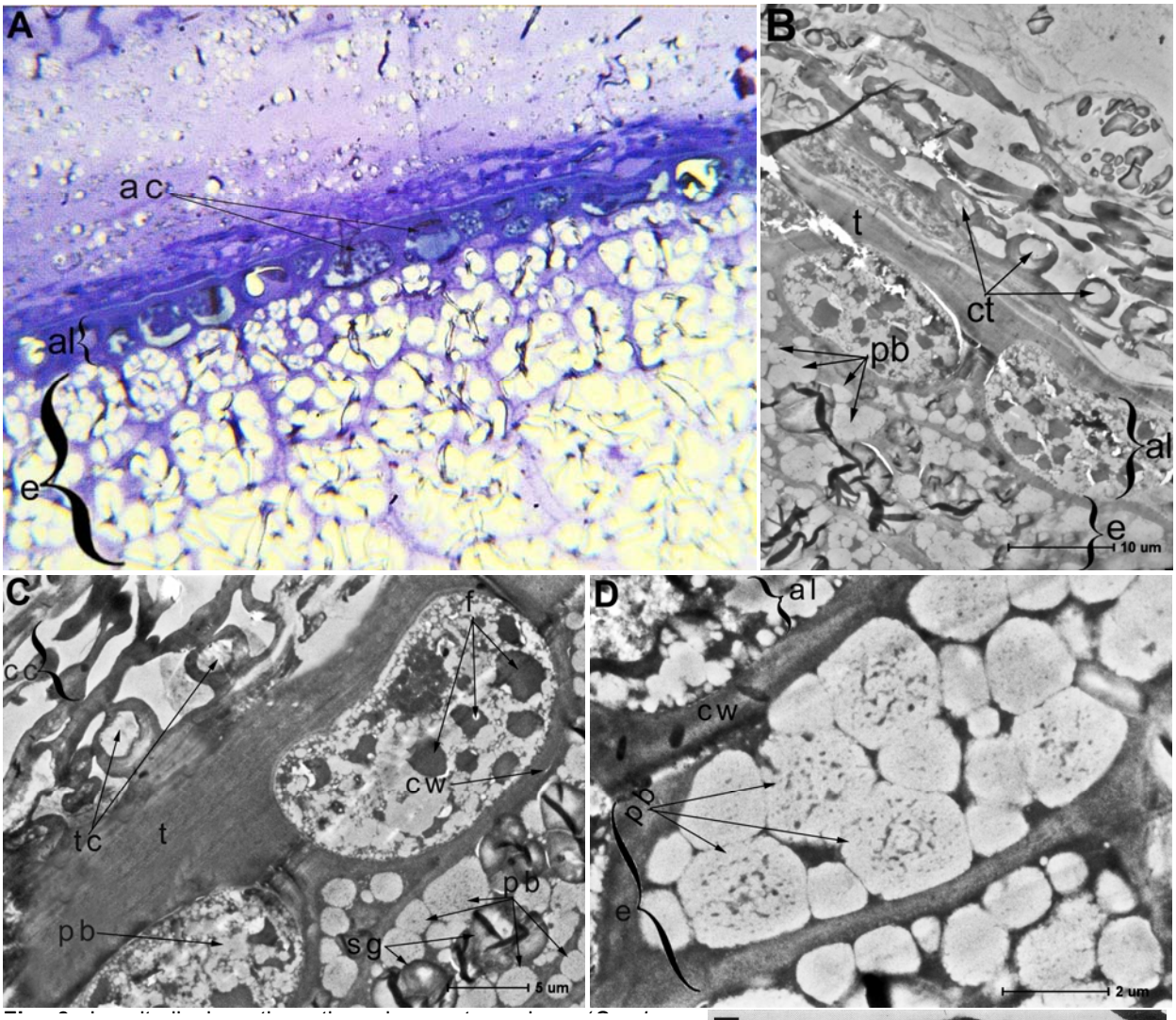
Glennie et al. (1983), made a thorough research regarding the biochemical aspects observed in the first 12 days of germination of the sweet sorghum caryopsis. They emphasized the mobilization of the endosperm deposit substances and the modifications happened in the aleurone layer. Thus, they observed that in the first phases of germination the scutellum, through its epithelium, adjacent to farinaceous endosperm, is the one with a major enzymatic function in the mobilization of protein adherent to starch particles of the farinaceous endosperm, and not in the aleurone layer. Upon the previously cited authors, the protein matrix is the first to disappear, and after that the starch granules and the protein corps are simultaneously degraded. *Glennie* and collaborators (1983) once again concluded that the aleurone layer cells don`t seems to have enzymatic production, this activity being specific to scutellum. After 12 days of germination, the content of endosperm cells are depleted, only the cell walls remaining untouched.

At sorghum, the aleurone layer cells are rich in mineral elements (*Glennie* et al., 1983), mainly phosphorus, element still present in these cells, in the 12th day of germination; but in the same cells the content of sulphur and potassium has decreased significantly. At the same time, in the 12th day of germination, generally, the aleurone layer content is heavily modified, the cells being drained off.

Consequent to the realized researches, after 12 hours of initiating the germination process in the sorghum caryopsis, the aleurone layer cells (Fig. 3A-D) were loaded of corpuscular formations, some electron-dense and some lightly colored. After 36 hours of germination (Fig. 4A and B), the cell content of aleurone layer was absent, but in exchange the numerous starch granules were find in the peripheral layer of the endosperm, near the aleurone layer cells. In the cells observed in the transmission electron microscopy, at 12 hours from germination, we identified in endosperm starch granules (Fig. 3B-D) and the protein corps(Fig. 3B-D). Some of the images taken by us (Fig. 3D), were described in the literature (Fig. 3F) (*Adams* and colab., 1975). Also, the pericarp structure we described is similar with the one presented by *Rooney* and *Miller* (1982) (see Fig. 2C and Fig. 3A-C). In the 5A and 5B figures, the aspects presented by *Glennie* and collaborators (1983) were observed at the scanning electron microscope, images illustrated where observed in the farinaceous endosperm of sweet sorghum caryopsis, in the 4th day of germination.

Interesting is the fact that, from histo-anatomical and biochemical analysis, referring to sweet sorghum caryopsis, the descriptions showing the embryonary zone, and scutellum transformations during germination. Generally in the articles, were described the important role of scutellum in elaborating enzymes, respectively of its epithelium, is emphasized, but it is not mentioned that the connection among embryo and scutellum is made through a connective tissue, which preserves the communication among the embryonary axis and the scutellum, as much as at 36 hours from germination the embryo is detached of the scutellum and the one connection of the embryo with the nutritive reserves from the endosperm, reserves which get to the radice and buds, is made is made through a tissue called by us "umbilical cord", which is missing in dicotyledonous plants.

Interesting is also the fact that, after the radice breakthrough the pericarp, already at its basis (Fig. 6C, D and Fig. 7A and B), one my observe – all around it – a dense mass of root hairs, with the purpose of insuring the water and the nutrients the plantlet need. On another hand, the plumule (Fig. 8A-D), surrounded in leaflets, break's through the coleoptile, which penetrates the caryopsis pericarp and becomes progressively parchment like.



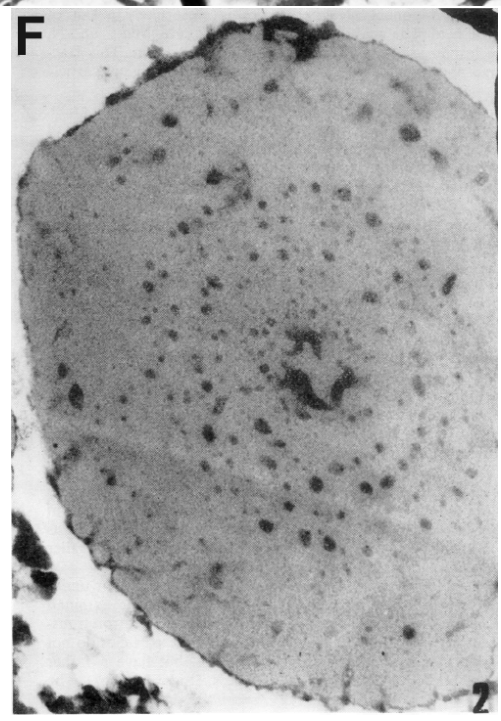


Fig. 3. Longitudinal sections through sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis, at 12*h* from germination: A - aleurone cells, optical microscop image, ob 40x; B, C - cells from aleurone layer, transmission electron microscopy images; D - cell from peripheral layer of endosperm tissue with corpuscular deposits of protein, transmission electron microscopy image; E - protein body showing areas of darker material arranged in concentric rings, X84,000 (after *Adams* et. all., 1975); *ac* - aleurone cells; *al* - aleurone layer; *cc* - cross cells; *cw* - cell wall; *f* - fitin; *e* - endosperm; pb - protein bodies; *sg* - starch granules; *tc* - tube cells; *t* - testa.

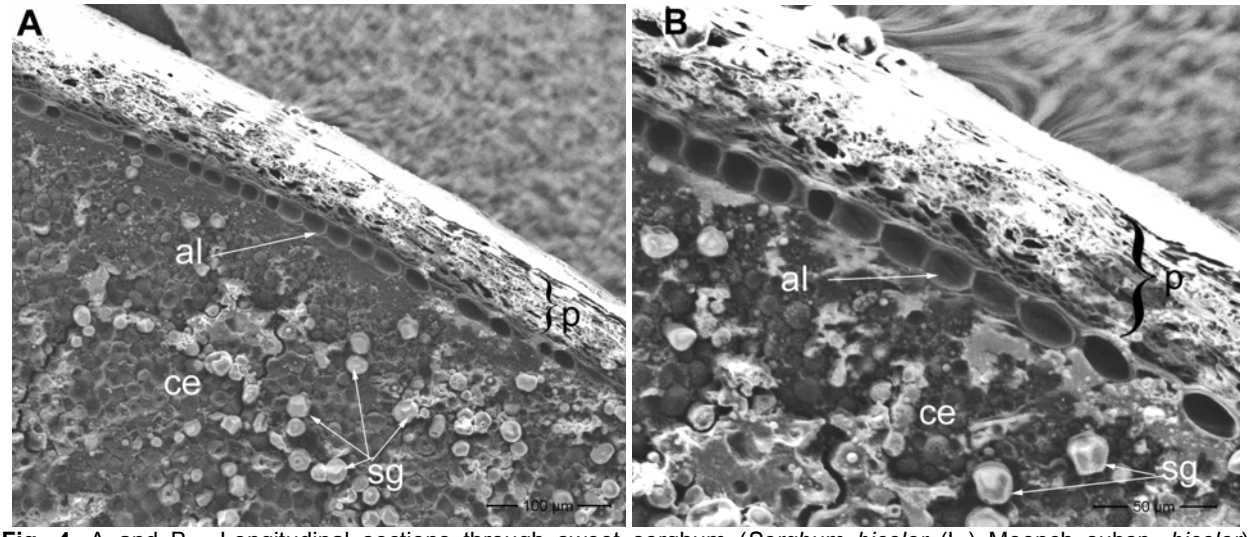


Fig. 4. A and B - Longitudinal sections through sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis, at *36h* from germination, scanning electron microscopy images: *al* - aleurone layer; *ce* - corneaus endosperm; *p* - pericarp; *sg* - starch granules.

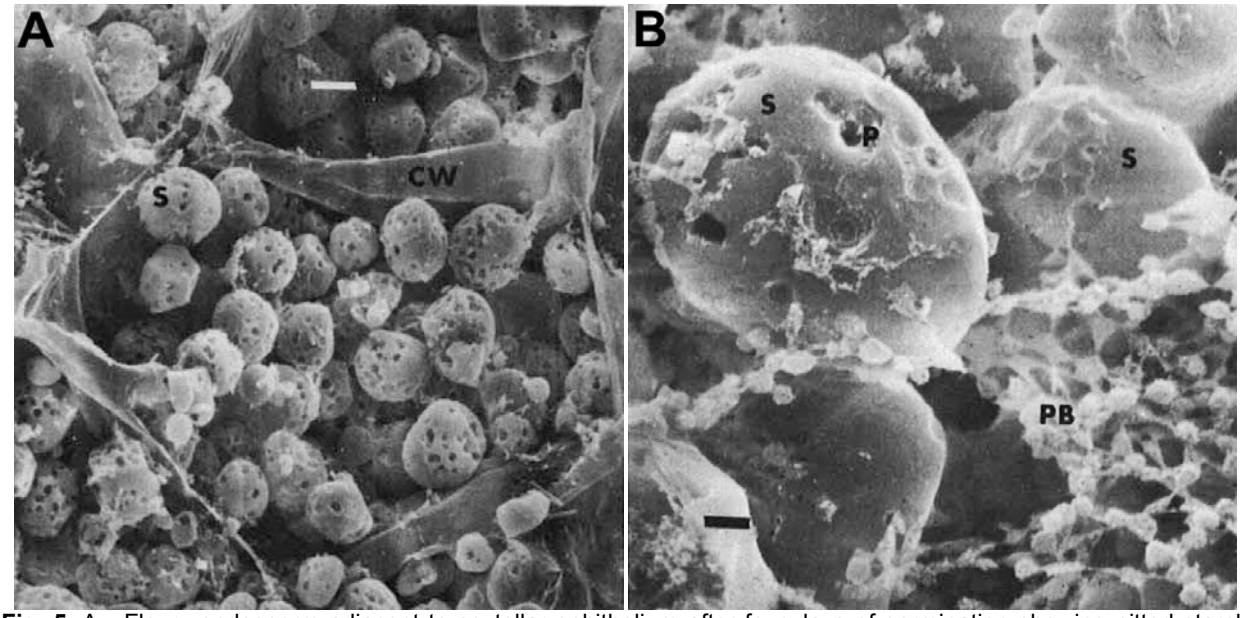


Fig. 5. A - Floury endosperm adjacent to scutellar ephithelium after four days of germination showing pitted starch granules and intact cell walls (Bar = 10 µm); B - Floury endosperm adjacent to scutellar ephithelium showing empty cell with intact walls. Bar =100 µm; *cw* - cell wall; *s* - sutellum, *pb* - protein bodies. (after *Glennie* et all., 1983).

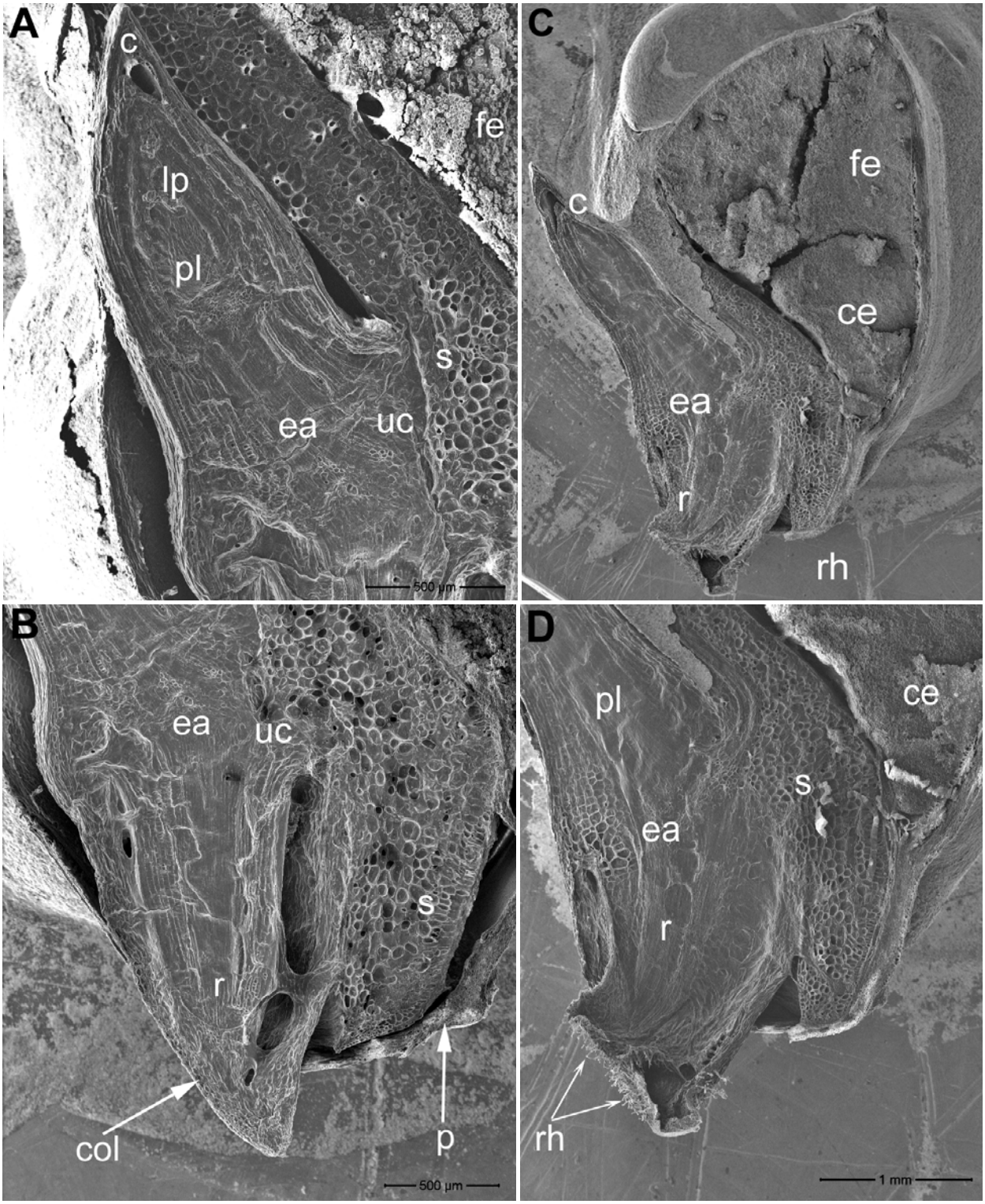


Fig. 6. Longitudinal sections through sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) caryopsis, scanning electron microscopy images: A, B - images taken at 24*h* from germination; C, D - images taken at 36*h* from germination; *c* - coleoptile; *ce* - corneaus endosperm; *col* - coleorhiza; *e* - embryo; *ea* - embryonal axis; *fe* - floury endosperm; *lp*- leaf primordia; *p* - pericarp; *pl* - plumule; *r* - radicel; *rh* - root hair; *s* - scutellum; *uc*- umbilical cord.

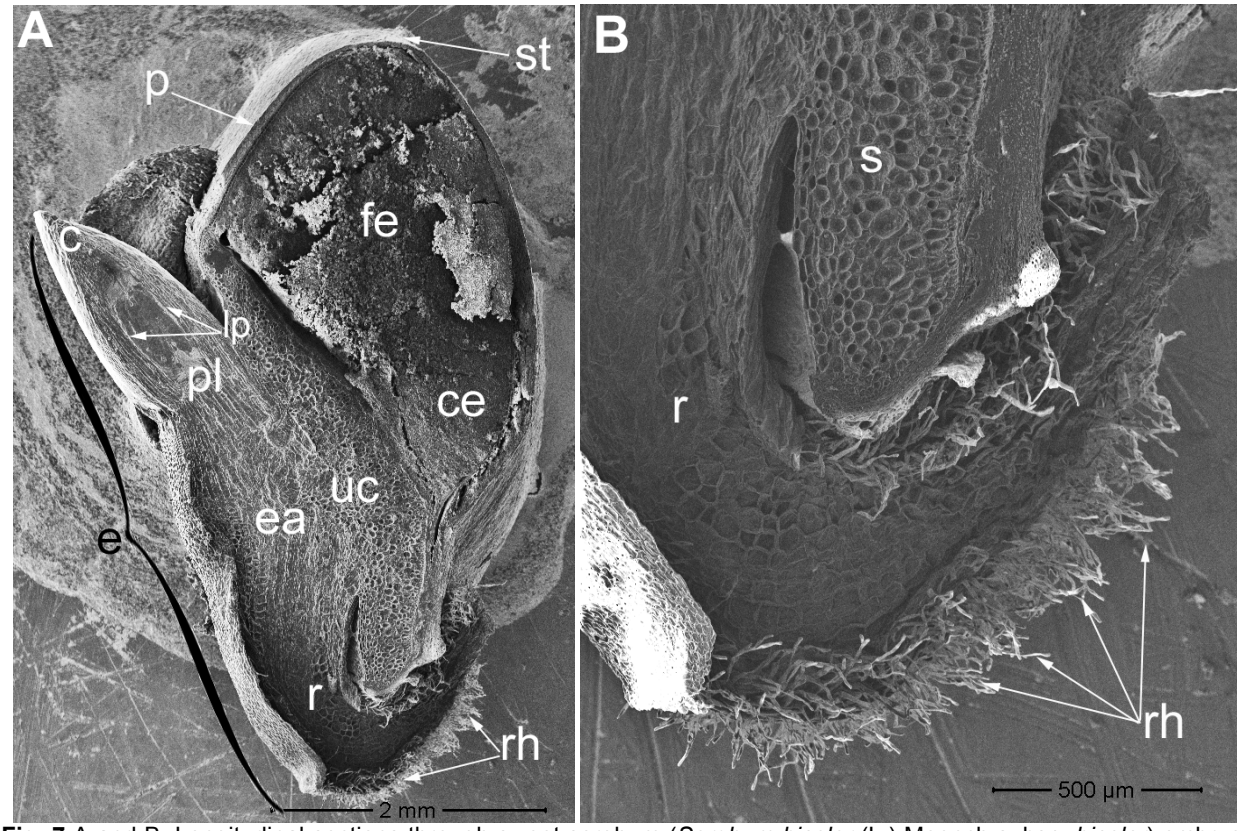
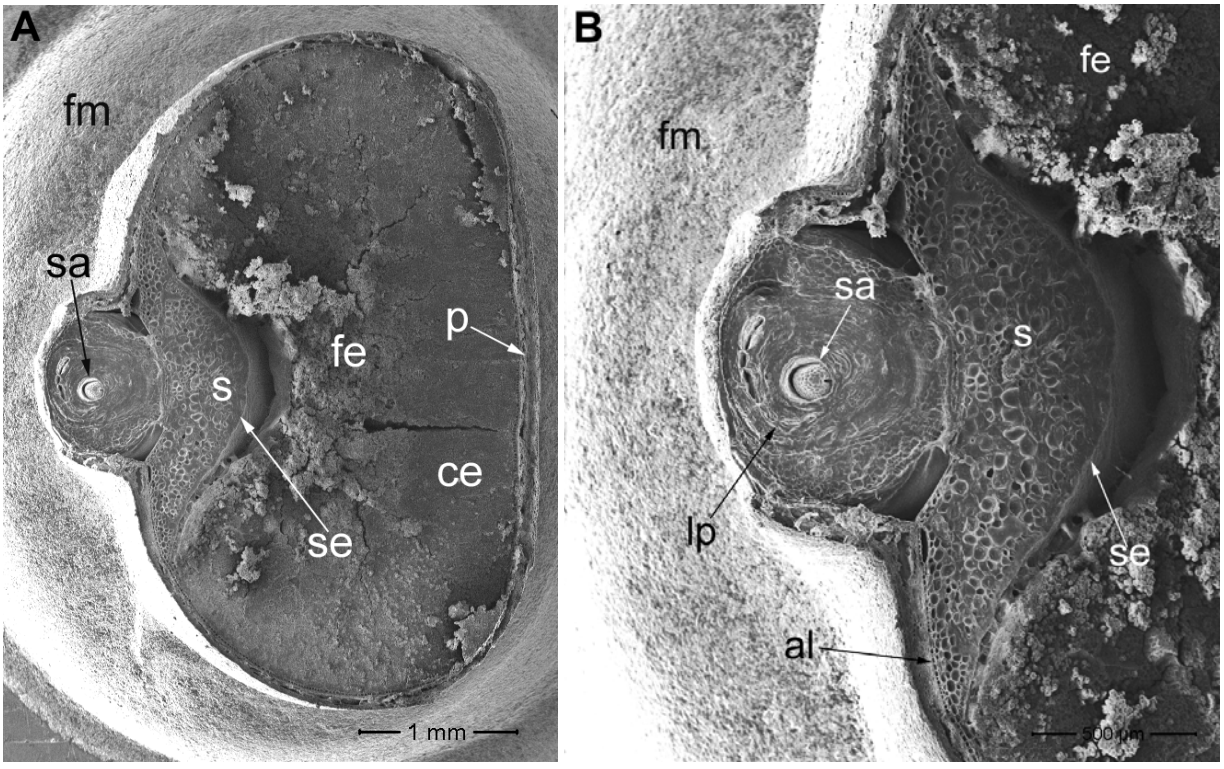


Fig. 7 A and B. Longitudinal sections through sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) embryo, scanning electron microscopy images, taken at 48*h* from germination: *c* - coleoptile; *ce* - corneaus endosperm; *e* - embryo; *ea* - embryonal axis; *fe* - floury endosperm; *lp*- leaf primordia; *p* - pericarp; *pl* - plumule; *r* - radicel; *rh* - root hair; *s* - scutellum; *st* - stylet; *uc*- umbilical cord.



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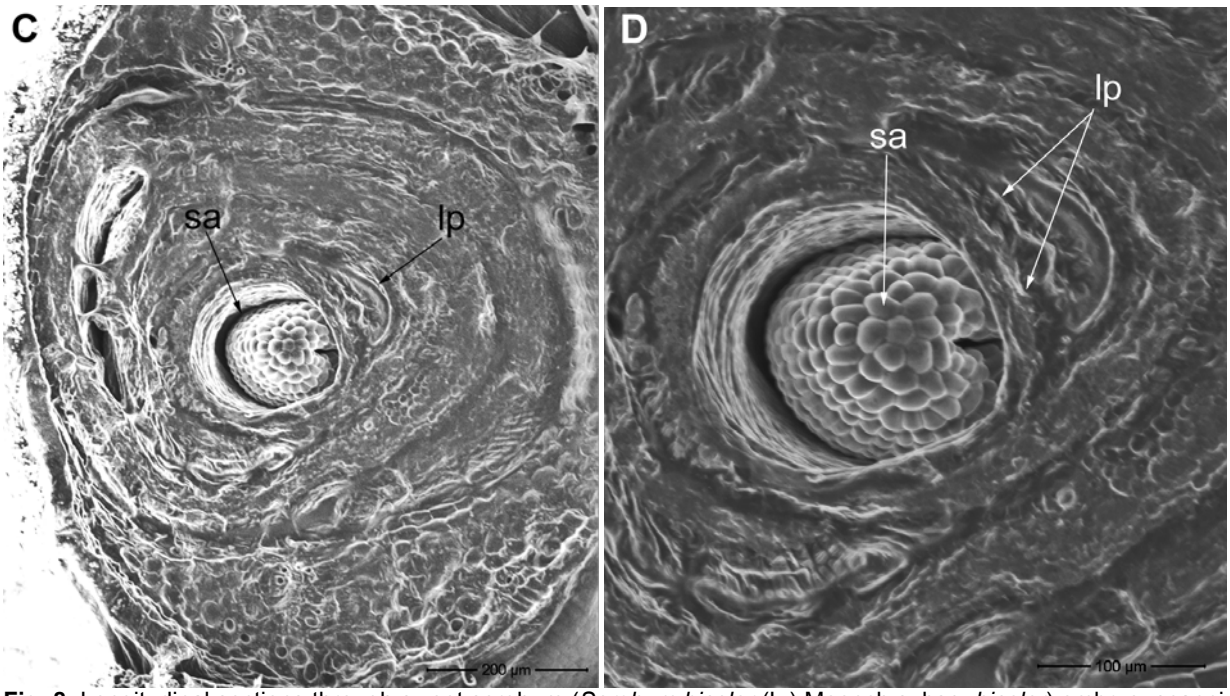

Fig. 8. Longitudinal sections through sweet sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) embryo, scanning electron microscopy images, taken at 36*h* from germination: *al* - aleurone layer; *ce* - corneaus endosperm; *fe* - floury endosperm; *fm* - fixation material; *lp*- leaf primordia; *p* - pericarp; *s* - scutellum; *se* - scutellum epithelium*; sa* - shoot apex.

The coleoptile is a morpho-anatomical formation that protects the cauline leaflets which are surrounding the plumula. There are a few scientific data regarding the structure of coleoptile. At sweet sorghum it looks like a finger glove (Fig. 1C and D; Fig. 6A and C; Fig. 7A), which enclose the surrounding leaflets which have at their base the cauline dome (Fig. 8C and D), made of cells of the apical meristem.

The coleoptile, after the leaflets and stem grow in about 3 days of germination, is penetrated by the tallest leaflet and, gradually, becomes parchment like and its physiologic function seems to end. In the 1C and 1D figures we present the aspect of a sweet sorghum plantlet developed from a caryopsis in the 4th day of germination. The coleoptile has a height of 10-15m and a reddish coloring, due to the presence of vacuole juice dissolved anthocyanin (Fig. 9A). For other Sorghum varieties, with non colored coleoptile, in the vacuole juice leuco-anthocyanin may be found, with no coloring. In the 7th day of germination, when the sorghum plantlets had 3 leaflets and a height of 15-17cm, the coleoptile cells were still alive, which in the presence of a saccharose concentrated solution were plasmolyzed. It's microscopic aspect is illustrated in the 9B figure.

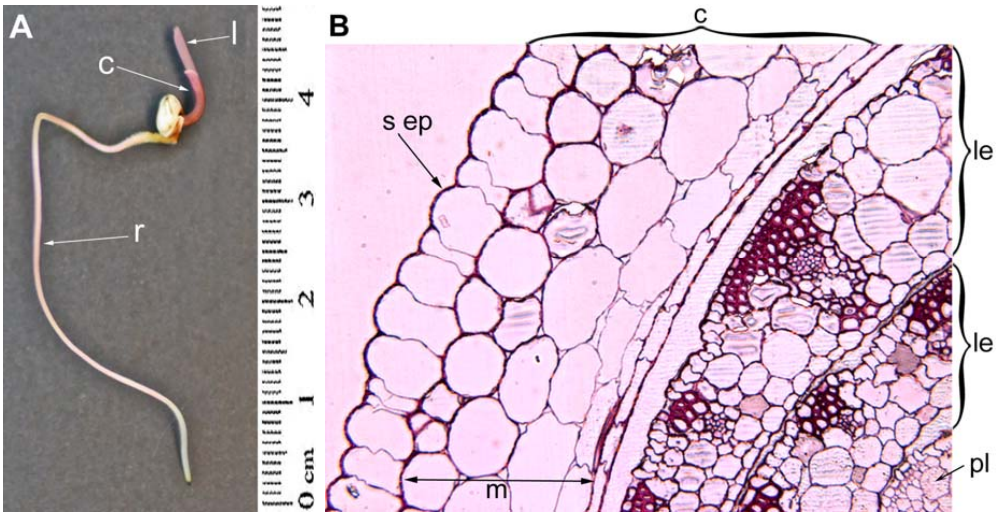

Fig. 9. A - Swett sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*) plantlet at 4 days from germnination; B - longitudinal sections through swett sorghum coleoptile, and the first two leafleats, optic microscopy image, ob. 40x; *c* - coleoptile; *le* - leafleats; *m* - mezocarp; *pl* - plumule; *r* - radice; *sep* - superior epiderma;

Interesting is the fact that, at the level of the 2 epidermal layers of the coleoptile (superior and inferior), there are no stomata. Details regarding the ultra-structure of optical microscope examined cells makes the object of future articles.

CONCLUSIONS

The fast germination of the sorghum caryopsis and the accelerated growth of the plantlets in the first 48 hours after putting the seeds to germination, may be due to the endosperm richness, mainly in proteins and starch. In the peripheral zone of the endosperm, in the neighborhood of the internal face of the aleurone layer, very abundant protein corpuscular formations were identified.

For the first time in scientific literature, we identified the existence of a pluricellular bridge, as an "umbilical cord", which makes the connection among the embryonary axis and the scutellum. This formation intermediates the access of hydrolysis products resulted from the endosperm reserve substances degradation, towards the cells of the growing embryo.

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