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Scanning electron microscope studies of *Avena* coleoptiles during primary leaf emergence

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Abstract

A scanning electron microscope study of the fine structure of coleoptiles of *Avena sativa* L. cv. Clintford has revealed information about the relationship between aspects of coleoptilar structure and the emergence of the primary leaf. Stomata are located on the lateral sides of the coleoptile and arch across the apex. The location of the stomata and associated vascular tissue may play a role in the splitting of the coleoptile pore and influence the manner in which the primary leaf emerges. Details of the surface fine structure of the coleoptile pore, its associated cells, stomata and guard cells are presented.

Key words: Avena coleoptile, coleoptile development, coleoptile fine structure, fluorophenylalanine, scanning electron microscopy

INTRODUCTION

Work in our laboratory has centered on studies of extension growth of excised *Avena* coleoptile apices. Hopkins and Bonnell (1969) and Hopkins and Orkwiszewski (1971) observed that the amino acid analogue D,L-p-fluorophenylalanine (p-FPA) significantly enhanced elongation of apical *Avena* coleoptile segments in darkness. Additional research (Orkwiszewski and Hopkins 1974, Orkwiszewski et al. 1976, Maksymowych and Orkwiszewski 1983, 1987) has refined our understanding of p-FPA growth stimulation. These authors have demonstrated that p-FPA exerts its effect by lowering the activity of L-phenylalanine ammonia-lyase (E.C. 4.3.1.5), inhibiting the production of potentially phytotoxic low molecular weight phenols and altering endogenous levels of auxin oxidase activity.

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Since the mechanics by which p-FPA growth is effected remain unclear, the initial purpose of our work was to examine the fine structure of etiolated *Avena* coleoptiles in an attempt to gain an understanding of the manner by which p-FPA stimulated growth enhancement occurs. In the course of this study we noted a number of structural features associated with the course of coleoptile development and emergence of the primary leaf. To the best of our knowledge, scanning electron microscope (SEM) studies of these aspects of coleoptile fine structure have not been reported in the literature. We feel that these data present valuable information with respect to the course of development of these organs in *Avena*.

MATERIALS AND METHODS

Unhusked seeds of *Avena sativa* L. cv. Clintford (The Stanford Seed Co., Denver, Pennsylvania) were grown as described by Hopkins and Bonnell (1969). Plant material used throughout this study was apical coleoptile segments which were excised by hand from etiolated seedlings.

For examination of any possible effects of p-FPA on coleoptile fine structure, 8 mm apical segments minus primary leaves were harvested and treated according to the methods described by Maksymowych and Orkwiszewski (1987). Coleoptiles incubated with 5 mM p-FPA were compared to control segments.

Further examination of coleoptile fine structure was performed on apical coleoptile segments containing the primary leaf. Coleoptile apices were excised from dark grown seedlings at various stages of development and rinsed with glass-redistilled water prior to fixation. All operations involving living tissue were conducted either in darkness or under a dim green safelight (Hopkins and Bonnell 1969).

At appropriate stages of development, *Avena* coleoptiles were fixed by one of two techniques. Coleoptile segments were placed in 3% gluteraldehyde in 0.3 M (piperazine-N-N'-bis-2-ethanesulfonic acid)-NaOH buffer (PIPES) overnight. The tissue was then dehydrated through a series of acetone and water solutions and left overnight in 100% acetone. Specimens were critical point dried in a Denton DCP-1 Critical Point Dryer, employing liquid CO_2, mounted on stubbs with silver paint, coated with gold in a Denton Desk Sputter Coater and observed with an Hitachi S-570 SEM.

An alternative technique was to fix *Avena* seedlings in a mixture of 3:1 ethanol:acetic acid for 12 hr. Samples were then dehydrated under a mild vacuum in a graded acetone series. The vacuum facilitated removal of air from within the tubular coleoptile structure. Samples were then critical point dried in a Denton DCP-1 Critical Point Dryer employing liquid CO_2. As described above, specimens were mounted on stubbs with silver paint, coated with gold

in a Denton Desk Sputter Coater and observed with an Hitachi S-570 SEM.

Studies comparing the two fixation techniques revealed no difference in effectiveness of either process. The latter technique was more rapid and efficient and therefore employed in these studies.

RESULTS

Initial studies of the surface fine structure of coleoptiles incubated with 5 mM p-FPA displayed no obvious differences in the disposition of cellulose cross-ribs or other structural aspects at the low magnification employed (Galanti 1985). Thus we pursued our investigation in a direction to determine whether or not further information could be obtained with respect to the course of coleoptile development and leaf emergence in *Avena*.

Figures 1-4 illustrate the general features of the *Avena* coleoptile prior to emergence of the primary leaf. The plants illustrated in these figures were fixed from 72 to 120 hr after planting. The coleoptile during this time period tapers toward the tip. The coleoptile pore (C.P.) is on the anterior flattened face (C.P. side, Bonnett 1961). The lateral sides are set off by the presence of stomata which may serve as hydathodal pores (Butterfass 1956, Esau 1965). The stomata are more numerous per unit area at the tip than more basally. In Figs. 1, 3 and 4 the C.P. is situated on the anterior flattened surface below the coleoptile tip. The slit-like opening of the C.P. is bound on each side by a cluster of small cells. In Figs. 3 and 4 a groove can be seen extending from just below the tip back to the pore and continuing basally. The C.P. and groove may be of significance in later stages associated with leaf emergence.

In the pre-emergence coleoptiles (Figs. 5-9), the stomata differ morphologically depending upon their distance from the coleoptile apex. The stomata are bounded by 2 cells (guard cells) that abutt at their ends and are arranged such that the pore is circular in outline toward the apex and slit-like more basally. In addition, many intermediate morphologies are shown in Figs. 7 and 8. In the basal portion, the 2 guard cells are elongated. Figures 5-9 demonstrate a variety of stomatal and guard cell shapes. In the stretched morphology, the guard eells are thinner than in the undistended cicular state.

Figures 10 and 11 show close-up views of the C.P. The C.P. can be seen to be bound by 2-3 rows of small cells morphologically distinct from the surrounding epidermis. Figure 12 shows a close-up a leaf at approximately the time of emergence (about 150 hr after planting). Figures 13 and 14 show a close up view of the C.P. cells just after the emergence of the leaf. The 2 halves of the pore have separated leading to a split through which the primary leaf is visible and will emerge during the course of development. In these figures, the same plant is illustrated, and the 2 distinct pads representing the old C.P. cells (surrounding 2-3 layers) can be seen at different mag-

PLATE I

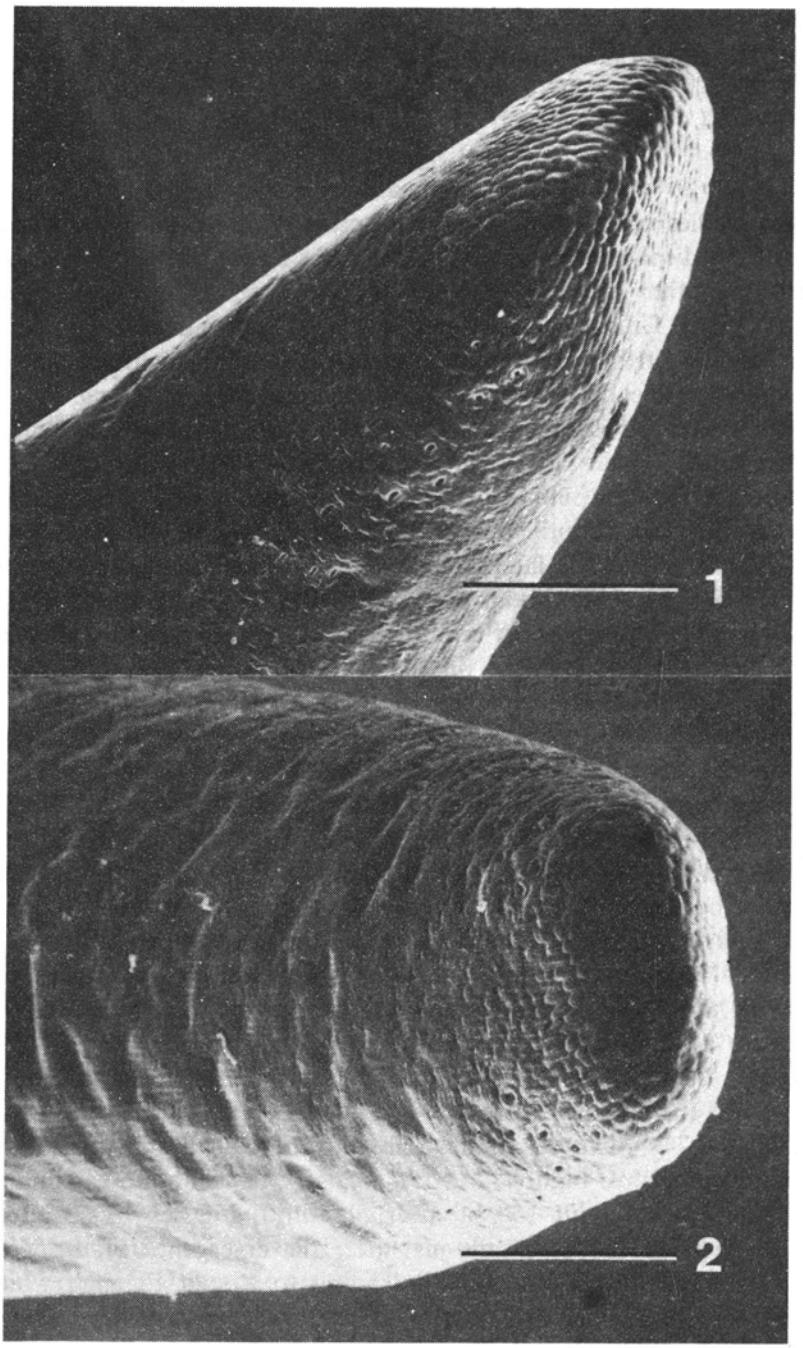


Fig. 1. Apex and right lateral surface of coleoptil fixed 96 hr after planting. Stomatal pores on lateral surface. Cells bordering coleoptile pore are visible on the anterior face. Scale bar: 0.3 mm.
Fig. 2. Posterior face of coleoptile 72 hr after planting. Stomatal pores can be seen intruding onto the posterior face on each side below the apex. Scale bar: 0.27 mm

PLATE II

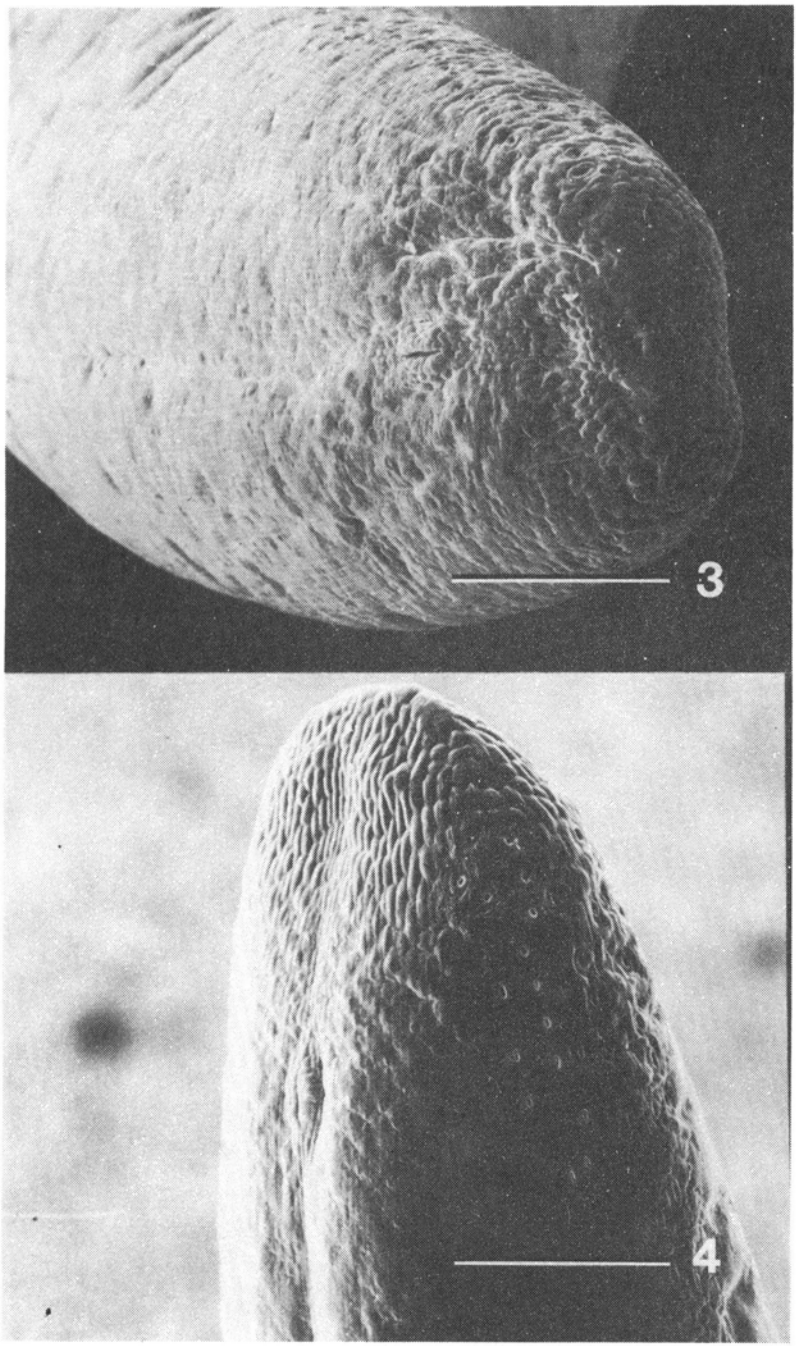


Fig. 3. Anterior face of coleoptile (96 hr after planting) showing the coleoptile pore below the apex and the central groove running basally from it. Stomatal pores are visible on the right side of the coleoptile. Scale bar: 0.3 mm. Fig. 4. Left side of coleoptile (120 hr after planting) bearing stomatal pores. Cells bounding the coleoptile pore are visible as a mound on the anterior surface. The central groove can be seen extending basally and apically from the pore area. Scale bar: 0.29 mm

PLATE III

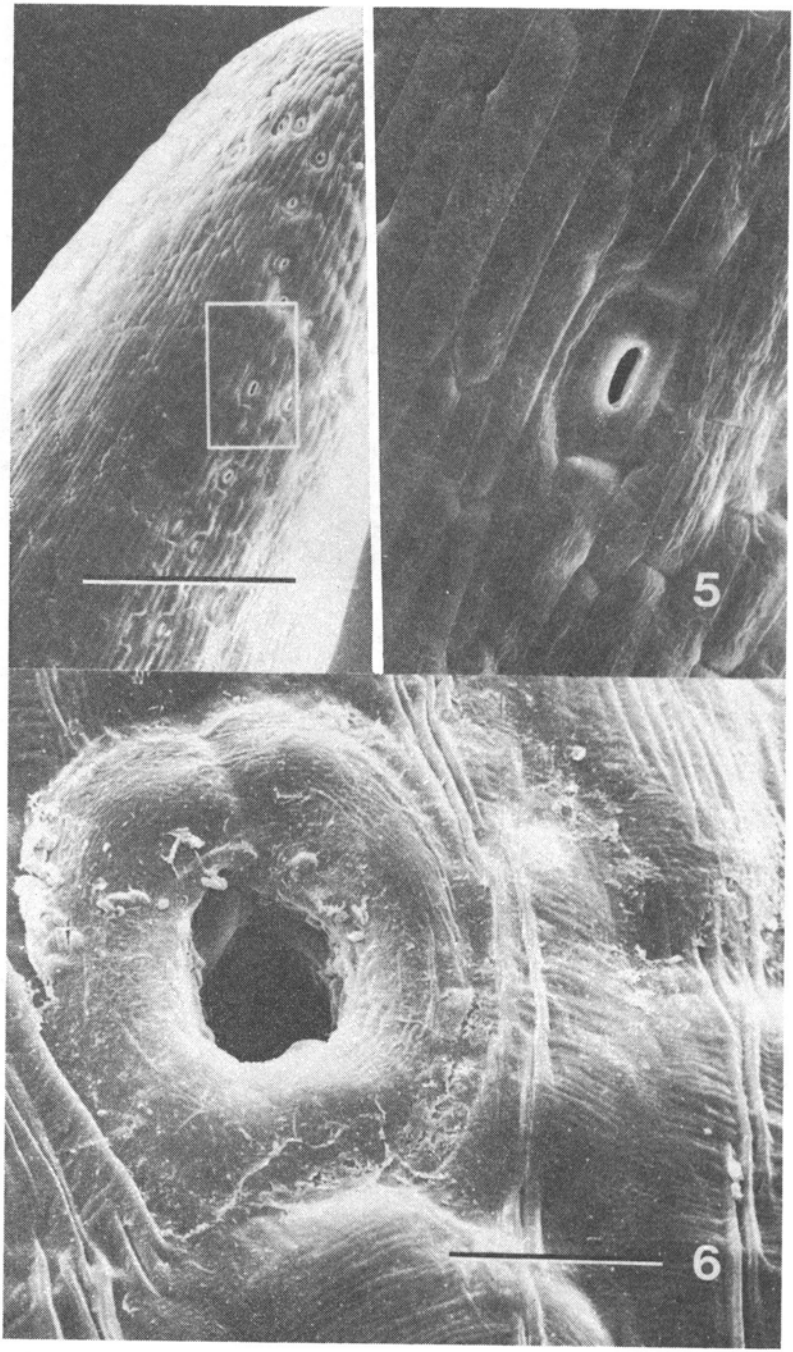
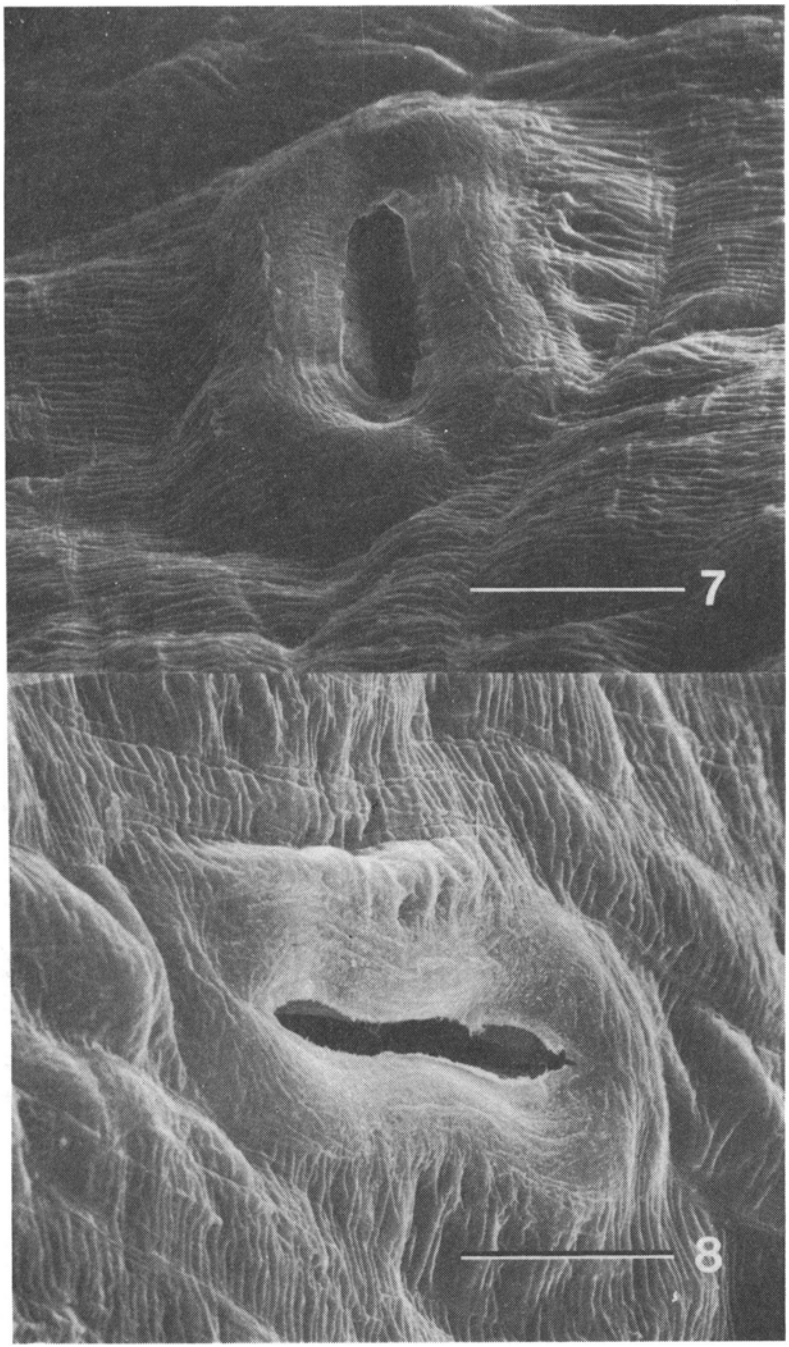


Fig. 5. Stomatal pores on the right side of the coleoptile with a higher magnification of one open pore and the 2 bounding cells (boxed area). Scale bar: 0.31 mm. Fig. 6. Open stomatal pore from near the apex of the coleoptile (144 hr after planting). Note the donut-like appearance of the structure. Scale bar: 15 μm

PLATE IV



Figs. 7 and 8. Pores from more basal regions of the coleoptile showing increasing distention of the pore and bounding cells as the base is approached. Scale bar: 13.6 μm for Fig. 7 and 13.9 μm for Fig. 8.

PLATE V

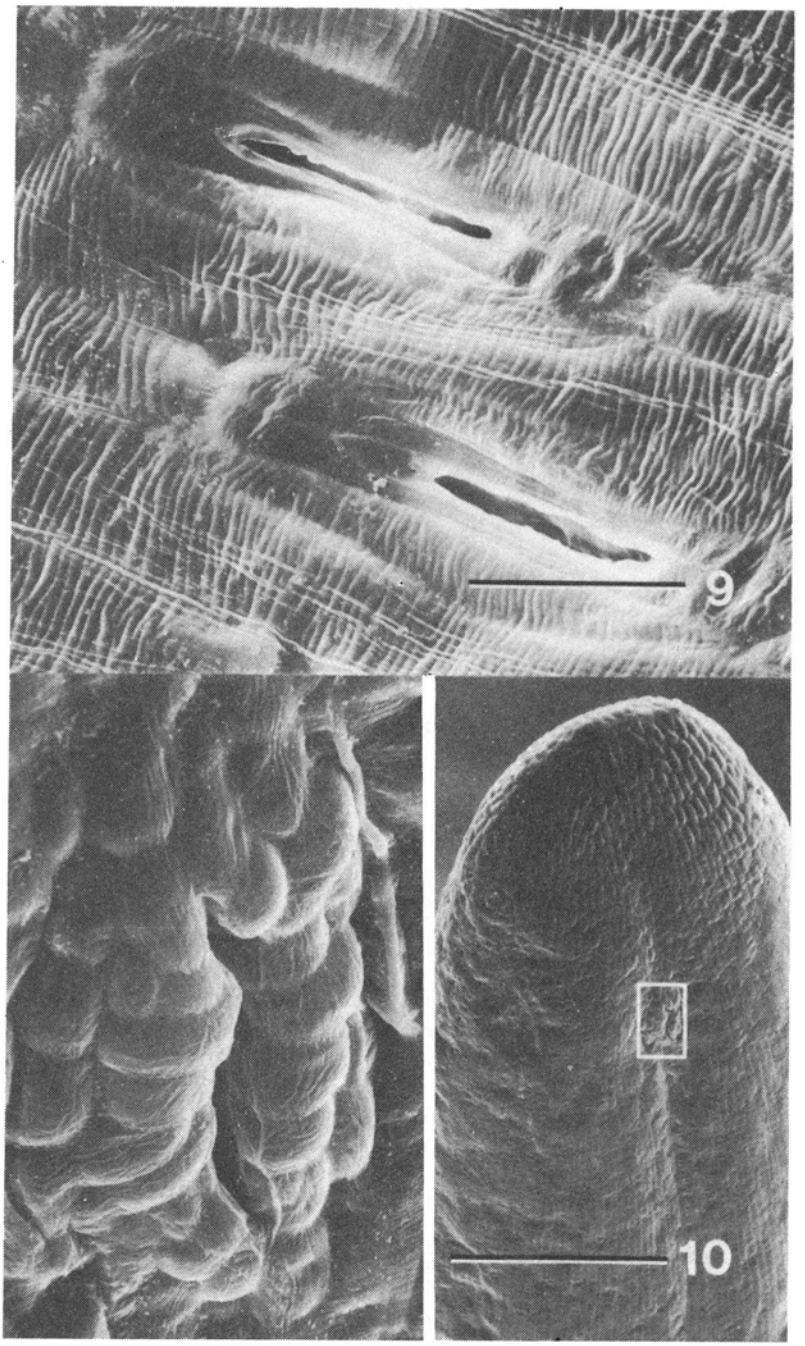


Fig. 9. Stomatal pore from near the base of a coleoptile showing the extremely elongate nature of the cells and the pore. Scale bar: 20 μm. Fig. 10. Coleoptile pore shown in position with a higher magnification of the boxed area to show detail. Each side of the pore is bound by 2-3 rows of small cells. Scale bar: 0.38 mm

PLATE VI

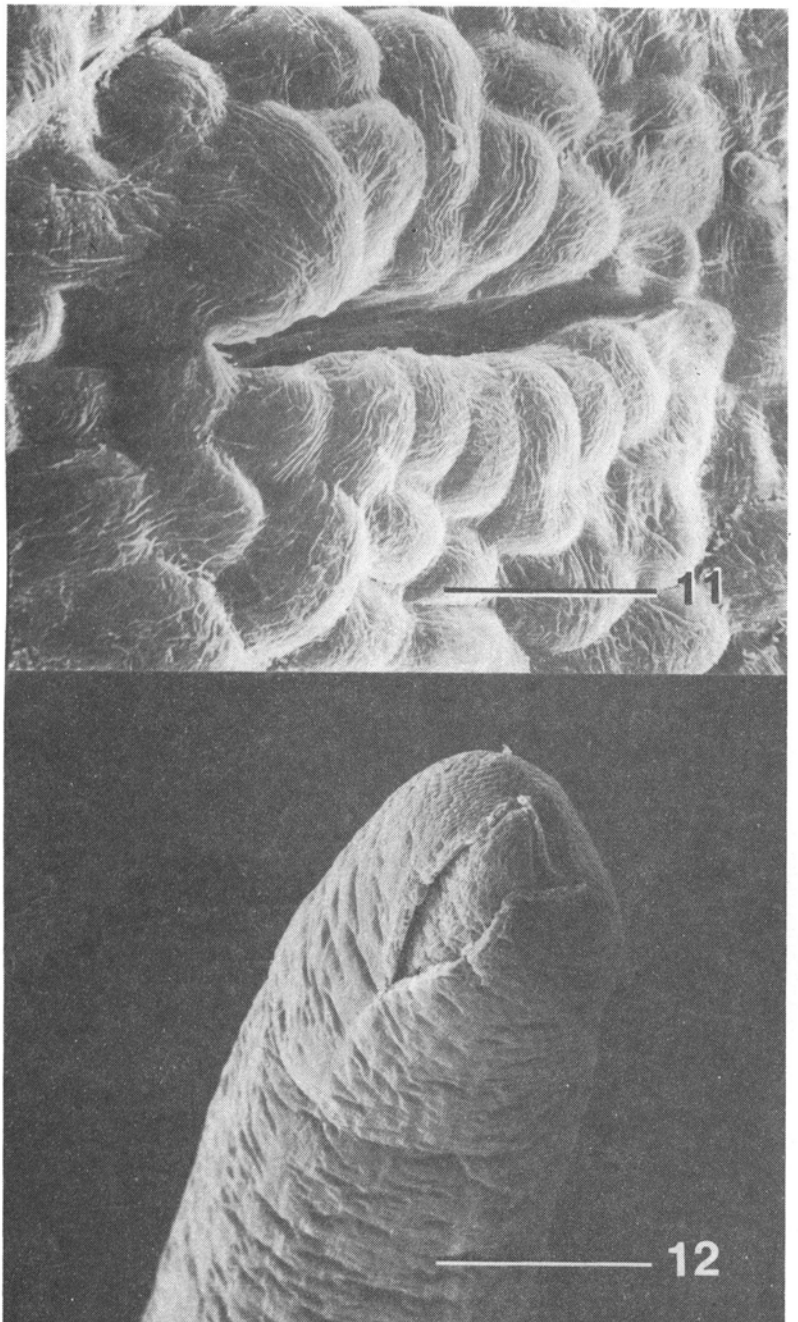
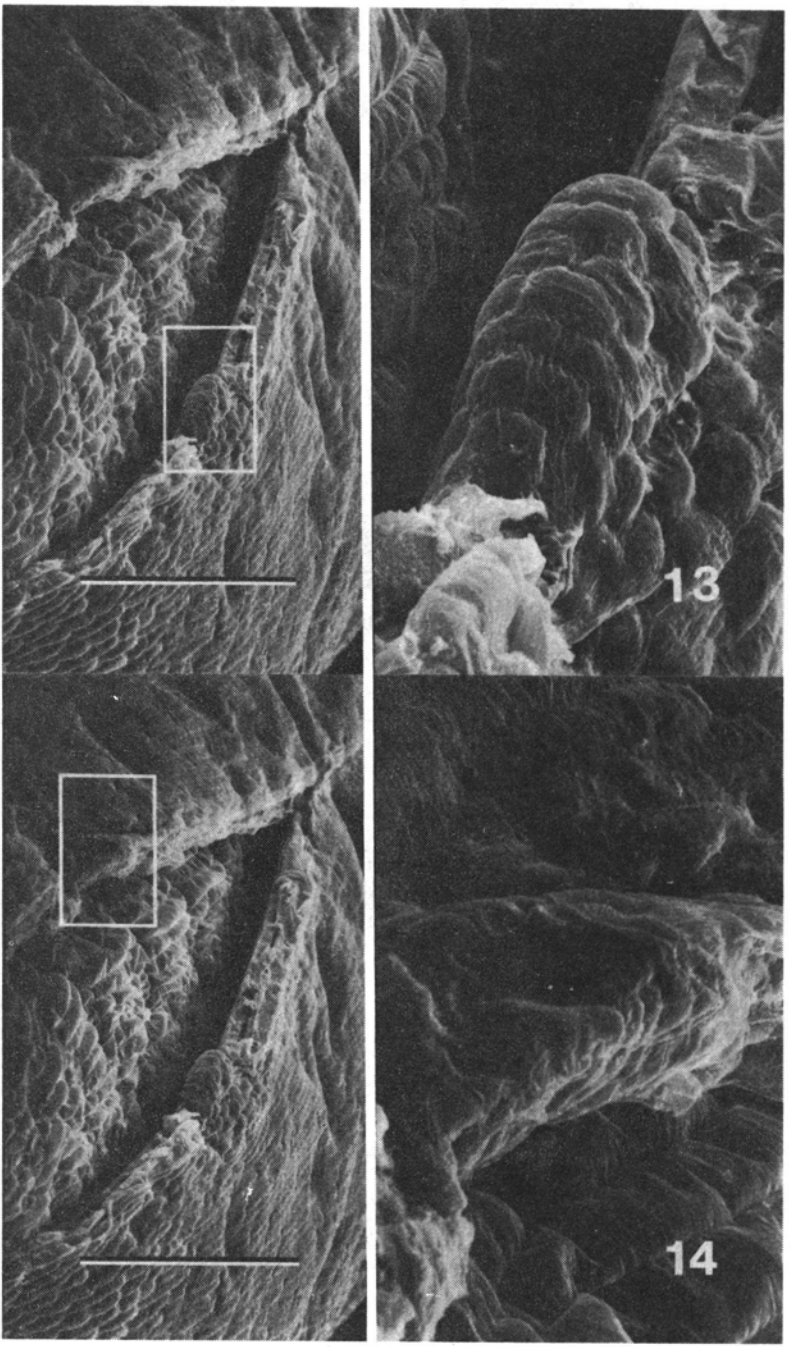


Fig. 11. View looking down into the coleoptile pore (96 hr after planting). Scale bar: 27 μm. Fig. 12. Early stage of leaf emergence viewed form the apical end of the coleoptile (about 150 hr after planting). The two masses of cells that once formed the boundaries of the pore are widely separated. A V-shaped split has developed toward the base and the leaf can be seen emerging anterior to the pore. Scale bar: 0.60 mm

PLATE VII



Figs. 13 and 14. Low and higher magnification views of cells that once bordered the coleoptile pore. Scale bars: 200 μm

PLATE VIII

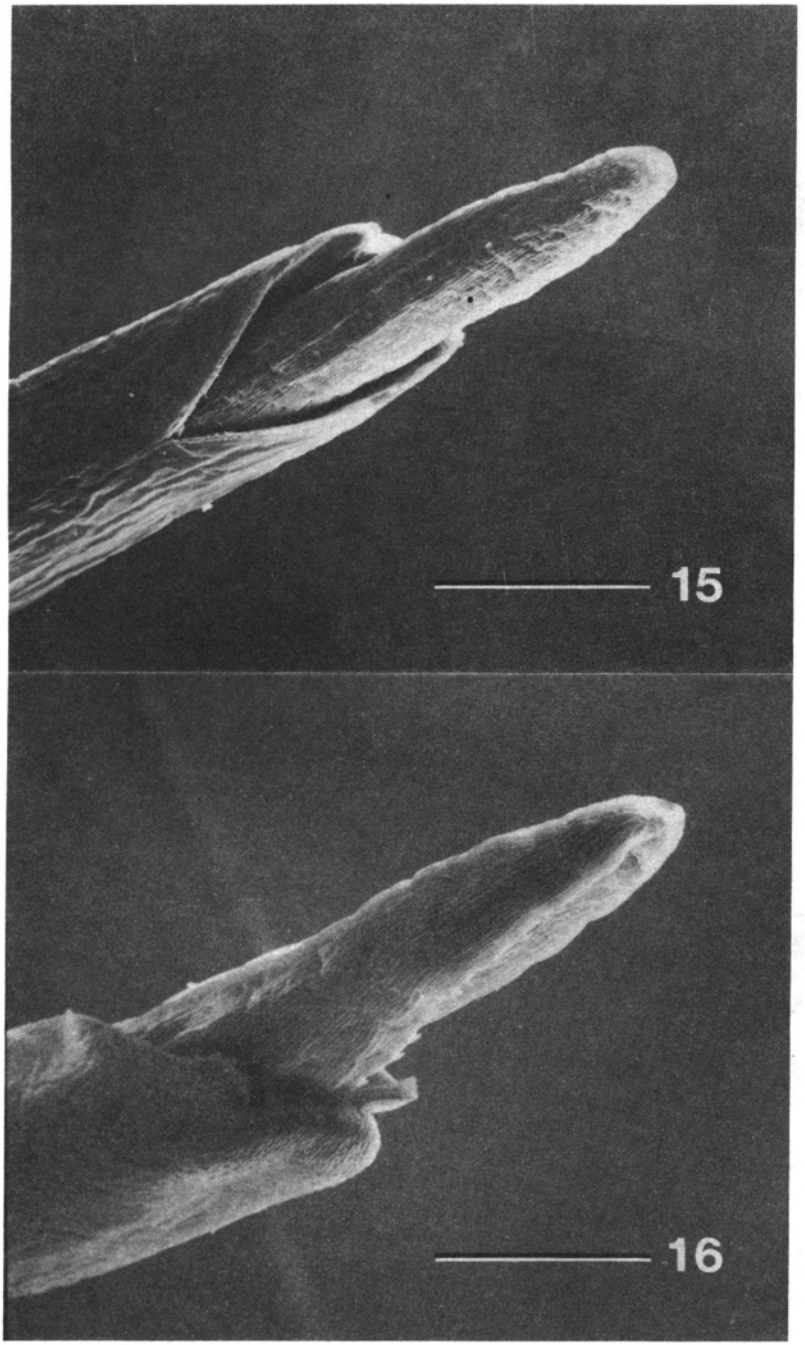


Fig. 15. Leaf has emerged and the collar-like nature of the apical part of the coleoptile is evident. View of the anterior face. Scale bar: 0.86 mm. Fig. 16. Lateral view (left side) of the emergent leaf and collar-like coleoptile. Scale bar: 0.55 mm

PLATE IX

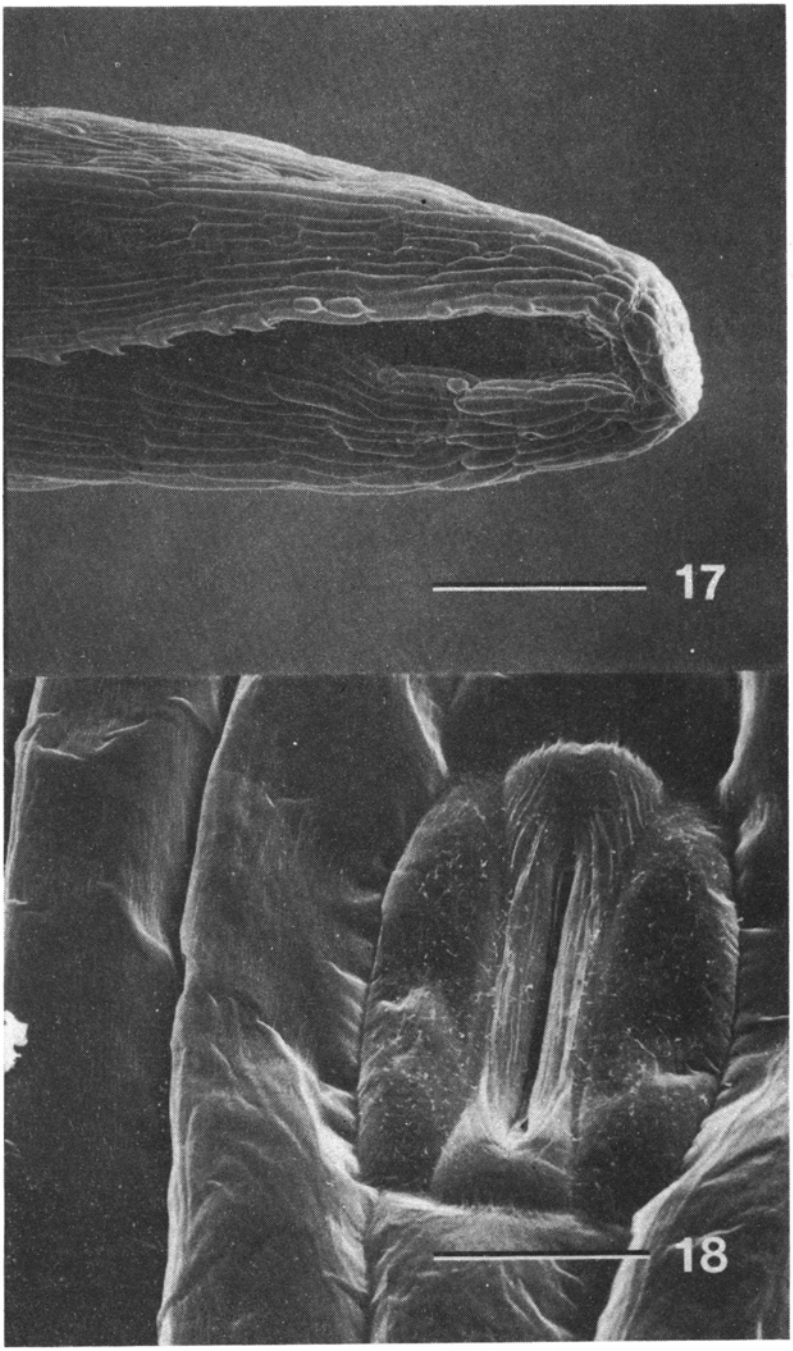


Fig. 17. Emerged leaf showing the rolled appearance and serrated margin. Scale bar: 250 μm. Fig. 18. Stomata of leaf showing bordering cells. Scale bar: 13.6 μm

nifications. At the apical end, Figs. 15 and 16, a collar-like arrangement is formed.

Figures 17 and 18 illustrate the nature of the leaf as it first emerges from the coleoptile sheath. The leaf emerges from the collar-like opening in a rolled condition (Figs. 15-17) much as what happens when one rolls the tongue while it passes through the lips. We include Fig. 18 in order to provide an illustration of stomata and guard cells of the primary leaf.

DISCUSSION

The coleoptile is generally regarded as a sheath which encloses the epicotyl and that elongates to the soil surface, forming a smooth passageway for the growth of the shoot (Raven et al. 1981, Mauseth 1988). It is an organ present in all grass embryos (Brown 1960). Histological studies (Bonnett 1961) have shown that in *Avena* the coleoptile originates from periclinal divisions in the protodermal layer of the proembryo. From its point of initiation, the coleoptile primordium develops to enclose the shoot apex in a circular ridge of tissue. At this early stage of development a pore is apparent and remains throughout coleoptile maturation (C.P., Figs. 10 and 11). Initial growth and development of the coleoptile results from cell divison. However, after germination coleoptile elongation results from cellular elongation and not from cell division (Bonnett 1961).

The vascular system of the *Avena* coleoptile is composed of 2 small laterally placed vascular bundles (Avery 1930, Esau 1965). The location of these vascular extensions conincide with the location of the stomata (hydathodal pores) seen in Figs. 1-4 which extend along the lateral surfaces and arch across the apex. The location of the vascular tissue (inferred from the disposition of the stomata) may well influence the nature of the splitting of the C.P. due to the emergence of the primary leaf and resulting in the collar-like arrangement seen in Figs. 15 and 16.

Butterfass (1956) employed fluorescent microscopy to study coleoptile stomata. He described the morphology of these stomata to be circular (open) or short slits (closed). In this study we have found the configuration of coleoptile stomata to be markedly variable. The morphologies described by Figs. 5-9 demonstrate a variety of stomatal and guard cell shapes, varying from approximately circular in apical regions to highly elongate in basal portions. These alterations in morphologies may be due to physical forces exerted by surrounding elongated epidermal cells in basal regions of the coleoptile.

Further studies by Brown and Johnson (1962) employing transmission electron microscopy indicated that the plastids of *Avena* guard cells are poorly developed. The surface data obtained in this SEM study do not allow us to corroborate these findings (Figs. 5-9, 17 and 18).

Bonnett (1961) noted that the cells bounding and adjacent to the C.P. differ from the other cells of the coleoptile in that they are irregular in shape and size. Figures 13 and 14 illustrate that the cells adjacent to the C.P. have different morphologies from the surrounding cells. The cells which make up the 2 to 3 layers bounding the C.P. on either side appear as elipses or oblate spheroids when visualized in SEM preparations.

Lastly, our initial studies on the effects of p-FPA on changes in coleoptile structure revealed no obvious differences between coleoptile apices treated with 5 mM p-FPA and control segments (Galanti 1985). Maksymowych and Orkwiszewski (1987) have presented presumptive evidence that p-FPA lowers auxin oxidase activity in *Avena* coleoptiles resulting in increased endogenous levels of indole-3-acetic acid (Maksymowych and Orkwiszewski 1988). Bergfeld et al. (1987) presented SEM figures which demonstrated that treatment of *Zea mays* L. coleoptiles with indole-3-acetic acid resulted in a change in the frequency and orientation of the cross-ribs of the outer epidermal wall. Clearly studies on the mechanics of p-FPA action warrant further investigation.

REFERENCES

Avery G. S., 1930. Comparative anatomy and morphology of embryos and seedlings of maize, oats and wheat. Bot. Gaz. 89: 1-39.

Bergfeld R., Speth V., Schopfer P., 1987. Reorientation of microfibrils and microtubules at the outer epidermal wall of maize coleoptiles during auxin-mediated growth. Bot. Acta 101: 31-41.

Bonnett O. T., 1961. The oat plant: its histology and development. Univ. Illinois Agri. Exp. Sta. Bull. 672: 96-112.

Brown W. V., 1960. The morphology of the grass embryo. Phytomorphology 10: 215-223.

Brown W. V., Johnson Sr.C., 1962. The fine structure of the grass guard cell. Amer. J. Bot. 40: 110-115.

Butterfass Th., 1956. Fluoroskopische Untersuchungen uber Anatomie und Funktion von Koleoptilen. Deut. Bot. Gesell. Ber. 69: 245-254.

Esau K., 1965. Plant anatomy. 2nd ed. John Wiley and Sons, Inc., New York-London-Sydney. pp. 613-614.

Galanti M., 1985. The effects of phenylalanine and halogenated phenylalanine analogues on *Avena* extension growth and phenolic metabolism. M.S. Thesis, Villanova University, Villanova, Pa.

Hopkins W. G., Bonnell K. F., 1969. *Avena* coleoptile elongation: stimulation by fluorophenylalanine. Plant Physiol. 44: 281-286.

Hopkins W. G., Orkwiszewski J. A. J., 1971. Inhibition of phenylalanine ammonia-lyase and enhancement of *Avena* coleoptile segment elongation by fluorophenylalanine. Can. J. Bot. 49: 129-135.

Maksymowych A. B., Orkwiszewski J. A. J., 1983. Specificity of fluorophenylalanine induced *Avena* coleoptile elongation. Amer. J. Bot. 70 (part 2): 88.

Maksymowych A. B., Orkwiszewski J. A. J., 1987. Studies of fluorophenylalanine induced *Avena* coleoptile elongation: possible modulation of auxin oxidase activity. Acta Soc. Bot. Pol. 56: 325-336.

Maksymowych A. B., Orkwiszewski J. A. J., 1988. Effect of fluorophenylalanine on indole-3-acetic acid levels in *Avena* coleoptiles. Acta Soc. Bot. Pol. 57: 499-505.

Mauseth J. D., 1988. Plant anatomy. The Benjamin/Cummings Pub. Co., Inc., Menlo Park, Ca., pp. 430-431.

Orkwiszewski J. A. J., Hopkins W. G., 1974. Effect of fluorophenylalanine on L-phenylalanine ammonia-lyase activity in *Avena* coleoptiles. Amer. J. Bot. 61: 433-436.

Orkwiszewski J. A. J., Koroly M. J., DiMichele L., Weber D. M., 1976. Similarities between L-phenylalanine and m-fluorophenylalanine as substrates for L-phenylalanine ammonia-lyase. Physiol. Plant. 41: 157-164.

Raven P. H., Evert R. F., Curtis H., 1981. Biology of plants. 3rd ed. Worth Pub. Inc., New York, N.Y., pp. 409-414.

Badanie w elekronowym mikroskopie skaningowym koleoptyli owsa podczas rozwoju pierwszego liścia

Streszczenie

Z badań w skaningowym mikroskopie elektronowym wynika, że jest związek między strukturą powierzchni koleoptyla a wyłanianiem się pierwszego liścia u *Avena sativa*, L. cv. Clintford. Szparki znajdują się na bokach koleoptyla i na łuku wierzchołka. Tak rozmieszczone szparki i związana z nimi tkanka waskularna może mieć znaczenie przy tworzeniu się szczeliny, przez którą wyrasta pierwszy liść. W koleoptylu opisano strukturę powierzchni szczeliny, komórek szparkowych i przyszparkowych.