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Growth and development of shoot apex in barley
I. Morphology and histology of shoot apex in vegetative phase

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Abstract

The vegetative phase of development of the main shoot apex lasts over 5 plastochrons after germination. The endosperm has a sufficient store of nutrition for this period. At the beginning of this phase the apex has a one-layer tunica. The cells of the latter divide above the level of bulge formation for leaf primordia, exclusively anticlinally, although somewhat lower within the leaf bulge periclinal divisions may occur. The cells immediately under the first tunica layer in the apical part grow tangentially to the surface. These cells divide only anticlinally forming gradually the second tunica layer. In the course of the entire phase the shape of the meristematic caulis from the tip to the 4th frustum remains unchanged.

INTRODUCTION

Investigations on mutagenesis and mutagenic barley breeding require a good knowledge of the organisation and development of the shoot apex in this species. The geneticist and breeder ought to know what is going on in mutated cells of the germ. The chances of mutation occurrence in the progeny of plants treated with mutagens at the germ stage depend on the fate of cell clones formed from various meristem cells. These in turn are dependent on the cell organisation and distribution of growth rate in the shoot apex. Knowing the distribution of the growth rate, we can estimate the drift of the clones (Hejnowicz, 1980 a; Hejnowicz, Nakielski, 1979).

In the first and second part in this series we deal with the shoot apex from the germination stage to initiation of the ear primordium, the third part will concern the shoot apex in the period of ear initiation,

the fourth answers the question which part of the mature shoot is formed from a particular part of the embryo shoot apex, the fifth will deal with formation of lateral shoot apices and the sixth with shoot initiation in embryogenesis.

The apex is considered as the upper part of the meristematic shoot consisting of an apical dome and shoot segments lying above the axil of the youngest leaf to overtop the apical dome. The apical dome is considered as the part above the axil of the youngest discernible primordium. Its basis is an imaginary cup-shaped surface intersecting both the dome surface and its axis orthogonally. Within the apex we distinguish the leaf primordia and the caulis. The latter is the axial part of the meristematic shoot consisting of the apical dome and the meristematic stem, the surfaces of which pass through the axils of leaf primordia. It units, below the apical dome, are frusta. Each frustum consists of the part of caulis from the insertion of the leaf primordium to the insertion of the next primordium below. The term plastochron is used in the sense of the temporal interval between the initiation of successive foliar primordia with no relation to the intervals in unfolding of the successive leaves which in barley seedlings are of much longer duration.

MATERIAL AND METHODS

The investigations concerned the barley variety 'Damazy'. The seeds were supplied by the Plant Breeding Station Łagiewniki from the 1976 and 1977 harvests. The seeds were soaked for 24 h at room temperature in well oxygenated water changed several times in this period. They were then placed on soil or perlite with which the containers were filled and covered with a 0.5-cm soil or perlite layer. The seedlings were grown in a controlled-environment room at 26°C and daylength 12 h and in the laboratory on a window sill at about 18°C in January and February and at about 22°C in September and October.

The apical part of the shoot comprising the apical dome and 3-4 youngest frusta was prepared out under a magnifying glass. For examination "*in toto*" the apical part of the shoot was placed on a slide in a drop of water so that the medial plane should be tangent to the glass surface. The slide was covered with a cover slip and photographed immediately with a × 63 objective. For anatomical studies the apical shoot part was fixed in 3 per cent glutaraldehyde — pH 7.2 for 8-h at room temperature and eventually stored in glutaraldehyde at 0°C. The apical parts of the shoot were treated individually taking note of the development stage of each of them. Dehydration was done in an acetone gradient and propylene oxide. The material was embedded in epon.

Semiultrathin (3 μm) sections were cut on an ultramicrotome. The sections were stained by the PAS method and with toluidine blue. They were photographed in a Docuval, Zeiss Jena microscope with a × 63 objective.

RESULTS

Noticeable differences were not found in the morphological development of the apex in different growth conditions, except for those in the rate of development which depends strongly on temperature. It should be noted that the whole phase of vegetative development of the apex occurs in the presence of the nutritional stores of the endosperm. The endosperm envelope containing the aleurone layer as a living part remains intact up to the beginning of the generative phase in the main apex.

Anatomical and cytological features of the apex in germinating seed

In the caryopse the meristematic part of the shoot consists of the coleoptile, primordia of 4 (sometimes 3) leaves distributed distichously and compactly on the short caulis ending in the apical dome. The medial plane is common to the leaves and passes through the caryopsis axis. Primordia of even leaves (numbered in the order of their diminishing age without coleoptile) lie on the scutellum side. The primordium of the first leaf is a relatively large hood adjacent to the inner epidermis of the coleoptile. Much smaller is the primordium of the second leaf, but it also is a hood covering the apex from the top. The primordium of the third leaf has a distinctly incised axil but does not reach to the top of the apical dome. The fourth leaf primordium is a bulge (1/2—2/3 of apical dome periphery) without an incised axil or with hardly marked incision (Fig. 1). We assume that initiation of the n-th primordium ends the n-th plastochron in the life of the apex. The apex in the caryopsis is thus usually in the 5th plastochron. If there are only three leaf primordia, the youngest one has usually an incised axil and the second one stands out above the tip of the apex. (In the embryo the youngest leaf overtopping the dome is the 2-nd one without counting the coleoptile). Mitoses appear within 24 h after seeding. It is difficult to prepare well stained and intact sections from unhydrated or poorly hydrated apices. However, the cellular organisation of a well hydrated embryo is undoubtedly the same as in the caryopse.

The apex of the germinating seedling is characterised by a one-layer tunica (Fig. 1). The underlying cells do not form a layer, but they are

distinctly connected with deeper lying cells, this leading to the conclusion that their mother cells divided before ripening of the seed both anticlinally and periclinally. The distal part of the apical dome is characterised by rather lighter-staining cells.

Formation of new primordia

New primordia appear as bulges on the sides of the apical dome, which before the stage of incised axill encompass about one half of the dome periphery. In formation of these bulges periclinal divisions take part, both in the subepidermal and surface cells (deriving from the 1-st layer of the tunica).

In the variety studied the eighth leaf is usually the flag leaf (sometimes it is the 9th or even the 10th). The leaf primordia with higher number such as 10 always belong to the ear. It is assumed that the transition to the generative phase occurs in the 9-10 plastochron.

The present paper thus deals with the apex in the course of 5 plastochrons elapsing from the moment of germination to the transition to the generative phase. These periods are: part of plastochron 5, and plastochrons 6, 7, 8, 9, 10. The number of the plastochron indicating the age of the apex is equal to that of the youngest leaf primordium on the apex plus one.

In the period of transition of the main shoot apex to the generative phase the seedling has two developed leaves and the nutrition store in the endosperm is almost exhausted.

When the 9th leaf is formed, the youngest leaf overtopping the dome is the 4th one. Thus, there is a significant increase of the apex size during the development.

Shape and dimensions of the apical dome and the caulis

During development of the seedling the apical dome while growing preserves its shape in its upper part (Fig. 2). This shape is not more variable in seeds of different ages than in even-aged ones. On the other hand, the dome height changes — it increases gradually during the plastochron and drastically decreases at the moment of transition from one plastochron to the next. This cyclic change in the height of the apical dome is superposed on the gradual increase of the dome height.

The shape of the caulis (Fig. 3) differs in the embryo and in the part formed after germination, but it is almost unchangeable in the latter part during its development. In the embryonal part, namely, the thickness of the successive frusta (from the apex) is distinctly larger and larger, whereas the caulis formed after germination is, below the young-

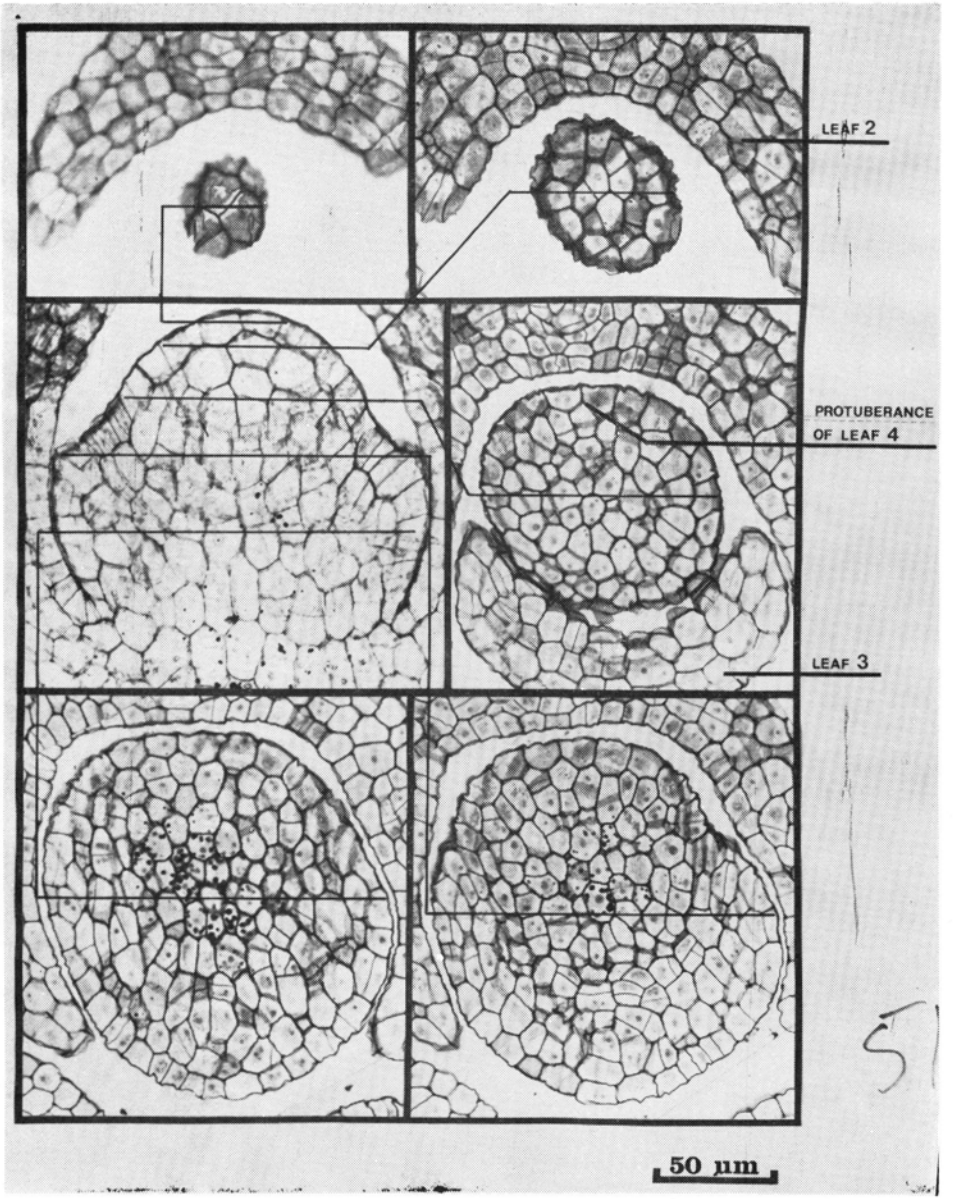

Fig. 1. Series of cross sections from various apex levels at beginning of germination and longitudinal sections in plane perpendicular to medial plane on which the levels of the cross sections are marked. Periclinally divided cells visible in leaf primordia

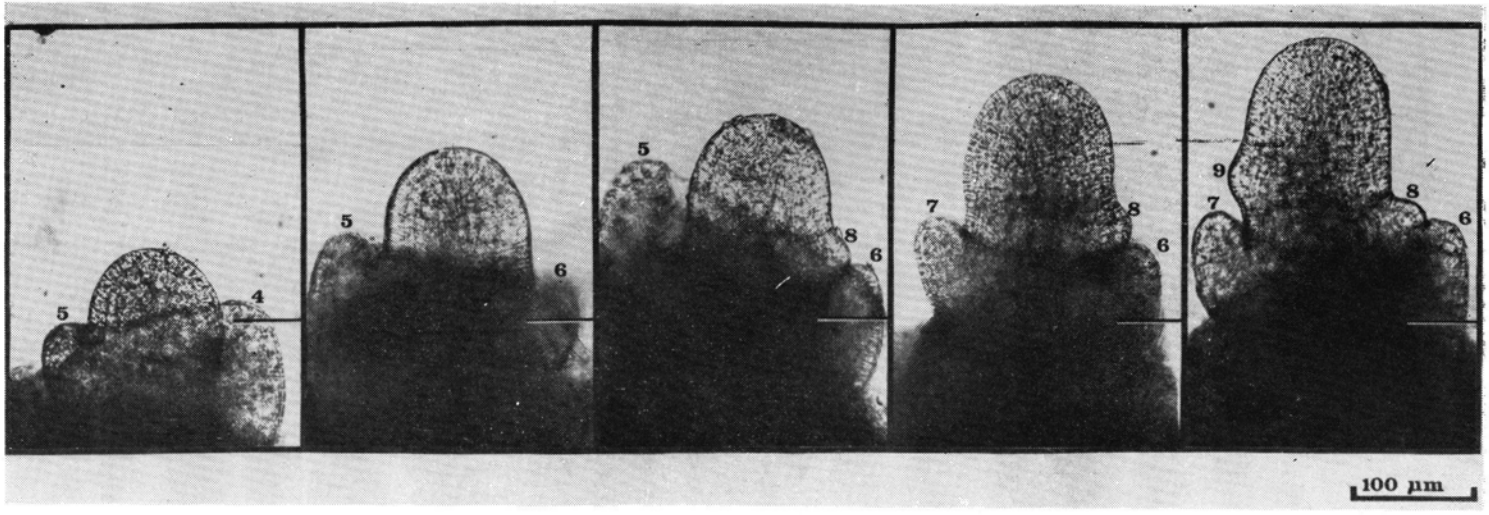

Fig. 2. Developmental series of barley shoot apex. Figures at primordia denote their serial number. Apices are so arranged that the axilis of the 6th leaves (no. 6) are at the same level

est frustum, of the same thickness at various levels. Thickening of the caulis occurs in this case as late as the 5th frustum; that is the part lying at the base of the apex.

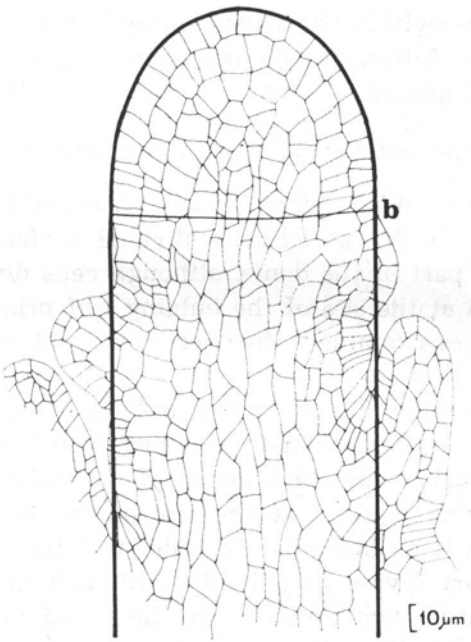

Fig. 3. Shape of caulis (bold line) in apex with initiated 9th primordium b — base of apical dome

We must now examine the caulis composed of the apical dome and the frusta which formed after germination. The average shape of this caulis is shown by the bold line in Fig. 3. Three-dimensionally the caulis is a figure of revolution. Its part below the first metamere is almost cylindrical, that is the frusta of the caulis do not grow in thickness. As mentioned, in the course of development the size of the apical dome increases this consisting in an increase in its height within the shape of the caulis, owing to the moving away of the leaf primordium formation level from the top. In the embryo starting to germinate this level lies in the vaulted part of the caulis, therefore the base of the apical dome is then distinctly cup-shaped. As this level goes down, the base of the apical dome becomes flatter (line b in Fig. 3).

The frusta initiated after germination remain short up to the transition to the generative phase and are contained within the apex, whereas within the frusta initiated in the embryo, internode development progresses in an order beginnig from the lowest one belonging to the coleoptile. The internode is initiated by transverse divisions of cells located in the basal part of the frustum. It is recognisable by the longi-

tudinal files of cells, each originating from a single cell by repeated cell divisions and by more dense starch grains (Fig. 4).

It should be noted that these internodes initiated in the vegetative phase of the apex do not reach such lengths as do those developing in the generative phase within the frusta formed after germination. In the latter, however, the internodes do not start to grow in the vegetative phase of apex development.

Anatomical and cytological features in growing shoot apex

In the apical dome of all apices a distinct surface layer of tunica was found (Figs 5 and 6). No periclinally divided surface cells were ever noted in the apical part of the dome, although cells dividing in this way are frequently seen at the site of the bulging leaf primordium. Although the number of apices examined after sectioning did not exceed forty, it should be stressed that the first layer of the tunica is well visible also on optic sections through living apices, and hundreds such apices have been examined. If cells periclinally divided would occur in the first tunica, in the top part of the apical dome, they would have been recorded as they can be recognized at the site of the forming leaf bulges. Neither do the cells lying immediately under the first layer of the tunica in the periaxial part divide periclinally, although in apices of age up to the 6th plastochron such division may be found in hypodermal cells on the sides of the apical dome.

Cells underlying immediately the first tunica layer in the periaxial part of the germinating seedling grow slowly, tangentially to the surface, dividing anticlinally. They develop gradually into the second layer of the tunica (Figs 6, 7, 8). Thus, they are initials of the 2nd tunica layer. They can be distinguished by their larger dimensions and poorer staining. Of similar character are the cells of the 2nd tunica layer developing from them. It may be added here in anticipation of further investigations that the initials of the 2nd tunica layer existing in germinating seed are at the same time cells initiating the generative lineage. Cells of this lineage grow in the vegetative phase relatively slow, they divide only anticlinally and the clone formed from them hardly reaches the base of the apical dome at the end of this phase.

The cells lying under the initials of the 2nd tunica layer, that is the initials of the corpus divide both anti- and periclinally. From these cells ribs running down the apex are derived.

DISCUSSION

Bonnet (1966) reports that the barley seed embryo contains primordia of 4 leaves beside the coleoptile and that the vegetative phase of main shoot apex development lasts to the end of growth of the second

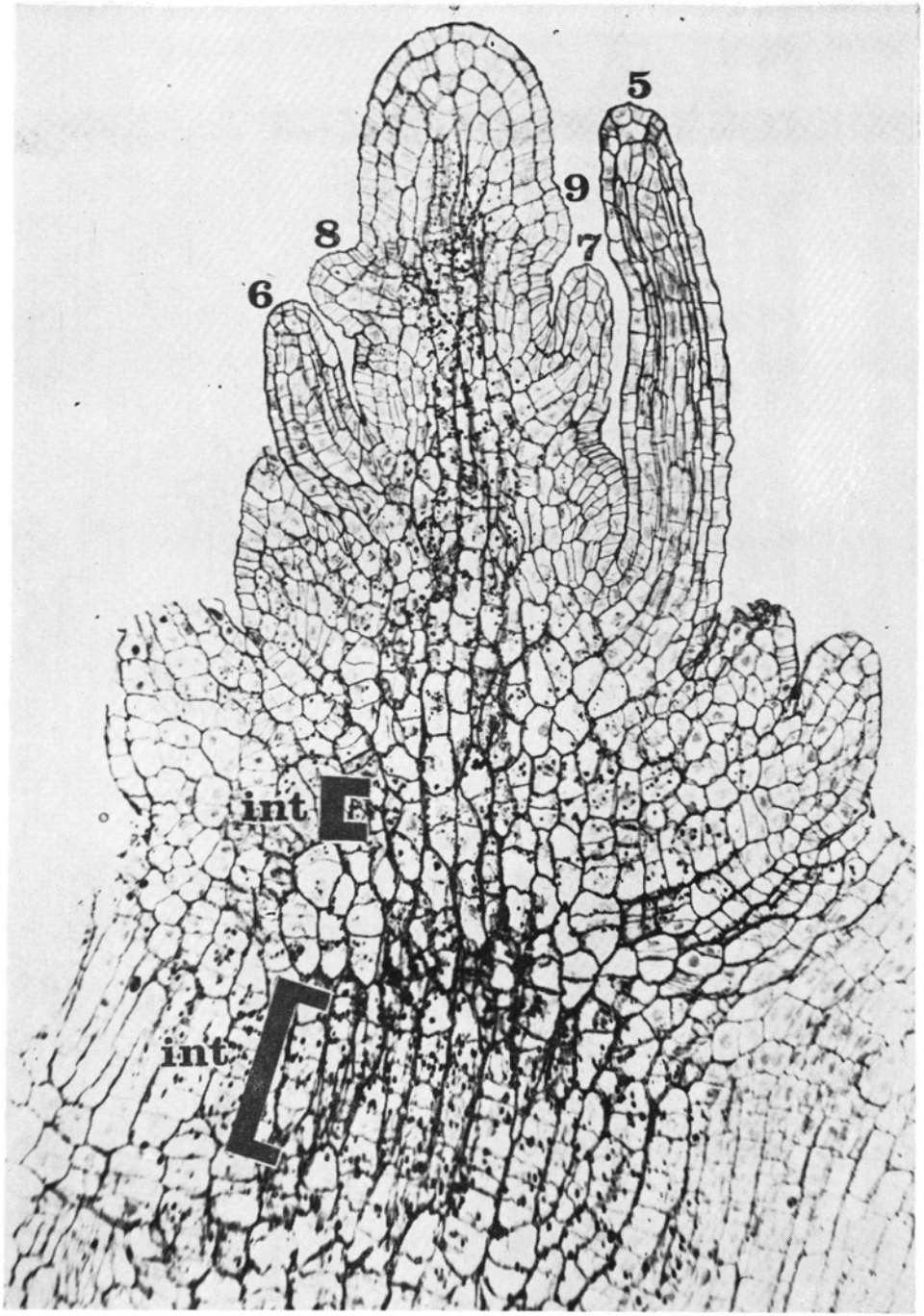

Fig. 4. Longitudinal section through apex in medial plane at plastochron 10 stage. Figures at primordia denote their number. Int — initiated internodes in frusta 4 and 3

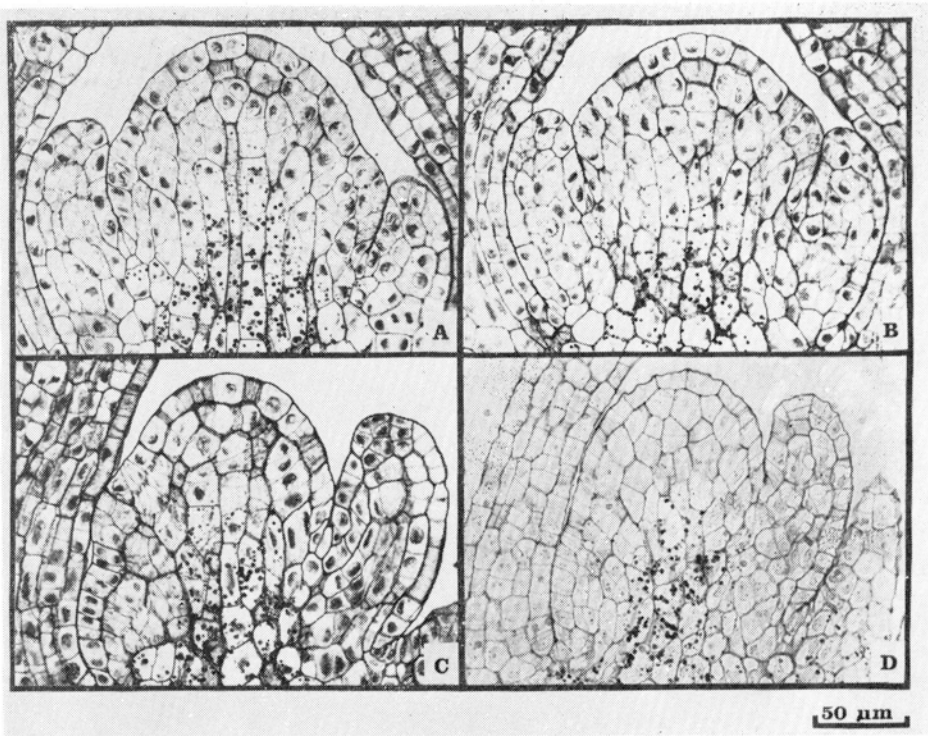

Fig. 5. Longitudinal section through apices 48 h after beginning of soaking in medial plane — C, D, and in plane perpendicular to it — A, B. One-layer tunica visible

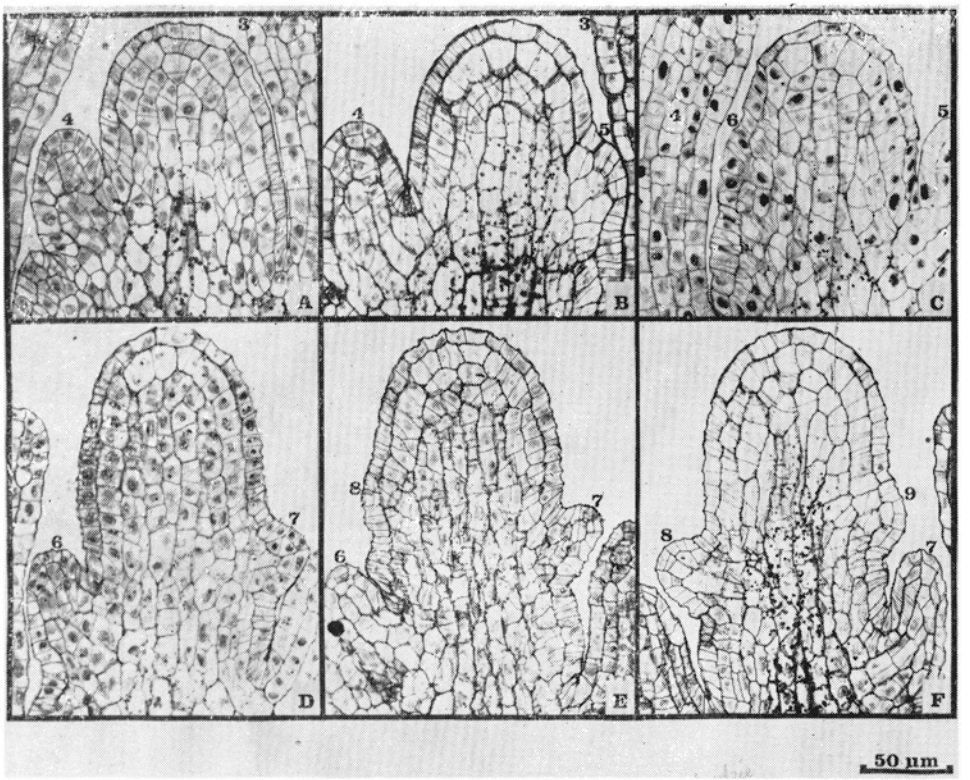

Fig. 6. Central cross sections through apex in medial or almost medial plane in various stages of vegetative phase of development of apex, beginning with end of plastochron 5 (A) to beginning of plastochron 10 (F). Figures at primordia indicate their number. Development of second tunica layer is noticable

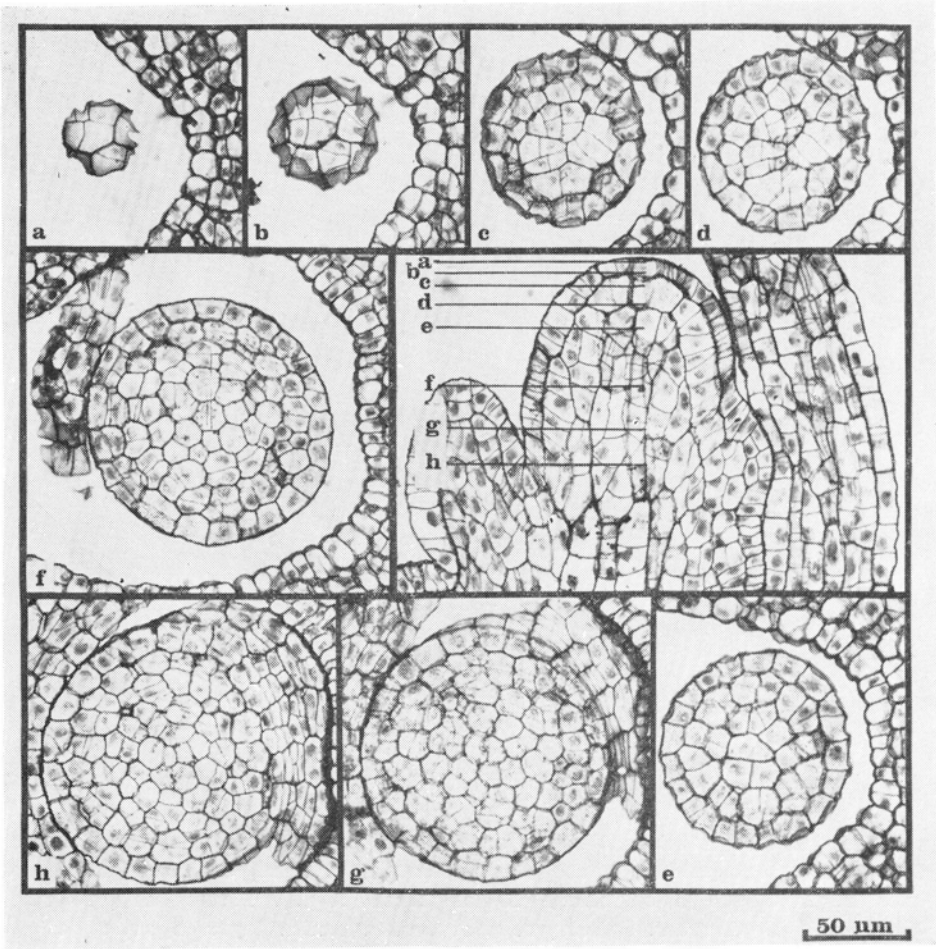

Fig. 7. Series of cross sections at various levels through apex at mid plastochron 6. Periclinally divided surface cells in primordium visible. Leaf overtopping apical dome is no. 3

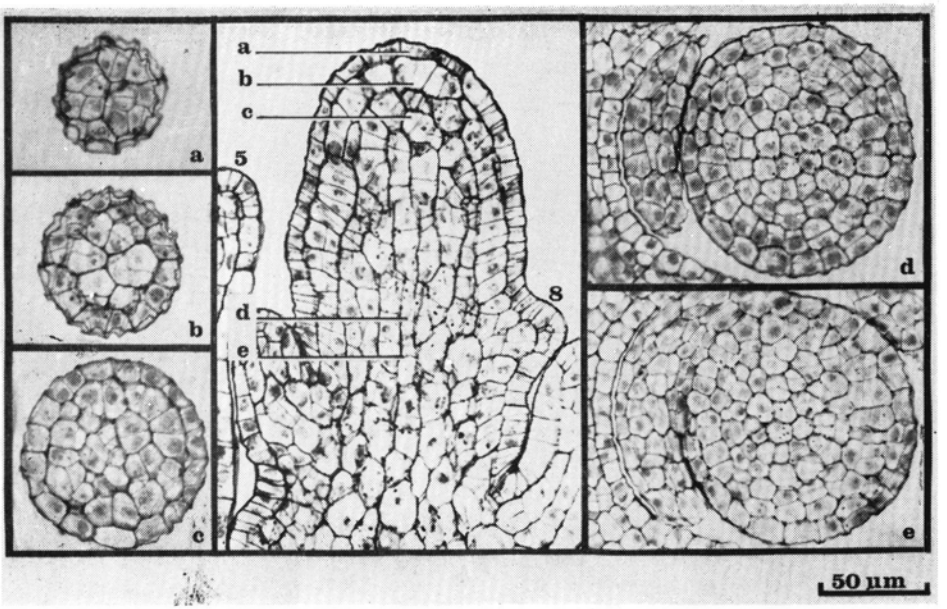

Fig. 8. Series of cross sections at various levels through apex at stage of 9th leaf primordium formation

leaf of the seedling, when the apex passes to the generative phase in which the ear is initiated. The existence of primordia for 4 leaves in the seed has also been reported by Jacobsen (1966) on crossections across the embryo. Investigations of this author on mutated sectors in different-rowed ears in plants developing from seeds treated with a chemical mutagen demonstrated that in the embryo of a ripe barley seed there are at least 6 independent meristematic groups. From these groups take origin the ear of the main shoot and ears of five lateral shoots (each independently). The mutational independence of these ears is closely connected with the existence of primordia of four leaves + + coleoptile in the seed, as already mentioned, since the apices of the lateral shoots form from cells lying above the leaf axil. The mutational independence of the meristem groups of the main and lateral ears was confirmed by Nishimura and Kurakami (1952) for rice.

The relatively large number of leaf primordia in the embryo of ripe seed is not a special character of barley, it is also noted in other grasses. Wheat has 3-leaf primordia (Williams, 1975), maize six (Stefffensen, 1968), according to Abbe and Phinney (1951) five or six.

Different developmental and growth responses of the apex in barley to variations in daylength or light intensity were observed by Aspinall nad Paleg (1963). Shading of the first leaf, which strongly affects the appearance of tillers and thus reduces over-all yield of grain, only slightly affects the course of early development of the main shoot, delaying slightly the initiation of the 8th and subsequent leaves (Dale et al., 1972). It seems, therefore, that the morphological development of the shoot apex in barley seedlings is homeostatic, except for the rate of development, the more so since the nutrition store in the endosperm is only exhausted after transition to the generative phase.

It is a common opinion that the apical dome undergoes changes in form and size (see Kaufman, 1959), however, it is not precisely defined what size means. Usually it is characterised by the height and diameter. It should be kept in mind that the apical dome is an open system and the change of height and width solely does not signify a change of shape of the system. For instance the portion of the paraboloid on the vertex side of the plane perpendicular to the axis will change the height and diameter in dependence on the position of the plane, but we can consider that the shape is the same as long as we deal with the same paraboloid. In this sense the shape of the apical dome in *Hordeum* is constant during the postgermination phase of development. Such a constancy of shape was observed by Rösler (1928) for *Triticum*. A constant shape of the dome with changing ratio of height/diameter was observed in *Agropyron repens* by Rogan and

Smith (1974) during the interval of plastochron 4-6 and a small change in shape with constant ratio in the interval 6-10. Obvious changes in the shape of the apical dome in seedling development were observed by Abbe and Phinney (1951) for *Zea* and Kaufman (1959) for *Oryza*. If the dome shape or that of the caulis is constant, we may consider the mean rate of dome growth as the rate of flux of substance produced within the dome through an imaginary transverse surface. If the shape changes, usually the rate of this change is very small as compared with the rate of the basal flux, and the former approach will still give a good approximation of the growth rate. Thus, from the point of view of mean rate of dome growth, or even of the growth rate distribution within the dome (Hejnowicz, 1980), one should rather try to find the approximate shape of the dome than stress its change. Thus, we stress the constancy of the caulis shape in the postgermination phase in barley, having in mind our study of the distribution of growth rate of the caulis (Hejnowicz, Włoch, 1980).

The shoot apices in angiosperms are characterised by the tunica--corpus organization so the chances of finding a way to the generative lineage are available solely to cells lying under the first layer of the tunica. As regards grasses, this condition, may, however, not be so strict, because periclinal divisions may occur in the shoot apices of these plants in the surface cells in the top part of the apex. The sporadic occurrence of periclinally divided surface cells in the top part of the apex has been observed by Sharman (1942) in *Zea*, Rösler (1928) in *Triticum*, Kleim (1937) in *Avena*. As a rule, however, a one-layer tunica has been found in grass apices, among others in *Triticum, Secale, Avena, Zea, Oryza* (Rösler, 1928; Kleim, 1937; Sharman, 1942; Kaufman, 1959). Rogan and Smith (1974) established that in *Agropyron repens* "a one-layered tunica may be distinguishable, but in many apices it is not clearly delimited from the corpus, particularly at the summit" up to the 6th plastochron, then, between plastochron 6 and 7 stratification takes place within the dome and generally a two-layered tunica is present in plastochron 8.

In some grasses the organisation of the shoot apex, as regards delimitation of the tunica is labile, for instance in *Erianthus* many apices have no tunica (Thielke, 1964a). In *Saccharum* the tunica is often absent in lateral apices, and a two-layer tunica develops in the course of further development (Thielke, 1964b). The barley apical shoot has a distinct surface layer of tunica from the embryo stage. During development of the apex after germination a second layer of tunica forms, namely, the cells lying in the apical part immediately under the first layer do not grow in anticlinal direction (do not divide periclinally),

but grow slowly in periclinal direction in agreement with the growth of the surface of the deeper lying part which corresponds to all the criteria for the corpus, that is they grow like initials of the second tunica layer. Consequently, the barley shoot apex enters the generative phase with a well developed, even cytologically distinct second tunica layer, although it does not have his layer at the beginning of germination. It would seem that it is not so much the existence of the ready tunica layers at the given moment that is important but the way of growth of the apex, leading to the development or preservation of these layers. From the initial cells of the 2nd tunica layer meiospores arise, thus they are cells of the generative lineage. The development of a second tunica layer indicates that there occur along this lineage only anticlinal divisions since the time of seed germination. Our investigations (Hejnowicz, Włoch, 1980) indicate that the top part of the apex grows relatively slow. The initials of the second tunica layer present there divide more rarely because as they grow they do not need to divide pariclinally. There is no doubt that, owing to the limitation of divisions to anticlinal ones and owing to the slow growth of the top of the apex, the number of cell divisions along the generative lineage in the vegetative phase is much smaller than it would be without this limitation and the reduction of growth rate in the apical part. At the same time the cells of this lineage are isolated from immediate contact with the outside by the first layer of the tunica.

The organisation of the apex of the type: two-layer tunica + corpus and the slow growth in the apical part seem to have a deep biological meaning from the viewpoint of protection of the cells of the generative lineage from changes in genetic information (Hejnowicz, 1980).

Cytohistological zonation in the apical zones — the occurrence of lightly staining cells with a lower number of thin recently formed partitions in the distal part of the apical dome as compared with the rib-shaped groups of more intensely staining cells with a higher number of thin newly formed partitions — seems to be related to the pattern of growth rates described in the next paper (Hejnowicz, Włoch, 1980), which is characterized by a much slower rate of growth of the distal part of the apical dome than in the proximal part. Such a pattern may well be the main system maintaining the cytohistological zonation and cell pattern in the shoot apex. Such a possibility is indicated by several authors (Johnson, 1951; Wardlaw, 1953; Green, 1976). This has not, however, been empirically tested since detailed investigations on the cytohistological zonality were not conducted jointly with studies on growth distribution in the shoot apex.

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Wzrost i rozwój wierzchołka pędu u jęczmienia. I. Morfologia i histologia wierzchołka pędu w fazie wegetatywnej

Streszczenie

Wegetatywna faza rozwoju wierzchołka głównego pędu trwa przez 5 plastochronów od kiełkowania. W ciągu tego okresu istnieją zapasy pokarmowe bielma. Na początku tej fazy wierzchołek ma jednowarstwową tunikę. Jej komórki dzielą się powyżej poziomu tworzenia uwypukleń dla zawiązków liści wyłącznie antyklinalnie, choć nieco wyżej w obrębie uwypuklenia liściowego mogą występować podziały peryklinalne. Komórki znajdujące się bezpośrednio pod pierwszą warstwą tuniki w części szczytowej rozrastają się stycznie do powierzchni. Dzielą się tylko antyklinalnie wykształcając stopniowo drugą warstwę tuniki. W ciągu całej fazy merystematyczny kaulis od szczytu wierzchołka do metameru obejmującego 4 liść zachowuje stały kształt.