HISTOCHEMICAL STUDIES OF THE SHOOT APEX IN BRASSICA CHINENSIS CULTIVAR TO-PE-TSAI[1]

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Abstract

RNA was observed uniformly in the cells of early stage apical meristem before morphological zonation. In adult vegetative apices, however, the central axis of leaf primordia and peripheral zone showed slightly higher concentration. In reproductive apices, pyroninophilia increased in corpus cytoplasms as well as nucleoli. The latters are exceptionally large in size. Peroxidase was present at the tip of leaf primordia, outer tunica layers and procambium during vegetative stage. When bolting begin, strong peroxidase activity was observed in elongating pith cells. Succinic dehydrogenase (SD) was higher in leaf primordia and peripheral zone compared to axial zone during vegetative stage. In reproductive apices, however, SD was strongest in corpus. AP was found only in the first and second tunica layers of a vegetative apex and pith tissue. The enzyme disappeared from the tunica layers as reproductive growth proceeds. Ascorbic acid was present in the tip of leaf primordia, peripheral zone and procambium of shoot apices. During transitional stage of flower-bud differentiation, highest ascorbic acid concentration was observed in the flower initial. Much starch grains were present in pith cells of vegetative rosette stem, but they disappeared as bolting commenced.

Introduction

The auther has previously described histological changes of the domes in shoot apices of *Brassica chinensis* cultivar To-pe-tsai during development and flower-bud differentiation (Wang, 1969a). The histological studies revealed the changes of the number of tunica layers during development, the activation of the corpus before flower-bud differentiation and that the leaf primordium initials were derived from tunica whereas flower primordium initials from corpus. Also, it was noted that the original cells of leaf and flower initials were stained strong with hematoxylin.

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The nucleic acids, enzymes and other metabolic substances, because of their role in metablism are the most important and interesting substances in studing the development and differentiation of shoot apices.

This report describes an ontogenetic change in the differential staining pattern of RNA, DNA, succinic dehydrogenase activity, peroxidase activity, alkaline phosphatase activity, protein-bound sulfhydryl groups, ascorbic acid and starch grains in cells of the dome of shoot apex.

Material and methods

The general procedures of growing plants for the studies were outlined in an earlier report (Wang, 1969b).

Slightly modified Brachet's pyronine method was employed to identify RNA as follows: tissues were embedded in paraffin by freeze-substitution. The sections were deparaffinized, brought to water, transferred into a solution composed of 0.5 g of pyronine B and 100 ml of acetate buffer at pH 4.7 for an hour, washed in running water, the excessive water removed with filter papers and finally placed in tertiary butyl alcohol. The sections were thus over stained and then decolorized in a solution containing equal parts of ethyl alcohol and tertiary butyl alcohol until a fine contrast was obtained. The preparations were then passed through tertiary butyl alcohol and xylene and mounted with canada balsam. DNA was identified by Feulgen reaction (Jensen, 1962). Peroxidase (PO) and succinic dehydrogenase activities (SD) were detacted with benzidine and nitro-blue tetrazolium methods respectively, according to the proceadures published in a separate article (Wang, 1971). Alkaline phosphatase activity (AP) was detected with cobalt sulfide method (Ross, 1951). Identifications of protein-bound sulfhydryl groups (SH-P) were made with tetazolium reaction method (Gomori, 1956). Ascorbic acid (AA) was fixed and identified by acidified silver nitrate solution (Chayen, 1953). Detection of starch grain was relied on IKI reaction (Johnsen, 1940).

Result

Before the morphological zones were formed, RNA was detected uniformly in the cells of apical meristem. The older vegetative apex showed more developed tunica, corpus and organized zonations. At this stage, the central axis of the leaf primordium (lp) showed slightly higher affinity for pyronine. These cells would eventually develop into procambium. Slightly pyroninophilic cells at both sides of the axial zone (az) were the peripheral zone (pz), which would develop into leaf primordium. As the flower-bud differentiation commences, the apex becomes higher and wider. At this stage pyroninophilia

prevails in cytoplasm and nucleolus in the entire area of the apex, partcularly the cells at the inner part of the peripheral zone (Fig. 3 arrow). In a more developed apex, there is a definite increase in RNA concentration in the peripheral zone, especially at the inner side of the zone (Fig. 4). These RNA rich cells are suspected to be the initials of flower bud.

Feulgen reaction demonstrated the presence of large and deeply stained nuclei in the peripheral cells of both vegetative and reproductive apices. The large nuclei are present in the outer layer of peripheral zone, especially that of leaf primordia in vegetative bud. They are, however, positioned in the inner part of peripheral zone during transitional phase. The sites of these large and deeply stained nuclei coincided with those of RNA rich cells during the same stage.

Reduction of tetrazolium salt, which appears blue, was employed to demonstrate the presence of SH-P groups. SH-P distribution in apices was similar during vegetative and reproductive stages. In an apex the cells of first and second tunica layers and peripheral zone are rich in SH-P whereas the cells of procambium, both in the stem and leaves, were unexpectedly poor in it.

PO was demonstrated by the occurrence of benzidine blue coloration. During the vegetative phase, the tip and epidermis of leaf primordia possess apparent PO activities (Fig. 13). Outer axial and peripheral zones showed even higher activities. The activities of the same enzyme in leaf and subapical region were weak, whereas in the inner peripheral zone, corpus, and pith cells are negative. Wang (1969a) has previously reported that before the apex tend to reproductive stage, the stem, especially the pith cell elongation would occur at the subapical region. PO increased rapidly in the elongating pith cells before flower bud are recognizable. There were no visible changes of PO in the apical dome during transition from vegetative to reproductive stage.

SD was demonstrated by the occurrence of insoluble formazan in blue coloration in the presence of the tetrazolium compound. During vegetative stage, higher SD was observed in the cells of pith initial, peripheral zone, cells between leaf initial and axial zone, and procambium (Fig. 15). In axial zone, especially corpus and pith cells, SD were relatively inactive. At the transitional stage, SD showed weak activity, and at reproductive stage, it was strongest in corpus (Figs. 16, 17).

AP was demonstrated by occurence of black precipitates, cobalt sulfide. In vegeative apex, AP was localized in the first and second layers of tunica and pith cells. It is less active in the procambium cells. In reproductive stage, however, AP activities disappeared from the first and second tunica layers.

AA was demonstrated by the occurence of black or brown reduced fine-grain silver precipitates. During vegetative stage, AA distribution was similar to that of RNA. At the transitional stage, marked addition of AA was observed along the inner part of peripheral zone and axillary bud initials. When the first flower initial was formed, AA activity was richest in the cells along the inner part of flower initial where the cells were dividing or going to divide (Fig. 20). The bract primordium and initials of axillary bud showed somewhat higher in AA. Between the axial and peripheral zone which would become the flower initials later, also possess high AA concentration.

A small number of starch grains were seen in pith cells at early stage of germination. In mature apices, a number of large starch grains were seen in pith cells, whereas in the tunica layers only small starch grains were distributed (Fig. 23). As the stem began elongation (bolting), starch grains disappeared from pith cells, but no change was observed at the tunica layers. In reproductive apices, scarcely any starch grains could be found in the elongated pith cells.

Discussion

In the early stages of apical development before the morphological zones are formed, nucleic acid and enzyme activities were observed uniformly throughout the apex. Characteristic localizations were seen only after the zonation was formed. Similar results were obtained by Fosket and Miksche (1966) for *Pinus lambertiana*, and by Abdul and Pathak (1968) for Rauwolfia. In early stages of growth, when the population of cells within the meristem is relatively homogeneous, the cells contain nucli and nucleoli of similar densities. Considerable cellular difference was found both morphologically and histochemically within two or three days after germination. At this time the leaf initial is derived from peripheral zone and concurrenty the tunica layers are formed. PO and SD activites are high in the peripheral zone and at the tip of leaf primordium whereas weak in the corpus. In *Pinus lambertiana*, according to Fosket and Miksche (1966), central mother cells and apical initials (equivalents of the corpus and axial zone of *Brassica chinensis*) possessed high SD activities. Obviously there are differences in SD activities between the axial zones of these two plants.

During transition from vegetative to reproductive phase, RNA, SD, and AA diminished in tunica layers whereas increased in corpus. It is believed that the active cells possess high respiratory activities because they are actively synthesizing new materials (Brown and Broadbent, 1950). This is why high RNA, SD, and AA are detected from these areas.

PO was localized in the areas where cell divisions were taking place or

could be expected to take place, for instance in the first and second layers of peripheral zone during the vegetative stage. Van Fleet (1952) has emphasized the association of PO and cell division. Vanden Boun (1963) has reported the same observation in white spruce and supported Van Fleet's conclusion that PO might be detected in advance or accompanying cell division. It is premature to conclude, however, whether the presence of PO can be used to identify meristematic tissues, though such tissues almost invariably yield a positive PO reaction, because similar results are also obtainable for other specialized tissues such as young and more mature phloem elements (Van Fleet, 1959). From its localization in subapical region and pith where rapid cell elongation takes place during bolting, it is suggested that PO may play a part in cell elongation. The relationship between PO and cell elongation has never been reported. It is not known whether this correlation is also true for other kinds of plant.

During vegetative stage, AP was most active in first and occasionally second tunica layer. The activity disappeared from first and second tunica layers before transitional stage and distributed uniformly throughout the apex. The pith cells, during both vegetative and reproductive stages demonstrated relatively high AP activity. Abdul and Pathak (1968), Fosket and Miksche (1966), Vanden Born (1963), and Van Fleet (1952) have reported the same results. The function of AP is closely associated with differentiation (Abdul and Pathak, 1963) and manuration (Avers, 1958), and appear to be significant in carbohydrate transformations taking place at critical stages during morphogenesis (Van Fleet, 1952). Vanden Born (1963) suggested that they play an important role in the paticular metabolic pathways involved in the differentiation of the apex. But the functions of high AP activity in first and second tunica layers, and disappeared from the same place after reproductive stage in our plant is not known.

Starch grains decreased as bolting commences. Gibberellins enhance amylase activities in barley endosperm (Leslie, 1960). Starch digestion therefore may be associated with gibberellin activity in pith cells during stem elongation. The same phenomenon has also been reported for *Chenopodium album* (Gifford and Tepfer, 1961).

In this investigation the auther has demonstrated prominent localizationg of RNA, SD and AA, but not on SH-P during apex developments. It is very difficult, if not impossible, to compare two different preparations with respect to their color densities under microscope without micro-colorimeter unless they have striking visual differences. Negative localization for SH-P reported in this paper, therefore, does not negate the possible quantitative changes of this group during apex developments,

Reference

ABDUL, J. MIA and S. M. PATHAK. 1968. A histochemical study of the shoot apical meristem of Rauwolfa with reference to differentiation of sclereids. Can. J. Bot. **46**: 115-120.

AVERS, C. J. 1958. Histochemical localization of enzyme activity in the root epidermis of Phleum pratense. Amer. J. Bot. **45**: 609-613.

BROWN, R. and BROADBENT, D. 1950. The development of cells in the growing zones of the root. J. Exp. Bot. **1**: 249-263.

CHAYEN, J. 1953. Ascorbic acid and its intracellular localization, with special reference to plants. Intern. Rev. Cytol. **2**: 78-132.

FOSKET, D. E. and J. P. MIKSCHE. 1966. A histochemical studies of the seedling shoot apical meristem of Pinus lambertiana. Amer. J. Bot. **53**: 694-702.

GIFFORD, E. M. and H. B. TEPFER. 1961. Ontogeny of the inflorescence in Chenopodium album. Amer. J. Bot. **48**: 657-667.

GOMORI, G. 1956. Histochemical methods for protein bound sulfhydryl and disulfide groups. Quart. J. Microscop. Sci. **97**: 1-10.

JENSEN, W. W. 1962. Botanical histochemistry. W. H. Freemen and company. San Francisco and London.

JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York.

LESLIE, G. PALEG. 1960. Physiological effects of gibberellic acid. I. On carbohydrate metabolism and amylase activity of barley endosperm. Plant Physiol. **35**: 293-299.

ROSS, M. H. and J. O. ELY. 1951. Alkaline phosphatase in fixed plant cells. Exp. Cell. Research. **2**: 339-348.

VANDEN BOUN W. H. 1963. Histochemical studies of enzyme distribution in shoot tips of white spruce (Picea glauca, Moench, voss). Can. J. Bot. **41**: 1509-1527.

VAN FLERT D. S. 1952. Histochemical localization of enzymes in vascular plants. Bot. Rev. **18**: 354-397.

WANG, P. J. 1969a. Changes in the anatomical structure of the shoot apex of To-pe-tsai (Brassica Chinensis L.) during transition from the vegetative to the reproductive state and the determination of the bolting. Jour. Japan. Soc. Hort. Sci. **38**: 52-59.

WANG, P. J. 1969b. Studies on the floral differentiation and fruiting in Brassica Chinensis cultivar To-pe-tsai. ibid. **38**: 144-149.

WANG, P. L. 1971. Simplified techniques for Enzymo-histochemical studies in plant shoot apex. Bot. Bull. Academia Sinica. **12**: 32-38

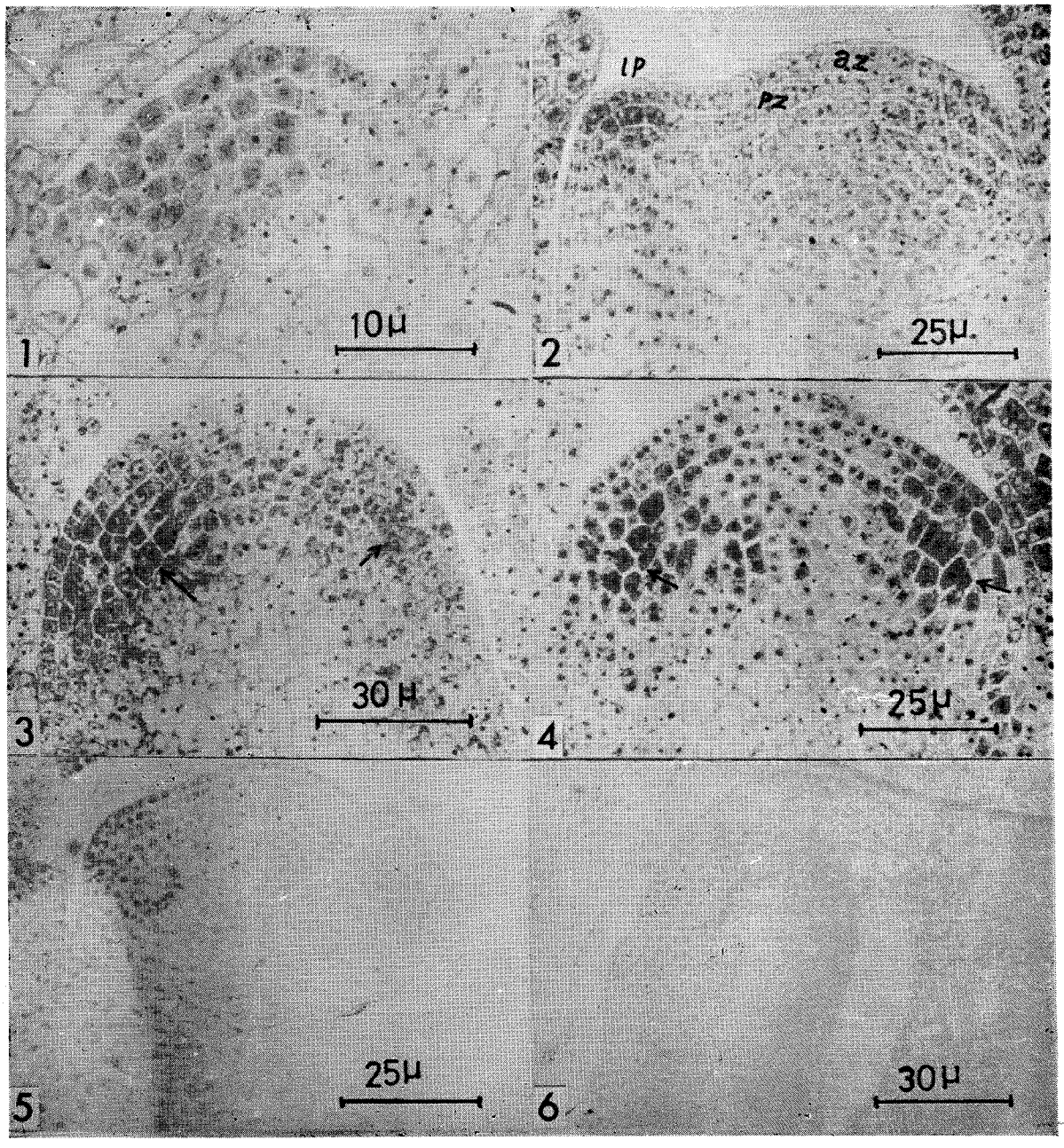

Fig. 1-4. Longitudinal sections of Brassica Chinensis L. apices stained with pyronin to reveal RNA.—Fig. 1. Early stage apex show uniformly localized RNA.—Fig. 2. A vegetative apex.—Fig. 3. An apex of transitional stage.—Fig. 4. An apex of during flower-bud differentiation, an arrow shows heavy stained cells, they are suggested as a flower bud initial. Fig. 5-6. Apices stained by Feulgen reaction to reveal DNA.—Fig. 5. A vegetative apex.—Fig. 6. An apex of transitional stage.

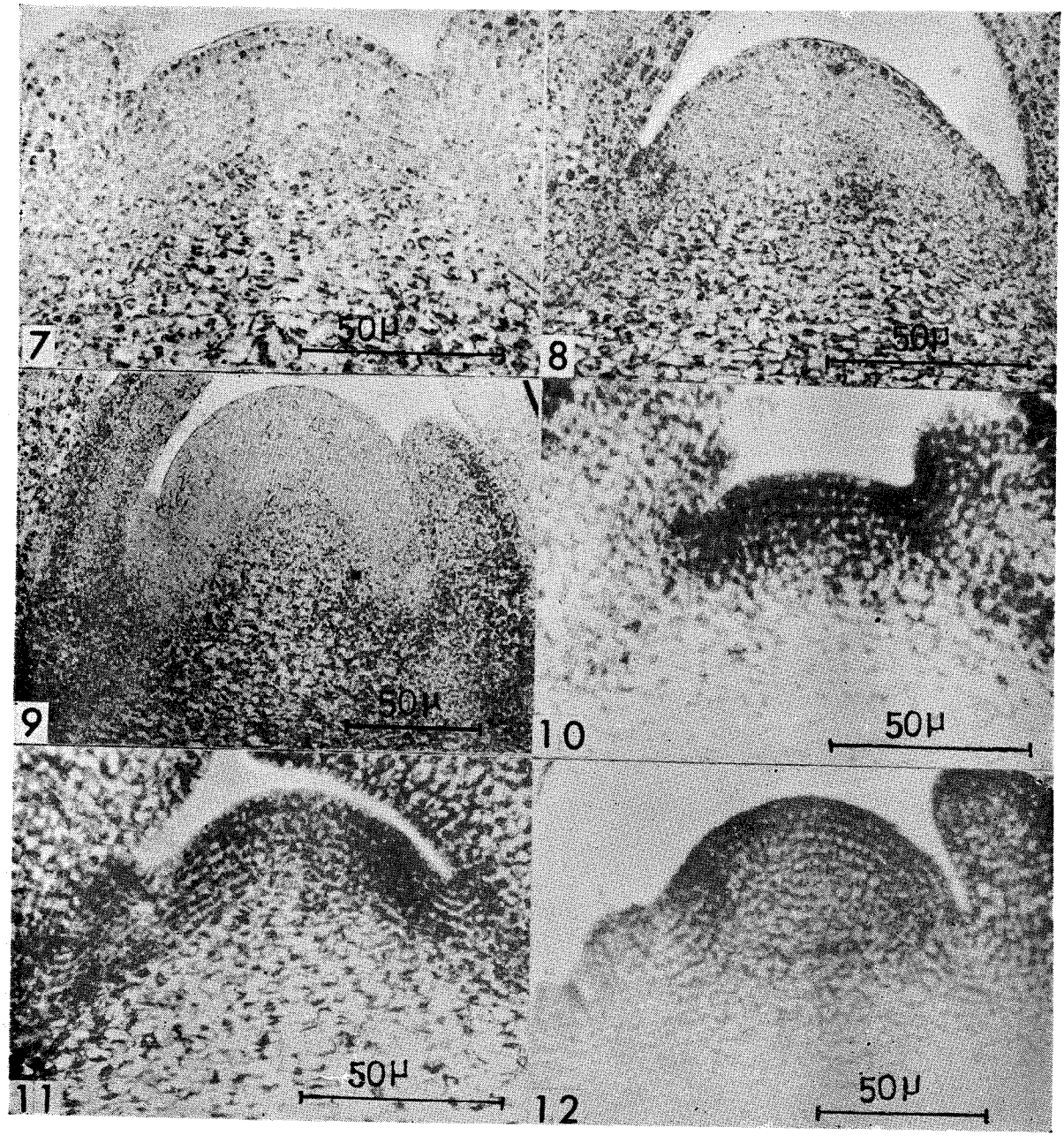

Fig. 7-9. Alkaline phosphatase activity in shoot spices of Brassica Chinensis L. Vegetative, transitional, and reproductive stages respectively. Fig. 10-12. Protein contains sulfhydryl-disulfide in shoot apices. Early apex, vegetative, and transitional stage respectively.

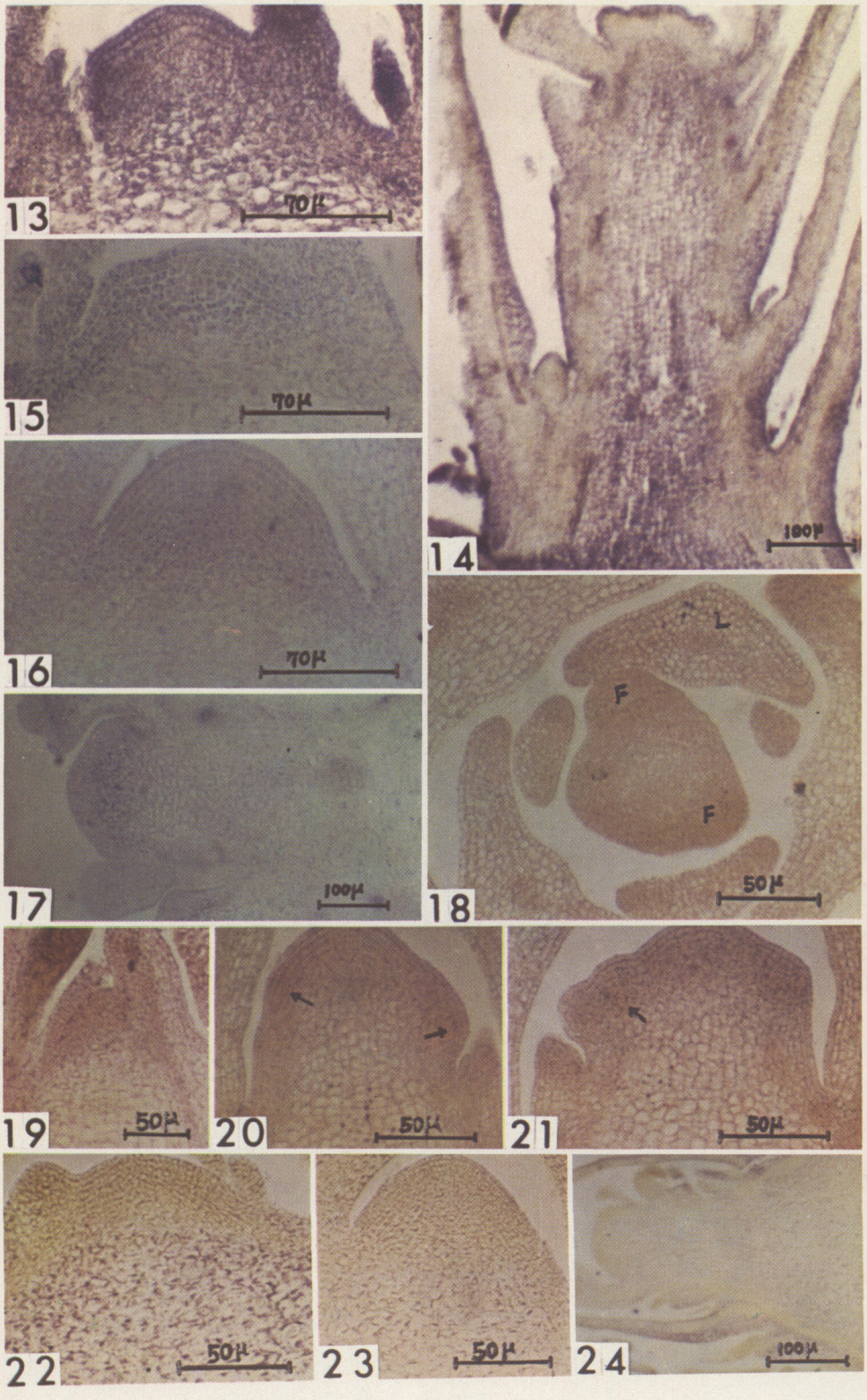
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Fig. 13-14. Peroxidas activity in shoot apices of Brassica chinensis L.—Fig. 13. A vegetative apex.—Fig. 14. Bolting pith cells shows high enzyme activity. Fig. 15-17. Succinic dehydrogenase activity in shoot apices. Vegetative, transitional, and reproductive stages respectively. Fig. 18-21. Ascorbic acid distribution.—Fig. 18. Transvers section of shoot apex showing ascorbic acid is localized at the flower primordia (F) and sides of leaf.—Fig. 19. Vegetative stage.—Fig. 20. Transitional stage.—Fig. 21. Reproductive stage. Fig. 22-24. Distribution of starch grains.—Fig. 22. Vegetative stage shows large and lot of starch grains in pith cells.—Fig. 23. Transitional stage to show starch grains decreased.—Fig. 24. Bolting pith cells to show starch grains disappeared.