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## Histochemical Studies on Germinated Seeds of *Setaria italica*

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Mobilization of reserve substances and utilization of these for the growth of embryonic areas like shoot and root apices is mediated through the embryonic axis. This process usually depends on the uptake of water, solubilization and hydrolysis of stored substances in the seed. The histochemical tests in this regard help in knowing and identifying the various metabolic constituents and their probable role in different tissues. Further, the structural differentiation is intimately connected with the metabolic changes. Since metabolic changes do not occur without a structural differentiation, it is clear that both are complementary aspects of the same situation. Keeping this in view, the present histochemical work on the seedling shoot and root apices and their axis in *Setaria italica* Beauv. has been undertaken. Such a study enhances our knowledge on plant growth and differentiation. The histochemical constituents assessed are: the insoluble polysaccharides, RNA, proteins, lipids and reducing sugars.

### Material and methods

Seeds of *Setaria italica* Beauv. were germinated in petri dishes and incubated at room temperature (27°C to 30°C) for about four days. Fixed and live materials were used. Daily one set of germinated seeds with shoot and root apices were fixed in Carnoy's fixative and dehydrated in ethanol-n-butanol series and embedded in paraffin. Microtome sections of one cell thickness were cut and mounted using gelatin adhesive (Jensen, 1962). Hand cut sections of live material was used to localize lipids and reducing sugars.

The histochemical assessment of tissue-sections, as adopted in the present study is of specific advantage over the generally followed extraction procedures. The former (the present method) offers biochemical composition of the component cell and/or tissue and possible chemical correlation among the cells of tissues in any given plant structure. The standard histochemical procedures and their control methods were followed and are listed in the following table:

Table 1:

Substances	Procedures	Indications
Polysaccharides	Periodic acid Schiff's (PAS) test (Jensen, 1962)	Purple colour
Starch	IKI test	Deep blue to black
Proteins	Mercuric bromphenol blue (Mazia <i>et al.</i> , 1953)	Deep blue
Lipids	Sudan black B test (Chiffelle and Putt, 1951)	Blue to black
Reducing sugars	Fehling's test (MacLean and Cook, 1952)	Orange red
RNA	Azure B test (Jensen, 1962)	Purple and blue
Control Procedures		
Polysaccharides	Avoiding periodic acid in PAS reaction (Cass and Jensen, 1970)	Negative
RNA	10% cold perchloric acid at 02°C for 18 hours (Kasten, 1965)	Negative in the cytoplasm
Lipids	Hot pyridine method (Baker, 1946)	Negative

## Results

*Polysaccharides:* Successive growth phases of the seedling were observed with respect to the distribution of insoluble polysaccharides. Generally, shoot apex of all stages lacks starch (Fig. 1), if present they are small and distributed in all the zones. The cell walls are PAS-positive (Fig. 1). However, irrespective of the age group of the seedling, the basal area of the shoot apex shows abundant starch (Fig. 2). The youngest leaf primordia show no starch, whereas the older ones contain a few small grains. The coleoptile at all stages of its growth contains starch (Fig. 3).

In the root, starch is generally present in the root-cap and cortex (Figs. 4, 5) and very thick PAS-positive deposition is observed along the periphery of the root epidermis (Fig. 4). Rarely, the root-cap initials show small PAS-positive grains (Fig. 5). The cell walls of the provascular strands are PAS-positive but contain no starch (Fig. 2). The cortex of the hypocotyl and the parenchymatous cells of the nodal plate shows rich starch deposition of varied sizes (Figs. 2, 6). Vascular tissues which connect the shoot, root and scutellum are devoid of starch, but their cell walls are PAS-positive (Fig. 6). Lateral root initials differentiate from the pericycle of the primary root and show the deposition of small starch grains, but their

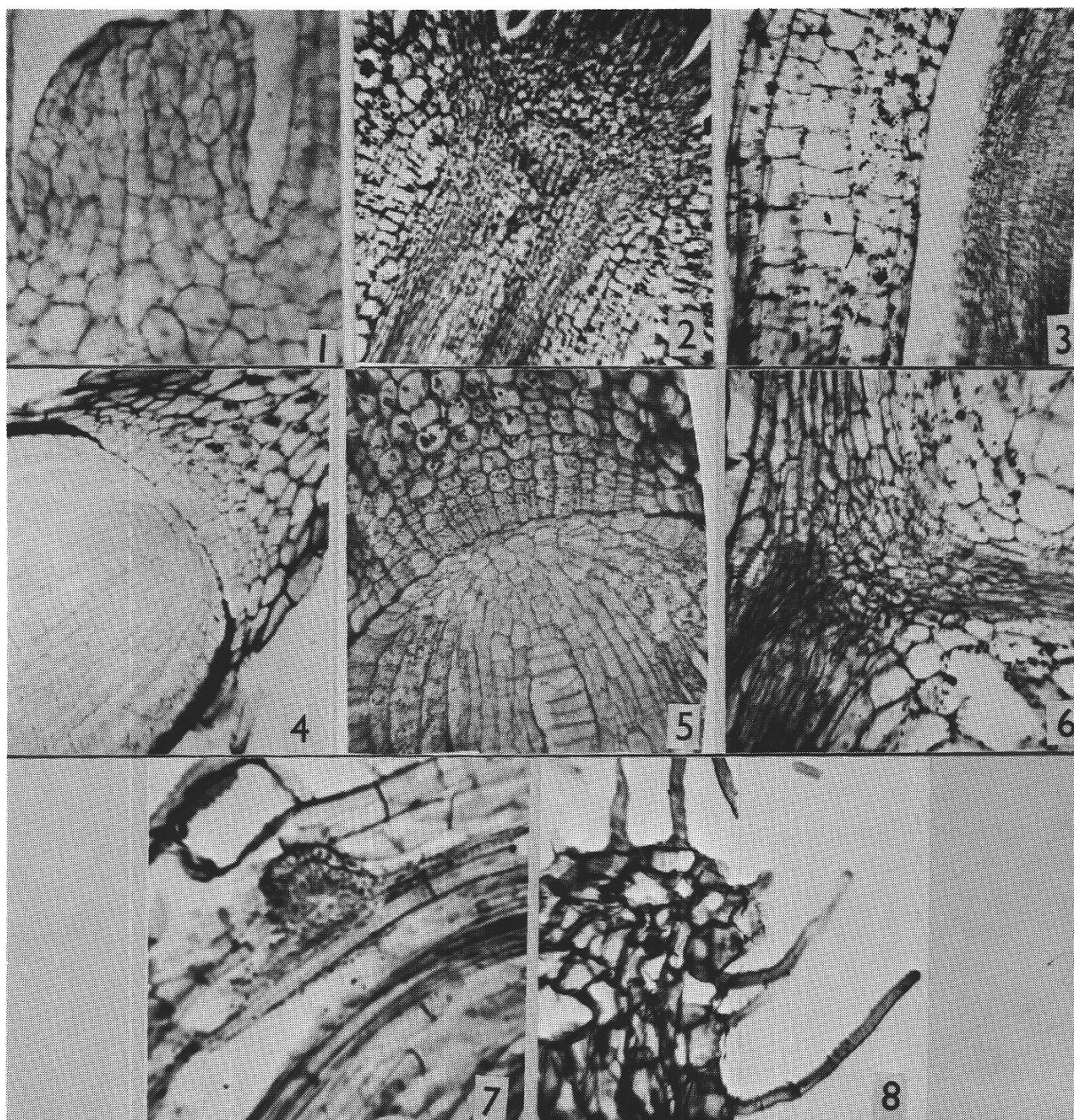


Plate I:

*Fig. 1–8.* Seedling sections at different regions tested for polysaccharides.

- Fig. 1. Shoot apex showing no starch but PAS-positive cell walls. x 900.
- Fig. 2. Starch is abundant in the shoot base and hypocotyl but absent in the provascular tissue. x 900.
- Fig. 3. Leaf and coleoptile. Starch is abundant in the latter only. x 900.
- Fig. 4 and 5. Root-cap and cortex with abundant starch. Small grains are present in the root-cap initials and thick PAS-positive depositions occur along the epidermis. Fig. 4: x 600, Fig. 5: x 900.
- Fig. 6. Nodal plate region with a high starch content. Cell walls of the vascular strands are PAS-positive. x 900.
- Fig. 7. Note the differentiation of lateral root primordium from the pericycle region containing starch. x 900.
- Fig. 8. Coleorhiza and its hairs. Cell walls of the former are thickly PAS-positive. x 700.



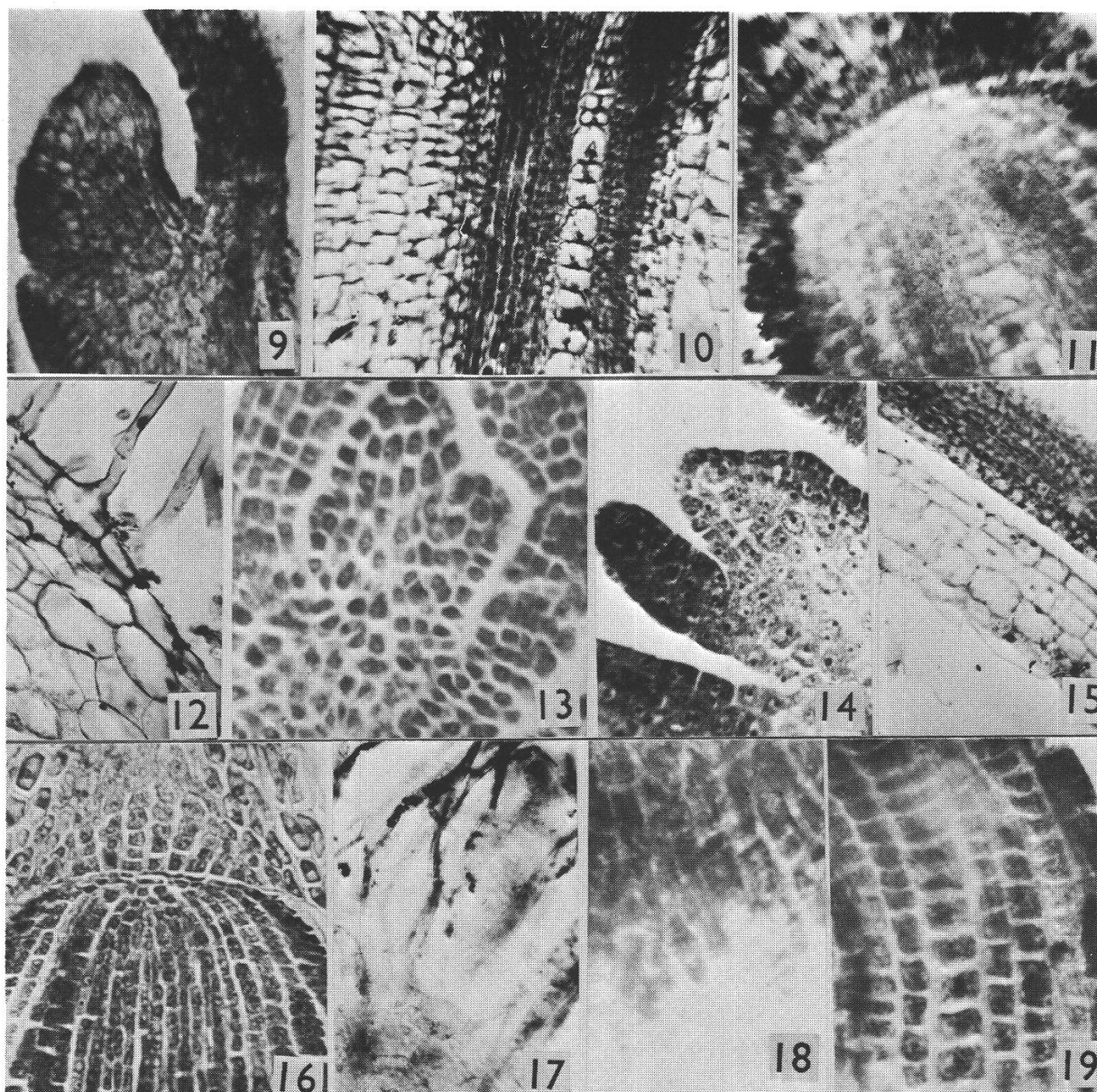


Plate II:

Seedling sections at different regions tested for RNA (*Fig. 9–12*), proteins (*Fig. 13–16*) and lipids (*Fig. 17–19*).

- Fig. 9. Shoot apex with highest RNA content in the flank zone and leaf but less in the apical region. x 400.
- Fig. 10. Hypocotyl region showing low RNA content when compared to provascular tissues. x 700.
- Fig. 11. Root with low RNA content in its apical initials. Root-cap initials and procambial strands are rich in RNA. x 700.
- Fig. 12. Coleorhiza and its hairs showing very little RNA. x 400.
- Fig. 13 and 14. Shoot apices with a high and uniform protein content (slightly lower in apical cells in fig. 13). Leaf primordia have the highest protein content. x 400.
- Fig. 15. Leaf and coleoptile, respectively, with high and low protein content. x 400.
- Fig. 16. Root with high and uniform protein content in all tissues, slightly less in mature root-cap cells. x 700.
- Fig. 17. Coleorhiza and its hairs with a high lipid content in the latter. x 500.
- Fig. 18. Root-cap cells rich in lipids. x 500.
- Fig. 19. Cortex of the hypocotyl showing a rich lipid storage. x 500.

neighbouring mature cells are devoid of starch (Fig. 7). Coleorhiza shows no starch, but its cell walls are thick and PAS-positive (Fig. 8). Similarly, its hairs and root hairs are PAS-positive (Fig. 8). Presence of starch in different tissues of the seedling was verified with IKI test.

**RNA:** In the shoot apex, RNA concentration is comparatively low in apical cells (Fig. 9) when compared to the flank zone cells which show highest RNA, although RNA based cytohistological zonations cannot be demarcated. The leaf primordia show highest RNA content (Fig. 9). The subtending ribmeristem zone, although poorly defined, shows comparatively low RNA concentration (Fig. 9). At the base of the shoot apex, the permanent parenchymatous cells show low RNA, except the provascular strands which are very rich in the substance (Figs. 9, 10). In the older seedling further reduction in RNA concentration is noticed in the hypocotyl and nodal plate regions. Older leaves and coleoptile at all stages of seedling growth show rich and low content of RNA, respectively.

In the mature root, the quiescent area has low RNA content when compared to its neighbouring tissues (Fig. 11). The procambial strands since their differentiation contain more RNA than the cortex (Fig. 11). From the meristematic zone there is a gradual basipetalous reduction in RNA concentration is observed reaching to very low level in the permanent tissues of the older root. The mature root-cap cells have comparatively low RNA, while its initials are rich in the substance (Fig. 11). Coleorhiza and its hairs show very low RNA content (Fig. 12), but its cell walls exhibit partially green colour with azure B.

**Proteins:** Distribution and concentration of proteins in the seedling resemble more or less to that of RNA. Generally, the shoot apex shows a high content of proteins at all stages of its growth (Figs. 13, 14). The flank zone retains a higher concentration similar to RNA whereas the leaf primordia show highest protein content (Figs. 13, 14). Coleoptile, the parenchymatous cells in the hypocotyl and the nodal plate regions show comparatively low protein content, but the provascular tissues are rich in the substance (Fig. 15).

In the young seedling, the root tip (quiescent area) is rich in protein (Fig. 16), but less when compared to the procambial strands which show highest protein content. Epidermis and cortex of the root are rich in proteins, but in older region these tissues show very low proteins. Root-cap initials are rich in proteins when compared to its mature cells (Fig. 16). Coleoptile in the germinated seed shows very low content of proteins.

**Lipids:** The shoot apex is uniformly rich in lipids. The coleoptile contains a few lipid bodies, while the young leaves are rich in these. Coleorhiza develops unicellular hairs which resemble root hairs in having rich, but smaller lipid bodies (Fig. 17). In the primary root, the peripheral cells of the root-cap contain abundant lipid bodies, more than that of starch content (Fig. 18). In the young seedling, all the tissues of the root including the root meristem and epidermis are rich in lipids. In the older root, lipids present only in the cortex. The root hairs are rich in small lipid bodies. The provascular strands show low lipid content whereas the parenchymatous cells of the hypocotyl are rich in them (Fig. 19).

*Reducing sugars:* Seedlings up to the 4th day of germination were examined for reducing sugars. The shoot apex and its subtending vascular tissues retain rich reducing sugars. The older leaves are devoid of sugars, whereas the younger ones are rich in these substances. The nodal plate region except the provascular tissues reacts negative to the test. In all the grades of seedling, root-cap initials show much reducing sugars, but its mature cells are devoid of these. The root earlier to its emergence from the coleorhiza shows a higher concentration of reducing sugars but after its emergence the older basal areas gradually lose the sugar content. The coleorhiza and its hairs show no reducing sugars. The lateral root initials are predetermined in the primary root showing rich reducing sugars while its neighbouring mature cells remain negative to the test. The organized lateral root resembles primary root in sugar content.

## Discussion

Histochemical studies reveal the pattern of distribution of biochemical substances and their possible role in tissue differentiation. Differentiation always begins in the embryonic structures which are typically shoot and root apices and consist of small cells, later by division and differentiation produce other cells which become involved in the function of the mature tissues and organs of the plant.

The shoot apex forms an active centre of utilization of biochemical substances from the proximal area where carbohydrates and lipids form principal substrates. The embryonic axis which connects the meristems with the scutellum appears to play an important role in providing the necessary biochemical requirements. In other words, the axis acts as a liaison tissue in drawing and translocating the hydrolysed products from the scutellum, as the latter contains rich concentrations of nucleic acids, proteins, lipids, ascorbic acid (AA) and polysaccharides and these substances decline gradually in older seedling (Panchaksharappa and Rudramuniyappa, 1975). Thus it provides an evidence for degradation of reserve substances present in the scutellum. Histochemically, the axis shows low RNA and proteins but more lipids and polysaccharides. Low RNA and proteins in this region show that these substances are actively involved in the production of enzymes which are necessary for the breakdown of food reserves. Small starch grains in this area suggest their degradation and release of energy, as reducing and non-reducing sugars are reported to be high in the axis of barley (Vyas, 1976). Therefore, the embryonic axis appears to become a physiologically active centre. It may function in controlling, initiation of metabolism and translocation of biochemical substances present in the scutellum.

The shoot apex of a plant is a locus of tremendous activity in initiating and controlling a variety of growth processes. In *Setaria*, generally, the shoot apex lacks starch, but its cell walls are PAS-positive. However, in other members of the Gramineae (Rudramuniyappa, 1973; Rudramuniyappa and Panchaksharappa, 1975) the changing pattern of starch distribution is difficult to understand. Similarly, in *Chenopodium* (Gifford and Tepper, 1962) starch was seen only in the young shoot and lost when 6th pair of leaf produced, but in *Paulownia* (Rickson, 1968) it appears after four days of germination and persists in the entire shoot including leaf pri-



mordia with bigger grains in the central mother cell zone. However, in *Brachyochiton* (West and Gunckel, 1968a) and *Pharbitis* (Healey and Jensen, 1968) the substance is present only in the central mother cell zone. Numerous spherosomes and lipid bodies are reported in the shoot apex of *Zea mays* (Trelease, 1968). Therefore, the present as well as the previous studies on shoot apex indicate that there is no consistency in starch distribution and concentration and the distribution appears to be specific with the plant.

In contrast to polysaccharides and RNA, proteins, lipids and reducing sugars are rich in the entire shoot apex. RNA content is comparatively low in the apical cells, but both RNA and proteins are equally high in the flank zone where the leaf primordia differentiate. Similar observations have also been made in *Paulownia* (Rickson, 1969), *Chenopodium* (Gifford and Tepper, 1962), *Brachyochiton* (West and Gunckel, 1968b), *Pharbitis* (Healey and Jensen, 1965) and *Panicum* (Rudramuniyappa and Panchaksharappa, 1975) where the flank zone shows highest RNA and protein contents when compared to the other areas of the shoot apex. Therefore, it appears that growth and differentiation of leaf primordia in the flank zone greatly depend on RNA, proteins, lipids and reducing sugars. Generally, high RNA content indicates the rate of cell division and differentiation, but its contribution appears to be very small to the differentiation of cytohistological zones in the shoot apex of the Gramineae (Rudramuniyappa and Panchaksharappa, 1975). Rich contents of macromolecular substances namely, RNA, proteins, lipids, reducing sugars, polysaccharides and AA suggest that the shoot apex is a metabolically active growth centre where a variety of growth processes occur.

Among the tissues which show abundant starch and lipids is the subapical area of the shoot. This region shows comparatively low RNA and proteins except the procambial strands which are rich in these substances. Therefore, the subapical region of the shoot apex in some way biochemically connects in translocation of metabolic substances to the formative region the shoot apex where maximum growth activity occurs. RNA and proteins in this region probably help in the synthesis of enzymes which in turn take part in breakdown of stored substances, as it has been known that starch and lipids form a distinct sources of energy for various biochemical reactions.

Unlike the shoot, the root apex is simple since no lateral appendages are produced from the growing zone and all the cells formed in the terminal meristem contribute to an axile system. The root contains root-cap, meristematic cells and cells in the early stages of enlargement, while the basal segment contains only fully expanded cells in the early stages of maturation. Cell division and growth of the root are accompanied by an increase in the histochemical substances (RNA, proteins, lipids and reducing sugars). High RNA, proteins, starch, lipids and reducing sugars in some members of the Gramineae (Rudramuniyappa, 1973) including *Setaria* and various enzymes namely, acid phosphatase, glucose-6-phosphatase and ATP ase in the cortical cells of many angiosperm roots (Shaykh and Roberts, 1974) suggest that root cortical cells play a part in conduction and in the storage of reserve substances. Localization of AA along the cell walls of the root in *Triticum* and *Pennisetum* (author's own observation) obviously suggests that AA has a functional relationship at the cell surface, perhaps the absorption and retention of solutes (Reid, 1941). Similarly, the presence of high glucose and fructose in cortical cells suggest a regulatory factor for



sugars concentration within the tissue and in concentration with root osmotic pressure (Patricia, 1968). Considering all these observations the root cortex undoubtedly plays a key role in conduction and secretion of metabolic substances. Differentiation of procambial strands into trachary elements in the root probably is influenced by rich RNA, proteins, sugars, lipids and enzymes like acid phosphatase,

The young root in Gramineae exhibits no quiescent zone, but as it grows older this zone is demarcated by showing low concentrations of RNA, proteins, insoluble polysaccharides and lipids (Rudramuniyappa, 1973; Rudramuniyappa and Panchaksharappa, 1975). Similarly, in many angiosperm roots (Shaykh and Roberts, 1974) enzymes like acid phosphatase, ATPase and glucose-6-phosphatase were not observed in this region; this gives an impression that quiescent centre is metabolically inactive. These as well as the present study support the concept of a quiescent centre in angiosperm roots. The exact functioning of this centre is not known.

Classically, the root-cap has been looked upon as a structure „protecting“ the apical cells of the root proper. However, its physiological nature has not been understood. Histochemically, its mature cells store abundant starch and lipids and its initials are rich in RNA, proteins, lipids and reducing sugars. Further, it has been reported that root-cap cells contain high concentrations of acid phosphatase and glucose-6-phosphatase (Shaykh and Roberts, 1974), lysosome like organelles rich in acid phosphatase and esterase (Patricia, 1968), and AA (authors own observation). It has been known that glucose-6-phosphatase and AA function in the biosynthesis of polysaccharides. The hydrolytic enzymes present in the lysosomes of root-cap cells degrade the reserve substances into simpler ones and the presence of RNA and proteins probably help in this regard. Therefore, it appears that with these metabolic constituents and enzymes the root-cap plays a very important physiological role in addition to a protective one. It may also play a role in supplying energy to the apical initials where rapid cell division and differentiation occurs. This is because of the occurrence of plasmodesmata connections between root-cap cells and root initials (Leech, *et al.*, 1963). Such connections help in translocation of biochemical substances from one system to the other.

The coleorhiza is a peculiar structure to the grass embryo. At mature embryo stage, it forms a protective covering for the entire root system but during germination this structure gradually disintegrates. Histochemically, it stores abundant starch since its inception to maturity (Panchaksharappa and Rudramuniyappa, 1972). During germination the coleorhiza shows low RNA, proteins and lipids, but no starch. Regarding its function much has not been known. However, it develops unicellular hairs during germination which are similar to that of root hairs, rich in lipids. Therefore, it is quite possible that the coleorhiza performs the function of absorption, as long as the primary root is enclosed within it. Functionally, the root hairs play a major role in the uptake of water and nutrients from the soil. Hence, the same function may be attributed to the coleorhiza hairs.

Coleoptile in the seedling is a colourless tubular structure enclosing within it the leaf primordia and shoot apex. Histochemically and morphologically, the coleoptile does not resemble the leaf. It shows low RNA and proteins, but no reducing sugars. However, these substances are abundant in the leaf. Starch is more abundant in the coleoptile than in the leaf. Hence, the present study strongly supports the contention that the coleoptile is not a leaf.

## Summary

The present study deals with the histochemical constitution and the developmental requirements of shoot, root and their axis in the seedling of *Setaria italica* (Gramineae). The substances localized are insoluble polysaccharides, RNA, proteins, lipids, and reducing sugars. The assessment is made on tissue-sections using the light microscope.

The shoot apex generally lacks starch but starch is stored at its base. RNA content is low in the distal end of the shoot but high in the flank zones. Unlike RNA, proteins, lipids and reducing sugars are generally abundant in the entire shoot apex. The leaf primordia and procambial strands are rich in all histochemical substances except that the latter tissue contains no starch. Coleoptile, nodal plate and hypocotyl contain more starch and lipids.

In the root, the cortex stores starch and lipids in addition to RNA, proteins and reducing sugars. However, these substances decline in mature tissues of the root. The older root apex exhibits a quiescent centre with low RNA, protein and polysaccharide content. The root cap contains abundant starch and lipids; its initials are rich in RNA, proteins and reducing sugars. Coleorrhiza and its hairs are rich in lipids. Based on earlier relevant data, the probable role of these substances in relation to their concentration and distribution in the seedling tissues is discussed.

## Zusammenfassung

Histochemische Untersuchungen an gekeimten Samen von *Setaria italica*.

In Keimlingen von *Setaria italica* (Gramineae) wurde die histochemische Zusammensetzung und Entwicklung von Spross, Wurzel und deren Achse untersucht. Die Verteilung von unlöslichen Polysacchariden, RNA, Proteinen, Lipiden und reduzierenden Zuckern wurde verfolgt. Die Untersuchungen erfolgten an Gewebeschichten unter dem Lichtmikroskop.

Im Spross fehlt Stärke in der Regel am Scheitel; sie kommt jedoch an der Basis vor. Der RNA-Gehalt ist am distalen Ende des Sprosses gering und in den Seitenzonen hoch. Im Unterschied dazu finden sich Proteine, Lipide und reduzierende Zucker im ganzen Sprossscheitel in hoher Konzentration. Blattanlagen und Prokambium sind reich an allen untersuchten Verbindungen; nur das Prokambium enthält keine Stärke. Coleoptile und Hypocotyl enthalten vor allem Stärke und Lipide.

In der Wurzelrinde finden sich Stärke und Lipide zusammen mit RNA, Proteinen und reduzierenden Zuckern. In alternden Wurzeln nimmt der Gehalt an diesen Verbindungen ab. In älteren Wurzelscheiteln findet sich ein wenig aktives Zentrum mit geringem Gehalt an RNA, Proteinen und Polysacchariden. Die Wurzelhauben enthalten reichlich Stärke und Lipide; ihre Anlagen sind reich an RNA, Proteinen und reduzierenden Zuckern. Die Coleorrhiza und ihre Haare weisen einen hohen Gehalt an Lipiden auf. Auf Grund dieser Ergebnisse und früheren Arbeiten wird die Bedeutung von Konzentration und Verteilung dieser Verbindungen in den Geweben des Keimlings diskutiert.

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