

## The Localization of Starch in Root Tips

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**Abstract.** As an extension of earlier studies performed in our laboratory on enzyme localization on tissue level, the distribution of starch was examined in seedling root tips of pea *Pisum sativum* L., broad bean *Faba vulgaris* MOENCH., maize *Zea mays* L., lupin *Lupinus albus* L., and pumpkin *Cucurbita maxima* DUCH., and in tips of adventive roots of onion *Allium cepa* L. by means of the alcian blue /JJK procedure applied to paraffin sections. In pea, some genetic (various cultivars) and eco-physiological (different harvests, various localities, diverse way of germination, uneven seedling age, unequal stage of cell growth) factors were studied concerning the present problem. In all these cases the starch localization pattern remains constant though the amounts of starch vary in particular cell complexes. From the evaluation of the starch localization at the level of the beginning cell growth in particular species and from comparison of the findings in different species it follows that starch localization and histogenesis patterns are different. This supports the view that the two phenomena are caused or controlled by uneven factors.

The unequal localization or distribution of enzymes and other substances on subcellular level is generally accepted. The differences in the presence of particular plant products within various organs of the plant body are regarded as trivial, though relevant information is still desired. Leaving aside some well known findings like the existence of idioblasts *etc.*, the research into the distribution of substances on tissue level seems to be greatly omitted. The suggestions from protoplasmatic anatomy or from studies on histophysiological gradients *etc.* have remained mostly without response.

Recently, a summary of results from our laboratory on the localization of hydrolytic enzymes in root tips has been compiled (BENEŠ 1975). In the course of this work the lack of comparable information on the distribution of different plant substances in various root cell complexes became evident. In this connection an attempt is made in the present paper to study the localization of starch\*\* or starch grains (starch granules) in the root tip and to evaluate the findings in relation to tissue differentiation, extending thus some of our previous works (HADAČOVÁ *et al.* 1973, KUTÍK 1977, KUTÍK and BENEŠ 1977a, b, c).

Received June 16, 1978; accepted September 19, 1978

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\*\* As mostly accepted (and in accordance with our previous papers), the term starch is understood here as a group name for polyglucosan complexes containing also some other substances, appearing in plant cells as characteristic particles, starch grains (starch granules): the polysaccharide material considerably dominates the mass of the initial plastid.

### Material and Methods

The objects of the present work were primary seedling roots of pea — *Pisum sativum* L., cv. Raman, broad bean — *Faba vulgaris* MOENCH., cv. Chlumec-ký, maize — *Zea mays* L., cv. Český bílý koňský zub, lupin — *Lupinus albus* L., cv. Pflug ultra and pumpkin — *Cucurbita maxima* DUCH., cv. Veltruská obrovská, and adventive roots of onion — *Allium cepa* L., cv. Hiberna. In the case of seedling roots, the seeds were hydrated overnight in running tap water and then allowed to germinate at 25 °C in dark in closed glass cylinders on plastic sheets covered with moist cotton wool for 2 (pea, lupin and maize) or 3 days (broad bean and pumpkin). The adventive roots of onion were obtained from bulbs placed with their bases into tap water changed occasionally (25 °C, dark).

The material was fixed overnight with FAA (JOHANSEN 1940), washed in 50% ethanol, through tertiary butanol series brought to paraffin and sectioned into 10 μm thick transverse or longitudinal sections. The starch grains were detected using the alcian blue/JJK procedure (KUTÍK and BENEŠ 1977c). Their presence was estimated under low (obj. 10×, eyep. 10×) and middle (obj. 40×, eyep. 10×) magnifications, considering their number and size on both per cell and per defined tissue volume or area basis, the results being expressed as absence, presence, abundance. The starch localization was studied and compared in different species on transverse sections at the level of the beginning of cell elongation. In contrast to our previous studies (ADÁMKOVÁ and BENEŠ 1966) the commencement of cortex cell elongation was marked on longitudinal median sections and the distance from the end of the root (the cap including) was measured. However, the regions approximately 1 mm acro- and basipetally from the beginning of cell elongation were also taken into account.

Since pea appeared as the most interesting of the objects studied, some physiological and genetic factors were followed, which could influence the distribution of starch.

In the way mentioned above, the root tips from seeds cv. Raman were compared from different harvests (1973, 1974, 1975) at the same locality (Čelechovice in Haná). Since the comparison was made simultaneously (1976) the seeds were of different age. Similarly, the seeds cv. Raman obtained in the same year (1974) from three different places (Čelechovice in Haná, Pěčice, and a not precisely known breeding station in south Moravia) were taken into the experiment.

Besides cv. Raman, used currently, the root tips from germinating seeds were studied of other cultivars: Meteor, Borek, Orion (harvest 1974, different localities).

Some other eco-physiological factors were followed with cv. Raman.

The localization of starch was checked in roots taken from seeds germinating for 1 and 3 days in comparison with the standard 2 days. Moreover, the presence of starch grains was examined in root tips of photosynthesizing plants: the seeds allowed to germinate in the standard way were transferred onto the modified Knop's solution (BALCAR *et al.* 1969) and kept there at 21 to 23 °C in 12 h dark/12 h light regime (white fluorescent tubes Tesla, Praha, ČSSR; 9000 lx at the level of leaves) for another 7 days. The fixation was performed in the midst of the light period. Here, the distribution of starch was compared in the main and lateral roots at the level 1 to 2 mm from the

root ends. Finally, starch localization was compared in the roots from seeds germinating in different ways. The seeds, soaked with water as given above, were allowed to germinate either in the usual way, *i.e.* free in the humid atmosphere, or over H<sub>2</sub>O so that the roots grew into it, or on the surface of a granular substrate (Perlit, Keramické závody, Praha), saturated with H<sub>2</sub>O or — finally — on H<sub>2</sub>O moistened filter paper in Petri dishes.

As already mentioned, the starch distribution was observed in root tips at the level of the beginning of cell growth. In addition, in the standard material the localization of starch grains was also studied in sections at the distance 10 to 12 mm from the end of the root.

## Results

Table 1 demonstrates the results on the distribution of starch in root tips of the different species studied at the level of the commencement of cell elongation. (The cell elongation begins 1.5 mm from the end of the root in pea, 2.0 mm in broad bean, in maize 2.0 mm, in lupin 1.7 mm, in pumpkin 1.0 mm and in onion 2.7 mm.) It appears that the localization of starch grains in the material studied follows a strange regularity. The greatest amount of starch appears in the root tip cortex in all species tested. In some cases (pea, broad bean; see Figs. 1, 2) it is altogether absent from the central cylinder: some small grains were found only in phloem poles. In other root tips (maize: see Figs. 3, 4) starch is abundant in both the cortex and the stele. In the adventive root of onion, the starch grains are absent in the studied region. They are present only in the cells of columella in the root cap. Though some differences in the amount of starch appear between particular individuals within the given species, the pattern of starch localization remains unchanged. Following the distribution of starch grains into the meristematic and cell elongation regions (about 1 mm on both sides from the level of the commencement of cell elongation as given above), the starch distribution reveals no substantial change. However, in meristematic cells the amount of starch is lower as well as in growing cells.

As far as the pea cv. Raman, seeds of different harvests and age and the seeds from various localities were studied concerning starch grains distribution in seedling root tips, no differences were observed. The same starch localization was also found in root tips of all the pea cultivars compared.

With pea cv. Raman some other eco-physiological factors were taken into account, which could influence starch localization in the root tips.

After 1 day of germination, when the seedling roots are about 1 cm long, practically no starch is present in their tips. The results given above relate to a 2 day-germination. Whereas the dissimilarity is striking between the root of 1 and 2 days germinating seeds, there is no difference in this respect comparing 2 and 3 days of seed germination.

In root tips of photosynthesizing plants cultured in modified Knop's solution, the amount of starch is low. The starch grains are visible in the endodermis and in the pericambium (mostly in phloem poles) and in sieve tubes and cells adjacent to them. Some starch is present in rhizodermis and in cortex. The obligate difference in starch distribution between cortex and central cylinder is not evident in this material. The distribution of starch in the tips of main and lateral roots is quite similar.

TABLE 1

Outline of starch localization in root tips at the level of the beginning of cell growth

	Pea	Broad bean	Lupin	Pumpkin	Maize	Onion
Root Cap	+	+	++	++	/	/
Rhizodermis	+	+	++	+	+	-
Cortex	++	++	++	++	++	-
Endodermis	-	-	+	+	++	-
Pericycle	-	-	+	+	+	-
Central cylinder (in general)	-	-	+	+	++	-

- absence, + presence, ++ abundance; / not afflicted.

The starch distribution pattern remained unchanged in roots taken from seeds allowed to germinate in the different ways given above.

Concerning the localization of starch grains in roots at the level of cell maturation (10 to 12 mm from the end), the results are qualitatively equal. However, confronting the presence of starch in particular parts of the root tissue, the proportions are dissimilar: there is no striking difference between cortex and stele. The amount of starch is lower in comparison with the level of the beginning of cell elongation.

### Discussion

The studies on starch were and still are extensive and many-sided, comprising works in food technology, plant products chemistry, plant physiology *etc.* Considerable progress was achieved in the field of starch formation from the viewpoints of both biochemistry and cytology. The cases of discrete localization of starch on tissue level are generally known: the cells of stomatal apparatus, the starch sheaths *etc.* Though starch is a common reserve substance, profound changes in its quantity and even presence in the particular cell complex are quite common. In both of these phenomena effective regulatory mechanisms must be involved. Besides the possibility of practical use, this seems to be the reason of high interest of many workers in starch formation and breakdown in leaves, storage tissues *etc.* After the vivifying work of THORPE and MURASHIGE (1968) many papers to similar purpose were published, some also from our laboratory, as mentioned above.

Concerning the roots, the localization of starch grains was studied many times. However, the present authors failed to find out satisfying information on the distribution of starch grains in root tips from the common compendia, handbooks and monographs on plant cytology, anatomy, histochemistry, biochemistry, and physiology. The problem of the presence and localization of starch is highly complex. It was therefore considered here in connection with the function of root, geotropism, production and action of growth substances, *in vitro* cultures, transport and occurrence of carbohydrates, their respiratory metabolism and regulatory functions, meristematic activity, cell growth and tissue differentiation *etc.* A thorough searching the literature has revealed that only separate findings on starch localization in root tips are scattered in the literature (HUŠEK 1903, NĚMEC 1910, ZIEGENSPECK 1924,

DYAR 1950, PILET and MARGOT 1953, PILET and TURIAN 1953, ROGOZINSKA 1965, NOUGARÈDE and PILET 1971, VASIL 1973, BARLOW and GRUNDWAG 1974, IVANOV 1964), but no comprehensive work was found. The exceptional plasticity — as mentioned — in the appearance of starch within the given tissue, which complicates any comparative evaluation of results, seems to be the reason for this *status questionis*.

The availability of a suitable method of starch detection (KUTÍK and BENEŠ 1977c) was a necessary prerequisite of any progress in our studies. It should be mentioned that the evaluation of slides was done under low and middle magnifications. This means that only developed starch grains were considered. It seems that starch formation proceeds as a two-step process: first, small inclusions appear within the plastid, which increase forming the "adult" starch gran in the second step. Two stages of amyloplast functioning were distinguished even in our earlier work (MANIRUZZAMAN *et al.* 1967). The metabolic processes of starch formation may differ within the two stages and may be controlled by different regulatory systems.

Before analyzing the results in root tips of different species, it seems useful to evaluate the factors which could influence the starch grain distribution in general.

As far as pea root tips were studied, the same starch localization was revealed in all cultivars tested. Dealing then with cv. Raman only, starch distribution was unchanged in roots obtained from the seeds of different localities, harvests and age. The mode of seed germination did not substantially influence the results. The presence of starch grains in particular cell complexes depends significantly on the course of growth and development: remarkable differences were recognized between tips of 1 and 2 or 3 days germinating roots and between seedling roots and roots from photosynthesizing plantlets. Even the findings at the levels of the beginning of cell growth and of maturing tissues are not quite equal. However, though the proportions of starch distribution were different comparing the given portions of tissue, the localization pattern remained unchanged. Moreover, as concerns the stability of the starch localization pattern the same localization of starch grains was roughly preserved even in pea roots cultured *in vitro* (KUTÍK and BENEŠ 1977a).

To be able to estimate the results in different species on a basis respecting the anatomical and physiological features of the particular objects, it was decided to study the root tips at the level of the beginning of cell elongation. Taking into account the classification of localization types (BENEŠ 1975), the distribution of the same substance (starch) is different in comparable material: continuous in maize, discontinuous in pea. The localization of starch within or in close proximity of sieve tubes in pea could be connected with their function, but in maize starch is abundant in xylem vessels. From the viewpoint of histogenesis (BENEŠ 1970, VORONKINA 1975, BARLOW 1976) pea and broad bean have transversal meristems with no distinct groups of initials, whereas in maize, particular initials have been distinguished for rhizodermis, cortex and central cylinder. The distribution of starch is quite opposite to what could be expected if histogenesis were the background for its localization. The same follows from tracing the line delimiting the root tip portions with and without starch in pea: the boundary between starch containing and starch lacking parts does not correspond quite exactly to the

margin between stele and cortex. It should be remembered that similar conclusions were reached on the relation between histogenetic and localization patterns in the case of several hydrolytic enzymes (BENEŠ 1975).

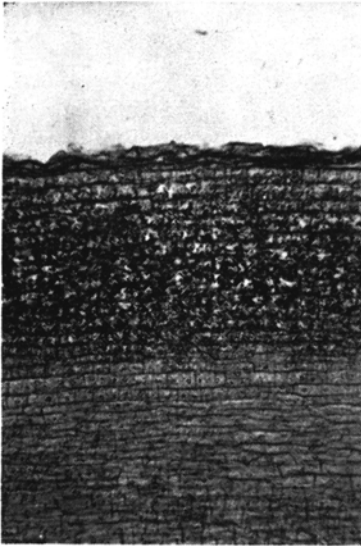
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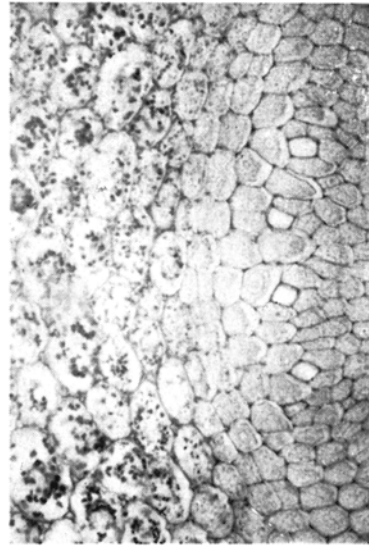
*Figures at the end of the issue.*

K. BENEŠ, J. KUTÍK  
LOCALIZATION OF STARCH

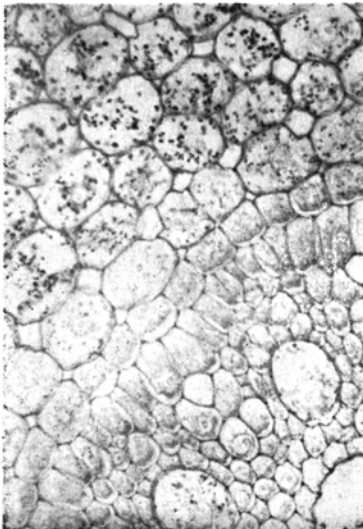
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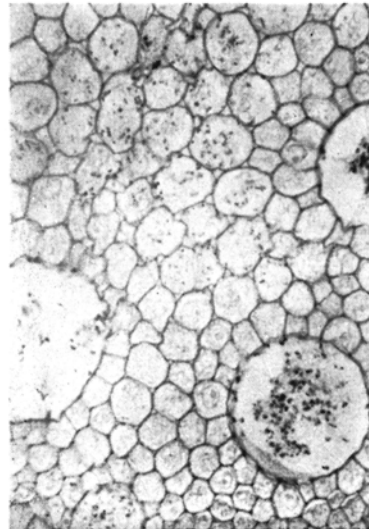
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3



4



The localization of starch in paraffin sections of FAA fixed root tips of pea and maize using alcian blue/JJK procedure. Figs. 1, 2 pea, Figs. 3, 4 maize. Fig. 1 longitudinal section, magn.  $160\times$ , Figs. 2, 3, 4 transverse sections, magn.  $320\times$ .

Note the absence of starch in almost the whole central cylinder in pea, and its presence in maize in xylem vessels and in pith.