CYTOPLASMIC INSOLUBLE POLYSACCHARIDES IN THE APICAL MERISTEMS OF *PHASEOLUS RADIATUS* ASSOCIATED WITH SEED GERMINATION*

SU-HWA TSAI CHIANG and TAN CHOU**

Abstract: Relative amounts and the distributional change of insoluble polysaccharide granules existing in the cells were observed in both root and shoot apical meristems of *Phaseolus radiatus* Linn. during seed germination. With the exception of the periblem and root cap, no polysaccharide granules were present in the resting embryo axis. They increased gradually and decreased again as the embryo grew. No more polysaccharides were seen except in the columella tip of the root cap in the materials longer than 60 hr after soaking.

INTRODUCTION

Apical meristems have long attracted the attention of botanists on account of their cellular contents as well as for other reasons. Their studies have clarified some of the conspicuous aspects in the differentiation of the root and shoot apical meristems relative to changes in the cell contents, such as: DNA, RNA, proteins etc. (Clowes, 1959; Jensen, 1958; Sunderland, Heyes and Brown, 1957; West and Gunckel, 1968a, b). But the study on the amount of change in carbohydrates is insufficiently known. In most cases, the workers have centered their interests on the root cap, and some associated with the perception of gravity (Iversen, Pedersen and Larsen, 1968; Juniper and French, 1970). The shoot investigators have also been more interested in observing the other contents rather than the carbohydrates. West and Gunckel's (1968) work indicated that the distribution of starch displays a differential zonation in the shoot than that in the root. In the previous papers, the zonation change of the root apical meristem at the OM level as well as the cytological changes in the promeristem at the EM level during the early stage of germination in *Phaseolus* were reported (Chiang and Tsou, 1974; 1975). The pattern of the distributional change of insoluble carbohydrates by histochemical technique in the cells of both root and shoot apical zones have been contrasted.

MATERIALS AND METHODS

Seeds and seedlings of *Phaseolus radiatus* Linn. were used. The source of the materials as well as the methods of soaking and sampling were mentioned in the previous reports (Chiang and Tsou, 1974; 1975).

Fixed in FAA, the excised tips were sectioned at 12 μ longitudinally after the traditional method of paraffin embedding procedure. Then the sections were stained with periodic acid-Schiff's (PAS) reation (Jensen, 1962) to detect the insoluble polysaccharides. In the present investigation, the observations were centered on the polysaccharides which were present inside the cells though both cell walls and cytoplasmic inclusions were fairly well stained.

RESULTS

I. Root Apical Meristem:

No polysaccharide granules could be identified in the promeristem until the root had been

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** 江蔡淑華 and 周淡, Professor and instructor in Botany, National Taiwan University.

soaked for more than 8 hr (Figs. 1, 2). The polysaccharide particles appeared in 8 hr root's promeristem. In 8 hr and 12 hr roots, they were numerous and distributed throughout the entire promeristem. In 24 hr root only a few particles were seen, and finally almost no PAS particles were visible in materials soaked longer than 48 hr (Table 1).

Table 1. Polysaccharide Distribution in Root Tips

hr after germination / tissue		0	4	8	12	24	48	60
periblem	outer	+	+	++	+	+		
	inner	+		++	+	+		
central procambium				+				
promeristem				++	++	+		
rootcap	periphery	+	+	+				
	columella tip	+	+	+	+++	+++	+++	+++

The polysaccharides were uniformly distributed in the periblem in the resting embryo (Fig. 1), and they showed a little decrease from the inner part (located next to central procambium). But they increased again in 8 hr root, then decreased gradually as the germination preceeded (Table 1).

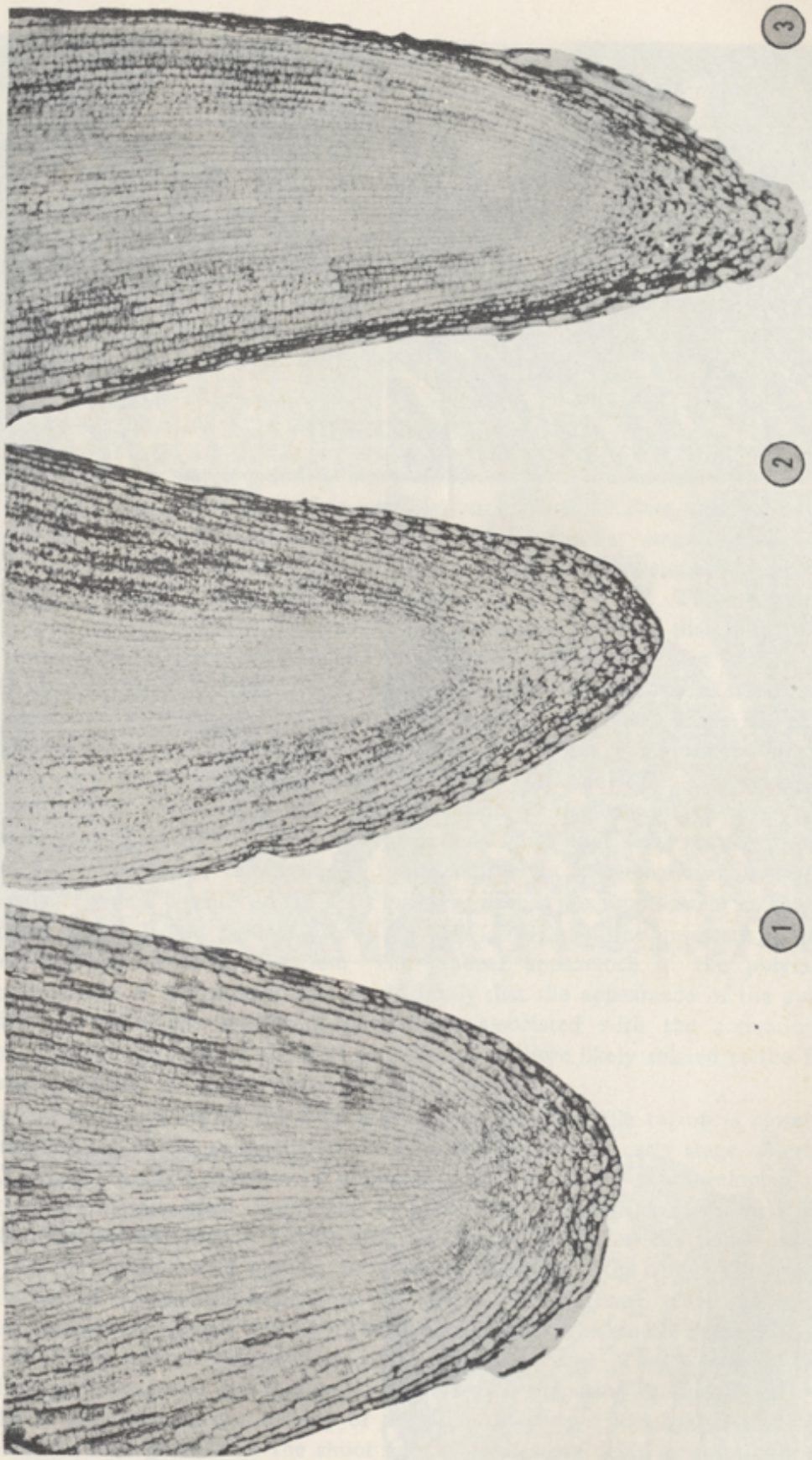
In the central procambium, the 8 hr material was the only stage containing PAS particles. On the other hand, the root cap was the only tissue which contained the polysaccharides in all the stages examined (Table 1; Figs. 1–3). The size of the polysaccharide granules in the cap cells appeared to be smaller than those found in either the promeristem or periblem. They were distributed uniformly throughout the entire root cap in the early stages of germination including the resting embryo. But in the 8 hr material, the PAS particles migrated gradually downward to the columella tip. In all the stages later than 12 hr, no PAS particles were seen in the root cap except in the columella tip (the columella excluding the region close to the promeristem). They increased in number in the 12 hr and 24 hr columella tip, then no conspicuous increase in number or redistribution was seen in the root caps after 24 hr of soaking (Table 1).

II. Shoot Apical Meristem:

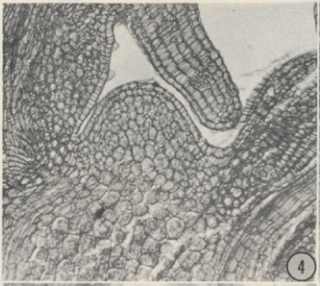
No polysaccharide granules could be seen in the entire shoot apex of the either embryo or the 4 hr stage. They first appeared in the corpus cells in the 8 hr shoot (Fig. 4; Table 2). In 12 hr material, they were distributed throughout the whole apex, and the ground meristem and the procambial area contained more particles than the promeristem (tunica and corpus). The size of the PAS particles seemed to be larger than at the earlier stages of soaking. In 24 hr stage, they tended to become more numerous in the entire apical region (Fig. 6). More

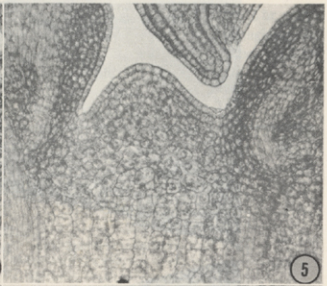
Table 2. Polysaccharide Distribution in Shoot Apices

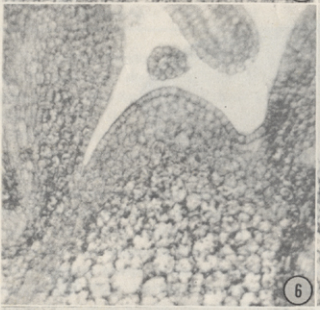
hr after germination / tissue	0	4	8	12	24	48	60
tunica				+	+		
corpus			+	+	++	+	(+)
ground meristem				++	+++	+	(+)
procambium				++	+++	+	

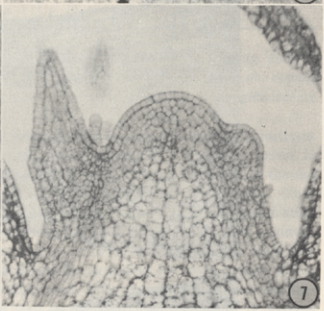


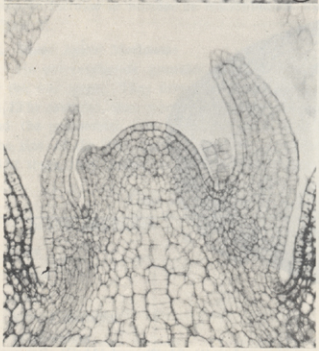
Figs. 1-3. Median longitudinal sections of the root tips from: resting embryo(1); 8 hr(2); and 12 hr(3) after soaking, all ×100.











Figs. 4-8. Median longitudinal sections of the shoot apices from: resting embryo(4); 12 hr(5); 24 hr(6); 48 hr(7); and 60 hr(8) after soaking. all ×175.

particles were located in the ground meristem and procambial area. In 48 hr shoot the central tunica became clearer in staining and the polysaccharides gradually decreased in the other parts. Finally they were visible only at the regions within the lower corpus and the upper ground meristem in 60 hr material (Fig. 8; Table 2).

In conclusion, the PAS granules in the embryo axis of this plant have changed during germination. No PAS particles were seen in the promeristems of either root or shoot of the resting embryo. But they appeared in these two promeristems as the embryo grew, and showed their maximum amount in the 8 to 12 hr roots and in the 24 hr shoots (Tables 1 & 2). The columella tip was the only structure which maintained insoluble cytoplasmic polysaccharides throughout all stages examined. The polysaccharides disappeared in all the structures other than the columella tip in the embryo axis after 60 hr soaking.

DISCUSSION

At the EM level, the starch granules were visible in the root promeristem in the stages from zero (resting embryo) to 24 hr after germination (Chiang & Tsou, 1975). But no PAS particles could be identified in the root promeristem at an earlier stage (before 4 hr stage). After the examination of the EM micrographs, the starch granules enveloped by plastids were the only granular polysaccharides in this plant (Chiang & Tsou, 1975). These starch granules were small in size in the materials of zero to 12 hr. This result indicates that the PAS particles in the root promeristem corresponded to the plastid-starch granules as seen in EM, and they could not be detected in OM level in zero to 12 hr roots because of their small size. The gradual disappearance of PAS particles in the later stages (after 48 hr) from the entire root tip (except the root cap) may have been correlated with the abrupt decrease in the diameter of the roots between 12 to 48 hr roots (Chiang & Tsou, 1974; Table 1). The apical meristem has been considered by some botanists be a continuation of the embryo of the plant which possesses an open type of growth. It is easy to understand that some changes could have occurred during the continuous development from embryo to later stages. One of the most conspicuous developmental changes in the early primary root is the establisment of the quiescent center (Clowes, 1958). It was pointed out by Clowes (1958) that the quiescent center in the primary roots appeared after germination. The gradual appearance of the polysaccharides during the germination in the promeristem could imply that the appearance of the cytoplasmic polysaccharides is one of the significant phenomena associated with the activation of the promeristem. And the decrease of the PAS particles seems more likely related to the formation of the columella (Chiang & Tsou, 1974).

Increase and accumulation of the PAS granules in the columella region is closely related with the establishment of the columella in the root cap during the early stage of germination (Chiang & Tsou, 1974). The presence of polysaccharide particles in the developing root (not the resting radicle) of this plant might act in some way as statoliths (Iversen, Pedersen & Larsen, 1968). Juniper and French (1970) also indicated that one of the important functions of the root cap, the perception of gravity, is served by the cells of the core. The redistribution and accumulation of the polysaccharides in cap cells in the present plant agrees with the description of Juniper and French (1970). They reported that the starch accumulated rapidly as soon as the cells ceased meristematic activity in root cap. The columella tip which accumulated polysaccharides as the root grew is the region preparing to slough off.

Generally at germination, the activity of the plumule always falls behind that of radicle. The change of the PAS granules in the shoot apex showed some delay as compared with that of the root tip (Tables 1 & 2). Polysaccharides showed their maximum amount in 8 to 12 hr stages in root, whereas it was 24 hr material in the shoot. Catesson (1953) indicated that the

young seedling cells of the tunica and corpus are more meristematic than the rest of the apex. And the rate of division is low at the summit of the apex in the later stage of growth (Buvat, 1958). So that the amount of change of the cytoplasmic polysaccharide particles in the shoot apical meristem may reflect the developmental status in seedling of the plant. On the well-developed shoot apices of *Brachychiton*, the large amounts of starch grains were found located in the central and pith rib zones including the corpus cells (West and Gunckel, 1968b). This different pattern of starch distribution from that observed in *Phaseolus* could be due to the different conditions of differentiation and perhaps the other factors.

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