ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 2: 160-169
1986

Distribution of insoluble polysaccharides in the shoot apex of *Rhododendron arboreum* Linn. during the annual growth cycle

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(Received: August 14, 1985. Accepted: October 28, 1985)

Abstract

Starch grains occur all over the dormant shoot apex of *Rhododendron arboreum* except in the bud scales. They are abundant in the peripheral, rib and pith meristem cells, as well as in the youngest leaf primordia. Tannin is present in the entire dormant bud but for the cells of the apical meristem and leaf primordia. Gradually, tannin degradation into numerous globules occurs. This is concomitant with the disappearance of starch grains and indicates the earliest structural manifestation of spring awakening by meristematic activity in the buds. The weak affinity of tannin globules to PAS is due to their hydrolysis which releases glucose for metabolic activities. Thus, a parallelism seems to exist between the metabolism of tannins and starch in relation to the various phases of bud development.

Key words: apical meristem, dormancy, pith meristem, polysaccharides, spring-bud awakening, tannin

INTRODUCTION

The polysaccharides, tannins and other metabolites usually accumulate in dormant buds of a number of temperate trees as stored food reserves also serve as a source of energy especially for spring shoot elongation (Kozlowski and Winget 1964). The earlier studies on the histochemical localization of insoluble polysaccharides in the vegetative shoot apices cover limited aspects, particularly from the standpoint of the seasonal variation during the year (Pillai and Chacko 1978, Hejnowicz 1979). While investigating the bud development of *Pinus silvestris*, the latter author analysed

the distribution of starch in relation to that of tannins during the yearly growth cycle, and concluded that the tannin of dormant buds could be one of the sources of starch synthesis during spring. Although an attempt was made by Hejnowicz (1979) to present a sufficently detailed analysis, no generalization could be made as to whether such a relationship doe exist in other temperate trees as well, particularly the angiosperms. Keeping this viewpoint, and paucity of reports available in literature, the present work has been undertaken on *Rhododendron arboreum* — a temperate, evergreen tree, covering localization of insoluble polysaccharides during different growth phases of the vegetative bud development.

The shoot apex (terminal vegetative bud — terminology adopted from Owston 1969) of this species shows marked seasonal variations, both morphologically and histochemically (Badola 1983). Its dormant phase is witnessed between the last week of October to March and is recognized by the absence of mitotic activity. The summer activity is observed by the last week of March in the entire vegetative bud owing to enhanced cell divisions, and continuous bud scale initiation takes place till the third week of August. The bud swells and sprouts in the first week of May, leading to rapid shoot elongation. By the third week of August, the bud scale initiation ceases and leaf initiation begins which ends by the last week of October, leading to the formation of overwintering buds.

MATERIAL AND METHODS

Terminal vegetative buds were collected weekly or bi-weekly from various branches situated approximately at the same crown level of mature trees, growing naturally in the Kandolia Forest Range, Pauri (U.P.), India (altitude 1800 m, 29°20′ to 30°15′ N latitude and 78°10′ to 79°10′ E longitude) from February, 1980 to January, 1982. These were subjected to the commonly employed microtechniques (fixed in FAA, embedded in paraffin and cut on a rotary microtome). The 6-9 μm thick, median longitudinal sections were obtained and stained with PAS reagent (Jensen 1962). Tannins were localized by $FeCl_3$ test.

Terminology: The description of basic zonation pattern of apical meristem encompasses three zones, viz. central, peripheral and rib meristem, adopting the views of Philipson (1954).

RESULTS

During the dormant phase, the cells of the shoot apex (used here synonymously with the terminal bud) showed enhanced affinity for PAS

reagent, including those comprising the apical meristem, indicating higher accumulation of insoluble polysaccharides (Fig. 1). The starch grains distinctly appeared in the cells of the latter, in the leaf primordia and the pith cells, but were absent or rare in the bud scales. The dormant shoot apex showed these in increasing size and quantity below the apical meristem, towards the pith cells (Fig. 5) up to a distance of 200-300 μm in addition to the presence of tannin-filled cells (Fig. 2). Below this region, the starch grains decreased both in size as well as density. The tannin cells gave a positive reaction to PAS, and stained reddish orange to dark brown or black (also stained deeply with $FeCl_3$). The pith cells stained magenta in colour. The cells at the base (constituting a transitory, spongy basal pith region between the bud and last year's shoot below) of the dormant buds had degraded protoplasts, and destroyed plasma membranes and cell walls which formed lysigenous cavities. These were completely devoid of starch grains but accumulated large amounts of tannins (probably non--hydrolysable type as indicated by their low affinity to PAS) and other secretory substances which did not show a higher affinity for PAS (Fig. 3).

In the apical meristem, the starch grains abounded in the peripheral (Fig. 4) as well as the rib meristem zone and below it (Fig. 5). Although very few and minute, these also appeared in the cells of the central zone, except those of the axial tunica layers which showed their presence prominently (Fig. 6). In the leaf primordia, these were frequently observed, especially in the cells at the tips, and in the surface layers (Fig. 4).

The spring bud awakening was witnessed by tannin fragmentation and disappearance of starch grains followed by enhanced cell divisions all over the bud, particularly in the pith meristem (Figs. 7, 8), leading to bud swelling. The degraded tannin globules could be seen all over in the pith cells which showed a weak affinity for PAS and stained orange. The pith cells now hardly showed any starch grains, but cytoplasm, especially close to walls, showed a weak reaction for PAS and stained pinkish-violet. As revealed from Fig. 9, during bud scale initiation, the cells of the apical meristem hardly exhibited any starch grains.

Late in the summer, in the pith cells of the current year shoot, the affinity for insoluble polysaccharides increased with only rare starch grains. By the beginning of July, i.e. when rapid elongation of shoots ceased, the pith at the base of the developing buds become demarcated due to enhanced accumulation of insoluble polysaccharides suggested by the darker stainability of cells with PAS. The starch grains also showed up in this region, but as the shoot elongation stoped these disappeared and an increasing accumulation of tannins as well as other metabolites started.

During the leaf initiaton phase, the entire bud gradually accumulated increasing amounts of polysaccharides but only a few starch grains appeared

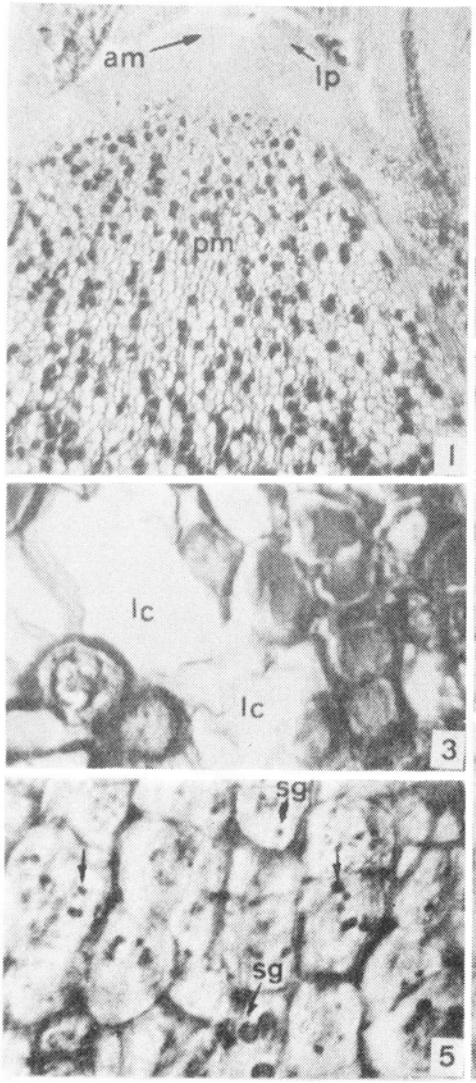
in the apical meristem. Towards late October (i.e. beginning of dormancy), the pith meristem cells showed only limited starch grains and augmented accumulation of tannins and those of the apical meristem did not indicate any appreciable amount of starch grains until the end of December, although later their amount increased.

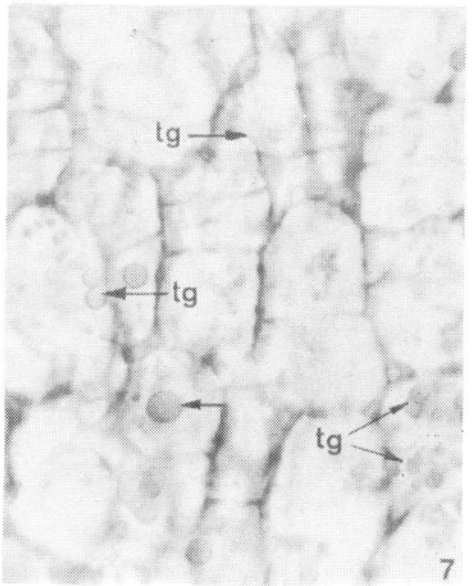
DISCUSSION

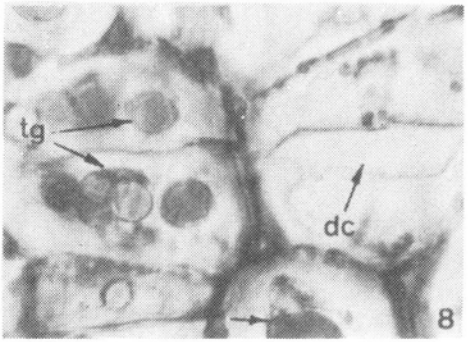
The higher amount of insoluble polysaccharides in the cells of a dormant shoot apex has also been recorded by a few earlier authors (Shah and Raju 1975, Pillai and Chacko 1978). Von't Hof (1968) stated that the cell wall carbohydrates are necessary for cell division, West and Gunckel (1968) associated their role with cell elongation. The deposition of polysaccharides at the basal region of the buds of *Picea* has been suggested by Romberger and Tabor (1975) on the basis of the data obtained from their tissue culture experiments. These authors have later (Romberger and Tabor 1976) stated that soluble precursors of the polysaccharides are gradually released by the tissue and get slowly deposited at sites remote from the living cells. In the present study, the deposition of insoluble polysaccharides and tannins occured at the base of the buds during new bud development. As the shoot elongation ceased (towards the dormant period), these cells showed granular deposits of tannins, with limited affinity for PAS. These are probably in the condition which eventually all tannin cells of the bud attain after complete hydrolysis of the tannin present in the cells (Hejnowicz 1979) and support the contention that the pith

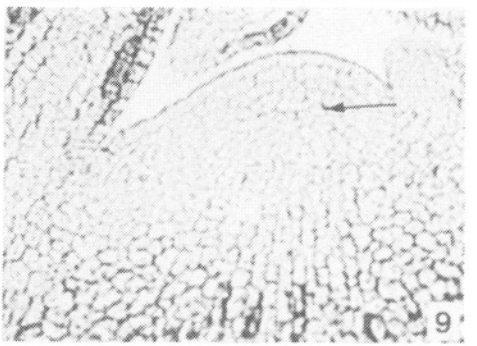
Figs. 1-6. Microphotographs of median longitudinal sections through the vegetative shoot apices during the dormant period, stained with PAS (am — apical meristem, lc — lysigenous cavity, lp — leaf primordia, pm — pith meristem, sg — starch grains, tc — tannin cells). Fig. 1. A PAS-positive reaction throughout the cells of the shoot apex; tannin is absent from the apical meristem and leaf primordia but is prominent in the pith cells. X 110. Fig. 2. Pith meristem cells at a distance of 200-300 μm from the apical meristem showing prominent starch grains and tannin filled cells, a PAS-positive reaction. X 1250. Fig. 3. A portion of pith region from the base of the dormant bud showing degraded protoplasm, damaged plasma membrane (formation of lysigenous cavities), and accumulation of secretory substances with reduced affinity to PAS. X 500. Fig. 4. Peripheral zone of apical meristem with outer tunica layer and lower surface layers of youngest leaf primordia with copious and prominent starch grains and PAS-positive stain reaction. X 1250. Fig. 5. Cells of the rib meristem zone exhibiting increasing size of starch grains towards the pith below; PAS-positive test. X 1250. Fig. 6. Cells of the central zone showing a few starch grains; the outer tunica cells possess these prominently. X 1450

PLATE – I









Figs. 7-9. Microphotographs of median longitudinal sections through vegetative shoot apices stained for PAS reaction (dc — dividing cell, tg — tannin globules). Figs. 7-8. Actively dividing pith meristem cells (April end) showing disappearance of starch grains, but fragmented tannin glubules can be seen all over the cells which stained poorly for PAS: a few cells have been enlarged to bring out tannin globules (Fig. 8). Fig. 7 — X 700, Fig. 8 — X 1200. Fig. 9. Least affinity to PAS in the cells of apical meristem, except corner thickenings of the cell walls in the central zone (June). X 220

region at the base of the dormant bud does not help in the bud/shoot elongation during spring, in most of the temperate trees.

Besides the deposition of insoluble polysaccharides in the dormant bud, abundant starch grains have also been recorded in its cells. Their presence below the basal pith region/crown region in the dormant bud of *Cedrus deodara* has been noticed by Pillai and Chacko (1978) who stated that these had no role in shoot elongation. In *R. arboreum*, these grains were mostly present in cells above the basal pith region, rarely below it an therefore seem to have a relationship with bud elongation as they become digested or disappear during the spring growth. Riding and Gifford (1973) observed starch grains in the pith meristem, rib meristem and the peripheral zone and related their abundance to the cell division activity. The restricted distribution of starch-containing plastids in the central zone of the vegetative shoot apical meristem of *Helianthus annuus* observed by Sawhney et al. (1981) exclusively in the two tunica layers, which were in fact most distinct in terms of both cell and nuclear volume and were physiologically different from the remaining central zone cells, is also worth noting. In *R. arboreum* as well, the situation is identical with regard to the axial tunica cells in the central zone. The accumulation of starch grains during dormancy reflects high energy requirements for the morphogenetic activities of the shoot apex. Contrastingly, Lynch and Rivera (1981) observed that the lack of starch grains in the dormant apex (a histological observation only) of *Rhododendron maximum* suggests a pattern of structural carbohydrate synthesis rather than their role as energy reserves. During the dormant period, prior to bud swelling in *R. arboreum*, the starch grains increased in quantity and as the activity started with the elongating bud, these become digested rapidly, suggesting that the bud swelling as well as the new shoot elongation depends upon the food reserves from the subtending, one-year-old shoots of the dormant bud (Krueger 1967).

The cells of the apical meristem indicate a decline in the polysaccharide content during the period when primordial initiation was rapid, and as the mitotic activity slowed down, these started increasing gradually. Cecich and Horner (1977) have observed that starch is digested just prior to the appearance of leaves. Similarly, Murakami (1960) has also observed reduced polysaccharide concentration during organogenesis and Riding and Gifford (1973) have stated that this decrease is due to the formation of needle primordia in *Pinus radiata*.

The tannins occurring in the dormant buds of the present species gave positive PAS reaction. This indicates that these are the hydrolysable type (Haslam 1966) having within their structure a glucose group. During the spring, they become fragmented into smaller globules, which show a weak PAS reaction. This, accompanied by the disappearance of starch grains, indicates thereby that tannins present in the vacuoles undergo hydro-

lysis and perhaps release glucose groups which are transported to the cytoplasm and utilized for metabolic activities in the same way as starch grains. This also supports the views of Block and Ball (after Hall et al. 1972) and Hejnowicz (1979), that the accumulation of tannins in the cells does not deprive them of mitotic activity. Hejnowicz (1979) could not observe any starch grains in dormant buds but noticed that their maximum quantity in the bud coincides with the period when tannin vacuoles are most fragmented (in spring). She formulated the probable role of tannin cells, towards the synthesis of starch grains. Although she pointed out that these do not seem to be the only source of synthesis of starch grains. By comparing the above two studies it seems that the dormant buds of *Pinus silvestris* (Hejnowicz 1979) were not rich in energy reserves (i.e. absence of starch). During spring activation of buds, the tannin vacuoles became converted into glucose after fragmentation and finally synthesized starch grains which also disappeared for metabolic activities. On the other hand, the dormant buds of *R. arboreum* are already rich in energy reserves (i.e. presence of starch grains as well as tannin vacuoles) and during spring the starch is utilized for morphogenetic processes. The tannins released glucose after fragmentation but did not synthesize starch grains, perhaps it was already present in abundance. The present study shows that more or less the tannins and starch grains run parallel metabolically during seasonal growth cycle of bud development.

Acknowledgment

Acknowledgment is due to the Council of Scientific and Industrial Research, New Delhi for the award of a „Research Associateship" to first author.

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Rozkład nierozpuszczalnych polisacharydów w wierzchołku pędu Rhododendron arboreum Linn. w czasie rocznego cyklu wzrostu

Streszczenie

Ziarna skrobi występują w całym zimującym wierzchołku pędu *Rhododendron arboreum* z wyjątkiem łusek pąków. Są one liczne w zewnętrznych, pasmowych i rdzeniowych komórkach merystematycznych, jak też w najmłodszych zawiązkach liścia. Tanina występuje w całym zimującym pąku, ale jest tylko w komórkach merystemu wierzchołkowego i w komórkach zawiązków liścia. Stopniowo zachodzi rozpad taniny na wiele ziarenek. Zjawisko to towarzyszy znikaniu ziaren skrobi i wskazuje na najwcześniejsze, strukturalne objawy wiosennego „budzenia się" aktywności merystematycznej w pąkach. Słabe powinowactwo ziarenek taniny do PAS jest związane z ich hydrolizą, podczas której jest uwalniana glukoza, potrzebna do aktywności metabolicznej. Tak więc, wydaje się że jest analogia między metabolizmem tanin i skrobi w różnych fazach rozwoju pąka.