Plant Physiol. (1974) 53, 398-401

Amyloplast Size and Number in Gravity-compensated Oat Seedlings[1]

Received for publication May 29, 1973 and in revised form October 15, 1973

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ABSTRACT

Gravity compensation by the horizontal clinostat increases the diameter of amyloplast starch grains of oat (*Avena sativa* cv. Victory) coleoptile parenchyma cells, as compared to vertically rotated and stationary controls. In dark-grown coleoptile tip parenchyma cells, measured starch grain sizes exhibit a wide distribution of diameters, from approximately 1.5 to approximately 8.0 μm, but fall into three prominent diameter classes. The compensated tissues from both the tip and the subapical region have more starch grains in the larger, and fewer in the smaller size classes, compared to controls. The total number of starch grains per cell, the total plastid number per cell, and cell volume are unaffected by gravity compensation. Amyloplasts with large starch grains are denser, as well as larger in diameter, than those with smaller starch grains. The amyloplast is considered as a geosensor with an active metabolic role in the geotropic transduction mechanism.

In recent years increasing emphasis has been placed on the clinostat as a substitute for the free-fall environment, or as a test situation preliminary to free-fall experiments utilizing orthogeotropic plants. Clinostats are devices designed to reorient the plant continuously by rotation on a horizontal axis, so that the tropic effect of gravity direction is nullified or "compensated." Gravity-compensated plants grow in the direction imparted by the original orientation of the plant or seed and show no directional response to the continuously changing directional vector of gravity. Furthermore, gravity compensation appears to increase shoot geosensitivity. If the shoots of compensated cereal grain seedlings are subsequently gravity-stimulated by placing them in a stationary horizontal position, they will curve more than shoots that were not compensated prior to geostimulation (6, 24).

In the cells of the geoperceptive regions of many plants, mobile, dense amyloplasts fall toward the physically lower wall during geotropic stimulation. The "statolith" amyloplasts observed in these geoperceptive regions are often ultrastructurally distinct (higher starch-stroma ratio) from those found elsewhere in the plant (14) and are believed by many to function as gravity sensors (1, 19, 26; but see 21).

[1] Research was supported by Grant W12792 from the National Aeronautics and Space Administration and by the United States Atomic Energy Commission.

[2] Solon A. Gordon died October 5, 1973.

Increased levels of starch and soluble sugars have been observed recently in the shoots of gravity-compensated oat seedlings (8). The carbohydrates were assayed using extraction methods, and thus it was not determined if the increase in starch was due to an increase in starch grain size or number. We present evidence here that indicates that elevated starch content in compensated oat shoots is the result of an increase in the size of amyloplast starch grains but not in their number.

MATERIALS AND METHODS

Seeds of *Avena sativa* cv. Victory were soaked in the dark for 2.5 hr in tap H_2O at 25 C. They were then drained and stored in the dark at 2 to 5 C for 18 hr. Following cold treatment the seeds were planted in 250-ml beakers containing 4 cm of quartz sand, 16% water content. Seven seeds were planted vertically and uniformly spaced in each 250-ml beaker with the embryo of the seed toward the bottom of the beaker. The beakers were covered with Saran Wrap and placed on single-axis clinostats rotating at 2 rpm, with the axis of rotation horizontal (compensation) or vertical (rotated controls). A third set of beakers was placed in a stationary vertical position (stationary controls). The plants were grown at 26 C for 72 hr in total darkness and harvested under a dim green safelight (23). The shoots (coleoptile plus its contained leaf) were immediately fixed in warm (70 C) FPA (formalin, 5%; propionic acid, 5%; 70% ethyl alcohol, 90%; all volume percentage) under aspirator vacuum, dehydrated in a tertiary butyl alcohol series (17), and embedded in Tissuemat. Sets of serial longitudinal sections, each 10 μm thick, were cut approximately perpendicular to the plane of the vascular bundles. Only sections within 50 μm of either side of the median plane of the shoot were used for counts and measurements. The sections were stained with IKI (1% I_2 in 1% aqueous KI), and mounted in a Karo syrup-IKI mixture (16). The "tip" refers to those parenchyma cells above the lumen; "below-tip" refers to the parenchyma cells from the top of the lumen down to 3 mm below the coleoptile tip.

For total plastid counts, the apical 3 mm of fixed coleoptiles, with the leaf removed, were rinsed in water and macerated in a 20% pectinase (Nutritional Biochemicals) solution at pH 4.5 for 24 hr at 25 C. Following maceration the tissue was rinsed and mounted in 80% aqueous Karo. The individual cells were spread by tapping the cover slip with a pointed wooden dowel. These preparations were examined and counted using Zeiss Nomarski optics. Measurements of entire compound starch grains were made to the nearest 0.5 μm using a calibrated ocular micrometer. For all measurements, significance of the difference between means was assessed by the *t* test. Since the means of the two controls (vertical rotation and vertical stationary) were not significantly different ($0.40 > P >$

0.50) for any of the measurements, these two groups of data were pooled in each set.

RESULTS AND DISCUSSION

In the IKI-stained preparations, the starch grains appear as round, uniformly dark bodies, apparently free within the cells. However, ultrastructural studies (15) of oat coleoptiles show that starch grains are always deposited within a plastid and are usually composed of two to six closely appressed polyhedral granules.

Figure 1 shows that the starch grain diameter is larger in compensated shoots, both in the tip and in the cells below the tip, than in noncompensated controls. The differences are highly significant ($P < 0.01$). The number of starch grains per 10 μm cell section in each region is not significantly different in compensated and control shoots (Table I). The shape of the distribution curves for starch grain diameters in the compensated and control tissues of the tip region exhibit a degree of similarity, but the starch grains of the compensated tissues fall into three prominent size classes, whereas the controls fall into only two. The compensated tips have more grains in the larger (4.5–5.0 μm), and fewer in the smaller (2.5–3.0 μm) prominent size classes. In the below-tip tissues the distribution curves of starch grain diameters in both compensated and control tissues are again similar to each other and exhibit somewhat normal size distributions with a single prominent size class for each of the treatments. In the compensated tissues the entire distribution curve is shifted toward the larger size classes. However, the two distribution curves of the tip region are quite dissimilar in shape from the corresponding curves of the below-tip region.

Amyloplasts are the only organelles that can be directly observed to settle to the physical "bottom" of living plant cells upon reorientation. Since amyloplast starch has a density (1.60–1.63; ref. 22) considerably greater than cytoplasm (approximately 1.05; ref. 18) or plastid matrix (1.2–1.3; ref. 22), amyloplasts with large starch grains will be more dense, as well as larger in diameter, than those with smaller starch grains, assuming a constant volume of plastid matrix. According to Stokes' law, the velocity of sedimentation of a body is proportional to the square of its radius times the difference in density between the particle and the suspending medium. Thus, an explanation can be suggested for the enhancement of geosensitivity in shoots by gravity compensation based on an increase in amyloplast size and density. Hertel *et al.* (11) and Filner *et al.* (7) have examined the geotropic responses of a number of mutants of maize with large or small starch grains in their amyloplasts. They have demonstrated a high, direct correlation between amyloplast size and both geotropic response and lateral redistribution of auxin. In contrast to the geotropic effect, phototropically induced lateral auxin asymmetry was not significantly different in the normal and mutant corn varieties. Amyloplast size is often directly proportional to starch content. However, the movement *per se* of amyloplasts, or any other organelle, such as the dictyosome (25), in geotropically stimulated plant cells does not explain the transduction mechanism producing the hormone imbalance that precedes geotropic curvature.

Our results are consistent with hypotheses proposing sedimented statolith amyloplasts as the source of some chemical or factor that stimulates transport (1, 2, 12). In this case, amyloplasts of the oat coleoptile tip parenchyma cells may function as physiologically active gravity sensors that polarize the cell by providing more starch-derived metabolites to the physically lower portion of the cell into which they settle. These amyloplasts are considered to continually secrete sugar as a result of an internal starch turnover mechanism (14). This localized supply of carbohydrate is probably different from the transport sugar (3) and could drive mechanisms such as hormone synthesis and active transport, resulting in differential secretion of hormone between the "up" and "down" side of the cell. Leopold and Hall (20) using mathematical models of auxin transport have shown that very small differences in secretion between two sides of a cell can be amplified into large differences in over-all transport upon integration through tissues. Conversely, under gravity compensation (9),

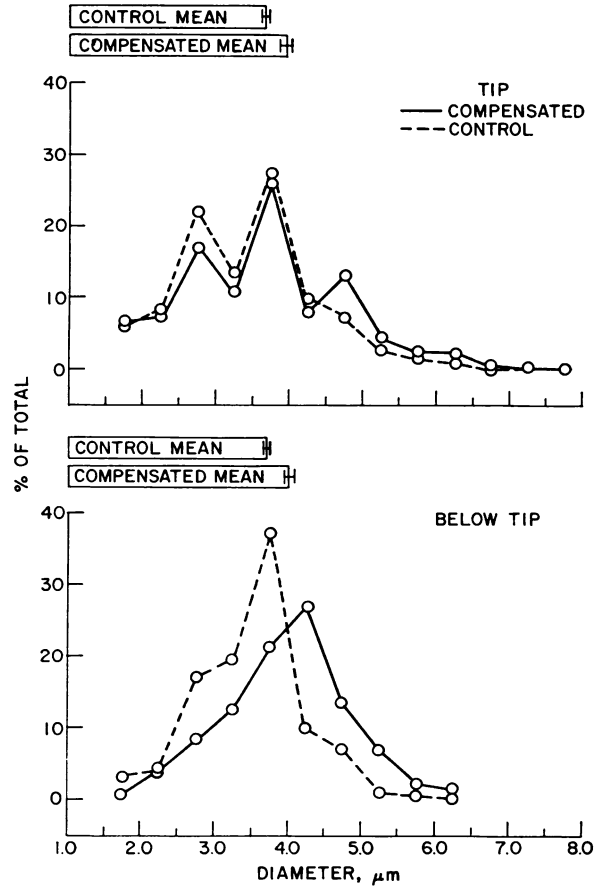

FIG. 1. Compound starch grain diameters in compensated (—) and noncompensated (--) (control) tissues of the tip and below-tip regions. The distribution curves show the percentage of starch grains in each of the 0.5 μm size classes. The histograms show the mean diameters ± SE (differences significant at the 1% level).

Table I. *Number of Starch Grains Per Cell and Cell Volume in Parenchyma Cells in the Tip and Subapical Regions of Compensated and Control Tissues*

Numbers in the same columns with different superscripts are significantly different ($P < 0.01$).

	No. of Starch Grains per 10 μm Parenchyma Cell Section	Volume of Parenchyma Cells
		μm^3
Tip		
Compensated	14.6 ± 0.7[a]	11,600 ± 770[a]
Control	13.6 ± 0.5[a]	14,200 ± 1200[a]
Below Tip		
Compensated	9.9 ± 0.5[b]	30,000 ± 1800[b]
Control	8.9 ± 0.3[b]	30,300 ± 1500[b]

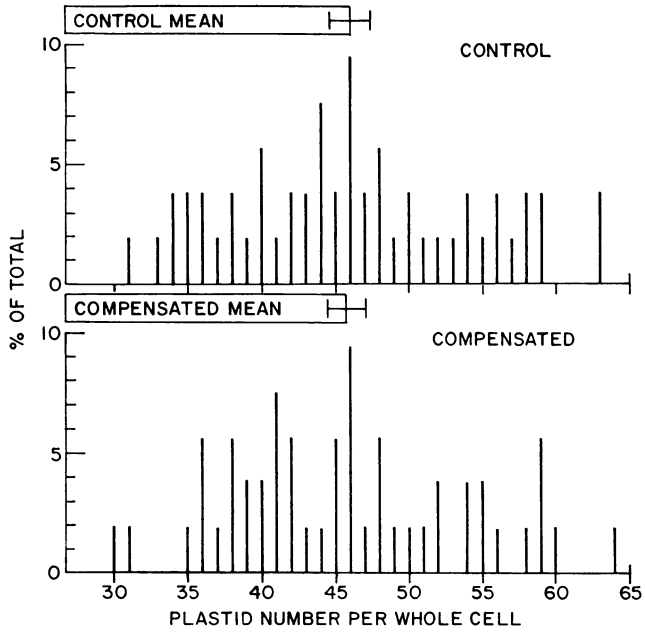

FIG. 2. Total plastid number per parenchyma cell in compensated and control tissues, expressed as the mean ± SE (difference not significant), and as a percentage distribution.

or under actual free-fall conditions (10) the amyloplasts become uniformly distributed and the organs show no georesponse, but do show increased rates of respiratory metabolism (5).

In compensated oat shoots, increases in starch and soluble sugars, in starch grain diameter, and in metabolic activity all suggest an enhanced availability of carbohydrate from a source external to the shoot. The only source of such carbohydrate in a 72-hr dark-grown seedling is the endosperm. A compensation-induced mixing action in the endosperm cavity may cause increased hydrolysis of the very large, free starch grains that comprise most of the semiliquid contents (13). The starch grains, degradative enzymes, and scutellum surface would make increased contacts with one another. Increased absorption and transport to the shoot could result in increased levels of carbohydrates as reported by Gordon (8), in increased amyloplast size following redeposition as starch, as reported here, and increased respiratory metabolism by the mechanism proposed by Dedolph (5).

Without distinguishing between amyloplasts and other plastid types, there is a statistically constant number of plastids per whole parenchyma cell in the 3 mm coleoptile tip regardless of treatment (Fig. 2). These counts were made on macerated tissue using Nomarski optics, under which all of the plastids appeared similar. The maceration procedure results in disruption of the tissue so that tip and below-tip cells could not be distinguished. It also affects the stainability of the starch grains, and may cause some hydrolysis of starch. Thus, this material could not be used for starch grain counts, but only for total plastid counts. The distribution of plastid numbers per cell are similar in the two treatments and range between 30 and 65 plastids per cell.

Although there is no difference in starch grain number between compensated and control cells in each of the two regions examined, there is a significantly ($P < 0.01$) greater number of starch grains per cell section in all of the tip cells compared to all of the cells below the tip (Table I). Since we have shown a constant number of plastids for all of the parenchyma cells studied (Fig. 2), the differences in amyloplast number may be partially explained by the larger average volume of the cells below the tip (Table I). A smaller fraction of the total number of plastids would be observed in each 10 μm section of the larger cells. Contributing to the larger number of starch grains observed in the tip cells may be the tendency of the amyloplasts there to maintain a full complement of starch beyond 72 hr of growth in darkness (14), whereas the amyloplasts of the parenchyma cells below the tip lose starch during this period. Starch is not lost uniformly from all of the below-tip amyloplasts. Rather, some amyloplasts retain starch grains while others become completely starch-free and thus are not visible in the sectioned material stained with IKI (15).

The starch mobilization (degradation or deposition) characteristics and ultrastructural appearance of the tip amyloplasts are quite distinct from those in the below-tip region. In many of the tip amyloplasts one or two of the starch granules comprising the dimeric to hexameric compound grain are observed to be preferentially mobilized, while the other granules are unaffected. Hypotheses explaining the ability to localize the degradative process to a particular area within the plastid have been proposed (14, 15). They suggest that a portion of the starch-mobilizing enzyme system is bound on, or within, the numerous plastid lamellar membranes that are observed in close proximity to, or within, the eroding grains. Coleoptile tip amyloplasts that maintain a uniform morphology and that show localized, lamellae-associated starch mobilization are observed throughout the first 96 hr of growth in the dark (14). These observations suggest a process of continuous carbohydrate turnover in these plastids. The degradation or deposition of an individual starch granule within a tip amyloplast is sufficient to shift the compound grain size by several of the diameter classes of Figure 1, and may help to explain the multiple peaks in the size distribution curves.

Whether or not starch-derived metabolites function directly in the gravity-sensing mechanism is still unresolved. However, from this study it is apparent that changes in the gravitational environment of oat seedlings result in changes in the size of amyloplast starch grains. The increases in starch grain diameter observed here under gravity compensation result in amyloplasts of greater size and density. These findings are consistent with reports (4, 6) that clinostat rotation increases the geotropic curvature in response to subsequent stimulation, and with the hypothesis that amyloplasts have a role in the geoperceptive mechanism.

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