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SUMMARY OF RESEARCH

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Title: **Re-Evaluation of the Role of Starch in Gravitropic Sensing**

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Re-Evaluation of the Role of Starch in Gravitropic Sensing

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SUMMARY

Plant organs grow toward or away from gravity as a way to orient those organs for optimizing growth. Starch has long been thought to be important in sensing the direction of the g-vector in gravitropism, but that hypothesis has also evoked controversy. We have previously shown that starch-deficient mutants of *Arabidopsis* (TC7, *pgm*) and *Nicotiana* (NS458, *pgm*) are impaired in their gravitropism. While this suggests that starch is not necessary for reduced gravitropism, it also indicates that the mass of the starch contributes to sensing when present and thus is necessary for full gravitropic sensitivity.

The research supported by this grant focused on three related projects, (1) the effect of light on hypocotyl gravitropism in NS458, (2) the effects of root phototropism on measurements of gravitropic sensitivity, and (3) the effects of starch overproduction on sedimentation and gravitropism.

Collectively, our results provide additional strong support for the importance of starch in gravitropic sensing. First, by accounting for negative phototropism in roots of two starchless mutants of *Arabidopsis*, *pgm* and *adg1*, we have established that these mutants are much less sensitive to gravity than previously thought. This work also demonstrates the importance of designing experimental protocols that remove the influence of root phototropism on measuring root gravitropism. Second, light apparently promotes gravitropism in starch-deficient *Nicotiana* hypocotyls by increasing the trace amounts of starch in the plastids, by inducing limited plastid sedimentation and thus by presumably increasing the signal provided by plastid mass. And finally, we show that excess starch in *Arabidopsis* seedlings has little effect on gravitropic sensitivity implying that the sensing system is already saturated. However, in light-grown stems where this mutation results in starch accumulation and where the wild-type practically lacks starch in the sensing cells, the mutant is much more sensitive than the wild-type again showing that the loss of starch depresses gravity sensing.

Context

Starch has long been thought to be important to gravitropic sensing, but the mechanism of sensing is still unclear. We have previously shown that starch-deficient mutants of *Arabidopsis* (TC7 = *pgm*) and *Nicotiana* (NS458 = *pgm*) are impaired in their gravitropism (reviewed in Sack, 1997). While this suggests that starch is not necessary for reduced gravitropism, it also indicates that the mass of the starch contributes to sensing when present and thus is necessary for full gravitropic sensitivity.

However, two problems arose regarding the TC7 mutant of *Arabidopsis* that we studied extensively. First, Dr. Kenneth Poff found evidence that the line of TC7 we were using might contain a second mutation that is responsible for impairing gravitropism and that this mutation was independent of a lesion in starch. If true, it should have been possible to separate out these two mutations genetically with the prediction that starchless plants should be normal in gravitropism and that plants abnormal in gravitropism should be normal in starch. We obtained all the seeds of the critical crosses from Dr. Poff and intended to do years of genetics to determine if his initial data were true. However, in the interim, Tim Caspar, who isolated the original TC7 mutant, also isolated a second *pgm* allele and in collaboration with John Kiss found that this mutant was also deficient in gravitropism (reviewed in Sack, 1997). Moreover, as described below, we found that gravitropism was also depressed in a second locus, *adg1*. The probability that three different mutations all contain the same second mutation responsible for depressing gravitropism is so remote as to not warrant performing all the genetic work. Thus, we conclude that the absence of starch depresses gravitropism, as originally predicted.

A second reason that we wanted to reexamine the role of starch was that it became clear that *Arabidopsis* roots were phototropic and that negative phototropism might have influenced our previous assessments of root gravitropism. After extensive study (see below) we concluded that root phototropism resulted in a gross overestimate of the gravitropic sensitivity of starchless mutants and that therefore the absence of starch depresses gravitropism much more than previously thought.

Other related projects that we explored are why light-grown stems of the *pgm* mutant of tobacco are much better at gravitropism than dark-grown stems, and also whether an overproduction of starch enhances gravitropism.

Light promotion of hypocotyl gravitropism of a starch-deficient Nicotiana sylvestris mutant correlates with plastid enlargement and sedimentation

Dark-grown hypocotyls of a starch-deficient mutant (NS458) of *Nicotiana sylvestris* lack amyloplasts and plastid sedimentation, and have severely reduced gravitropism. However, gravitropism improves dramatically when NS458 seedlings are grown in the light. To determine the extent of this improvement and whether mutant hypocotyls contain sedimented amyloplasts, gravitropic sensitivity (induction time and intermittent stimulation) and plastid size and position in the endodermis were measured in seedlings grown for eight days in the light. Light-grown NS458 hypocotyls are gravitropic but are less sensitive than the wild type. Starch occupies 10% of the volume of NS458 plastids in both the light and the dark, whereas wild-type plastids are essentially filled with starch in both treatments. Light increases plastid size twice as much in the mutant as in the wild type. Plastids in light-grown NS458 are sedimented, presumably due to their larger size and greater total starch content. The induction by light of plastid sedimentation in NS458 provides new evidence for the role of plastid mass and sedimentation in stem gravitropic sensing. Since the mutant is not as sensitive as the wild type, NS458 plastids may not have sufficient mass to provide full gravitropic sensitivity.

Interaction of root gravitropism and phototropism in Arabidopsis wild type and starchless mutants

Arabidopsis roots are negatively phototropic (grow away from the light), and gravitropism-impaired mutant roots (*aux1*) displayed stronger phototropism than the wild-type (WT; Okada & Shimura 1992 Aust J Pl Physiol 19: 439). In many gravitropism experiments, the light came from above, a configuration that could exaggerate the effect of gravitropism if phototropism were not taken into account.

Many data indicate that starch-filled amyloplasts trigger gravitropic sensing. However, roots of the starchless mutant, *pgm-1* (TC75), have been reported to be gravitropic, especially when grown in the light. But studies at threshold g-doses have determined that *pgm-1* roots are about twelve times less sensitive than WT roots (Kiss et al. 1989 Planta 177: 198-206). These results demonstrate that starch is not necessary for some gravitropism but is required for full sensitivity. However, these data were obtained with light from above and thus it is possible that the contribution of root phototropism led to an overestimation of the gravitropic sensitivity of either or both genotypes.

To determine the contribution of phototropism to the measurement of apparent root gravitropism, various measures of gravitropism were performed so that the responses with light from above or below were compared in the WT and in the starchless mutants *pgm-1* and *adg1-1* (TL255) of *Arabidopsis*. Light from above exaggerated apparent root gravitropism in almost all measurements including extended growth in continuous light, downward curvature following reorientation to the horizontal (a time course), and estimates of sensitivity (presentation and perception times). The effect of light position was especially noticeable in the starchless mutants where overhead light masked defective gravitropism and extended illumination from below caused roots to grow above the horizontal. But wild-type roots were also affected by phototropism since they curved down more slowly in circumlateral light. Estimates of sensitivity were obtained by placing roots in darkness during brief periods of horizontal exposure. All three genotypes had longer presentation times but the relative sensitivities were comparable to light from above. However, new measurements of the perception time show that roots of the starchless mutants are significantly less sensitive than previously estimated. These results provide additional support for the importance of amyloplasts in gravitropic sensing and also point to the need for consideration of light position in the design of gravitropism experiments.

Gravitropism and sex1

The *sex1* (starch excess) mutant TC265 of *Arabidopsis* accumulates extra starch apparently through inactivation of a hexose transporter in the plastid envelope. Various tissues including the presumptive gravity sensing cells of the root, hypocotyl, and inflorescence stem were examined to determine whether the mutation alters amyloplast sedimentation and starch content. The peripheral root cap and the body of the root had noticeably more starch in *sex1* compared to the wild-type (WT). However, stereological analysis of electron micrographs of the central cap cells did not reveal any differences between *sex1* and WT in amyloplast size or position. Root gravitropic sensitivities were comparable. *sex1* seedlings also contained excess starch in cotyledons, hypocotyls, root hairs and the root/hypocotyl transition zone. Sedimenting amyloplasts were found in the root columella, endodermis of stems and hypocotyls and the petioles of cotyledons. In the starch sheath of hypocotyls, both absolute and relative (*sex1* vs WT) plastid sizes varied with the light regime used for cultivation. Seedlings grown two days on light and one day in the dark had amyloplasts two to three times smaller (*sex1* 15-35% larger than the WT) than those grown four days in dark (where *sex1* amyloplasts were 12-22% smaller than in the WT), and 1.5 to 3 times smaller than in hypocotyls grown 7 days in light (where *sex1* plastids were 100% larger than in the WT). Gravitropic sensitivity of hypocotyls grown under different light regimes was not proportionate to the amyloplast size. However, *sex1* hypocotyls grown 7 d on light, which had the largest amyloplasts, also had the highest gravitropic sensitivity.

In the stem endodermis, amyloplasts were 20-40 % larger in *sex1* compared to the WT, but the plastids were sedimented over the same length of the stem in both genotypes. Plastid size varied with the distance from the apex in a manner similar in both WT and *sex1*.

Ectopic sedimentation of plastids was observed in outer layers of cortex in about 30% of *sex1* hypocotyls grown under continuous light for seven days.

These data indicate that the *sex1* mutation affects different tissues differentially. And because the *sex1* is impaired in starch degradation, the data suggest that exposure of seedlings to light triggers changes in starch synthesis/degradation pattern in various tissues. Moreover, amyloplast sedimentation is not simply a function of plastid size but is also regulated by cell-specific factors. Ectopic sedimentation observed in some *sex1* hypocotyls indicate the threshold plastid size to allow sedimentation in non-specialized cells of cortex.

In summary, in most organs and in three cultivation regimes (varying amounts of light and dark) the presence of extra starch (and thus extra mass) in the gravity sensing cells had no effect on sensitivity. However, *sex1* hypocotyls grown in the light for 7 days were much more sensitive than light-grown WT hypocotyls, i.e., at least 90 min of gravitropic stimulation was needed to cause significant curvature in the WT, while the estimated presentation time of *sex1* hypocotyls was only 6 min. Thus we conclude that *"sex* only rarely improves gravitropism" and only in those cases where this mutation causes starch content to persist whereas in the wild-type starch is degraded.

Conclusion

Collectively, our results provide additional strong support for the importance of starch in gravitropic sensing. First, by accounting for negative phototropism in roots of two starchless mutants of *Arabidopsis*, *pgm* and *adg1*, we have established that these mutants are much less sensitive to gravity than previously thought. This work also demonstrates the importance of designing experimental protocols that remove the influence of root phototropism on measuring root gravitropism. Second, light apparently promotes gravitropism in starch-deficient *Nicotiana* hypocotyls by increasing the trace amounts of starch in the plastids, by inducing limited plastid sedimentation and thus by presumably increasing the signal provided by plastid mass. And finally, we show that excess starch in *Arabidopsis* seedlings has little effect on gravitropic sensitivity implying that the sensing system is already saturated. However, in light-grown stems where this mutation results in starch accumulation and where the wild-type practically lacks starch in the sensing cells, then the mutant is much more sensitive than the wild-type again showing that the loss of starch depresses gravity sensing.

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