Reduced gravitropic sensitivity in roots of a starch-deficient mutant of *Nicotiana sylvestris*

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Abstract. Gravitropism was studied in seedlings of *Nicotiana sylvestris* Speg. et Comes wild-type (WT) and mutant NS 458 which has a defective plastid phosphoglucomutase (EC 2.7.5.1). Starch was greatly reduced in NS 458 compared to the WT, but small amounts of starch were detected in root-cap columella cells in NS 458 by light and electron microscopy. The roots of WT are more sensitive to gravity than mutant NS 458 roots since: (1) in mutant roots, curvature was reduced and delayed in the time course of curvature; (2) curvature of mutant roots was 24–56% that of WT roots over the range of induction periods tested; (3) in intermittent-stimulation experiments, curvature of mutant roots was 37% or less than that of WT roots in all treatments tested. The perception time, determined by intermittent-stimulation experiments, was ≤5 s for WT roots and 30–60 s for mutant roots. The growth rates for WT and NS 458 roots were essentially equal. These results and our previous results with WT and starchless mutant *Arabidopsis* roots (Kiss et al. 1989, Planta 177, 198–206) support the conclusions that a full complement of starch is necessary for full gravitropic sensitivity and that amyloplasts function in gravity perception. Since a presumed relatively small increase in plastid buoyant mass (*N. sylvestris* mutant versus *Arabidopsis* mutant) significantly improves the orientation of the *N. sylvestris* mutant roots, we suggest that plastids are the likeliest candidates to be triggering gravity perception in roots of both mutants.

Key words: Amyloplast – Gravitropism – Mutant (gravitropism) – *Nicotiana* (gravitropism) – Plastid and graviperception – Starch and graviperception – Statolith

Introduction

For the past century, the starch-statolith hypothesis has been used to explain gravity perception in plants. This theory proposes that the displacement of and-or pressure from amyloplasts is the initial signal for gravity perception (for reviews see Volkman and Sievers 1979; Björkman 1988; Sack and Kiss 1989b). Starch-deficient mutants have been employed in tests of the starch-statolith hypothesis (Hertel et al. 1969; Roberts 1984; Moore and McClelen 1985; Moore 1987). However, since data about the molecular nature of the mutations are not available, it is difficult to make conclusions about the gravitropic sensitivity of these mutants relative to the wild-types (WT). Recently, Caspar and Pickard (1989) studied gravitropism in a mutant of *Arabidopsis* which is starchless as a result of a deficiency in plastid phosphoglucomutase (EC 2.7.5.1). They concluded that starch was not necessary for gravity perception in *Arabidopsis* and questioned the starch-statolith hypothesis (Caspar and Pickard 1989). However, using the same mutant, we demonstrated that the absence of starch markedly decreased gravitropic sensitivity in the roots (Kiss et al. 1989).

In this paper, we further clarify the relationship between starch and gravitropic sensitivity by studying gravitropism in the WT and in a starch-deficient mutant of *Nicotiana sylvestris*. This mutant, isolated by Hanson and McHale (1988), is also deficient in plastid phosphoglucomutase. We
report here that roots of the *N. sylvestris* mutant are less sensitive to gravity than are WT roots. These results are consistent with the conclusion that amyloplasts function in gravity perception.

**Material and methods**

Plant material and culture conditions. Seeds of the WT of *Nicotiana sylvestris* Speng. et Comes, and of the starch-deficient mutant NS 458 (F3 generation from the first backcross) were generous gifts of Dr. Kenneth R. Hanson (Connecticut Agricultural Experimental Station, New Haven, USA). NS 458 has a recessive mutation in a single nuclear gene which makes it grossly deficient in the activity of plastid enzyme phosphoglucomutase (Hanson and McHale 1988).

Culture conditions were essentially as described in Kiss et al. (1989). Briefly, for curvature and growth studies, seeds were surface sterilized, and seedlings were grown under sterile conditions in Petri dishes on 1% (w/v) agar containing nutrients and 1% (w/v) sucrose (under continuous illumination of 90–100 μmol photons m⁻² s⁻¹ from 40-W “cool-white” fluorescent lamps).

Seeds were used for curvature and growth studies two to ten months after harvesting. Wild-type and NS 458 roots were used when they were 3–6 mm long, approx. 6–7 d after sowing (except the experiments summarized in Table 3).

Curvature and growth studies. These studies were performed in continuous illumination since we determined that the roots were not phototropic. In all experiments, for each root, curvature was measured as an increment over the starting value. Procedures used were as described in Kiss et al. (1989) except that; (1) in the induction experiments (Fig. 7; Table 2, 3), the horizontal exposures ranged from 5 to 60 min, and curvature was measured after a 2-h rotation on the clinostat; (2) growth rates were calculated from increases in root length over an 8-h period; (3) seedlings were excluded from all samples if their roots were not 3–6 mm long (except the experiments summarized in Table 3).

Microscopy. Light and electron microscopy were performed as described by Sack and Kiss (1989a). Briefly, the seedlings were fixed with glutaraldehyde and paraformaldehyde, and postfixed with osmium ferricyanide. For light microscopy, 1.5-μm sections were stained with toluidine-blue, and for electron microscopy, silver to gold sections were stained with lead citrate and uranyl acetate. In order to test for the presence of starch, fresh tissue was stained with iodine potassium iodide (IKI) and examined using light microscopy (O'Brien and McCully 1981).

Reagents. Agar and chemicals used for electron microscopy were purchased from Polysciences (Warrington, Penn., USA). All other biochemicals were obtained from Sigma Chemical Co. (St. Louis, Mo., USA).

Results

Starch content of the WT and mutant NS 458. Although Hanson and MeHale (1988) biochemically characterized the amount of starch in the WT and mutant, they did not use microscopy to test for starch. Since the columella cells of the root cap are the probable sites of gravity perception in the root (Behrens et al. 1985; Sack and Kiss 1989b), we determined the starch content of these cells by microscopy. Starch was detected in electron micrographs of plastids of the columella cells in both the WT (Fig. 1) and the mutant (Fig. 2). The mutant contains much less starch than the WT, but starch grains were present in most sections and positive staining with IKI confirmed the presence of reduced starch in these plastids. Based on microscopic observations of roots, we estimated that mutant plastids contain 10% or less of the starch of WT plastids. Small amounts of starch also were detected in the hypocotyl and cotyledons of the mutant seedlings by IKI staining (data not shown).

In addition to the plastoglobuli (presumably composed of lipid), the plastids in the root-cap columella cells also contained larger, membrane-bound, osmiophilic structures (Figs. 1, 2). Although initially more obvious in mutant plastids because of reduced starch (Fig. 2), these larger structures were also present in WT amyloplasts (Fig. 1). Vigil and Ruddat (1985) have demonstrated that these structures are protein bodies in *N. tabacum*. We found that the protein bodies were similar in size in WT and mutant plastids.

Columella cell polarity. Columella cells in WT *N. sylvestris* have a polar organization similar to that found in columella cells of other plants (Volkmann and Sievers 1979; Sack and Kiss 1989a). The nucleus was found in the proximal (top in vertical roots) part of the cell, and the plastids were sedimented in the distal (bottom) part of the cell in the WT (Fig. 3). NS 458 plastids can be identified in the light microscope in toluidine-blue-stained sections (Fig. 4). Based on qualitative observations of light- and electron-microscopic sections, the mutant plastids were not found to be sedimented to the bottom of columella cells in vertically oriented roots.

Vertical growth of seedlings. Both WT and mutant seedlings had roots that were closely oriented around the gravity vector during vertical growth. The distribution around and the extent of deviation from the gravity vector of vertically grown roots are shown in Fig. 5. Both WT and mutant roots had essentially the same negligible mean divergence from the vertical (1.4° and 1.3° for WT and mutant, respectively). The standard deviation for mutant roots (± 12.8°) was slightly higher than for the WT (± 10.8°).

Growth rate. The growth rates of roots of both genotypes were essentially equal for vertically