EFFECT OF ENVIRONMENTAL DENSITY AND BUOYANCY ON GROWTH AND GRAVITROPIC RESPONSES IN MAIZE ROOTS

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The ability of roots to perceive and respond to gravity has been investigated by botanists for many years (Moore & Evans, 1986). Charles and Francis Darwin (1881) were the first scientists to extensively examine the gravitropic response of primary roots. They observed that the removal of the root cap eliminated the gravitropic response of primary roots. This observation was confirmed by many researchers since the Darwins. The widely accepted theory for the perception of gravity by plant roots is based on the sedimentation of statoliths. This theory currently states that sedimentation of amyloplasts in columella cells of the root cap, which is accompanied by calcium redistribution on the lower side of the root cap, initiates the gravitropic response.

However, there are many graviresponding plants which do not have statoliths. In these plants, the plasma membrane or other cell organelles may act as statoliths (Wayne et al, 1990; Staves et al, 1997 a, b, c; Staves, 1997). Edwart (1903) observed the polarity of the rotational cytoplasmic streaming in Characean cells during the gravitropic response. The influence of gravity on cytoplasmic streaming was confirmed by Hayashi (1957). The hydrostatic gravisensing model has been proposed by Wayne et al. This hypothesis suggests that plants sense the gravitational vector via a pressure differential exerted on the cell membrane. This pressure differential results from the action of gravity on the entire contents of the cell instead of specific organelles within the cell. Thus, the entire cell instead of organelles within a cell would function as statoliths. This study investigates the effects of solutions of various densities which alters the environmental buoyancy of cells on growth and graviresponse of primary roots of maize.

MATERIAL & METHODS.

Plant Material. Primary roots of maize (Federal Hybrid RK112-1, Elgin, IA) with a length of about 1 cm were used. The caryopses were placed on moist paper towels on trays and germinated at 24 C.

Videography and Digitization. The roots were placed in 200 ml polystyrene chambers. The density and buoyancy of the environment in the chambers was modified using air, water, sucrose, sucrose/polyethylene glycol (PEG 4000), polyethylene glycol (PEG 8000) and Ficoll PM 400 (polysucrose; Amersham Biosciences, Piscataway, NJ). The rates of growth and gravitropic curvature were monitored using a video camera (Sharp VL-AH150 ViewCam®). The camera signal was multiplexed and archived using analog time-lapse video (Mace® Model ER960TCN Time-lapse Video recorder) and digital video and still images (AverMedia® UltraTV-USB400). The digitized video frames were processed using HandyAVI® software to produce digital AVI format videos (5 sec intervals frames; 30 frames/sec) and BMP still images.

Data Analysis. Curvature and growth rates were measured from BMP images using *UTHSCSA Image Tool 3.0®*.

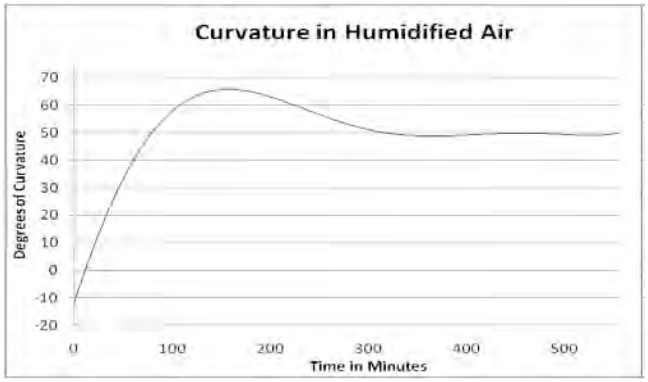

Figure 1. *Representative curvature of the primary root in humidified air. Humidified air provides minimal density of external media and minimal buoyancy of protoplasts. (n=25)*

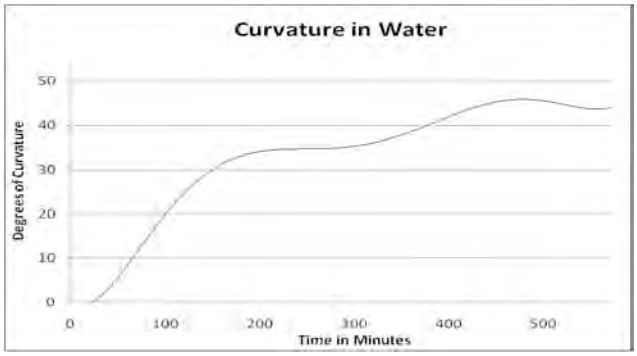

Figure 2. *Representative curvature of the primary root in RO water. Conditions provide an environment that is less dense than the cytoplasm and less than neutral buoyancy for the cytoplasm. (n=147)*

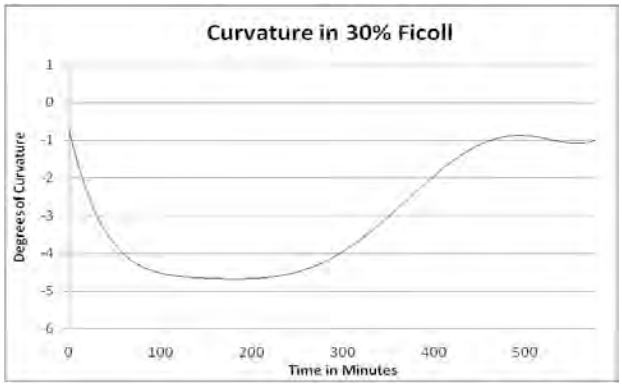

Figure 3. *Representative curvature of the primary root in 30% Ficoll PM 400. Conditions provide an environment with reduced osmotic activity when compared to sucrose or PEG solutions while maximizing density and buoyancy effects of the environment. (n=12)*

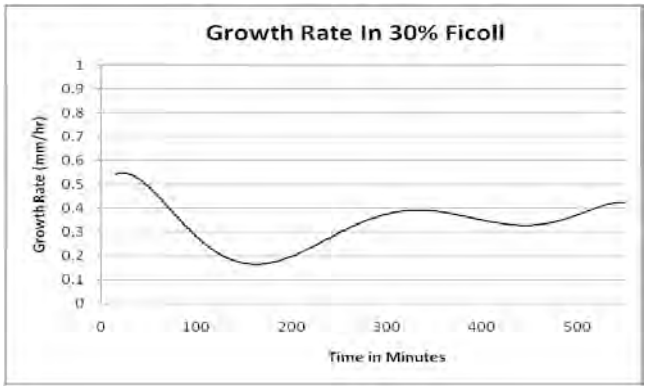

Figure 4. *Representative growth rate of the primary root in 30% Ficoll PM 400. Conditions provided minimal osmotic suppression of growth rate. (n=12)*

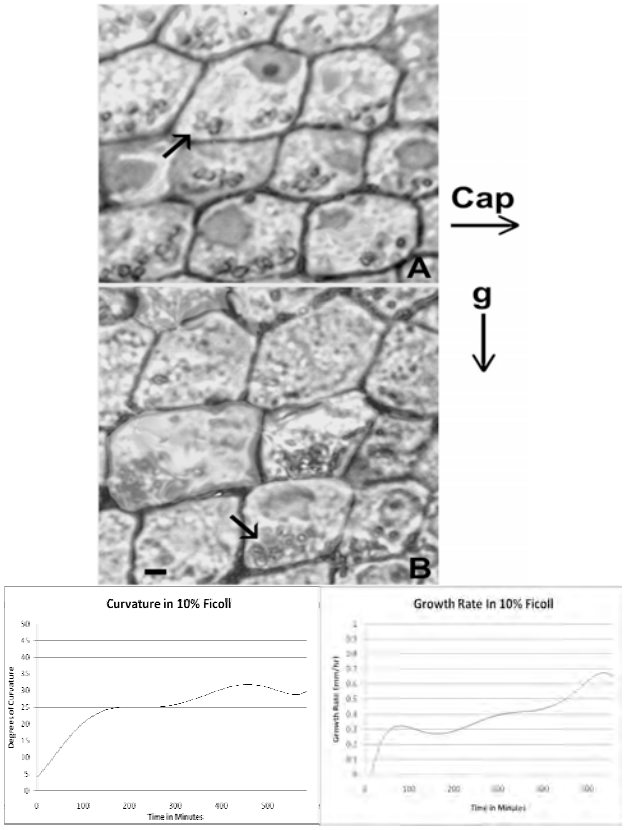

Figure 5. *Micrograph of maize root cap cells. A: Roots in RO water (n=5). B: Roots in 10% w/v Ficoll PM 400 (n=5). Arrows: point to clusters of amyloplasts, i.e. statoliths; G = gravity vector; Cap = direction of root cap. Line in lower left of panel B = 10 µm. Lower pair of graphs are representative curvature and growth rate of roots in 10% Ficoll PM 400.*

DISCUSSION & SUMMARY

- Primary roots of maize exhibit a stronger curvature response in humidified air than in water (Fig. 1 & 2) without a significant effect on elongation rate (data not shown). The decreased rate and degree of curvature in the higher density water environment (Fig. 2) supports the hydrostatic gravisensing model.
- Primary roots of maize exhibit decreasing amounts of curvature with increasing concentrations of sucrose-PEG. There is suppression of elongation by increasing concentrations of sucrose-PEG. Growth rates decrease 30% in 10% sucrose/PEG solution, 60% decrease in 15% sucrose/PEG solution and 90% decrease in 20% sucrose/PEG solution than the control. The inhibition of growth with increasing sucrose concentrations appears to be due to increasing osmolality of the sucrose solution. Solutions of sucrose and of PEG 8000 support these observations (data not shown).
- Ficoll PM 400, a polymer of sucrose, exhibits significantly less osmotic effects while providing increased density of the solution. Data with Ficoll indicate that there is no significant inhibition of elongation at concentrations up to 30%, but there is increasing suppression of gravicurvature as the Ficoll concentration is increased (Fig. 4 & 5).
- Light micrographs of statoliths sedimentation in root cap cells indicate that increasing density of the external environment does not significantly alter sedimentation of the statoliths (Fig. 5).

In conclusion, the hydrostatic model is supported with these data. When the density of the external media was changed, it produced a change in the gravitropic response of roots. Low osmolarity solutions proved to be the most tolerable environment for roots for such experiments. Tolerable environments were best created using Ficoll PM 400 over environments of only sucrose or a combination of it with PEG.

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