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Final Report on NASA grant NAGW- 3565:

MAGNETOPHORETIC INDUCTION OF ROOT CURVATURE

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The last year of the grant period concerned the consolidation of previous experiments to ascertain that the theoretical premise apply not just to root but also to shoots. In addition, we verified that high gradient magnetic fields do not interfere with regular cellular activities.

Our working premise has been that in high gradient magnetic fields (HGMF) substances with different magnetic susceptibilities are affected by ponderomotive forces. For weak magnetics, the magnetic ponderomotive force acting on a body of volume V of magnetic susceptibility $æ_b$, immersed in a liquid of magnetic susceptibility $æ_l$ in a nonuniform magnetic field H equals:

$$\vec{F}_m=(æ_b-æ_l)*V*\nabla(H^2/2)=\Delta æ*V*\nabla(H^2/2)$$

To substitute the gravity force $\vec{F}_{gr}=(\rho_b-\rho_l)*V*\vec{g}=\Delta\rho*V*\vec{g}$ by a magnetic force Fm, it is necessary to produce a high gradient magnetic field (HGMF) with the dynamic factor:

$$\nabla(H^2/2)=\frac{\Delta\rho}{\Delta æ}*\vec{a}$$

For the system starch/water the parameter $\Delta\rho/\Delta æ$ = -6*106 $(g/cm^3)/emu$. Therefore, if a is to be of the same magnitude as g, a HGMF with a dynamic factor $\nabla(H^2/2) = 10^9\text{-}10^{10}\ Oe^2/cm$ is required.

Previous results have established that
a) intracellular magnetophoresis is possible
b) HGMF lead to root curvature.

The continuation of these investigations showed that plant shoots (coleoptiles and hypocotyls) also respond to HGMF and in contrast to previous reports by Audus et al. (1973) the shoots curved toward the gradient. As if responding in a negatively gravitropic manner. This part of our investigations confirms that the presumptive gravisensing mechanism functions similarly in roots and shoots and starch displacement results in curvature. Coleoptiles of barley (*Hordeum vulgare*) were investigated in a high gradient magnetic field (HGMF, dynamic factor $\nabla H^2/2$ of 10^9 to $10^{10}\ Oe^2/cm$, generated by a ferromagnetic wedge in a uniform magnetic field) and rotated on a 1-rpm clinostat. After 4 hours 90% of coleoptiles had curved toward the HGMF. The cells affected by HGMF showed clear intracellular displacement of amyloplasts. Coleoptiles in a magnetic field next to a non-ferromagnetic brass wedge showed no preferential curvature. The small size of the area of non-uniformity of the HGMF allowed mapping of the sensitivity of the coleoptile tips by varying the initial position of the wedge relative to the coleoptile apex. The curvature response decreased as the ferromagnetic wedge was placed at a greater distance from the apex and at 1 mm below the tip only 58% of the coleoptiles curved toward the wedge indicating that the cells most sensitive to intracellular displacement of amyloplasts and thus gravity sensing are confined to the top one mm portion of (barley) coleoptiles. Interestingly, this effect could be mimicked by the removal of tip tissue (Table 1).

Table 1: Growth rate and curvature of vertically and horizontally oriented barley coleoptiles during 3.5 hours after removal of various lengths of apical tissue (values ± SE).

	vertical		horizontal		
mm removed	growth rate, mm/hr	n	growth rate, mm/hr	curvature rate, deg. /hr	n
zero	2.92±0.35	20	0.84 ± 0.09	26.3 ± 1.3	21
0.3	2.53±0.27	23	0.77 ± 0.07	21.1 ± 1.7	25
1	2.00±0.17	29	0.56 ± 0.07	17.6 ± 1.4	26

Similar experiments with tomato hypocotyls (*Lycopersicum esculentum*) also resulted in curvature toward the HGMF. The data strongly support the amyloplast-based gravity sensing system in higher plants and the usefulness of HGMF to substitute gravity in shoots. These studies are in press (Kuznetsov OA and Hasenstein KH, 1997, Magnetophoretic Induction of Curvature in Coleoptiles and Hypocotyls. J Exp Bot.).

Measurements on the physical properties and changes of amyloplasts have shown that the density is less than that reported for starch (1.4 *vs.* 1.53 g/cm^3). Using particle magneto-graviphoresis (upward movement due to HGMF and sedimentation due to gravity) changes in the magnetic susceptibility of amyloplasts was measured and compared to the gravisensitivity of roots. Measuring the velocity of amyloplasts movement in an HGMF with known dynamic factor $\nabla(H^2/2)$ (magnetophoresis) and the rate of sedimentation in the absence of the HGMF permits the determination of the ratio $\Delta x/\Delta\rho$, where Δx is the difference of magnetic susceptibilities and $\Delta\rho$ the difference of densities between the amyloplasts and medium. Amyloplast movement in a vertically mounted capillary between poles of a magnetic system ($\nabla H^2/2 = 9.4\times10^9$ Oe^2/cm) was observed through a video microscope and the vertical velocities were determined. The density of amyloplasts was measured by isopycnic centrifugation. The magnetic susceptibility x of amyloplasts from primary roots of flax, corn, sunflower, and barley seedlings was close to x for starch. The ratio $\Delta x/\Delta\rho$ for all tested amyloplasts was 3.2±0.2*10-7 emu*g-1*cm3. Removing membranes from amyloplasts (1 h in 0.5% SDS) did not affect sedimentation but magnetophoretic velocities increased 18%. Loss of starch due to growth in darkness was measured after 15 d. Sedimentation decreased by 23% and magnetophoretic velocities dropped by 29%, leading to a decline of $\Delta x/\Delta\rho$ by 11%, presumably due to reduced starch content. Since magnetophoresis can detect the loss of starch or removal of membranes, magnetic susceptibility measurements are suitable for the determination of amyloplasts composition and gravitropic sensitivity. In addition to the study of the density and composition of amyloplasts, these results open new avenues for the study of the rheology of the interior of (plant) cells.

In order to investigate whether HGMF affect the assembly and/or organization of structural proteins, we examined the arrangement of microtubules in roots exposed to HGMF. The cytoskeletal investigations were performed with fomaldehyde-fixed, non-embedded tissue segments that were cut with a vibratome (see Blancaflor and

Hasenstein, 1993). Microtubules (MTs) were stained with rat anti-yeast tubulin (YOL 1/34) and DTAF-labeled antibody against rat IgG. Microfilaments (MFs) were visualized by incubation in rhodamine-labeled phalloidin. The distribution and arrangement of both components of the cytoskeleton were examined with a confocal microscope. Measurements of growth rates and graviresponse were done using a video-digitizer. Since HGMF repel diamagnetic substances including starch-filled amyloplasts and most bulk protein it is conceivable that HGMF also affect other cellular compounds such as the cytoskeleton. To test this notion and to gather evidence for force sensing structures that are not associated with amyloplasts, we examined corn roots exposed to HGMF for changes in the organization of MTs about 2 mm from the root cap junction (Fig. 1). The organization of MTs was not affected by the HGMF because the cell within the HGMF and on the opposite side of the same root showed similar orientation. However, there were some varietal differences since roots of Golden Cross Bantam showed oblique MTs at a distance of about 3 mm from the tip which is about half the distance observed in Merit roots.

The second aspect of the work includes studies of the effect of cytoskeletal inhibitors on MTs and MFs. The analysis of the effect of micotubular inhibitors on the auxin transport in roots showed that there is very little effect of MT-depolymerizing

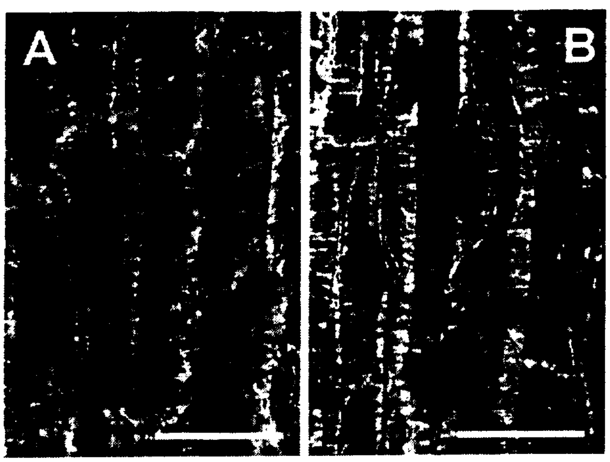

Figure 1. Organization of microtubules in cortical cells of maize roots after exposure to HGMF. There is no difference between the side exposed to a HGMF (A) and the distal (control) side of the same root (B). MTs appear in their normal transverse orientation. Bars 50 µm

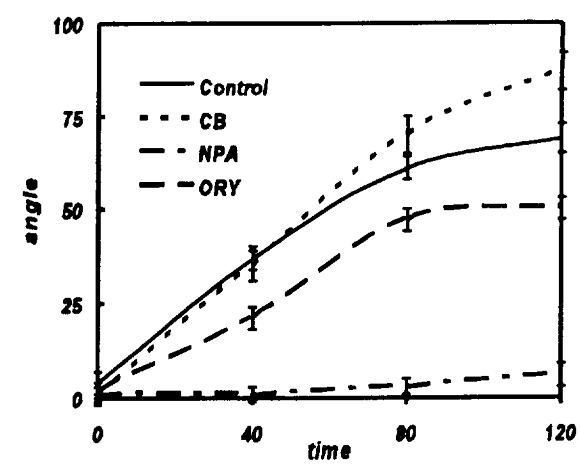

Figure 2. Gravicurvature of horizontally placed primary roots of *Zea mays* L. after 3 hours pretreatment with buffer (control), cytochalasin B (CB, 50 µM), naphthylphthalamic acid (NPA, 5 µM) or oryzalin (ORY, 5 µM) in vertical orientation. Means ±SE.

or stabilizing drugs on auxin transport. This is in line with observations that application of such drugs is not immediately affecting the graviresponsiveness of roots. In a second set of studies we found that the actin cytoskeleton of cortical cells in the elongation zone of roots is not affected during the graviresponse. Likewise, treatments that inhibit auxin transport and graviresponse such as naphthylphthalamic acid do not affect the actin filaments. In vertical roots bundles of MFs in the elongation and maturation zone were oriented parallel to the longitudinal axis of cells. MFs in the vascular parenchyma cells were more abundant than in the cortex and epidermis. Epidermal and proendodermal cells in the meristematic region contained transverse cortical MFs. The organization of MFs of graviresponding roots was similar to vertical roots. Application of cytochalasin B or D; resulted in extensive disruption of MFs in the cortex and epidermis but only partially affected MFs in the stele. Despite the cytochalasin-induced depolymerization of MFs, gravicurvature was not inhibited (Fig. 2). In contrast, the auxin transport inhibitor N-1 naphthylphthalamic acid suppressed root curvature but had no observable effect on the integrity of the MFs. The data indicate that MFs may not be involved in the graviresponse of maize roots.

List of Publications During the Funding Period

Blancaflor EB, Hasenstein, KH Microtubule orientation and growth of maize roots after osmotic shock. Plant Physiol. 108:39

Wan Y, Hasenstein KH Affinity purification of ABA-binding proteins. Plant Physiol. 108:80

Kuznetsov OA, Hasenstein KH PArticle magnetophoresis as a tool to study the gravity sensing system Plant Physiol. 108:136

Kuznetsov OA, Hasenstein KH Investigating plant gravi-sensing by magneto-graviphoresis of amyloplasts. ASGSB Bulletin 9:38

Hasenstein KH, Lee JS, Blancaflor EB Auxin transport and graviresponse of maize roots treated with microtubule inhibitors ASGSB Bulletin 9:37

Blancaflor EB, Hasenstein KH The graviresponse of maize roots is independent of the actin cytoskeleton. ASGSB Bulletin 9:51

Gordon Conference "Gravitational Effects of Living Systems" New London, NH, Invited Speaker: Magnetophoretic induction of curvature in plants

Hasenstein KH, Kuznetsov OA, Blancaflor EB (1996) Induction of plant curvature by magnetophoresis and cytoskeletal changes during root graviresponse. 6th European Symposium on Life Sciences Research in Space. Trondheim, Norway

Baluška F, Hasenstein KH Root Cytoskeleton: its Role in Perception of and Response to Gravity. International Workshop on Plant Biology in Space, Bonn, Germany

Wan Y, Hasenstein KH Abscisic acid and a putative receptor protein are not restricted to plants

Blancaflor EB, Hasenstein KH Actin microfilaments are not involved in the graviresponse of maize roots. (Later published in Plant Physiology 113: 1447-1455)

Kuznetsov OA, Hasenstein KH Magnetophoretic response of barley coleoptiles

Kuznetsov OA, Hasenstein KH Magnetophoretic Characterization of the Plant Gravity Receptor. First International Conference on Scientific and Clinical Applications of Magnetic Carriers. Rostock, Germany

Wan Y, Hasenstein KH (1996) Purification and Identification of ABA Binding Proteins and Antibody Preparation. Journal of Molecular Recognition, 9:722-727

Blancaflor E, Hasenstein KH 1995 Time Course and Auxin Sensitivity of Cortical Microtubule Reorientation in Maize Roots. Protoplasma 185: 72-82

Blancaflor E, Hasenstein KH 1995 Growth and microtubule orientation of maize roots subjected to osmotic stress. Internat. J. Plant Sci. 156: 794-802

Kuznetsov OA, Hasenstein KH 1996 Magnetophoretic induction of root curvature. Planta 198: 87-94