USE OF ROTATO/RANDOM POSITIONING MACHINE (R/RPM) TECHNOLOGY TO INVESTIGATE GRAVITY SENSING AND THE GRAVITROPIC MOTOR RESPONSE OF MAIZE ROOTS

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The Random Positioning Machine (RPM, 3-D clinostat) has been useful for simulating μg on Earth (Hoson et al., 1992). A more recent advance for gravitropism studies is a device referred to as ROTATO, a video analysis system with feedback control of plant orientation allowing the maintenance of any given target segment of a root in a fixed position relative to gravity (Mullen et al., 2000). We have combined these technologies into the ROTATO-RPM (R-RPM). The R-RPM allows a seeding to be rotated randomly in three dimensions while examining the behavior of and controlling the orientation of the root. This device can be used to expose roots not only to simulated μg but also to simulated hypo-g. To simulate hypo-g we modify the software of the RPM so that the rotation favors orientation of the target in a particular direction for a specified fraction of the rotation time (Figure 1). Favorable orientation in direction (relative to the organ axis) “x” for 20% of the time, for example, would simulate a 0.2 g stimulus in that direction.

![Figure 1](image-url). Examples of g stimulation on the R-RPM. Left: There is no rotation and the net stimulus is 1 g. Center: Rotation is random simulating μg. Right: Rotation favors downward orientation 20% of the time, simulating 0.2 g. Arrows show gravity (acceleration) vectors.

ROTATO is used to maintain the target tissue in a fixed orientation so that the integrated stimulus can be applied to a fixed target. The R-RPM has great potential for investigating gravity sensing since it can apply simulated hypo-g to any given target section of the root at any angle within that target section. It may be particularly instructive to use the R-RPM to compare the effects of directional fractional stimuli on expression of gravity related genes in the root tip (Masson et al., 2002).

Operation of the R-RPM requires that the analysis/mechanical feedback system operate smoothly while the entire system is rotating in three dimensions. This places added demands on the stability of the video analysis software. Because of the importance of this aspect of the R-RPM system, we recently developed software based on the same concept utilized to control ROTATO, but built upon our original software (Ishikawa and Evans, 1997) for image acquisition and tracking.

The new software has improved reliability and precision. It detects false tracking within a second, and adjusts to allow uninterrupted tracking. Another advantage of this software is that it operates on images recorded during operation of the R-RPM thus allowing temporal separation of image collection during plant manipulation from the task of data analysis.

To test this new software we used it in an investigation of the early kinetics of maize root gravitropism, with special attention to the relationship between the angle of constant stimulation and the kinetics of the response. Historically, dose-response aspects of gravitropism have been investigated by measuring responses to different angles of gravistimulation (Kiss et al., 1997). For the experiments described here, we used roots of 3-d-old seedlings of the cultivar, Merit, (Ishikawa and Evans, 1993).

Figure 2 shows sample kinetics of the response to gravistimulation at a constant angle of 30° compared with the response to constant 90°. In both cases the root was rotated to the indicated angle and then the root tip was held at that angle by ROTATO throughout the response. We were surprised to find that the response to gravistimulation was consistently faster for the weaker stimulation (lower angle of stimulation).

In Figure 3, the dashed line compares the average latent period of the gravitropic response over a wider range of stimulation angles. It is clear that the response time increases with increasing angle of stimulation for angles up to 90°. As the angle increases above 90° the latent period of the response declines again. This pattern appears to follow the “sine rule” (Iino et al., 1996) for gravitropism dose response relationships.

We considered the possibility that the delayed response as the angle of stimulation approaches 90° may indicate that such angles cause “over-stimulation” of the gravity sensing system requiring some period of adaptation before the motor mechanism can be activated. Sudden shifts in angle from 0° to 90° would be rare under natural conditions. To test this idea, we attempted to “pre-adapt” roots to large displacement by rotating them (120° per min, tip as pivot point) for 9 minutes in a vertical orientation prior to stimulation at a fixed angle. The results are shown in Figure 3 (solid line). With the exception of the data points at 120° the response was quicker for pre-stimulated roots than for roots that were not pre-stimulated in all cases. However, the general pattern of quicker responses for smaller angles of stimulation was still evident even in pre-stimulated roots. Experiments with pre-stimulated roots at angles higher than 120° are currently underway.

The finding that gravitropic responses are delayed at strong (near 90°) angles of stimulation and that this delay...
can be reduced by pre-adapting roots to radical
displacement suggests that the common experimental
practice of stimulation at 90° may induce an over-
stimulation. Strong stimulation may be inappropriate for
studying "normal" gravitropism. Prior studies of
quantitative parameters of gravistimulation such as
presentation time or presentation dose (Perbal et al.,
2002), for example, if done using large angles of
stimulation, could lead to results different from those
expected using milder stimulation.

![Figure 2A](image1)

**Figure 2A.** Sample responses to constant stimulation at
90° (Fig. 2A) and 30° (Fig. 2B). In each case the upper part
represents changes in the angle of the cap, the middle part
changes in the angle of sequential 1 mm segments beginning
with the cap and the lower part the time course of ROTATO
activity required to keep the root cap at the indicated angle
*e.galls the time course of gravitropism.*

With the completion of the R-RPM and its supporting
software, researchers in gravitropism will have a powerful
new tool for controlling quantitative aspects of
gravistimulation and will realize the opportunity to
simulate directional gravitational forces between µg and 1
in ground based laboratories. This capability may be of
increasing significance in view of the limited
opportunities for space flight experiments.

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