Communication

Diagravitropism in Corn Roots

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ABSTRACT

The diagravitropic behavior of Merit corn (*Zea mays* L.) roots grown in darkness provides an opportunity for comparison of two qualitatively different gravitropic systems. As with positive gravitropism, diagravitropism is shown to require the presence of the root cap, have a similar time course for the onset of curvature, and a similar presentation time. In contrast with positive gravitropism, diagravitropism appears to have a more limited requirement for calcium, for it is insensitive to the elution of calcium by EGTA and insensitive to the subsequent addition of a calcium/EGTA complex. These results are interpreted as indicating that whereas the same sensing system is shared by the two types of gravitropism, separate transductive systems are involved, one for diagravitropism, which is relatively independent of calcium, and one for positive gravitropism, which is markedly dependent on calcium.

The usual characteristic of primary roots directing their growth toward the force of gravity is lacking in some cultivars of corn until the roots have experienced red light or other environmental stress (6, 7, 9). Instead, these dark-grown roots tend to orient themselves into a position transverse to gravity, a phenomenon called diagravitropism. In numerous plant species, this transverse gravitropism is expressed in creeping roots, stems, or rhizomes.

The diagravitropic behavior is an appealing experimental item because of its sensitivity to red light, because it has received very little attention, and because it seems to be intractable to explanation by the classic Cholodny-Went theory of tropistic behavior as being a simple redistribution of auxin.

We have attempted to characterize the dynamics of diagravitropism, comparing them with the dynamics of positive gravitropism in each case. We will show that the dynamics of the two tropisms are very similar, except for the extent of curvature. We find that there is a difference in the role of calcium, diagravitropism being notably insensitive to elution or supplementation of the calcium supply.

MATERIALS AND METHODS

Seeds of Merit corn (*Zea mays* L.) from Asgrow Seeds, Kalamazoo, MI, were germinated with the radicle end down within rolls of wet germination papers (Anchor Paper, St. Paul, MN) in a 25°C darkroom, in 80% RH. After 40 h the seedlings were selected for uniform root lengths between 1.0 and 1.5 cm. The seedlings were mounted in plastic holders folded over with wet cheesecloth, with the root extending 1 cm from the edge of the plastic holder, 10 seedlings per holder (7). The holders were magnetically attached to plastic boxes lined with wet paper, and the orientation of the seedlings was controlled by manipulation

of the position of the plastic boxes. Prior to gravistimulation, the roots were kept in the downward pointing direction (180°). From this position, the roots do not show any tropistic bending. All manipulations were done under dim green safelight.

For chemical treatments before gravistimulation, the holders were placed over troughs of solutions such that the terminal 1 to 2 mm of the root tip were immersed in the solution for the time periods specified. Gravistimulation was achieved by placing the boxes on a rack such that the roots were pointing at an angle 45° from upright vertical. Curvature was measured by a goiometer on a video screen which provided convenient enlargement of the images. Standard errors are given for all data points; where the standard error was smaller than the graphed point, it is not visible.

Red light treatments were given to some experiments, using two 40W red fluorescent bulbs (GE F 40R) continuously during the gravitropic treatment.

RESULTS

Positive gravitropism in Merit corn roots is specifically dependent upon the presence of the root cap; examination of this characteristic for diagravitropism was made by decapping with a razor blade. Subsequent gravistimulation for 2 h resulted in a curvature of 61.9 ± 7.2° for intact roots and −1.5 ± 3.2° for decapitated roots, indicating that the diagravitropic response requires the presence of the cap, as does positive gravitropism.

If the decapped roots were allowed 24 h to regenerate a new cap, the diagravitropic sensitivity was restored, giving 23.6° ± 2.8 curvature after 2 h orientation at 45° from upright vertical.

The simple kinetics of diagravitropic and positive gravitropic curvature were compared by following the time course of curvature for the two gravity responses under continuous gravistimulation. In every case, the gravity stimulation was given with the roots pointing at 45° from upright vertical. The curvatures obtained by dark-grown vs red-light-treated roots are shown in Figure 1, where it is evident that both types of materials show considerable curvature after 1 h of stimulation. The diagravitropic material hovers at the horizontal orientation (45°) of curvature, whereas the positive gravitropic material continues on past the horizontal. From shorter-term experiments, we conclude that the commencement of curvature for both situations occurs at about 40 min (data not shown). It is consistently true that the diagravitropic material tends to overshoot the horizontal orientation (45° in Fig. 1), and subsequently loses curvature to restore the horizontal position.

The presentation time (the minimal duration of gravity stimulation needed for the development of subsequent curvature) was determined for both the diagravitropic and the positive gravitropic materials. Roots were exposed to the 45° orientation for various periods of time, and then returned to the downward-pointing orientation to allow for curvature development. In each instance, curvature was read at 60 min from the start of the
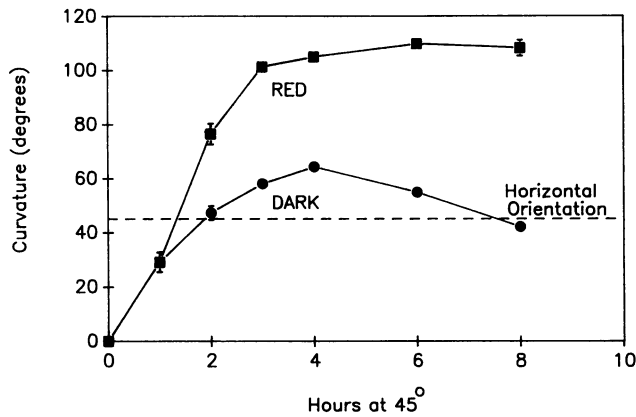
FIG. 1. The development of gravitropic curvature of Merit corn roots in dark and red light conditions. The roots were continuously stimulated at 45° from upright vertical from time zero. A curvature of 45° brings the root to a horizontal orientation (dashed line).
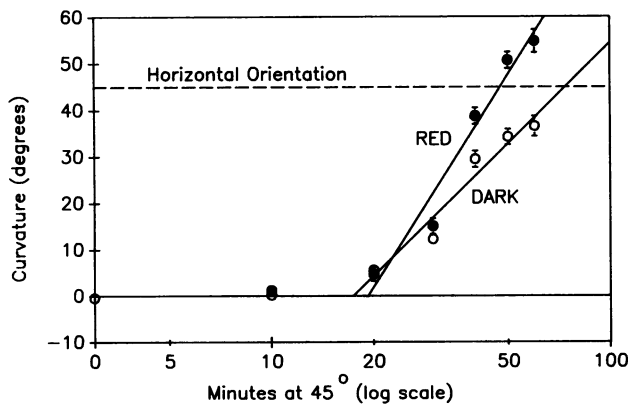

FIG. 2. Determination of the presentation times for gravitropic curvature in dark and red light conditions. Both lines intercept the abscissa between 14 and 19 min; r^2 values are 0.872 and 0.942, respectively.
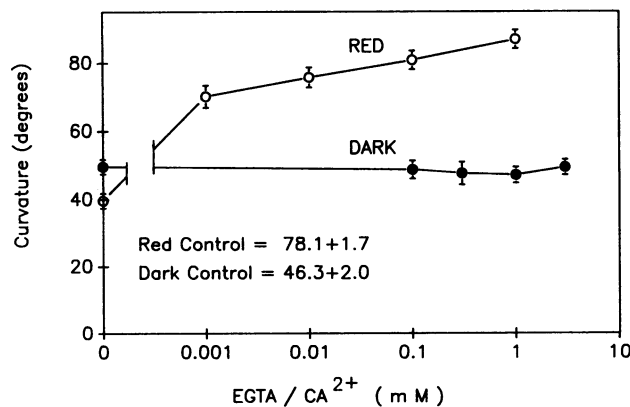

FIG. 3. Effects of calcium-altering treatments on gravitropic curvature in dark and red light conditions, including pretreatment with EGTA, and subsequent treatment with various concentrations of Ca^{2+}/EGTA complex (4/1 μmol). Curvatures for controls without EGTA treatment are printed out; treatments with EGTA alone are plotted on the ordinate; subsequent addition of EGTA/Ca^{2+} at concentrations from 0.001 to 3 mM are entered on plotted lines.
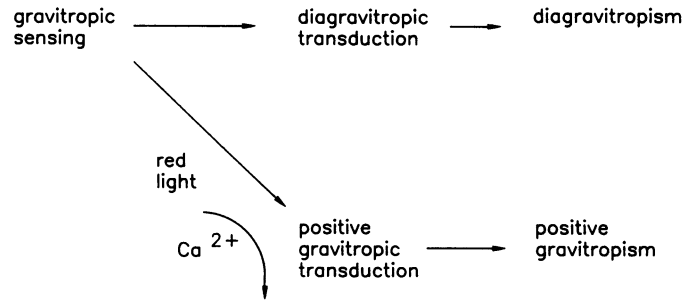

FIG. 4. Diagrammatic representation of hypothesis regarding the qualitative differences between two transductive systems.

gravity stimulus. The data obtained are presented in Figure 2 on a log time scale. The intercepts for the two types of material are seen to be in the vicinity of 14 to 19 min, indicating that the diagravitropic and the positive gravitropic materials have similar presentation times.

In order to compare calcium requirements for the diagravitropic and positive gravitropic system, root tips were immersed in 1 mM EGTA for 1 h before gravistimulation. In the absence of red light, the chelator treatment resulted in 49.6° curvature, which represented no inhibition below that of the buffer control (46.3°). With red light treatment the chelator reduced the gravi-response from 78.1 to 39.6°, an inhibition of about 50% (Fig. 3). The subsequent application of various concentrations of a Ca/EGTA complex did not increase the diagravitropic response but did increase the positive gravitropic curvature. Similar failure to increase diagravitropic curvature was obtained when mM $CaCl_2$ was applied after the chelator treatment. Parallel experiments with EDTA as a calcium chelator also lacked inhibitory action on diagravitropism (data not shown).

DISCUSSION

The two states of corn root gravitropism—diagravitropic and positive gravitropic—are shown to share a requirement for the root cap, presumably because they share the same sensing system. The similarity of the presentation times for the two states is also consistent with the sensing system being the same. The presentation time obtained in these experiments is markedly longer than we reported for roots stimulated at 90° (10). We interpret the difference as being due to complexities associated with the stimulus being at 45° and events occurring during the development of curvature in the present experiments.

The two states of gravitropism show striking differences in their requirement for calcium. In contrast with positive gravitropism, diagravitropism is essentially unaltered by pretreatment with a chelating agent, EGTA, and likewise is unaffected by the subsequent addition of calcium salt or chelate.

We suggest that the conversion of corn roots from the diagravitropic to the positive gravitropic state by red light involves an alteration of the transduction system—that is, the step which occurs after sensing, and which results in the development of some physiological gradient across the root. This hypothetical gradient subsequently can lead to the motor response: differential growth and tropistic bending of the root. In the case of diagravitropism, this physiological gradient would be qualitatively different from that involved in positive gravitropism. Thus, we would identify the red light control as a control of the transductive step of gravitropism.

We have published evidence (7) suggesting that the red light effects on gravitropism involve a substantial change in calcium pools, and these findings are consistent with the numerous reports of changes in calcium distribution during gravitropism (9). We suggest that there are qualitative differences between the

two transductive systems, one leading to diagravitropism and the other leading to positive gravitropism. The calcium role appears to be specific to the latter. Our hypothesis can be diagrammed as shown in Figure 4.

The widely observed requirement for a calcium flux in positive gravitropism is interpretable as a component of the positive transduction process, as has been suggested by Feldman (4). The electric current shift with gravity stimulation reported by BJÖRKMAN and LEOPOLD (2) is sensitive to calmodulin inhibitors, which may well have been a characteristic of the transductive step. The lack of sensitivity of diagravitropism to calcium chelators or calcium additions would suggest that the role of calmodulin may be more specific to the positive gravitropic transduction than to the sensing step.

It would seem unlikely that calcium redistribution is essential for gravitropism as claimed by numerous authors (3, 5, 8); instead, the calcium redistribution may more properly be essential only for positive transduction and may be minimal or even absent from diagravitropic transduction in Merit corn roots.

In the case of the diagravitropic rhizome of *Aegopodium*, Bennet-Clark and Ball (1) have suggested that the horizontally oriented growth might be regulated by balances between a growth promoter and a growth inhibitor. In subsequent decades, there has been no confirming evidence for such a hormonal mechanism. There seems to be no simple way to adapt the hormonal regulatory system of the classical Cholodny-Went theory to account for diagravitropism.

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