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Stimulation of Root Elongation and Curvature by Calcium[1]

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ABSTRACT

Ca^{2+} has been proposed to mediate inhibition of root elongation. However, exogenous Ca^{2+} at 10 or 20 millimolar, applied directly to the root cap, significantly stimulated root elongation in pea (*Pisum sativum* L.) and corn (*Zea mays* L.) seedlings. Furthermore, Ca^{2+} at 1 to 20 millimolar, applied unilaterally to the caps of Alaska pea roots, caused root curvature away from the Ca^{2+} source, which was caused by an acceleration of elongation growth on the convex side (Ca^{2+} side) of the roots. Roots of an agravitropic pea mutant, *ageotropum*, responded to a greater extent. Roots of Merit and Silver Queen corn also responded to Ca^{2+} in similar ways but required a higher Ca^{2+} concentration than that of pea roots. Roots of all other cultivars tested (additional four cultivars of pea and one of corn) curved away from the unilateral Ca^{2+} source as well. The Ca^{2+}-stimulated curvature was substantially enhanced by light. A Ca^{2+} ionophore, A23187, at 20 micromolar or abscisic acid at 0.1 to 100 micromolar partially substituted for the light effect and enhanced the Ca^{2+}-stimulated curvature in the dark. Unilateral application of Ca^{2+} to the elongation zone of intact roots or to the cut end of detipped roots caused either no curvature or very slight curvature toward the Ca^{2+}. Thus, Ca^{2+} action on root elongation differs depending on its site of application. The stimulatory action of Ca^{2+} may involve an elevation of cytoplasmic Ca^{2+} in root cap cells and may participate in root tropisms.

Despite strong arguments that Ca^{2+} mediates inhibition of root growth (6, 7, 10, 11), there is no consensus as to how, where, or when the cation has its effect (4, 10, 15). The proposed inhibitory action of Ca^{2+} has been emphasized particularly in studies of root gravitropism (7, 10–12). Such action has led to a theory that a redistribution of Ca^{2+} occurs in the root cap in response to gravistimulation, leading to an inhibition of root elongation by increasing the level of an inhibitor or by changing tissue sensitivity to it at the zone of elongation (11). An example of evidence supporting this theory is that roots have been shown to curve toward the higher Ca^{2+} side of an artificial Ca^{2+} gradient when applied unilaterally to the capless roots of corn (17). The level of Ca^{2+} is also reported to increase asymmetrically on the bottom side of the tip of horizontally oriented roots (18, 21). Downward or positive gravitropic curvature of roots could thus occur by growth inhibition on the bottom side of the horizontally oriented roots, which might be regulated by a Ca^{2+} asymmetry in the root cap. The inhibition theory is proposed to be consistent with the hypothesis that root gravitropism may be regulated by a growth inhibitor from the root cap (9, 24). However, recent studies of the effect of Ca^{2+} on root elongation show both inhibition and stimulation of growth due to Ca^{2+} application (6, 11). In addition, the inhibition theory alone cannot account for the growth kinetics of graviresponding roots because a number of studies show that an acceleration of elongation growth also takes place following gravistimulation (13, 14, 26).

Elevation of cytoplasmic Ca^{2+} in root cap cells has been proposed to be a key event in the signal transduction of gravity. In Merit corn roots which require red light for orthogravitropism, a Ca^{2+} ionophore, ABA, and osmotic shock, each proposed to elevate cytoplasmic Ca^{2+} levels in roots, do substitute for the light effect and induce an orthogravitropic response in the dark (19, 23, 25). The cytoplasmic Ca^{2+}/calmodulin system is also reported to play a role in such signal transduction of gravity (2, 27). Each of these responses is considered to take place in the root cap. Thus, Ca^{2+} in the root cap is very likely one factor responsible for modification of root growth and tropisms. However, the mechanism of action in growth and the relationship between cytoplasmic Ca^{2+} and its redistribution in gravistimulated roots require further elucidation. To answer such points, it is crucially important to obtain detailed information regarding the root cap and the effect of Ca^{2+} on root elongation and curvature.

Contrary to the inhibition theory, we report here evidence for the mediation of root cap Ca^{2+} in the promotion of root growth and curvature in pea and corn seedlings. The agravitropic roots of the pea mutant, *ageotropum*, are also highly sensitive to the stimulatory action of Ca^{2+}, which may provide further information concerning the mechanism of Ca^{2+} action in root growth and root tropisms.

MATERIALS AND METHODS

Seeds of Alaska pea (*Pisum sativum* L.) were obtained from W. Atlee Burpee & Co. (Warminster, PA), and seeds of a pea mutant, *ageotropum* (3), were provided by Watanabe Seed Co. (Kogota, Japan). Corn seeds of Merit and Silver Queen (*Zea mays* L.) were obtained from Asgrow Seed Co. (Kalamazoo, MI) and Southern States Corp., Inc. (Richmond, VA), respectively. All seeds were germinated on wet filter paper in a covered glass container (19 cm i.d., 7 cm deep) at 24 ± 1°C

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(mean ± SD) in the dark. Seedlings with straight roots 25 to 30 mm long were used for experiments when they were 2 to 3 d old. Seedlings were placed vertically with the roots oriented downward by mounting the seeds to a block of Styrofoam (20 × 13.5 × 4 cm) with insect pins. The Styrofoam block with eight to 14 seedlings was then placed in a glass aquarium chamber (41 × 24 × 23.5 cm), so that approximately the apical 15 mm of the root was suspended vertically in moist air. Humidity inside the chamber was nearly saturated (as measured by an HMI 31 humidity and temperature indicator with a probe, HMP 36/0904, Vaisala Sensor Systems, Helsinki, Finland) using layers of wet filter papers attached to the inner surface of the chamber. Experimental seedlings were maintained in total darkness with experimental manipulations performed under a green light sufficiently dim that it was not gravitropically inductive. When the roots were exposed to light, the vertically oriented seedlings were placed under white light at the irradiance of 3.2 W m^{-2} obtained from 34-W white fluorescent tubes (F40CW/RS/EW-II; Philips, Bloomfield, NJ). All experiments were performed at 24 ± 1°C.

$CaCl_2$ (Fisher Scientific Co., Pittsburgh, PA) and ABA (Sigma) at various concentrations were prepared in 1.2% agar (purified agar; Sigma). All chemical preparations with agar contained 2 mM Mes buffer at pH 6.4. Agar blocks, 1 × 1 × 1 mm in size, were placed unilaterally over the 1-mm root cap of an intact root or suspended from the cap end for the symmetric treatment. In some experiments, approximately 1 mm of the apical tip was removed with a razor blade, and an agar block was placed unilaterally to the side of the cut end of the detipped root. Control blocks contained only 2 mM Mes buffer. A Ca^{2+} ionophore, A23187 (Sigma), was dissolved in a small amount of DMSO (Sigma) and diluted with 2 mM Mes buffer at pH 6.4 to prepare a 2 mM stock solution. An 8-μL droplet containing 20 μM A23187 and 0.04% (v/v) DMSO in 2 mM Mes buffer at pH 6.4 was suspended from vertically placed roots, 1 to 2 mm from the tip, so that the entire root cap was immersed in the solution droplet for 1 h before Ca^{2+} application. DMSO alone in Mes buffer served as the control.

In experiments comparing the Ca^{2+} effect in the light and dark, irregular responses of roots were obtained depending on pretreatment with water. When the root tips were maintained excessively wet, smaller and irregular responses resulted from application of asymmetric Ca^{2+}. On the other hand, roots maintained in an atmosphere of 97 to 99% RH for the same period of time responded regularly and to a greater extent. Water balance in the root has been proposed to regulate cytoplasmic Ca^{2+} levels and has been reported to be significant for the induction of gravitropic response of light-requiring orthogravitropic roots in the dark (16, 19). Accordingly, excess water or swollen mucilage on the root surface, of particular importance in corn (20, 29), was carefully removed with filter paper before treatment with agar blocks, while using care to avoid drying during experimental manipulations.

To measure the growth of roots, 250-μm glass beads were attached on both sides of the vertically placed root with approximately 1-mm intervals for apical 10-mm length of the root, or the same apical 10-mm portion of roots were marked using India ink. The roots were photographed at time zero and 3 to 12 h after the application of agar blocks. Growth of both sides of the root was calculated from the changed distances between beads or marks by measurement of enlarged photographs. Root curvature was shadowgraphed at designated times after treatment and measured with a goniometer. Mean curvature of <1° is designated as "no curvature" in the tables.

Each Ca^{2+} treatment was repeated at least three times (often more, depending on the cultivar). Ionophore or ABA experiments were also repeated at least three times. Results of repetitions gave comparable results but varied with the treatment and the cultivar used. Data presented are, therefore, the mean ± SE of representative experiments.

RESULTS

To examine the effect of root cap application of Ca^{2+} on root elongation, dark-grown roots of pea and corn were exposed to white light in a vertical position for 2 h before Ca^{2+} application. Agar blocks containing 10 or 20 mM Ca^{2+} in 2 mM Mes buffer or the buffer alone were then placed over the cap end of the roots symmetrically. Elongation growth of the roots was measured at designated times following the application of blocks. As shown in Table I, the growth of roots treated with 10 mM Ca^{2+} was approximately 30% greater than controls for a 3.5-h period following Ca^{2+} application to Alaska pea roots and approximately 80% greater than control for 12 h following the treatment in *ageotropum* pea. However, the growth of Alaska pea roots did not differ from that of control roots when measured 12 h after Ca^{2+} treatment. Roots of Silver Queen corn also showed an increase of approximately 70% in growth 3 h following the application of 20 mM Ca^{2+} (Table I). Such symmetrical treatment of root caps with Ca^{2+} did not cause curvature of the roots.

Corn roots have been reported to curve toward Ca^{2+} when applied unilaterally to the root tips, although some varieties

Table I. *Root Elongation following Symmetrical Application of Ca^{2+} to the Cap*

Agar blocks containing Ca^{2+} or buffer alone were placed symmetrically on vertically oriented roots. All roots were exposed to light for 2 h before Ca^{2+} application. Apical 10-mm lengths were marked by India ink or by attachment of glass beads. The roots were photographed at time zero and after 3.5 and 12 h for Alaska pea, 12 h for *ageotropum*, and 3 h for Silver Queen corn. Root growth was calculated from the differences in distances between marks by using enlarged photographs.

Plant and Time of Measurement	Treatment	Growth Increment (mean ± SE)	% of Control
		mm	
Alaska (3.5 h)	Control	1.72 ± 0.14 (n = 24)	
	10 mM Ca^{2+}	2.28 ± 0.11 (n = 24)[a]	133
Alaska (12 h)	Control	6.84 ± 0.84 (n = 8)	
	10 mM Ca^{2+}	6.84 ± 0.60 (n = 8)	100
ageotropum (12 h)	Control	5.40 ± 0.72 (n = 8)	
	10 mM Ca^{2+}	9.84 ± 0.60 (n = 8)[a]	182
Silver Queen (3 h)	Control	0.69 ± 0.03 (n = 20)	
	20 mM Ca^{2+}	1.17 ± 0.03 (n = 20)[a]	170

[a] Statistically significant from control by Student's t test ($P < 0.01$).

curve away from it (12). Because this report is in disagreement with the growth stimulatory action of Ca^{2+} found in the present study, we reexamined the effect of Ca^{2+} on the induction of root curvature by applying agar blocks containing various concentrations of Ca^{2+} to the root cap of pea and corn roots. As shown in Figure 1, unlike the previous report (12), Ca^{2+} applied unilaterally to the root cap caused unequivocal root curvature away from the Ca^{2+} source in these two pea and two corn roots as well as all other cultivars or varieties used in the present study (see "Discussion"). When exposed to white light for 2 h before Ca^{2+} application, Alaska pea roots showed slight curvature away from 1 mM Ca^{2+} 4 h after the application (Fig. 1A). The curvature was greater as the Ca^{2+} concentration increased to 10 mM. On the other hand, roots of the pea mutant, *ageotropum*, showed strong curvature away from 1 mM Ca^{2+}, and the response was significantly greater than that of Alaska pea roots at all higher concentrations tested (Fig. 1A). Unlike peas, roots of Merit and Silver Queen corn did not curve significantly at concentrations of 1 or 5 mM of Ca^{2+} (Fig. 1B), but the roots curved unequivocally away from 10 mM and 20 mM Ca^{2+} in both cultivars. The degree of curvature increased when the Ca^{2+} concentration was increased from 10 to 20 mM in corn roots. Roots treated with control agar blocks did not show any curvature in either pea or corn roots. The consistent curvature away from Ca^{2+} was observed only when the root cap of intact roots was treated unilaterally with Ca^{2+}, and the response was concentration dependent. Unilateral application of Ca^{2+} to the elongation zone, 2 to 3 mm above the root tip, or to the side of the cut end of detipped roots failed to induce root curvature away from the Ca^{2+} in both Alaska and *ageotropum* peas (data not shown). Instead, there was a very slight trend to curve toward Ca^{2+} when applied to the elongation zone or the detipped roots (data not shown).

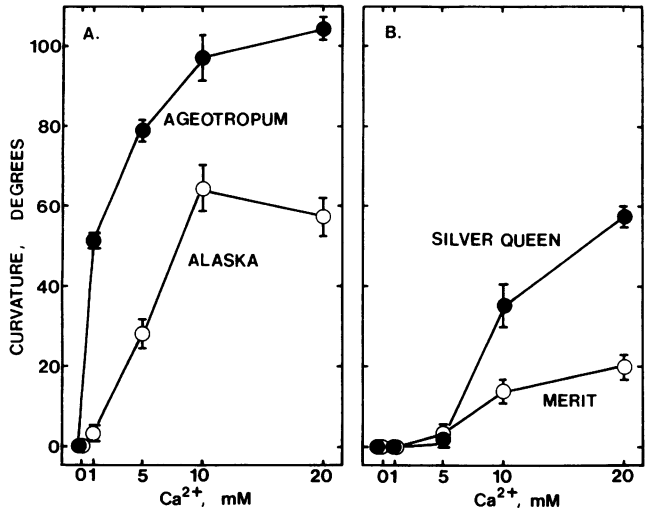

Figure 1. Curvature of pea and corn roots caused by unilateral cap application of Ca^{2+} at various concentrations. Dark-grown roots were placed in a vertical position and exposed to light for 2 h before unilateral application of Ca^{2+} to the cap. Curvature of pea and corn roots was measured 4 and 3 h after Ca^{2+} application, respectively. A, Alaska and *ageotropum* peas; B, Merit and Silver Queen corn. Each concentration point includes 16 to 18 roots for Alaska pea, 9 to 11 roots for *ageotropum* pea, 20 to 21 roots for Merit corn, and 10 to 11 roots for Silver Queen corn. Data represent curvature away from the Ca^{2+} source. Error bars, ± SE.

Table II. *Differential Growth of Roots Caused by Unilateral Application of Ca^{2+} in Alaska and ageotropum Peas*

Dark-grown roots were placed in a vertical position and exposed to light for 2 h before unilateral application of Ca^{2+} to the caps. The 10-mm apical portion of the root was marked by placing 250-μm glass beads on the side of the block application and opposite to it. The growth was calculated from the distances between beads on enlarged photographs. Data are growth of 10-mm apical portion during 4 h following Ca^{2+} application. Four roots of Alaska pea and six of *ageotropum* were used for the measurement. Roots treated with Ca^{2+} curved away from it.

Treatment	Location	Growth Increment (mean ± SE)[a]	
		Alaska	*ageotropum*
		mm	
Control	Agar side	2.10 ± 0.44*	1.46 ± 0.32*
	Opposite side	2.02 ± 0.47*	1.53 ± 0.36*
Ca^{2+}	Ca^{2+} side	3.26 ± 0.45**	2.29 ± 0.20**
	Opposite side	2.54 ± 0.28*	1.70 ± 0.16*

[a] Data with * are significant from those with ** by Student's *t* test ($P < 0.05$).

Because the cause of the Ca^{2+}-stimulated curvature could result from the combination of differences between the maintenance of growth or growth stimulation, inhibition, and cessation on the Ca^{2+} treated and opposite sides (14), the growth of both sides of the root was measured for 4 h following cap application of Ca^{2+}. As shown in Table II, roots of both Alaska and *ageotropum* peas curved away from 10 mM Ca^{2+} because of an acceleration of elongation growth on the convex side (Ca^{2+} side) and without inhibition on the opposite, concave side, of the root. The growth of the Ca^{2+}-applied side was approximately 28% greater than that of the opposite side in Alaska pea and approximately 35% greater in *ageotropum*. The growth of the concave side was not less than the side of the straight control root in either pea.

Agar blocks containing 10 mM Ca^{2+} placed asymmetrically on the root cap and maintained in the dark consistently caused curvature away from the Ca^{2+} source in Alaska and *ageotropum* pea roots (Fig. 2). In *ageotropum*, the curvature became distinct between 1 and 2 h and reached a plateau 4 h after the treatment. Exposure of the roots to white light for 2 h before Ca^{2+} application significantly increased the response to Ca^{2+} (Fig. 2A). Alaska pea roots also curved away from the 10 mM Ca^{2+} agar block immediately after its application but less so in the dark than *ageotropum* (Fig. 2B). The slight curvature of Alaska pea roots due to Ca^{2+} was transient; the roots regained their straight vertical position after 4 h. Again, preexposure of Alaska pea roots to white light substantially enhanced the curvature away from Ca^{2+} (Fig. 2B). For the first 4 h, the curvature of the light-exposed roots of Alaska pea was virtually the same as that of *ageotropum*, although this response was also transient (Fig. 2). The transient response of Alaska pea roots lasting only 4 h and the continued curving of *ageotropum* for 12 h was consistent with the straight growth

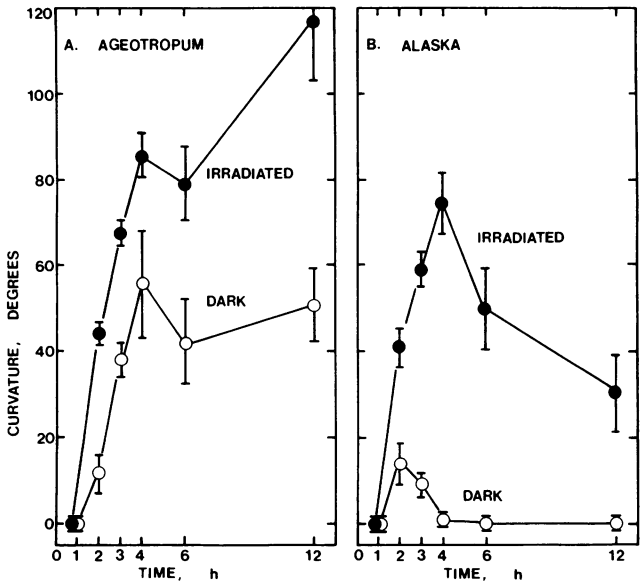

Figure 2. Time-course studies of root curvature caused by unilateral application of Ca^{2+} to the cap in Alaska and *ageotropum* peas. Agar blocks containing 10 mM $CaCl_2$ and 2 mM Mes buffer were placed unilaterally on the apical 1-mm cap of vertically oriented roots. A, *ageotropum* pea roots; B, Alaska pea roots. ○, Ca^{2+}-treated roots in the dark; ●, light-exposed and Ca^{2+}-treated roots. Each time point includes eight to 10 roots. Control agar blocks containing Mes buffer alone did not cause curvature in either root. Data represent curvature away from the Ca^{2+} source. Error bars, ± SE.

responses induced by symmetrical application of Ca^{2+} to the two pea roots (Table I). Although for experimental reasons a 2-h light treatment was normally used, exposure of the roots to white light for as little as 10 min was sufficient to cause saturation of its effect on Ca^{2+}-stimulated curvature (Table III). Control agar blocks placed asymmetrically on the root cap did not cause curvature in either dark-grown or light-exposed roots of either pea variety (Table III).

The light effect on gravitropism of light-requiring orthogravitropic roots is considered to be mediated by phytochrome in the root cap (8), and agents such as the Ca^{2+} ionophore, A23187 (23, 25), and ABA (19) are reported to substitute for the light effect in inducing orthogravitropism in the dark. Ca^{2+}-stimulated curvature of Alaska pea roots was also enhanced by those agents. When the cap region of Alaska pea roots was pretreated with 20 μM of the ionophore for 1 h, Ca^{2+} applied unilaterally to the cap caused strong curvature away from the Ca^{2+} source in the dark (Table IV). Ca^{2+}-stimulated curvature of pretreated roots was 33.2 ± 1.8° in 4 h, whereas roots without pretreatment showed no curvature. The curvature of light-exposed roots was 46.2 ± 1.9° (Table IV). ABA also substituted partially for the light effect and enhanced the Ca^{2+}-stimulated curvature of Alaska pea roots in the dark (Table V). Agar blocks containing both the Ca^{2+} and ABA, which were applied unilaterally to the root cap, induced substantial curvature away from the agar blocks in the dark. The curvature increased as the concentration of ABA increased from 0.1 to 100 μM. Unilateral application of agar blocks containing Ca^{2+} alone at 10 mM or ABA alone at 0.1 to 10 μM did not cause significant curvature, although the treatment with ABA alone at 100 μM induced curvature away from it to some extent. Roots did not bend at all when the elongation region was unilaterally treated with ABA.

Table III. *Ca^{2+}-Induced Curvature of Alaska Pea Roots Exposed to Light for Various Time Periods*

Dark-grown roots were placed vertically under 3.2-W m^{-2} white light for various durations before unilateral application of Ca^{2+} to the cap. Data represent curvature away from Ca^{2+}, measured 4 h after the application.

Duration of Light Exposure	Ca^{2+} Treatment	Curvature (mean ± SE)
min	*mM*	*degrees*
0	0	No curvature (n = 16)
0	10	5.4 ± 3.1 (n = 16)
10	10	36.4 ± 2.8 (n = 16)
15	10	29.3 ± 8.3 (n = 8)
30	10	26.3 ± 4.4 (n = 8)
90	10	26.4 ± 6.1 (n = 8)
180	0	No curvature (n = 8)
180	10	27.6 ± 4.0 (n = 8)
Continuous light	0	No curvature (n = 8)
Continuous light	10	38.1 ± 4.1 (n = 8)

DISCUSSION

The effect of Ca^{2+} on root elongation has been reported to be both stimulatory and inhibitory (4, 6, 10). In those initial studies, however, the whole root was treated with Ca^{2+}. Because the site of action for Ca^{2+} in gravitropism is considered to be root cap rather than the zone of elongation, it is necessary to focus on the role of the Ca^{2+}/cap interaction in root growth as well as in gravitropic responses. In the present study, we found that Ca^{2+} at 10 or 20 mM applied to the cap end of pea and corn roots mediated elongation growth of roots for at least 3 to 4 h following treatment (Table I). It was also found that unilateral application of 1 to 20 mM Ca^{2+} to the root cap always induced unequivocal curvature of roots away from the Ca^{2+} source in Alaska pea and to a greater

Table IV. *Enhancement of Ca^{2+}-Stimulated Curvature of Alaska Pea Roots by the Ca^{2+} Ionophore, A23187, in the Dark*

An 8-μL droplet containing 20 μM A23187 or the buffer alone (control) was suspended from the tip of vertically placed roots, 1 to 2 mm from the tip, for 1 h before unilateral application of Ca^{2+} to the cap (see text for details). When pretreated with light, vertically placed roots were exposed to white light for 2 h before Ca^{2+} application. Data are curvature away from the Ca^{2+} source, measured 4 h after application.

Treatment	Light Conditions	Curvature (mean ± SE)
		degrees
Control	Dark	No curvature (n = 12)
10 mM Ca^{2+}	Light	46.2 ± 1.9 (n = 12)
10 mM Ca^{2+}	Dark	No curvature (n = 12)
10 mM Ca^{2+}/20 μM A23187	Dark	33.2 ± 1.8 (n = 12)

Table V. *Enhancement of Ca^{2+}-Stimulated Curvature of Alaska Pea Roots by ABA*

Dark-grown roots were placed vertically in the dark. Agar blocks containing Ca^{2+} at 10 mM and/or ABA at 0.1 to 100 μM were then unilaterally placed over the root cap or the zone of elongation. Data represent curvature away from the Ca^{2+}/ABA agar blocks, measured 4 h after the application.

Treatment	Site of Application	Curvature (mean ± SE)
		degrees
Control	Cap	No curvature (n = 12)
10 mM Ca^{2+}	Cap	2.8 ± 2.5 (n = 12)
0.1 μM ABA	Cap	No curvature (n = 12)
0.1 μM ABA/10 mM Ca^{2+}	Cap	4.5 ± 1.9 (n = 12)
1 μM ABA	Cap	No curvature (n = 12)
1 μM ABA/10 mM Ca^{2+}	Cap	13.5 ± 3.4 (n = 12)
10 μM ABA	Cap	No curvature (n = 12)
10 μM ABA/10 mM Ca^{2+}	Cap	22.1 ± 2.7 (n = 10)
10 μM ABA	Elongation zone	No curvature (n = 10)
100 μM ABA	Cap	13.2 ± 2.3 (n = 10)
100 μM ABA/10 mM Ca^{2+}	Cap	33.3 ± 5.1 (n = 10)
100 μM ABA	Elongation zone	No curvature (n = 10)

extent in the roots of the agravitropic mutant, *ageotropum* (Figs. 1 and 2). Roots of Merit and Silver Queen corn also always curved away from Ca^{2+} applied to the cap, although a somewhat higher concentration was required for the response than in pea roots (Fig. 1). In addition, roots of all other cultivars tested (Trucker's Favorite corn and pea cultivars Sugar Snap, Sweet Snap, Wando, and Dwarf Grey Sugar) always showed consistent curvature away from Ca^{2+} (data not shown). The curvature away from the unilateral Ca^{2+} source on the cap was caused by an acceleration of elongation growth on the convex side (Ca^{2+} side) of pea roots (Table II). These results show a strong correlation between an increase of Ca^{2+} levels in the root cap and stimulation of root elongation. The results are in contrast to the previously proposed model that an increased level of Ca^{2+} in the root cap mediates inhibition of root growth (12).

However, increased levels of Ca^{2+} in the elongation zone may inhibit pea root elongation to some extent because Ca^{2+} applied unilaterally to the zone of elongation or to the cut end of detipped roots caused either no curvature or slight curvature toward Ca^{2+} (data not shown). It has also been reported that decapped roots of corn curved toward Ca^{2+} when applied unilaterally to the tip (17). These observations suggest differing actions of Ca^{2+} on root growth depending upon its site of application. Evans *et al.* (6) measured elongation growth of corn roots immersed in a 1 mM Ca^{2+} solution. They found inhibition of root growth almost immediately after Ca^{2+} treatment, but they also found that by 60 to 80 min the growth rate of Ca^{2+}-treated roots exceeded that before Ca^{2+} treatment. They did not discuss the stimulatory action of Ca^{2+} in detail, but the lag times for inhibitory and stimulatory action of Ca^{2+} may be explained by immediate and direct inhibitory action of Ca^{2+} in the elongation region and by indirect stimulatory action of Ca^{2+} there, which involves the root cap and factor(s) that are transmitted from the cap to the zone of elongation. These two separate responses might have occurred in their experiments because the entire root was immersed in a Ca^{2+} solution. In our experiments, Ca^{2+}-stimulated curvature of pea roots became apparent between 1 and 2 h after the treatment to the cap (Fig. 2), suggesting a similar lag time for the stimulatory action of Ca^{2+}.

In Alaska pea roots, stimulation of elongation growth due to symmetrical cap application of Ca^{2+} was evident 3.5 h following Ca^{2+} treatment, but no significant difference in the total growth between the Ca^{2+}-treated and control roots was observed 12 h after the treatment (Table I). On the other hand, the growth of the Ca^{2+}-treated *ageotropum* roots was much greater than that of the control for as many as 12 h after the treatment (Table I). These results of straight growth for the two peas appear to be parallel their pattern of Ca^{2+}-stimulated root curvature kinetics, *i.e.* Ca^{2+}-stimulated curvature in Alaska pea roots was transient with the peak 4 h after treatment, whereas *ageotropum* pea roots continuously responded to Ca^{2+} for at least 12 h following the treatment (Fig. 2).

It is of particular interest that agravitropic roots of *ageotropum* pea responded to cap application of Ca^{2+} and did so by curving strongly away from Ca^{2+} in the dark and at lower concentrations of Ca^{2+} than the Alaska pea. Roots of Alaska pea required exposure to light and relatively higher concentrations of Ca^{2+} for a full but, nevertheless, smaller response (Table III, Figs. 1A and 2). Although the mutation is not understood at the molecular level, the involvement of a Ca^{2+}/cap interaction appears to agree with the observation that agravitropism in *ageotropum* pea roots is due to cytological and physiological disruptions in the signal perception and transduction step(s) in the cap itself (5, 22). This mutant should be an excellent tool for further studies directed at the understanding of the Ca^{2+}/cap interaction in root growth.

It is significant that light substantially enhances the root response of curving away from Ca^{2+} (Table III, Fig. 2). The period lasting approximately 4 h for the Ca^{2+}/light enhancement effect in Alaska pea agrees with time intervals of other light-regulated responses, including root gravitropism, in which light is thought to stimulate an increase in cytoplasmic Ca^{2+} through phytochrome mediation (8, 19, 30). Furthermore, in such responses, a Ca^{2+} ionophore may substitute for the light effect (23, 25). When the cap region of Alaska pea roots was pretreated with 20 μM A23187 for 1 h, Ca^{2+} applied unilaterally to the cap caused strong curvature away from the source in the dark (Table IV). In addition, ABA, previously proposed to substitute for the effect of red light in inducing root gravitropism by increasing cytoplasmic Ca^{2+} (19), was also found to enhance the Ca^{2+}-stimulated curvature of Alaska pea roots in the dark (Table V). Light pretreatment is required for a full orthogravitropic response of roots in Alaska pea and Silver Queen corn, as well as Merit corn (data not shown). The requirement of light for orthogravitropism of Silver Queen corn roots was also reported by LaMotte and Pickard (16). The light effect on gravitropism and Ca^{2+}-stimulated curvature of roots may well be associated, at least in part, with the same mechanism. Thus, the stimulation of root elongation or curvature by cap application of Ca^{2+} could be mediated, at least in part, by an increase of cytoplasmic Ca^{2+}

in the cap, which could, in turn, be enhanced by light, an ionophore, or ABA. The alteration of cytoplasmic Ca^{2+} levels remains to be measured *in situ* by direct means.

Elevation of cytoplasmic Ca^{2+} levels in the root cap, previously proposed to play an important role in signal transduction of gravity (2, 19, 23, 25), could participate in gravitropic curvature of roots through its stimulatory action because many studies have shown that an acceleration of root elongation takes place following gravistimulation (13, 14, 26). In this regard, root curvature away from a Ca^{2+} source appears to be the opposite of reports that there is a Ca^{2+} redistribution with more accumulation on the bottom side of the graviresponding roots curving down (18, 21). However, it has recently been reported that saturation of the apoplast with external Ca^{2+} promotes rather than prevents gravitropic curvature of sunflower hypocotyls, indicating that it is not apoplastic Ca^{2+} asymmetry that is necessary for the gravitropic response (1). We have observed similar results in roots of Alaska pea; light is required for a full orthogravitropic response in the roots, but immersion of the root tip in a 20 mM Ca^{2+} solution for 1 h accelerated gravitropic curvature in the dark (data not shown).

In their comprehensive study of the effectiveness of metal ions in inducing root curvature, Hasenstein *et al.* (12) reported that roots curve away or toward the applied ions such as Al^{3+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, and Ca^{2+}. They observed that two of five corn cultivars showed root curvature away from a unilateral Ca^{2+} source applied to the cap. However, they concluded from their studies that in most cases corn roots curve toward Ca^{2+} (12). In fact, one of the cultivars that showed curvature away from Ca^{2+} was Merit corn, a finding consistent with our results. In similar experiments with the corn cultivar, Golden Cross Bantam 70, Suzuki (28) also observed root curvature away from Ca^{2+}. Hasenstein *et al.* (12) interpreted the difference in the Ca^{2+} responses by different tissue sensitivities to factor(s) controlling gravitropism in different corn cultivars. Differences in responsiveness of roots to Ca^{2+} may also result from different water conditions at the tip as described in "Materials and Methods" (20, 29). However, it is now evident that roots of many cultivars of corn and pea, including all those tested in the present study, curve away from Ca^{2+} when applied unilaterally to the root cap and that the response is concentration dependent (Fig. 1). Thus, the proposed model for Ca^{2+} action in inducing root curvature through growth inhibition (11, 12) needs to be restated and further studied.

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