Effects on Zea Mays Seedlings of a Strontium Replacement for Calcium in Nutrient Media [1, 2, 3]

William H. Queen[4], Harvey W. Fleming[5], & Joseph C. O'Kelley

Department of Biology, University of Alabama, University, Alabama

It has been reported that growth is supported in several species of algae when Sr is substituted for Ca in the nutrient media (10, 12). Sr also has been reported to almost completely replace Ca in the vegetative but not in the reproductive growth of several higher plant species (13). It is probable that in these instances Sr supported or stimulated growth in the presence of minute quantities of Ca. McHargue (6) reported that Sr could not completely replace Ca in certain higher plants, but found that Sr, if present in moderate quantities (5–10 g added to soil of about a 6 inch pot), brought about an increase in the growth of winter wheat and oats, in the presence of $CaCO_3$. Responses such as those described by McHargue but involving sodium and potassium have been designated as resulting from partial replacement (8); presumably the term partial replacement, or partial substitution, could also be applied to this response to Sr.

The present study was carried out to learn if Sr could partially replace Ca in the growth of maize and tomato and if partial replacement would produce morphological changes. Tomato seedlings died within 2 days after they were placed in a Ca-free solution and the addition of Sr did not prolong their life. The remainder of this paper deals with the behavior of maize.

Two related investigations were undertaken to ascertain the manner and the extent to which Sr could replace Ca in maize. In one, nutrient solutions were prepared with graded concentrations of Ca and Sr, and observations were made on growth of seedlings in these solutions. In the other, Ca^{45} was supplied, and its absorption, distribution, and loss by seedlings was determined by autoradiography and other isotope techniques.

Materials & Methods

Solution Culture of Seedlings. *Zea mays* L. seeds of the variety KYS (or Truckers Favorite Sweet Hybrid, which behaved similarly) were germinated in acid-washed sand for 3 days, rinsed with deionized water, and transferred to nutrient solutions. Culture vessels were one liter polyethylene bottles covered externally with aluminum foil; seedlings were held in place in paraffin-coated wooden lids. Seedlings transferred to nutrient solutions were randomly selected, and all plants were grown side by side on a greenhouse bench under similar conditions of humidity and temperature. Solutions were not mechanically aerated.

The basal nutrient solution contained 0.004 M KCl, 0.0018 M $MgSO_4 \cdot 7H_2O$, 0.0021 M $Ca(NO_3)_2 \cdot 4H_2O$, 0.0022 M KH_2PO_4, 0.07 mM Fe as Geigy Agricultural Chemicals Sequestrene 330 Fe, 0.08 mM $MnCl_2$, 0.037 mM $ZnCl_2$, 0.082 mM H_3BO_3, 0.004 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 0.013 mM $CuCl_2$ in distilled and deionized water. The Sr solution contained an equivalent weight of Johnson, Matthey and Co. Specpure $Sr(NO_3)_2$ substituted for $Ca(NO_3)_2$; the Na solution, an equivalent weight of $NaNO_3$ for $Ca(NO_3)_2$. For spectroscopic determinations 50 ml of each of the two replacement solutions (Sr & Na) were evaporated in Pt on 50 mg Specpure granular carbon; 40 mg were exposed in a d-c arc and the emitted light was integrated on infra red plates between 8087 and 3130 A. Estimation of content was made by the dilution method of Mitteldorf (9).

Three graded series of nutrient solutions were prepared in which there were, respectively, stepwise replacements of Ca by Na, Ca by Sr, and Sr by Na. There were 12 replicate seedlings per treatment. Measurements of shoot and root length were taken at several periods while the plants were in the nutrient solutions. Plant root tip samples were taken at intervals of growth, fixed, and examined microscopically both before and after being sectioned and stained. After the plants had grown for 24 days, they were removed from the nutrient solutions, and determinations of fresh weights of shoots and roots were made.

Radioactivity of Seedlings Supplied Ca^{45}. Truckers Favorite Sweet Hybrid variety maize was used; Ca^{45} was obtained in the form of $CaCl_2$ in HCl solution from Oak Ridge National Laboratory.

---

[1] Revised manuscript received Nov. 20, 1962.

[2] This study was supported by research grant A-3680 Nutr. from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, and by facilities provided by the University of Alabama Research Committee.

[3] Cooperative work done by Mr. L. W. Clements, U. S. Bureau of Mines Tuscaloosa Metallurgy Research Center, University, Alabama.

[4] Present address: Botany Department, Duke University, Durham, North Carolina.

[5] Present address: Science Department, Delta State College, Cleveland, Mississippi.

Loss of Ca from roots of developing seedlings was studied by soaking dry seeds for 24 hours in basal nutrient solution containing Ca^{45}; at the end of this period seeds were rinsed and germinated in petri dishes on filter paper wet with distilled water. Upon germination, 3 days after the seeds were soaked, root systems of 9 seedlings each were immersed in 100 ml of nonradioactive nutrient solution, either the basal Ca-containing solution, the Sr-replacement solution, or the Na-replacement solution. Radioactivity of each solution was checked daily by conventional counting procedures. Counts, uncorrected for self absorption, were also made of radioactivity of whole root systems before immersion in the nonradioactive solutions and at the end of the 7-day immersion period.

A second set of seeds was similarly exposed to Ca^{45} and germinated. In this instance the seedlings were immersed in a larger volume of nonradioactive nutrient solution (2250 ml), 9 seedlings in each kind of solution. After immersion, seedling root systems were harvested at intervals, rinsed, and dried under light pressure. When dried, they were placed against Eastman No-screen X-ray film.

Results

Solution Culture. Observations and measurements were made on seedlings as they developed in the three series of nutrient solutions. At age 6 days seedlings cultured in the Na solution, free of Ca and Sr, showed necrosis of leaves and roots; all these seedlings were dead at age 12 days. Plants supplied Sr, or Ca or both remained alive for 24 days or longer.

Table I

Effect of Changes in Proportional Parts of Ca & Sr on the Length & Fresh Weight of Maize Seedling Roots & Shoots

Proportional parts (Ca/Sr, Ca/Na, or Sr/Na)*	1.0/0	0.1/0.9	0/1.0
Ca/Sr			
Shoot (cm)**	16.8	14.2	11.4
Root (cm)	15.8	14.4	8.1
Shoot (g)	2.8	2.4	0.9
Root (g)	1.2	1.1	0.4
Ca/Na			
Shoot (cm)	17.2	13.5	Dead
Root (cm)	22.1	14.6	Dead
Shoot (g)	3.0	2.6	Dead
Root (g)	1.2	1.3	Dead
Sr/Na			
Shoot (cm)	11.2	9.5	Dead
Root (cm)	8.9	8.4	Dead
Shoot (g)	0.7	0.5	Dead
Root (g)	0.4	0.4	Dead

* Changes in proportional parts (Ca/Sr, Ca/Na, or Sr/Na) of 0.9/0.1, 0.8/0.2, 0.5/0.5, to 0.2/0.8 had little effect.

** All length measurements made at age 12 days, and all weights at age 24 days.

Measurements of shoot and root length, made at age 12 days (table I), showed that both shoot and root lengths were less when Sr replaced Ca. Except for smaller size, the seedlings in Sr at these ages did not differ macroscopically in appearance from those in Ca. At age 24 days the seedlings were harvested and root and shoot wet weights were determined (table I). At this age plants in Sr were significantly lower in wet weight. Some seedlings in Sr showed necrosis and browning of root tips while roots in Ca appeared healthy. Secondary roots of healthy root systems in Sr appeared to be more numerous than those on the control seedlings in Ca. Therefore, the full-Ca and full-Sr treatments were replicated with 16 seedlings in Ca and 20 in Sr, all selected carefully for uniformity in size. These seedlings were grown for 14 days, at which time root system lengths were determined and the total number of roots on each seedling was counted. The mean root system length in Ca was 17.7 cm; in Sr it was 12.6 cm. The average number of root tips per seedling in Ca was 97 and in Sr it was 119. The differences between both sets of means were shown by the t-test to be significant at the 1 % level.

Microscopic examination of longitudinal root tip sections taken from similar plants, age 14 days, showed that cells of Sr-supplied roots were smaller than those of Ca-supplied roots. Measurements of root diameter were made and those in Sr were found to have a mean diameter about 75 % of those supplied Ca. As suggested by macroscopic observations, cell elongation also was strongly depressed in the Sr-supplied roots. Normal mitotic figures were found in both types of root tips, apparently with equal frequency. The root tips of Sr-supplied plants differed from the Ca-supplied tips in that differentiated xylem elements and secondary root primordia were found very near the meristem region in Sr-supplied root tips, and only at a considerable distance from this region in Ca.

Spectroscopic analysis for Ca contaminating the Sr- and Na-substitution nutrient solutions indicated that the Ca content of each solution was about 0.8 μg/ml; there was no detectable difference in Ca content between the two substitution solutions.

Radioactivity of Seedlings Supplied Ca^{45}. Autoradiograms of maize seedlings, made of plants which had been supplied Ca^{45} during germination and then transferred to nonradioactive nutrient solutions, were examined to determine Ca^{45} distribution in the roots. In the inactive Ca-solution roots, elongation continued and, as the days progressed, the root ends possessed progressively less radioactivity (fig 1, 2). In the inactive Sr-solution roots, elongation also continued and the pattern of radioactivity was similar (fig 3). Roots in the inactive Na-solution elongated much less and Ca^{45} activity in the tip region remained higher (fig 4).

Loss of Ca^{45} from the seedlings to the nonradioactive solutions was relatively rapid for the first 24 hour period (table II). Following this, a plateau

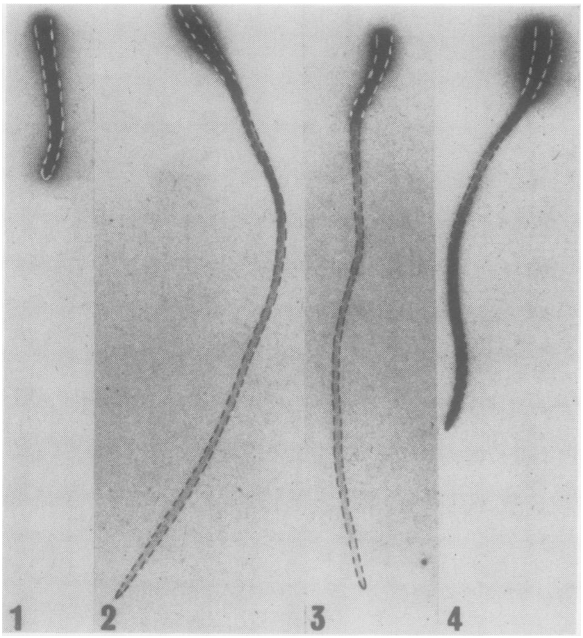

FIG. 1–4. Autoradiogram (× 2) of primary roots of maize seedlings produced from seeds soaked 1 day in Ca^{45}-containing nutrient solution, germinated on filter paper wet with distilled water, and transferred at age 4 days to nonradioactive nutrient solutions as indicated. FIG. 1. Root 4 days old, before transfer. FIG. 2. Root 8 days old, after transfer to Ca-containing solution. FIG. 3. Root 8 days old, after transfer to solution with Sr replacement for Ca. FIG. 4. Root 8 days old, after transfer to solution with Na replacement for Ca. Dashed lines indicate size and extent of roots.

was reached, particularly in the Ca solution, until day six, after which net loss of activity from roots to solution increased again. The amount of Ca^{45} lost to the Na solution was somewhat higher on day seven than the amount lost to the Ca and Sr solutions. By this time physical damage to the roots in Na was apparent. A comparison of the Ca^{45} activity of root systems before immersion in nonradioactive Ca-solution with their radioactivity seven days later, when they were removed from the solution, showed that these root systems retained approximately 95 % of the original radioactivity, and that only a small proportion of the Ca^{45} had been lost to the nutrient solution in which they were immersed.

Table II

Loss of Radioactivity from Seeds Previously Soaked in Basal Nutrient Medium Containing Ca^{45}

Days after transfer of seedlings to solution	Specific activity of nutrient solutions in which radioactive roots were immersed, c/min/ml.		
	Ca^{++}	Sr^{++}	Na^{+}
	c/min/ml	c/min/ml	c/min/ml
1	58	81	72
2	56	63	69
3	61	76	87
4	63	73	88
5	62	81	91
6	84	113	124
7	131*	130	177

* Represents approximately five per cent of the Ca^{45} initially present in the roots.

Discussion

The maize seedlings grown in nutrient solutions free of both Ca and Sr exhibited severe injury resembling the Ca-deficiency symptoms described by Bamford (1) and others (2); roots remained short and failed to branch, growth ceased, and the roots turned brown and gelatinous. In those seedlings supplied Sr in place of Ca, less shoot and root length and smaller root cell size were the only readily detectable symptoms that have been attributed to Ca deficiency (3, 4, 5, 11). Burstrom (3, 4) observed a diminution in cell size in wheat root tips, and also observed that the total cell number was less in deficient roots. He concluded that the Ca requirement for cell enlargement of wheat root cells is higher than for other growth processes in this root. It appears that in maize roots cell enlargement requires Ca specifically in relatively large quantities.

The occurrence of differentiated xylem elements and secondary roots and root primordia near the root tips when Ca was replaced with Sr suggested that Sr may stimulate differentiation. The age of the xylem initials is not known and their occurrence so near the tip may have been the result of inhibition of cell elongation. However, the data obtained clearly indicated that seedlings in Sr produced more secondary roots than those in Ca.

Since the spectroscopic analyses showed that the Ca contamination of the Sr-substituted solution was not detectably greater than of the Na-substituted solution, it is concluded that the Sr supplied these seedlings allowed in some way for a more efficient use of the limited quantity of Ca available from the seed. Various possible ways to explain how this might have been achieved by the plants have been examined.

Leaching of mineral elements from roots into nutrient solutions has been studied before, but there does not appear to have been a specific study of the effect of Sr in nutrient solutions on the leaching of Ca from roots. The growth response of these maize seedlings to the Sr-replacement for Ca, as compared to the Na-replacement, suggested the need for such a study. One possible explanation for the continued development of seedlings in Sr was that the Sr in solution allowed the maize seedlings to retain Ca, whereas Na in solution did not. Results of the experiment on loss of Ca^{45} from seedlings, however, showed that loss was not greatly different in the three nutrient solutions. These and the results of autoradiography of the root tips served as a basis for rejecting this explanation for the growth stimulation by Sr.

The possibility that Sr, supplied to the Ca-deficient seedlings, allowed for an internal redistribution of Ca toward the growing tip, which did not occur when Na replaced Sr, was also considered and investigated by autoradiography of seedling roots previously supplied Ca^{45}. Some Ca^{45} was found in the meristem of the Sr-replacement plants (also of the nonradioactive Ca-solution plants) after these roots had continued growing; however, this Ca^{45} was interpreted as being there because of dilution accompanying growth, rather than by redistribution to the tip. The low amount of Ca^{45} in the Sr-substituted tips led, also, to the conclusion that these tips had grown by utilization of Sr in place of some of the Ca normally required.

Root tips in nonradioactive Na solution contained larger quantities of Ca^{45} than did those in nonradioactive Sr or Ca. This was surprising, in that roots in Na ceased growing, while those in Sr, with less Ca^{45} in the tips, continued to elongate. The autoradiograms, with the observations on root growth, verify an earlier observation of Bamford (1), that roots normally need a continued external Ca supply for growth and development. They also confirm earlier observations that the mobility of Ca within deficient plants is very low, and that Ca, once bound, does not readily become redistributed (7).

Summary

When Sr replaced Ca in the nutrient medium, maize seedlings remained alive when seedlings in a Na-replacement for Ca died. In Sr, elongation of roots and shoots was inhibited; however, development of secondary root primordia was stimulated, and differentiated xylem elements appeared near the root apical meristem. In the absence of external Ca in the nutrient medium, Sr, as compared to Na, did not appear to slow down the rate of loss of Ca from seedling roots, nor did it appear to allow for redistribution of Ca from older parts of the root to the root tip. It is concluded that Sr can replace part of the Ca ordinarily required by these seedlings.

Acknowledgments

The authors wish to thank Professor Orville T. Bonnet, University of Illinois, for valuable advice concerning suitable maize varieties for root development studies, and for the KYS variety seeds used.

Literature Cited

1. Bamford, R. 1931. Changes in root tips of wheat & corn grown in nutrient solutions deficient in calcium. Bull. Torrey Botan. Club 58: 149–78.
2. Baumeister, W. 1958. Hauptnahrstoffe. Handbuch der Pflanzenphysiologie. IV: 482–557.
3. Burstrom, H. 1952. Studies on growth & metabolism of roots. VII. Calcium as a growth factor. Physiol. Plantarum 5: 391–402.
4. Burstrom, H. 1954. Studies on growth & metabolism of roots. X. Investigations of the calcium effect. Physiol. Plantarum 7: 332–42.
5. Davis, E. 1949. Some effects of calcium deficiency on the anatomy of *Pinus taeda*. Am. J. Botany 36: 276–82.
6. McHargue, J. S. 1919. Effect of certain compounds of barium & strontium on the growth of plants. J. Agr. Res. 16: 183–95.
7. Meyer, B. S. & D. B. Anderson. 1952. Plant Physiology. 2nd ed. D. Van Nostrand Co., Princeton, New Jersey.
8. Meyer, B. S., D. B. Anderson, & R. H. Bohning. 1960. Introduction to Plant Physiology. D. Van Nostrand Co., Princeton, New Jersey.
9. Mitteldorf, A. J. 1957. Semi-quantitative spectrochemical analysis. Spex Speaker II, No. 2. April.
10. O'Kelley, J. C. & W. R. Herndon. 1959. Effect of strontium replacement for calcium on production of motile cells in Protosiphon. Science 130: 718.
11. Sorokin, H. & A. L. Sommer. 1940. Effects of calcium deficiency upon the roots of *Pisum sativum*. Am. J. Botany 27: 308–18.
12. Walker, J. B. 1953. Inorganic micronutrient requirements of Chlorella. I. Requirements for calcium (or strontium), copper, & molybdenum. Arch. Biochem. Biophys. 46: 1–11.
13. Walsh, T. 1945. The effect on plant growth of substituting strontium for calcium in acid soils. Proc. Roy. Irish Acad., Sect. B, 50: 287–94.