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Screening Corn for Al Tolerance Using Different Ca and Mg Concentrations[1]

R. Dean Rhue and C. O. Grogan[2]

ABSTRACT

Cations such as Ca and Mg can protect roots from the toxic effects of Al. However, little use of this fact has been made in screening for Al tolerance. This study was undertaken to determine the effects of Ca and Mg concentrations in screening solutions on the toxicity of Al in roots of corn inbreds (*Zea mays* L.). Two-day-old corn seedlings were grown for 12 days in dilute nutrient solutions containing 0.25 m*M* Al. Treatments consisted of Ca or Mg at concentrations of 0.5, 1.0, and 2.0 m*M*. The pH was adjusted to 4.60 initially with no further adjustments. The large solution: root volume ratio resulted in only small pH changes during the 12-day screening. These small pH changes had little or no effect on the toxicity of Al in corn roots. Increasing the concentration of Ca decreased the severity of Al toxicity in roots of corn inbreds. However, there were marked differences among inbred lines in the ability of their roots to grow in these screening solutions at all levels of Ca. For example, roots of the inbred lines W153R and Va17 were susceptible and tolerant, respectively, to Al at all three levels of Ca, whereas A239 showed symptoms of severe Al toxicity at 0.5 m*M* and 1.0 m*M* Ca but produced good root growth at 2.0 m*M* Ca. Thus, a wide range in tolerance to Al was observed in these screening solutions by varying the concentration of Ca. At equal concentrations, Mg was as effective as Ca in protecting corn roots from Al. This technique could be utilized for screening species where a knowledge of the range in tolerance among individual genotypes is desired.

Additional index words: pH, Roots, Toxicity, *Zea mays* L.

THE first observable effect of Al in most plants is on root elongation. Clarkson and Sanderson (1969) showed that elongation of onion (*Allium cepa* L.) roots was reduced within 3 hours by 10^{-3} *M* Al and had stopped completely within 8 hours. Henning (1975) treated wheat (*Triticum aestivum* L.) roots with 10 ppm Al and found that root elongation of an Al-sensitive variety was completely stopped within 12 hours. In both the onion and wheat roots, the number of mitotic figures within the root meristem decreased concomitantly with root elongation.

Species and varieties within species differ in the degree to which a given concentration of Al interferes with root growth (Foy, 1974; Henning, 1975; Kerridge et al., 1971; Reid et al., 1971). When placed in the same solution, an Al-sensitive variety may show severe inhibition of root growth but root growth of an Al-tolerant variety may be little affected. This differential root growth is a precise measure of differential tolerance to Al and, at the same time, provides a quick and reliable method of comparing plants for Al tolerance.

Factors which affect the toxicity of Al are the concentration of Al, pH, temperature, and concentration of salts, particularly those of Ca and Mg (Vidal and Broyer, 1962; Moore, 1974; Foy, 1974).[3,4] In general, any degree of toxicity can be obtained with a given genotype by varying one or more of these factors. The biggest problem in developing a screening technique capable of separating plants clearly on the basis of differential root growth is finding an appropriate combination of these factors to use. One approach is to start with solutions in which Al is very toxic and, by varying one or more of the above factors, gradually reduce the toxicity. At some reduced level of stress, roots of the more tolerant genotypes will grow quite well while those of the more sensitive genotypes will remain severely damaged until a lower level of stress is obtained. In this way, the relative tolerance of plants over a wide range of stress conditions can be observed.

The degree of stress imposed on roots by a given concentration of Al can be controlled to a large extent by the concentration of Ca and Mg present in solution. Ali[3] showed that Al concentrations which normally resulted in severe damage to wheat roots had little or no effect when the concentration of Ca or Mg was increased to higher levels. Conversely, roots of the most Al-tolerant cultivars, such as 'Atlas 66', could be severely damaged by relatively low concen-

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[3] Ali, S. M. E. 1973. Influence of cations on aluminum toxicity in wheat (*Triticum aestivum* Vill. Host). Ph.D. thesis, Oregon State Univ., Corvallis.

[4] Zandstra, H. G. 1971. Aluminum toxicity in some highly weathered soils of the tropics. Ph.D. thesis, Ithaca, Cornell Univ., Ithaca.

Table 1. Ca and Mg treatments used to screen corn inbreds for Al tolerance.

Treatment	$Ca(NO_3)_2$	$MgSO_4$
	mM	
1	0.5	0.5
2	1.0	0.5
3	2.0	0.5
4	0.5	1.0
5	0.5	2.0

Table 2. Composition of screening solution.

$Ca(NO_3)_2$	--
$MgSO_4$	--
KNO_3	0.5 mM
$(NH_4)_2SO_4$	0.05 mM
KH_2PO_4	0.1 mM
Mn SO_4	2.0 μM
Cu SO_4	0.3 μM
Zn SO_4	0.8 μM
Na Cl	30.0 μM
Fe CDTA†	10.0 μM
Na_2MoO_4	0.3 μM
H_3BO_3	10.0 μM
$Al_2(SO_4)_3$	0.125 mM

trations of Al provided the concentration of Ca and Mg were also low.

The purpose of this paper is to describe a technique for screening corn (*Zea mays* L.) inbreds for Al tolerance in which Ca and Mg were used to control the degree of Al toxicity. A wide range in tolerance to Al was observed among corn inbreds when grown in 0.25 mM solutions containing different levels of Ca and Mg. The principles involved in this screening procedure should be applicable to screening other plant species where a knowledge of the range in tolerance to Al within the species is desired.

MATERIALS AND METHODS

Corn seeds, which had been soaked overnight in aerated tap water, were placed between layers of moist paper towel in petri dishes and incubated at approximately 30 C. Within 24 to 36 hours, these seeds had germinated and had produced radicles ranging from 2 to 5 mm long. These 2-day-old seedlings were then grown for 12 more days in complete nutrient solutions containing Al.

Treatments consisted of Ca as $Ca(NO_3)_2$ or Mg as $MgSO_4$ at the levels given in Table 1. All other nutrients plus Al were added to each solution in accordance with Table 2. Al was added as $Al_2(SO_4)_3 \cdot 18H_2O$. Results of experiments by the senior author showed that substitution of $CaSO_4$ for $Ca(NO_3)_2$ had virtually no effect on the results as reported here. Nutrient solutions containing Al are referred to in this paper as screening solutions.

Four seedlings of each of nine inbred lines were grown together in 20 liters of screening solution containing one of the Ca or Mg treatments listed in Table 1. All treatments were replicated at least twice. The plants were harvested and the lengths of the primary roots measured. Relative root lengths were calculated for each inbred line by dividing the root length obtained in a given Ca or Mg treatment by that obtained in a control solution which had received no Al. The data were analyzed by an analysis of variance for a completely randomized factorial experiment.

The pH of a screening solution immediately after dissolution of the $Al_2(SO_4)_3$ was about 4.0. The pH was adjusted to 4.60 with KOH immediately before placing the seedlings in these solutions. Solutions received no further pH or nutrient adjustments during the 12-day screening but pH was measured and recorded at frequent intervals.

Screening was done in plastic buckets placed on the floor of the laboratory. Each bucket was fitted with an acrylic lid which supported three acrylic trays. Each tray had a plastic screen

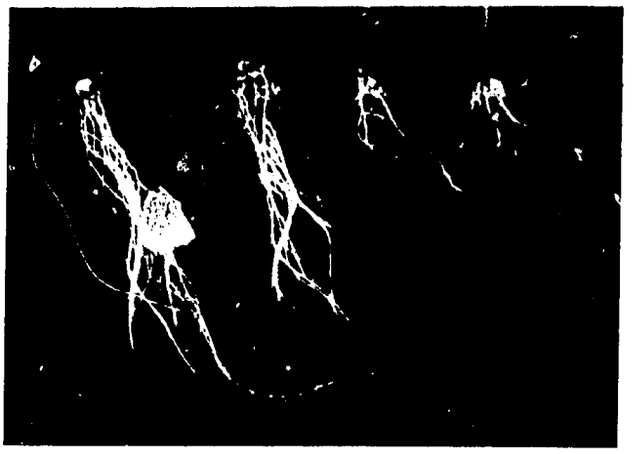

Fig. 1. Differential effects of Al on root growth of two inbred lines of corn: left, R158; right, NY511.

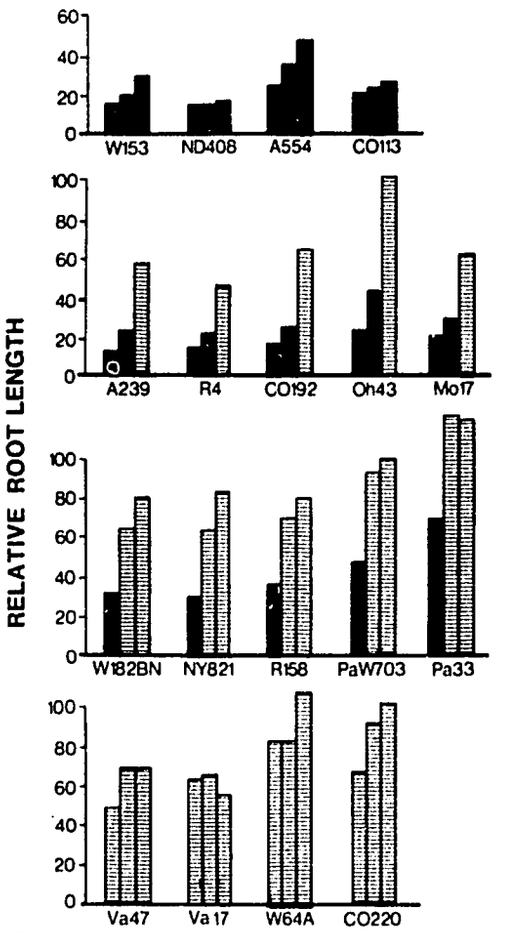

Fig. 2. Effect of increasing concentrations of $Ca(NO_3)_2$ on relative root length of inbred lines. For each inbred line, the concentrations were, from left to right, 0.5, 1.0, and 2.0 mM. Solid bars indicate roots severely damaged by Al; cross-hatched bars indicate no visible evidence of Al toxicity. The LSD (0.05) for relative root length is 0.14.

fastened over the bottom which was maintained in direct contact with 20 liters of screening solution. The germinated seeds were placed directly on these plastic screens with their radicles protruding through the screen into the solution below.

A light source consisting of two 1,000-W metal halide lamps was suspended approximately 1.5 m above the surface of the nutrient solutions. The two lamps were on a time clock set to

give 12 hours of continuous light per day. The spectral distribution and crop response to these lamps under greenhouse conditions was reported by Duke et al. (1975).

Temperature has a marked effect on the toxicity of Al in solution (D. P. Moore, personal communication). For this reason, a temperature-controlled environment was preferred for screening. Our laboratory temperature is maintained with only a small variation. Solution temperatures which occurred during all experiments reported herein were 24±1 C.

RESULTS

At the end of 12 days in these solutions, root systems of various inbred lines ranged from severely damaged to well developed and fibrous. Figure 1 shows the differential effects of Al on roots of two inbred lines of corn which had been grown together for 12 days in a screening solution with 1.0 m*M* $Ca(No_3)_2$ and 0.5 m*M* $MgSO_4$. The roots of NY511 showed symptoms which are typical of Al toxicity. The roots were severely stunted, thickened, and discolored. Other studies suggest that in roots this badly damaged by Al, much of the epidermal, cortical, and meristematic tissue was dead (Keser et al., 1975; Henning, 1975). Lateral roots were also severely damaged and failed to grow after emerging from the root surface. Roots of R158 showed none of these symptoms, even though both inbreds had been grown together in the same solution. Roots of R158 were able to grow quite well at this level of stress.

It is this differential ability of roots to grow at a given level of Al stress that can be used to separate Al-tolerant from Al-sensitive inbred lines. Using screening solutions containing 1.0 m*M* $Ca(No_3)_2$ and 0.5 m*M* $MgSO_4$, one could screen large numbers of inbred lines and classify them as tolerant or sensitive based on differences in root growth illustrated in Fig. 1.

However, screening at a single level of stress will fail to provide useful information about the range in tolerance which may exist among the genotypes of interest. In order to obtain this additional information, one should vary the degree of Al stress and screen many genotypes together under a wide range of stress conditions. Although this is often done using solutions with different Al concentrations, it should be possible to obtain the same results by varying the Ca concentration.[3]

Effect of Ca on Al Toxicity in Corn Roots

Increasing the concentration of Ca as $Ca(NO_3)_2$ in these screening solutions reduced the toxicity of Al in corn roots. The reduced toxicity was expressed as 1) an increase in relative root lengths and 2) a decrease in the number of inbred lines whose roots showed visible symptoms of Al toxicity. Figure 2 shows the effect of increasing Ca concentration on relative root lengths of 18 inbred lines.

At the lowest concentration of Ca, only 4 of the 18 inbreds shown in Fig. 2 had roots which were able to grow in these solutions: Va47, Va17, W64A, and CO22O. Roots of the other 14 showed symptoms of severe Al toxicity, i.e., swelling, stunting, and discoloration. At the highest concentration of Ca, there were four inbreds with roots still showing symptoms of severe Al toxicity (e.g., W153R, ND408, A554, and C0113). Between these two extremes, at least two other groups of inbreds could be identified: 1) those whose roots grew well in screening solutions with 1.0 m*M* Ca but showed symptoms of severe Al toxicity in solutions with 0.5 m*M* Ca (e.g., W182BN) and 2) those whose roots grew well in screening solutions with 2.0 m*M* Ca but showed symptoms of severe Al toxicity in solutions with 1.0 m*M* Ca (e.g., A239).

Although relative root lengths generally increased as the level of Ca was increased, the greatest increases were associated with a change in the ability of the roots to grow in these solutions. The most striking examples of this were the 58% increase for Oh43 when the Ca concentration was increased from 1.0 m*M* to 2.0 m*M*, and the 53% increase for Pa33 when the Ca concentration was increased from 0.5 m*M* to 1.0 m*M*. Above and below the point where the roots were able to make this transition, the changes in relative root length with increase in Ca concentration were much smaller.

Figure 3 shows four of the 18 inbreds whose root data are given in Fig. 2. ND408 and Va17 represent the extremes in tolerance identified by this technique. Neither showed striking responses to increasing levels of Ca in the screening solution. The response of A239 was in marked contrast to that of W153R, which showed symptoms of severe Al toxicity even at the highest concentration of Ca used. This Figure also illustrates the effect of Ca concentration on the relative tolerance of certain inbreds. For example, at the lower Ca concentrations, A239 appeared as sensitive to Al as W153R, but at the higher Ca concentration, A239 appeared as tolerant as Va17.

We have found that a concentration of 4.0 m*M* Ca will protect roots of inbreds such as W153R and ND408 from the Al in these screening solutions. At the other extreme, roots of the more tolerant lines such as Va17 are severely damaged by 0.25 m*M* Al solutions provided the Ca concentration is also reduced to 0.25 m*M*. Thus, a wide range in tolerance among inbred lines of corn could be observed in these screening solutions using Ca concentrations ranging from 0.25 m*M* to 4.0 m*M*. These results are similar to those of Ali[3] in that a wide range in tolerance to Al was also observed among wheat varieties as the concentration of Ca in Al toxic solutions was increased.

Effect of Mg on Al Toxicity in Corn Roots

Magnesium as $MgSO_4$ appeared to be as effective in protecting corn roots from Al as Ca when used at equal concentrations (Fig. 4). The increases in relative root length were not only similar to those obtained with an equivalent concentration of Ca, but Mg was also equally effective in preventing the appearance of Al toxicity symptoms. Thus, the ranking of corn inbreds with respect to Al tolerance was the same regardless of whether Ca or Mg was used to control the degree of Al toxicity.

pH Changes in Solutions With Different Ca and Mg Levels

pH has a marked effect on the toxicity of Al in solution. The pH of these screening solutions was adjusted to 4.60 initially with no further pH adjustments. The pH changes shown in Fig. 5 were typical of those which occurred in screening solutions using

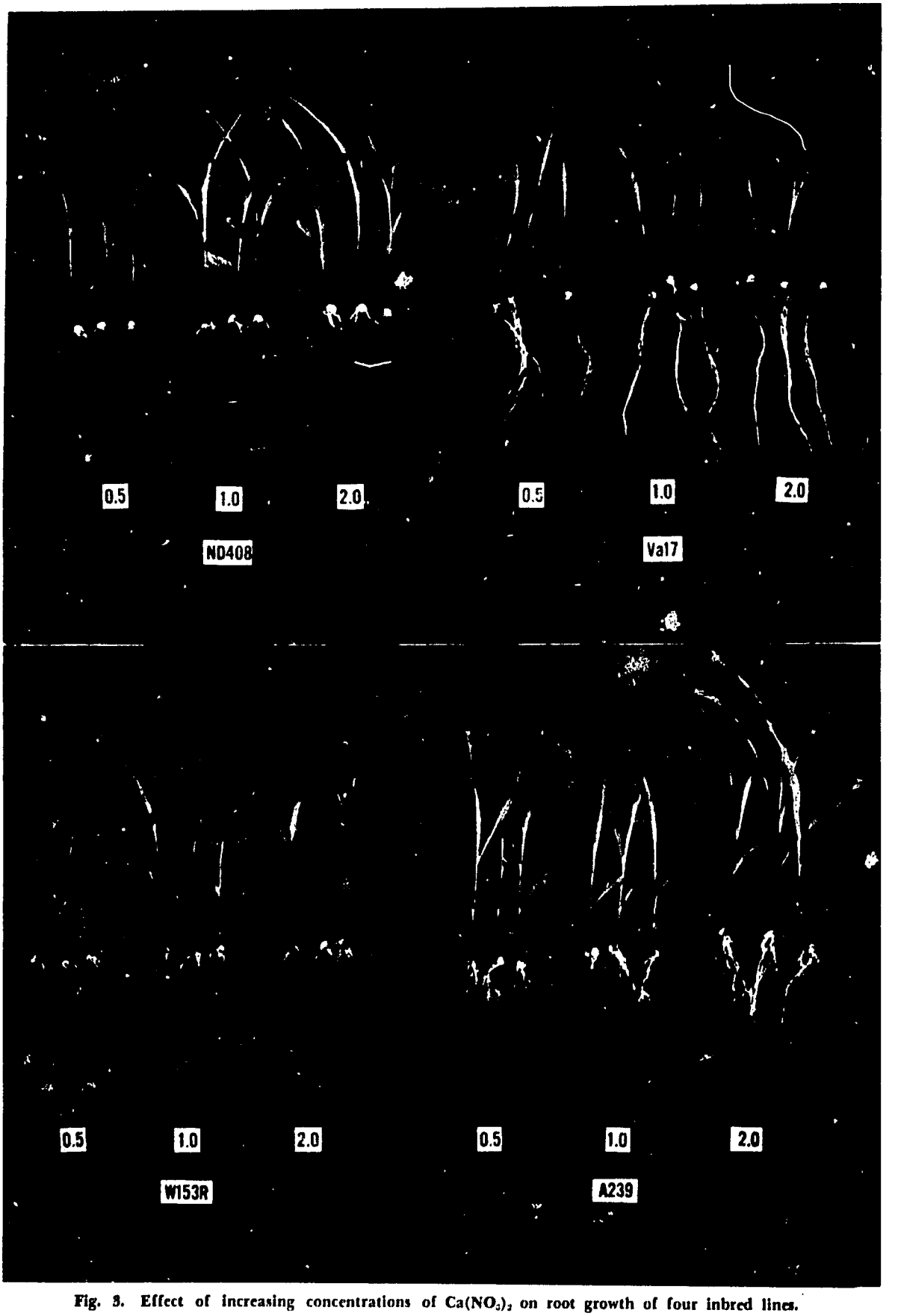

Fig. 3. Effect of increasing concentrations of $Ca(NO_3)_2$ on root growth of four inbred lines.

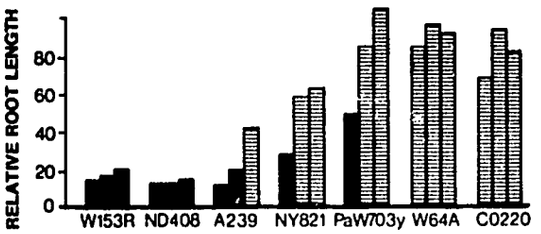

Fig. 4. Effect of increasing concentrations of $MgSO_4$ on relative root length of inbred lines. From left to right: 0.5, 1.0, and 2.0 m*M*. Solid bars indicate roots severely damaged by Al; cross-hatched bar indicate no visible evidence of Al toxicity. The L.S.D. (0.05) for relative root length is 0.14.

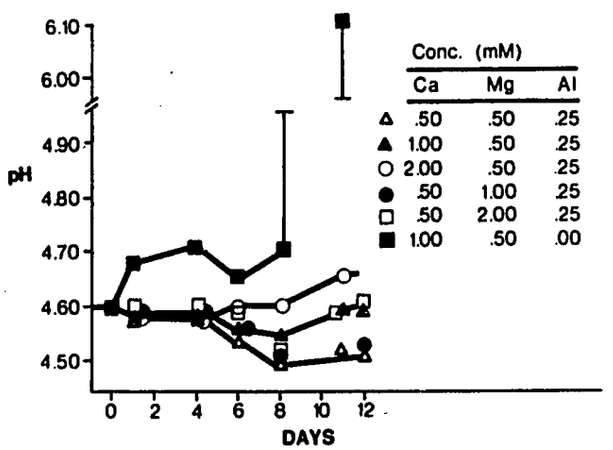

Fig. 5. pH changes which occurred in screening solutions with various Ca and Mg concentrations.

the Ca and Mg treatments in Table 1. Included for comparison are pH changes which occurred in a control solution which contained no Al. Note that pH increased with increasing Ca or Mg concentration and that pH was slightly higher in the Ca treatments than in the corresponding Mg treatments. In order to determine to what extent the pH differences in Fig. 5 might have contributed to the reduced toxicity of Al in solutions receiving additional Ca or Mg, experiments were conducted in which corn inbreds were screened in solutions with a constant level of Ca and Mg but with different pH's.

Screening solutions with 1.0 m*M* Ca $(NO_3)_2$ and 0.5 m*M* $MgSO_4$ were adjusted to the following initial pH's: 4.50, 4.55, 4.60, and 4.65. The Al was added and pH adjusted with KOH immediately before placing the seedlings in these solutions. No additional pH adjustments were made during the 12-day screening but pH was measured and recorded periodically throughout the screening. An additional treatment was included to determine what effect aging of a screening solution at pH 4.60 would have on the subsequent toxicity of Al. Changes in both pH and the relative amounts of polynuclear and crystalline phase Al species occur when Al solutions to which small amounts of base have been added are aged (Smith and Hem, 1972). Those changes occurring within the first 24 hours of aging could affect both pH and toxicity of Al during a subsequent 12-day screening. Therefore, an additional solution was made up 24 hours in advance and the pH adjusted to 4.60. At the end of 24 hours, the pH was readjusted to 4.60 and the seedlings placed in these aged solutions as described previously. Each treatment was replicated twice.

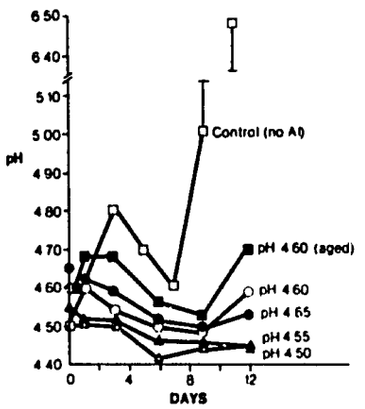

Fig. 6. pH changes which occurred in screening solutions of initial pH 4.50, 4.55, 4.60, and 4.65.

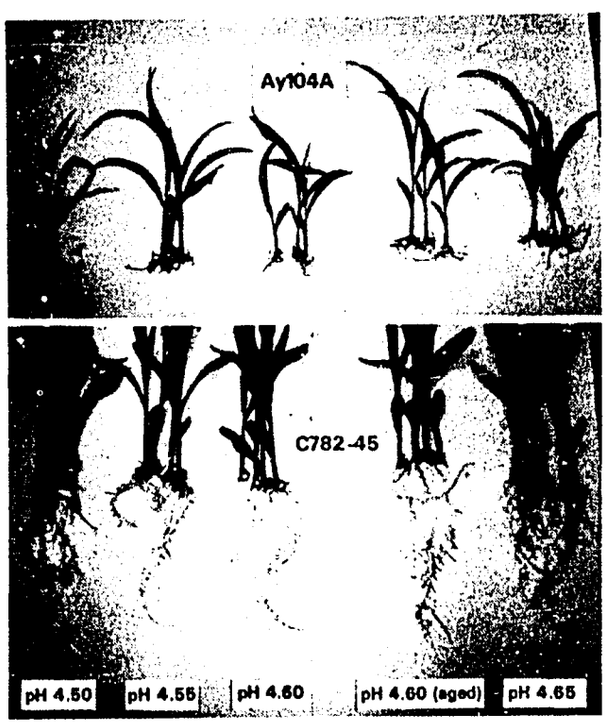

Fig. 7. Root growth of two corn inbreds in screening solutions of initial pH 4.50, 4.55, 4.60, and 4.65.

The pH changes which occurred in these solutions are shown in Fig. 6. Each data point in Fig. 6 is the average of two replications. Generally, solutions of lower initial pH maintained a lower pH throughout the screening than solutions of higher initial pH. Aging a screening solution at pH 4.60 for 24 hours before adding the germinated seeds had a marked effect on the subsequent pH. The range in pH among the solutions in Fig. 6 (i.e., 4.42 to 4.70) was slightly greater than that observed in Fig. 5. The effect of

these pH treatments on corn root growth is shown in Fig. 7. Regardless of the pH, roots of C782-45 were more tolerant to the Al in these solutions than those of Ay101A. Even in the lowest pH range, root growth of C782-45, particularly in terms of lateral root development, was far superior to that of Ay101A. These results indicate that pH in the range from 4.50 to 4.65 had little effect on the severity of Al toxicity in roots of corn inbreds. Thus, it is concluded that the marked response of corn roots illustrated in Fig. 2 and 4 resulted largely from the additional Ca and Mg and not from the pH differences measured among these solutions (Fig. 5).

DISCUSSION AND CONCLUSIONS

The ability of Ca and Mg to control the degree of Al toxicity in roots has been known for some time but this information has not been used to any extent in developing techniques for screening plants for Al tolerance. Results presented here show the importance of Ca and Mg concentrations in controlling the degree of Al toxicity in roots of corn inbreds growing in screening solutions.

Figures 2 and 4 show the advantage of screening plants under a wide range of Al toxicity; namely, it clearly indicates the range in tolerance existing among the genotypes of interest. However, screening large number of genotypes in a breeding program using numerous stress levels would involve considerable time, labor, and expense. Knowledge of the probable range in tolerance among genotypes would, however, allow one to choose a single stress level with some rational basis. Our data for corn inbreds show that there are many stress levels within a wide range of tolerance that produce marked differences in root growth and that any one of them could be selected arbitrarily for screening inbred lines. However, the degree of Al stress obtained in screening solutions containing 1.0 m*M* $Ca(NO_3)_2$ and 0.5 m*M* $MgSO_4$ divided the 18 inbreds in Fig. 2 into two groups of nine each; i.e., nine had roots which were tolerant and nine had roots which were sensitive to the Al in these solutions. Approximately 150 additional inbred lines have been screened at this level of stress and these, too, were observed to fall into two groups of almost equal numbers. These 150 lines constitute an exceptionally broad germplasm base, having originated in such diverse geographical locations as the USA, Canada, Eastern and Western Europe, and Africa. This level of stress, therefore, would seem to be a logical choice for future screening of large numbers of inbred lines.

An important question from a breeding standpoint is, to what extent are differences in tolerance among corn inbreds under genetic control? We are still seeking a precise answer to this question, but the preliminary evidence suggests that Al tolerance in corn is controlled at a single locus and that tolerance is dominant. The range in tolerance among inbred lines (Fig. 2) and within maize composites (unpublished data) suggest further that Al tolerance is controlled by a multiple allelic series. These findings will be reported later.

Figure 4 demonstrates that the effect of Ca on the toxicity of Al is a non-specific cation effect. If Ca were specifically required to protect these roots from Al, Mg would not be able to replace Ca in protecting these roots. However, Mg appeared to be equally effective. The same argument can be made against specificity for Mg. These results are in agreement with those of Ali[3] who showed that Ca and Mg were indistinguishable in their ability to protect wheat roots from Al.

Finally, we have observed that a wide range in tolerance to Al is usually found within any group of inbreds released from a single experiment station (Fig. 2). Two examples are the Wisconsin lines, W153R and W64A, and the Canadian lines C0113 and C0220. Many other examples could be cited. This range in tolerance among lines from a common breeding program is perhaps not surprising when one considers the great diversity of germplasm used to produce inbred lines. However, wide differences in tolerance among lines developed from a common breeding program also suggest that there has been little or no selection for Al tolerance. Consequently, screening and selection of Al-tolerant inbred lines may be essential for the rapid development of Al-tolerant corn hybrids.

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