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Rapid Screening of Maize for Tolerance to Aluminum in Breeding Varieties Better Adapted to Acid Soils

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SCREENING FOR AL TOLERANCE IN MAIZE[1]

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INTRODUCTION

Two major problems of soil fertility are tied together in acid soils: P deficiency due to some form of fixation, toxicity to the growth of some plants caused by excess of soluble Al and Mn, and in some cases Fe. The magnitude of the Fe toxicity problem is not yet well defined.

The conventional approach to these mineral imbalances in acid soils has been the use of fertilizer phosphate, and/or soil amendments to

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increase the pH of the soil. These solutions to the fertility problem in acid soils are temporary and in less developed countries very often uneconomic. Moreover, most of the amendments do not reach the depth of soil where most crop plant roots penetrate, and the amendments may generate other problems, such as some plant diseases.

A promising way of approaching the acid soil fertility problem is to manipulate the plants genetically, developing varieties better fitted to acid soil conditions. To improve crop varieties by adaptation to acid conditions, it is necessary first to develop practical techniques to separately evaluate the response of crops to the mineral stresses that affect acid soils.

Aluminum tolerance is probably the more intensely studied of the mineral stresses in acid soils. Considerable advances have been made in the techniques for screening Al tolerance in wheat and rice. In these species there are numerous studies of Al response of cultivars, not only in short-term experiments, but also for the whole life cycle of the plant in acid soils. In this way a link has been established between root symptoms in short-term laboratory experiments and the response of the plant in actual acid field conditions.

Most maize studies have been on the effects of Al on the growth of the primary root of seedlings, but the link between this response and the response in long-term field or solution culture experiments, or in soils under greenhouse conditions, has not yet been established. This situation is particularly unfortunate, considering the complex nature of the maize plant root system.

Maize seedlings tend to vary more in their response to the same Al treatment than does wheat which is self-pollinating. The variability

is less with inbred seeds, but this is not always the type of material required in a screening program. Variability effects can be overcome by increasing the number of replicates or treatments. However, these procedures tend to limit the scaling up of the method.

In the stain method described here, 6 Al levels are used and 3 seedlings are treated in each level. In the 14-day nutrient solution method, 4 Al treatments and 3 seedlings per treatment are used. These procedures permit reliable results in spite of the variability of the material.

When grown in nutrient solutions, some maize strains develop a "corkscrew" tip in the primary root. This type of growth tends to make it difficult to score these strains with both methods described here. Increasing the P content of the nutrient solution tends to alleviate this condition, but one cannot increase the P concentration without risking Al solubility problems. Therefore, for the rapid staining method, 0.4 mM of P was provided during the growing period in nutrient solution, but not during the overnight Al treatment.

In the 14-day nutrient solution method, a level of 0.1 mM P was utilized to decrease the "corkscrew" effect in some strains.

The stain method is scored in 5 days, requires illumination during the day and a temperature of 25 C. After an overnight Al treatment, the roots are stained and scored according to the pattern of staining. The results with this method show a good correlation with those obtained with the 14-day method.

The 14-day method uses a nutrient solution with 4 Al treatments. This method requires 3 days for germination and 11 days of growth in nutrient solution. The test is run under light and dark periods at about

25 C. Four changes of nutrient solution permit the maintenance of pH with less than 0.5 pH unit of variation in the first 6 days. In the last 3 days, the variations are wider, but for periods less than 1 day. Differences in root growth response by different strains were clearly observable with acceptable reproducibility. At the end of the growing period the root growth is evaluated by measuring the length of the primary root and by estimating the effects of the Al treatment in the growth of secondary roots.

It must be kept in mind that no laboratory methods, including these, have as yet been correlated with field results.

METHOD I. STAINING METHOD FOR MAIZE

Materials

One set of materials for screening 20 strains of maize includes:

20 plastic vials with lids of 38 ml capacity. The plastic vials are perforated with a drill 2/16 inch (0.32 cm) diameter as shown in Fig. 1.

2 shallow pans of 14 x 18 x 1 inch (35.6 x 45.7 x 2.5 cm)

1 plastic tray 13 x 10 x 6 inches (33 x 25.4 x 15.2 cm)

6 strips of styrofoam 3.6 x 29 x 0.7 cm. Each of these strips is drilled with 20 rows of 3 holes of 0.6 cm diameter (Fig. 2a).

6 trays 12 x 5 1/4 x 4 1/2 inches (30.5 x 13.3 x 11.4 cm)

For screening 60 varieties at once, triple the materials listed, except for the last item.

Solutions

Solution A, Stock Nutrient Solution: Dissolve in about 900 ml of distilled water 22.2 g of $CaCl_2$ anhydrous; 32.86 g of KNO_3; 25.41 g of $MgCl_2 \cdot 6H_2O$; 0.66 g of $(NH_4)_2SO_4$; and 1.60 g of NH_4NO_3. Dilute to 1 liter. When 20 ml of this solution are diluted to 1 liter, the concentrations will be 4 mM of $CaCl_2$; 6.5 mM of KNO_3; 2.5 mM of $MgCl_2$; 0.1 mM of $(NH_4)_2SO_4$; and 0.4 mM of NH_4NO_3.

Solution B, 0.1 M Al: Dissolve 24.15 g of $AlCl_3 \cdot 6H_2O$ in about 900 ml of distilled water. Dilute to one liter.

Solution C, 0.25 M HCl (approx.): 20.83 ml of concentrated HCl diluted to 1 liter.

Solution D, 0.2% Hematoxylin: Dissolve 2 g of hematoxylin anhydrous in about 900 ml of water, add 0.2 g $\pm$ .01 g of sodium iodate ($NaIO_3$), and dilute to 1 liter of volume.

Solution E, KH_2PO_4, 1 M: Dilute 136.1 g of KH_2PO_4 to 1 liter with distilled water.

Procedure

Place about 40 seeds of maize in each of 20 numbered perforated vials. Place the vials in a large container with aerated flowing tap water at 30 C for 24 hours. Then spread the seeds from each vial on the surface of a moistened filter paper in a shallow pan. (The filter paper is first divided into 10 numbered sections with an ordinary lead pencil.) Then cover the pan with transparent plastic taped to the edge of the pan to keep an atmosphere saturated with water during the germination period (Fig. 3). Place the pan with the seeds in an incubator at 25 C. Keep in the dark for 2 days. Afterwards, plant

germinated seeds of similar root length of each variety in the nutrient solution. Measure 9.8 liters of deionized water in the large plastic tray. Add 200 ml of Solution A and 4 ml of Solution E. With a serological pipet, adjust the pH to 4 with Solution C and record the volume of this solution required, for future use.

Place six strips of styrofoam floating in the tray. Plant three seeds of each strain in three different strips of styrofoam (Fig. 2b). In this manner three treatments of Al with three seedlings each will be tested. Label and number appropriately each styrofoam strip. Leave the seedlings in this nutrient solution for 24 hours and then transfer them to the Al treatments.

The Al treatments consist of nutrient solutions with 0.15, 0.25, 0.35, 0.45, 0.55, and 0.65 mM of Al. Each nutrient solution is prepared by placing in each of 5 trays 2.50 liters of water and adding to each 50 ml of Solution A. Then to each of the trays, add 3.75, 6.25, 8.75, 11.25, 13.75, or 16.25 ml of 0.1 M $AlCl_3$ to make the 0.15 to 0.65 mM Al solutions. Then adjust the pH of each solution to 4 with Solution C, and record the amount required, for future use.

For the Al treatment and after washing about 30 seconds in flowing distilled water, transfer one set of the styrofoam strips containing all the varieties to each of the containers with the Al treatments (Fig. 4). After about 20 hours (overnight), place all the styrofoam strips in a common tray with 10 liters of aerated distilled water for 30 minutes. Afterward, put the strips in a shallow layer of hematoxylin solution, deep enough to cover the roots (Fig. 5). Move the tray intermittently and after 15 minutes wash the plants by placing the styrofoam strip under flowing distilled water for about 30 seconds. Next,

put all the strips in a common tray with 10 liters of aerated distilled water for 30 minutes and then score them. Detach the stained tips of the primary roots with tweezers and put them on a moistened filter paper. It is convenient to assign a number to each variety and to indicate the Al treatment on the filter paper. This can be done by typing (use Polycarbon black ribbon) the figures on the filter paper before moistening it. After all the stained roots are displayed on the filter paper, take a photograph. Also, the filter paper with the roots can be covered with clear plastic film or a sheet of Lucite 1/16 inch thick and a print of the results can be made with a copier. A rather high contrast reproduction is convenient.

The stain method for maize requires a temperature of about 25 C during the test. After planting the germinated seeds in the nutrient solution and until the completion of the test, provide alternate periods of 12 hours of dark and light per day. Incandescent light 85 μ Einsteins $sec^{-1}m^{-2}$ (400-700 nm wave band) or any other suitable greenhouse lamp can be used for this purpose.

METHOD II. 14-DAY NUTRIENT SOLUTION CULTURE

Materials

For screening 20 maize varieties the following materials are required:

20 plastic vials with lids of 38 ml capacity and perforated as in Method I (Fig. 1)

2 shallow plastic trays, 14 x 18 x 1 inch (35.6 x 45.7 x 2.5 cm)

4 plastic trays, 13 x 10 x 6 inches (33 x 25.4 x 15.2 cm)

4 pieces of styrofoam 18 x 28 x 1.9 cm. Each piece has 20 holes where a plastic tube of 3 cm diameter carrying a nylon screen can be fitted (Fig. 6)

1 piece, 60 x 80 of nylon screen (hexagonal opening 0.35 to 0.40 cm)

Stock Nutrient Solutions

Solution A: Major elements - Dissolve 85.0 g of $Ca(NO_3)_2 \cdot 4H_2O$; 19.20 g of NH_4NO_3; 14.3 g of KCl; 23.7 g of $MgSO_4 \cdot 7H_2O$; and 3.27 g of KH_2PO_4 in about 5 liters of distilled water and complete volume to 6 liters. When 25 ml of this solution is diluted to 1 liter, the final concentration of salts will be: 1.5 mM of $Ca(NO_3)_2$; 1.0 mM of NH_4NO_3; 0.8 mM of KCl; 0.4 mM of $MgSO_4$; and 0.1 mM of KH_2PO_4.

Solution B: Minor elements - Dissolve 2.214 g of $MnSO_4 \cdot H_2O$; 0.235 g of $CuSO_4 \cdot 5H_2O$; 0.966 g of $ZnSO_4 \cdot 7H_2O$; 2.275 g of H_3BO_3; and 0.178 g of MoO_3 in about 800 ml of distilled water and complete volume to 1 liter.

Solution C, 0.3% Iron: Dissolve 30 g of sequestreme 330 Fe (Geigy 10% Fe) in about 800 ml water and complete to 1 liter.

Solution D, 0.1 M Al: Dissolve 24.15 g of $AlCl_3 \cdot 6H_2O$ in about 800 ml of water and complete volume to 1 liter.

Solution E, 0.25 M HCl (approx.): Dissolve 20.83 ml of concentrated HCl to 1 liter.

Procedure

Proceed as in the previous method until germination of the seeds.

Fill plastic trays with 10 liters of nutrient solution prepared by adding to about 5 liters of distilled water, 250 ml of Solution A,

5 ml of solution B and 10 ml of Solution C, and making up the volume to 10 liters. Tape labels of 0, 0.03, 0.06, and 0.09 mM of Al to each tray and add 0, 3, 6, and 9 ml of Solution D to the corresponding treatments in each tray. Then, with a serological pipet, add to each tray enough Solution E to bring the pH to 4 and record the volume of acid used. This volume will be added every time the solutions are prepared again.

Then in each tray, put one piece of styrofoam carrying 20 planting holes (Fig. 6) with the screens attached. Select for each strain 12 seedlings with uniform roots and plant 3 in 1 hole of each styrofoam piece (Fig. 7). Place the planted trays under light (incandescent, 8.6 Klux at the top of the plants, 12 hours per day) at 25 C.

Replace the solution of the trays with a fresh one the 4th, 7th, 9th, and 10th day after planting. Aerate the solutions constantly. The 11th day after planting, score the experiment by measuring the length of the roots in each treatment and by observing the growth of lateral roots.

EVALUATION OF RESULTS IN METHODS I AND II

Evaluation of results in the hematoxylin test is based upon the staining pattern of the root tip. Aluminum tends to accumulate in the surface cells of that part of the root that actively grew during the Al treatment. Thus, every tip develops a "stainable region" extending to about 2 cm from the root cap. As the concentration of Al increases, a stained region distant from the root cap develops and increases in size. At an Al level that is characteristic of each maize strain the entire "stainable region" is covered. As the stained region grows in size,

symptoms of damage in the surface cells can be seen. Apparently, because of unequal growth rates of inner cells and the epidermis, the latter breaks, leaving bands of unstained inner cells visible.

The staining pattern of the roots in Method I correlates with the effect of Al treatments on root growth in Method II. For example, Figure 8a shows Cargill 3, an Al tolerant strain, grown with Method II. The growth of the primary roots is little affected by the Al treatments. Some moderate increase in the growth of secondary roots is observable at the 0.06 and 0.09 mM Al treatment. The staining of the roots with Method I (Figs. 8b) was obtained in two separate experiments. In both tests a clear region can be seen behind the root cap. The hematoxylin stains only a region at a certain distance from the root tip and the clear gap between this region and the tip does not disappear, even after the 0.55 mM Al treatment.

Figure 9a shows PX485, an Al sensitive strain. In this case with Method II there is a marked reduction in the length of the secondary roots in all of the Al treatments and especially with the 0.06 and 0.09 mM Al. The decrease in secondary root growth is also apparent in the 0.03, 0.06 and 0.09 mM Al. In Method I, from 0.25 mM Al and above, the surface cells of all the growing tips are damaged and breaks in the epidermal layers leave exposed inner layers of cells unstained. In the 0.45, 0.55 and 0.65 mM Al treatment, some roots show a large area unstained behind the tip that corresponds to inner unstained cells.

Figure 10a shows an intermediate case in which there is some decrease in the growth of the primary roots in the zero Al treatment and a substantial reduction in the secondary root growth in 0.06 and 0.09 mM Al. Method I (Fig. 10b) shows an unstained and undamaged region behind the

root cap until treatment in 0.45 mM Al. In this treatment, the clear regions behind the small undamaged area correspond to unstained inner cells exposed by rupture of the epidermis.

On routine testing, the criteria for estimating the Al tolerance are as follows:

Method I - For the hematoxylin method the quantitative scoring reduces to estimating the Al concentration at which complete staining of the tip can be detected. Thus, in Figures 8b, 9b, and 10b, these values are 0.65 for Cargill, 0.55 for PX74, and 0.35 for PX485. Maize seedlings that show a clear and undamaged region behind the root cap above 0.55 mM Al are tolerant according to the nutrient solution test.

Method II - Estimate the average length of the primary roots of each treatment. Compute the relative root length ratio as the ratio of the average root in each Al treatment to the length in the zero Al treatment. For the evaluation of secondary root growth, take the growth in the zero Al treatment as equal to unity and as 0.75 or 0.50 if the growth of secondary roots is moderately or intensely decreased with respect to the control treatment. Then multiply these coefficients by the corresponding relative root length ratio to obtain a value representing the response of the roots to the Al treatments. Table I shows the values of root length, secondary root coefficient and composite values for Cargill 3, PX74, and PX485.

LEGEND FOR FIGURES

Fig. 1. Perforated plastic vial for water imbibition of maize seeds.

Fig. 2a. Styrofoam strip with holes for planting maize seedlings.

2b. Styrofoam strips each carrying the same 20 strains.

Fig. 3. Shallow pan with seeds on 2 layers of filter paper are covered with a film of plastic.

Fig. 4. Maize seedlings being treated with one Al treatment.

Fig. 5. After the Al treatment, the seedlings are treated for 15 minutes in a shallow layer of hematoxylin.

Fig. 6 Piece of styrofoam with 20 holes with plastic tubes carrying a nylon screen. The 3 tubes at the left of the front row show in sequence the positioning of the tubes and the screen.

Fig. 7. Four styrofoam pieces each in a different Al treatment, showing the growing maize seedlings.

Fig. 8a. Each group of plants corresponds to Cargill 3 treated, from left to right, with 0, 0.03, 0.06, and 0.09 mM Al.

8b. Staining of roots of Cargill 3 with hematoxylin, showing the "stainable region" partially stained in all the treatments.

Fig. 9a. Four groups of plants of PX485 treated, from left to right, with 0, 0.03, 0.06, and 0.09 mM Al.

9b. Hematoxylin treatments of PX485 showing staining over all the surface of the stainable region above 0.25 mM Al. Breaks in the surface cells leave exposed, unstained inner cells.

Fig. 10. Four groups of plants of PX74 treated, from left to right, with 0, 0.03, 0.06, and 0.09 mM Al. A clear unstained and undamaged region is shown up to 0.45 mM Al.

TABLE I

Al Conc. (mM)	Cargill 3			PX74			PX485		
	Length (cm)	Sec. Root Coef.	Composite Value	Length (cm)	Sec. Root Coef.	Composite Value	Length (cm)	Sec. Root Coef.	Composite Value
0.	21	1	1	19	1	1	33	1	1
0.03	22	1	1	22	0.75	0.86	22	0.5	0.33
0.06	22	0.75	0.76	19	0.5	0.50	10.5	0.5	0.16
0.09	22	0.75	0.76	17	0.5	0.44	11.0	0.5	0.16

Fig. 1

Fig. 2 a

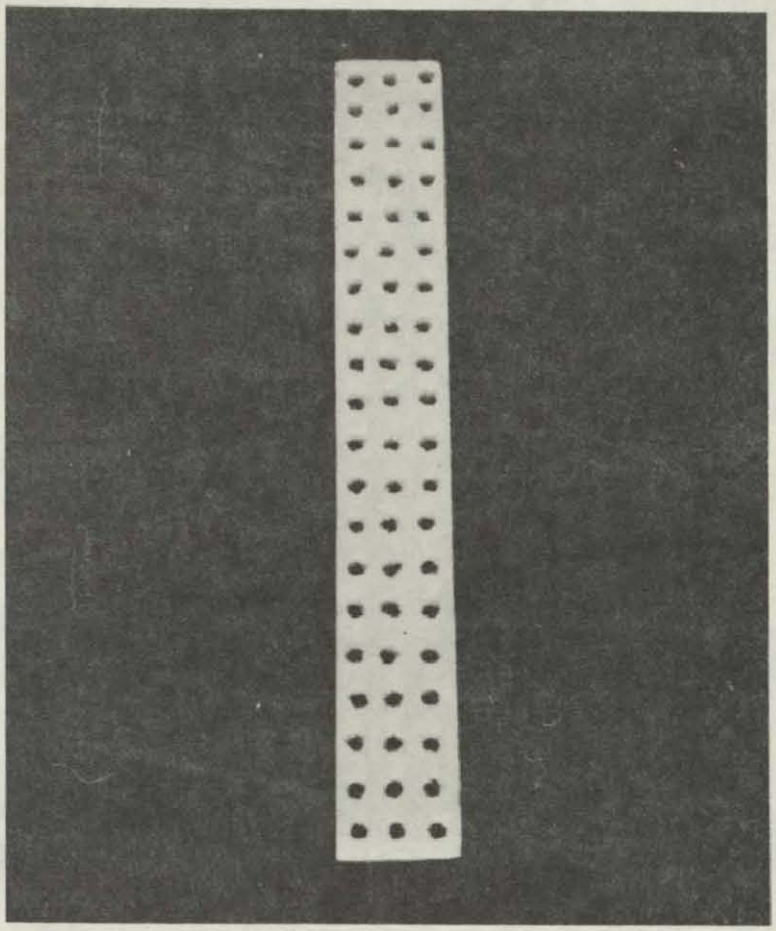
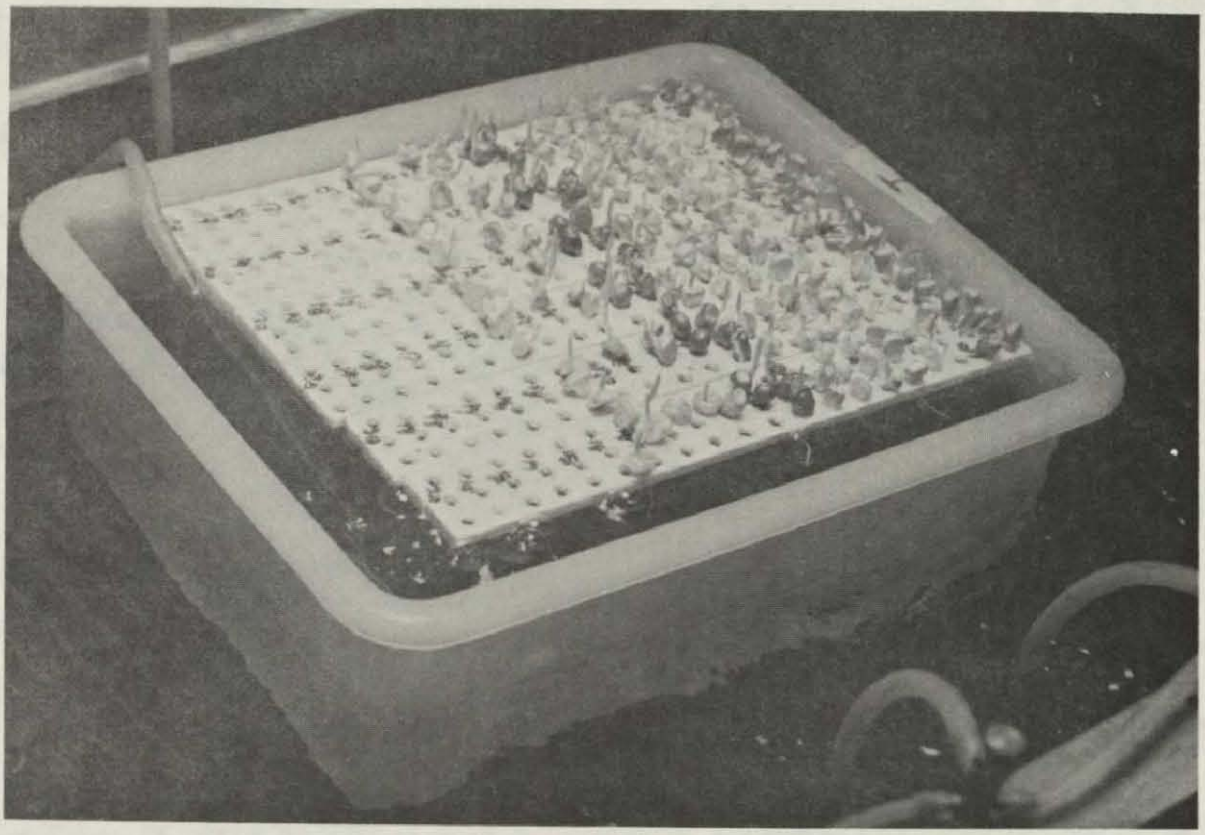


Fig. 2 b



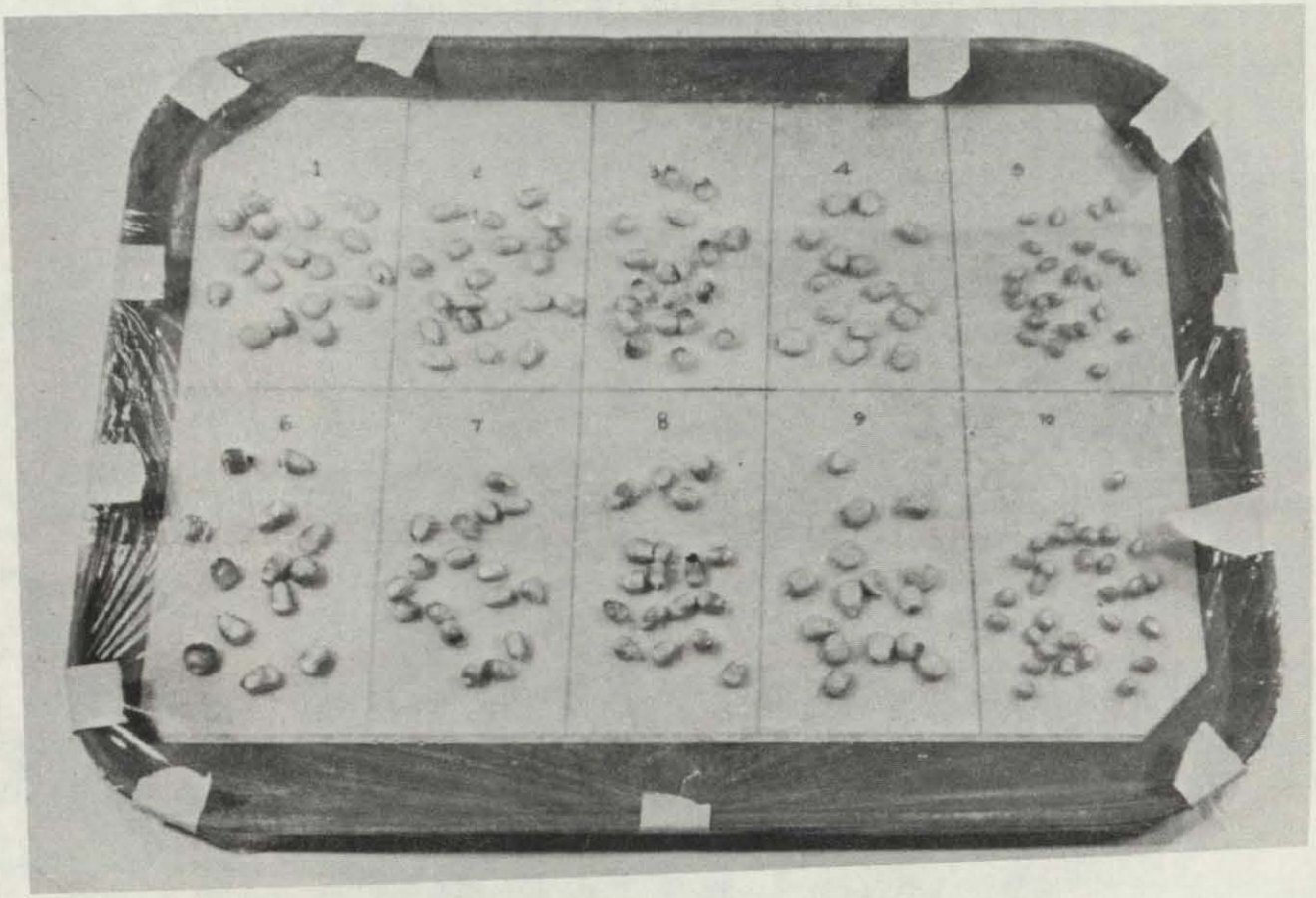


Fig. 3

Fig. 4

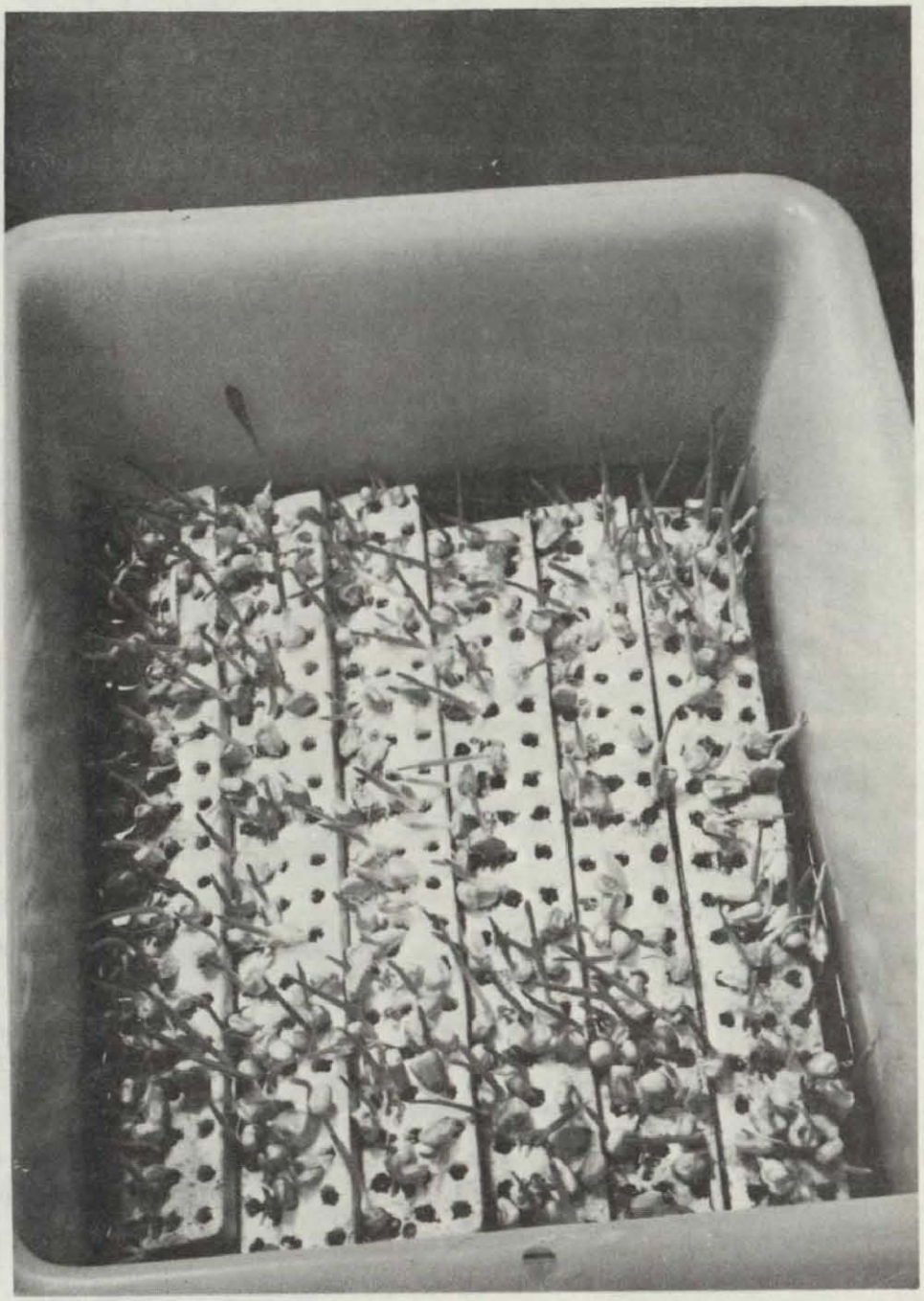


Fig.5

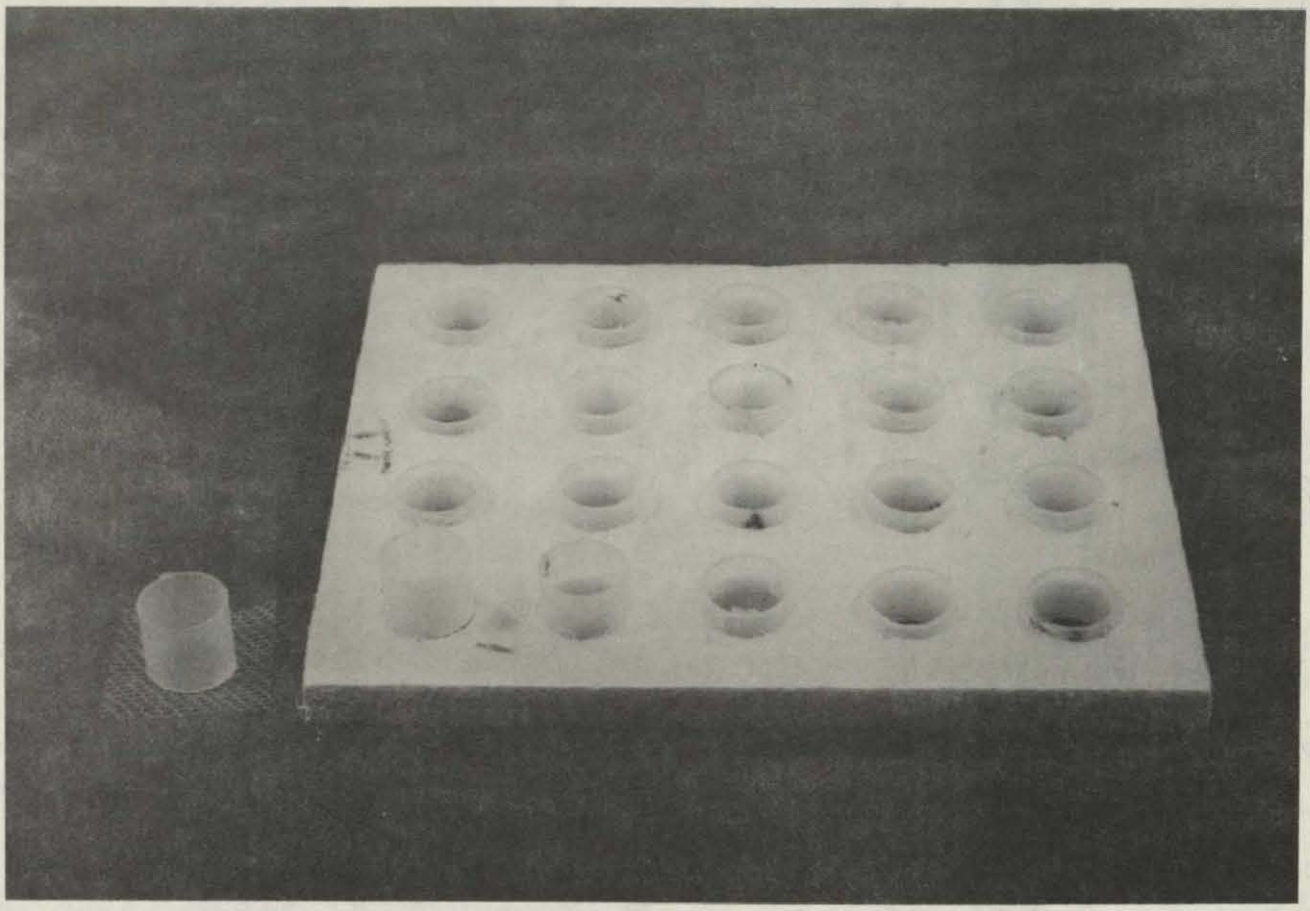


Fig. 6

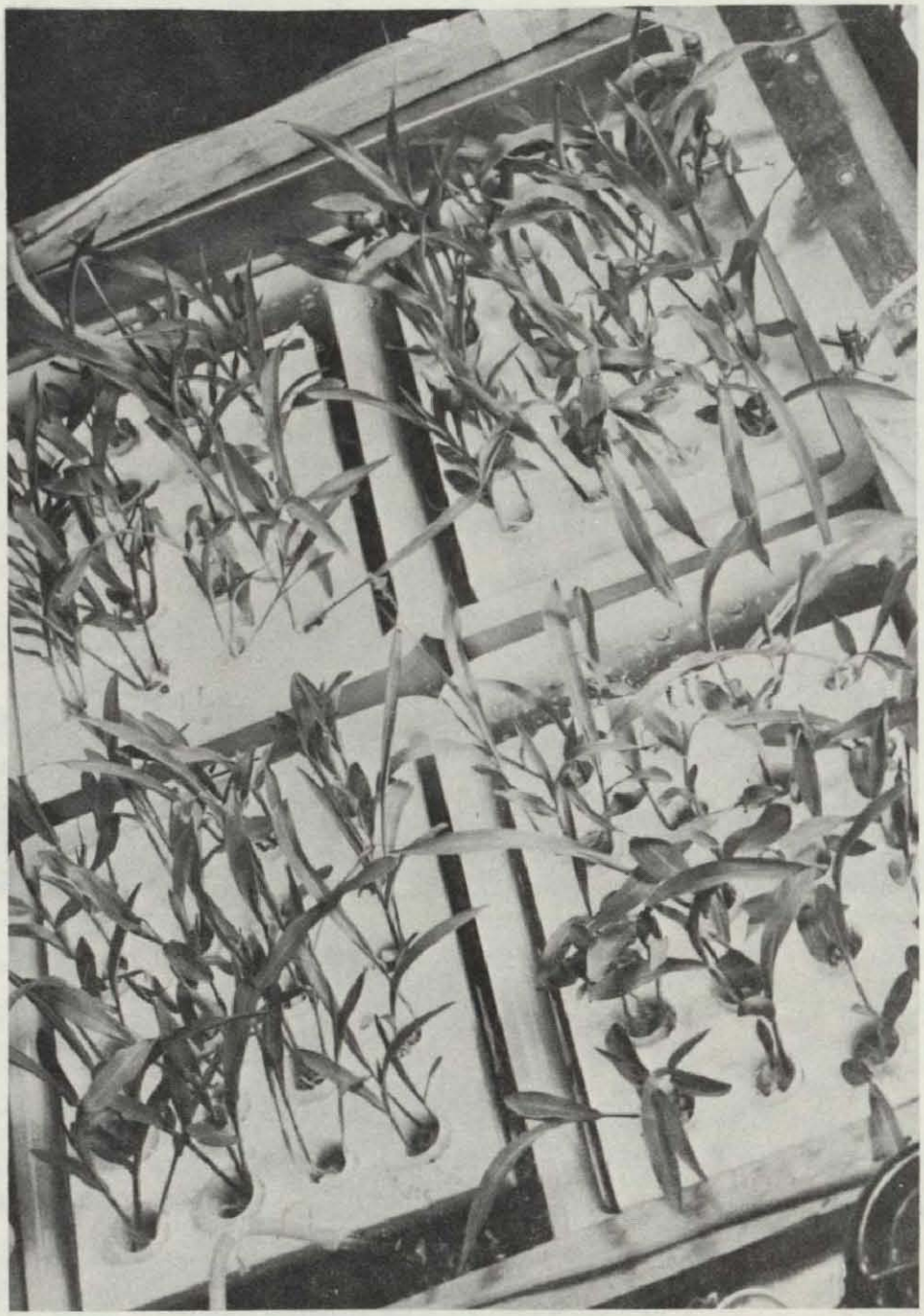


Fig. 7

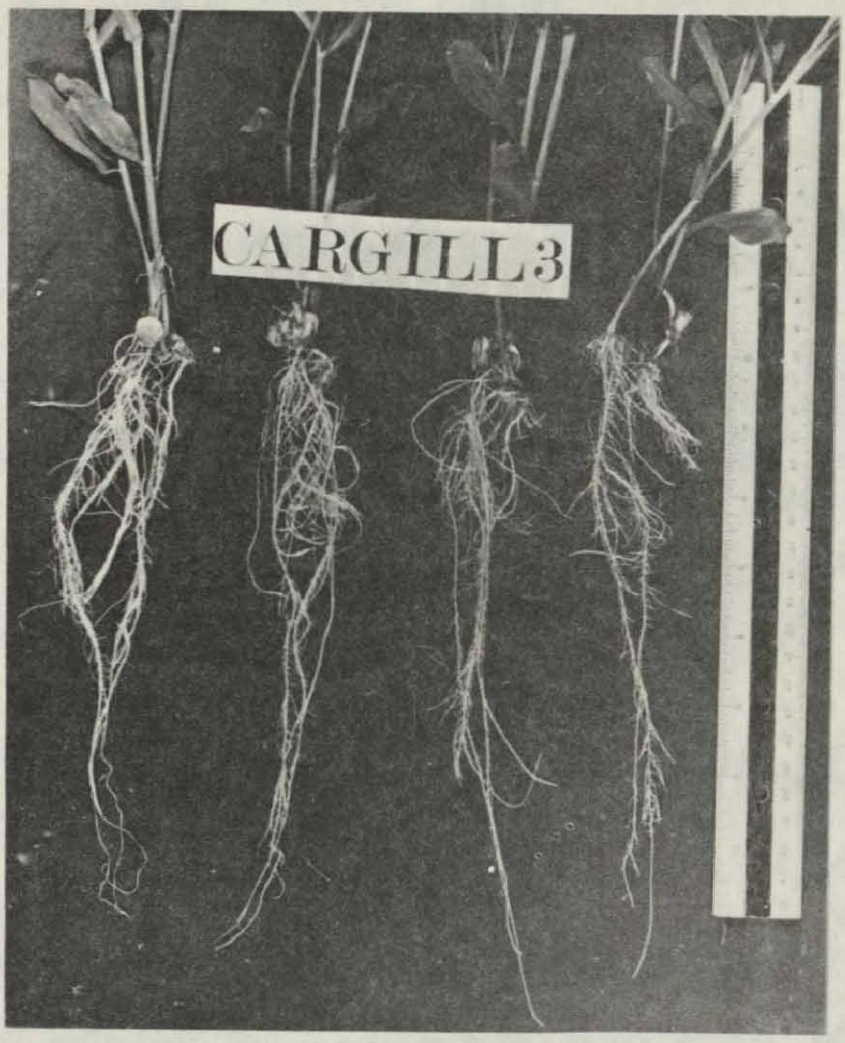

CARGILL 3

Fig. 8 a

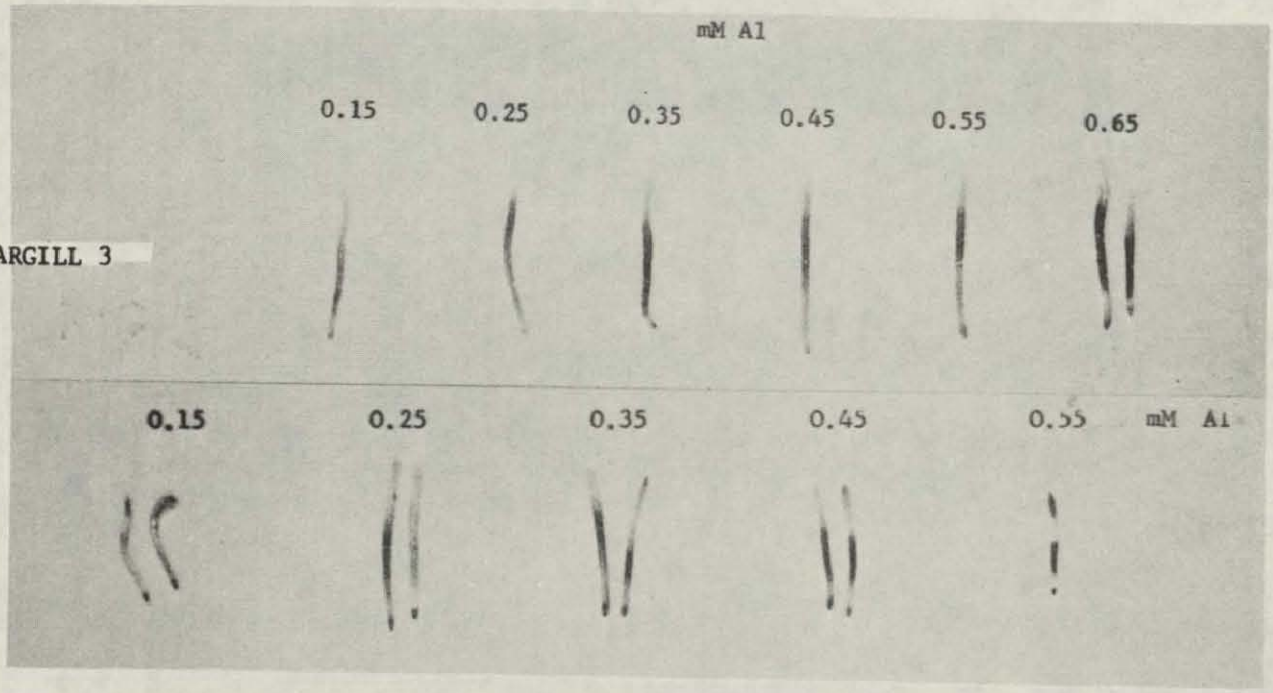

mM Al
0.15
0.25
0.35
0.45
0.55
0.65
CARGILL 3
0.15
0.25
0.35
0.45
0.55
mM Al

Fig. 8 b

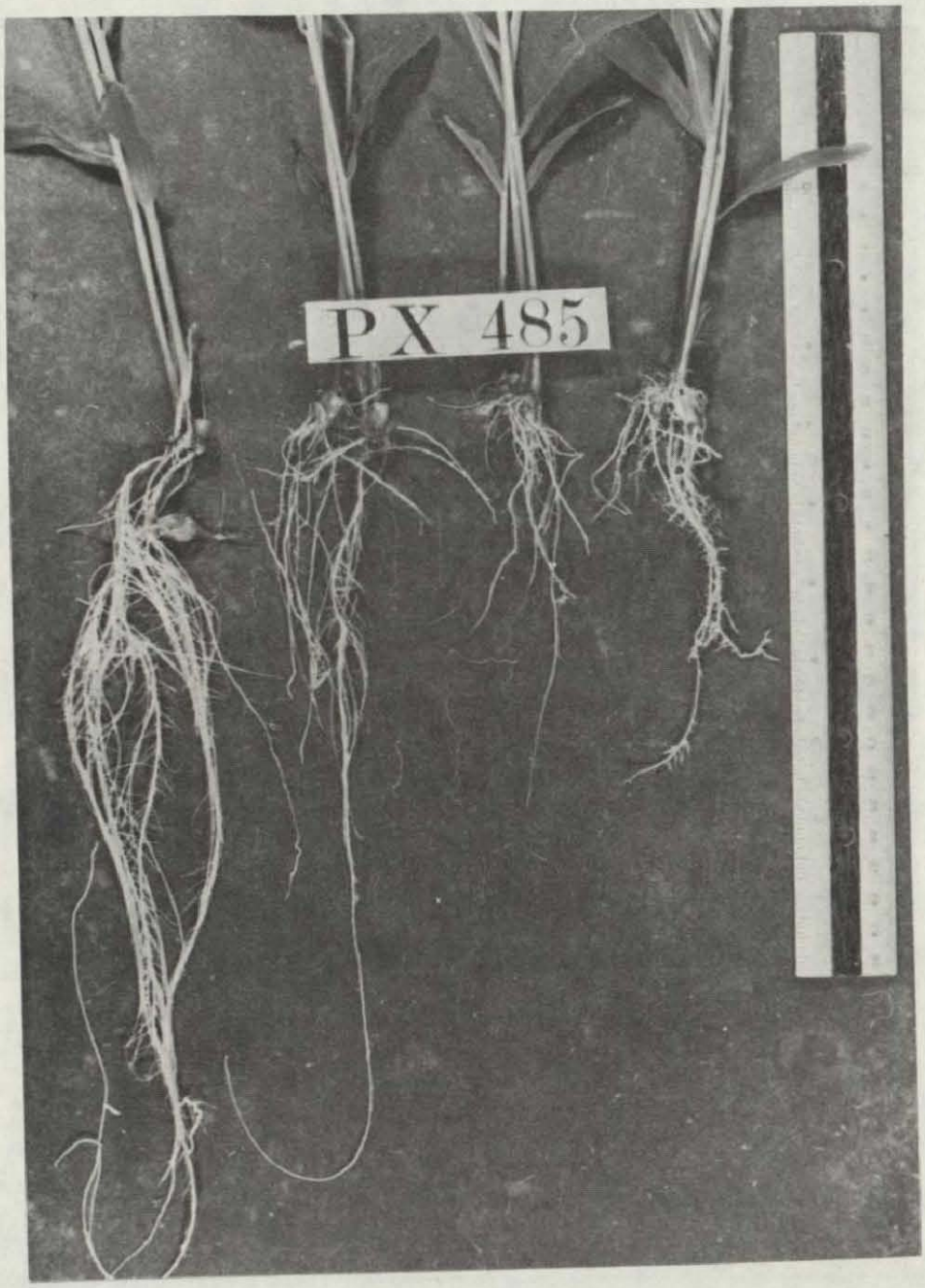
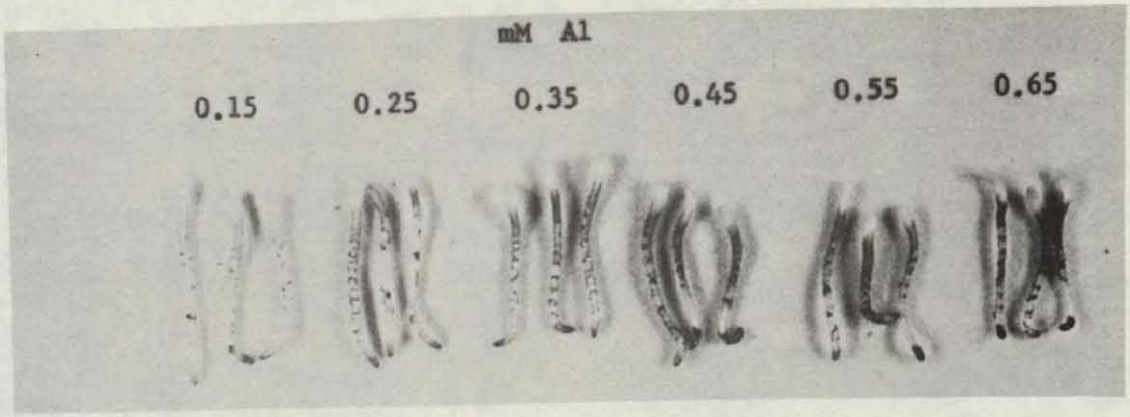


Fig. 9 a



PX 485

Fig. 9 b

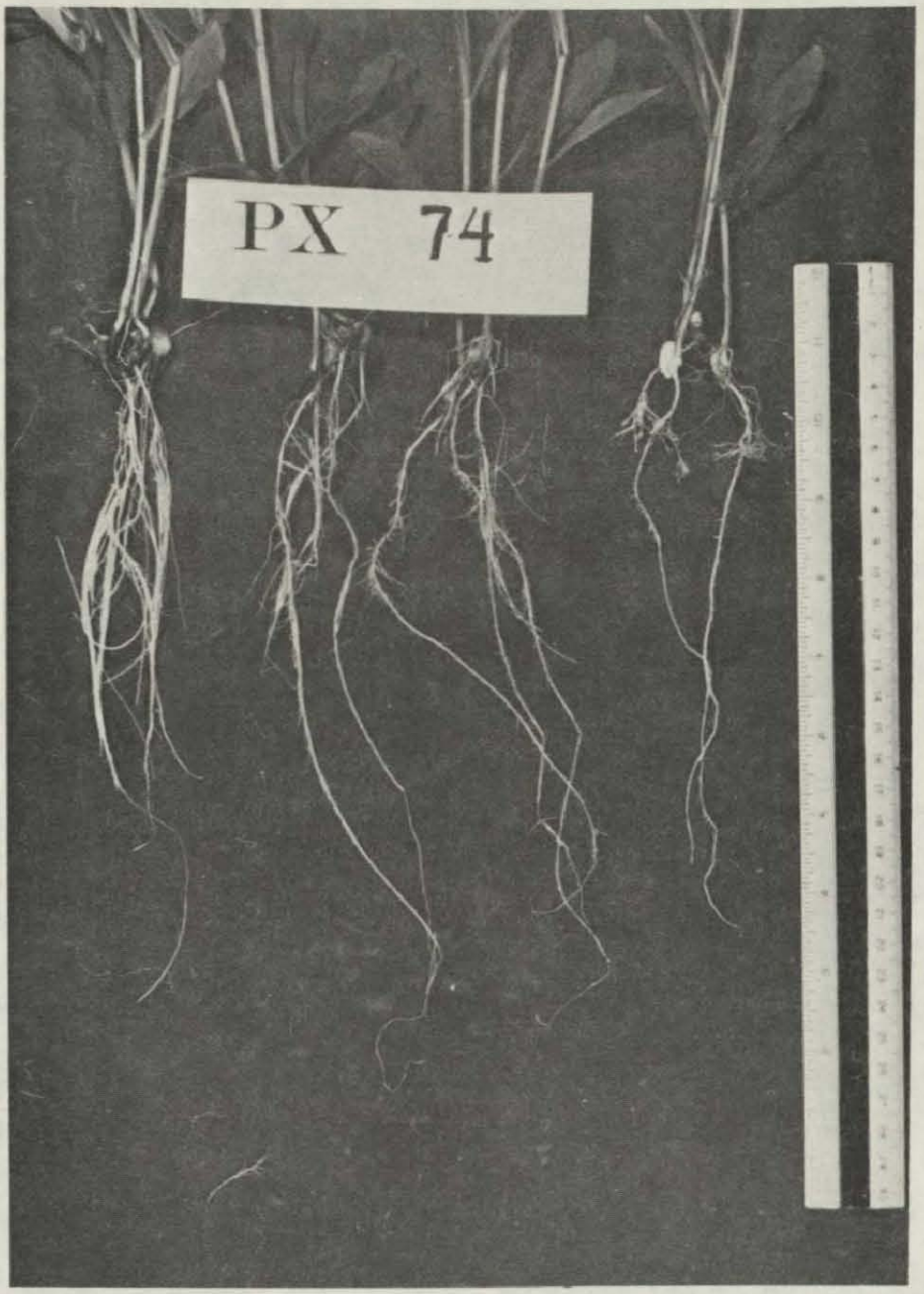


Fig. 10 a

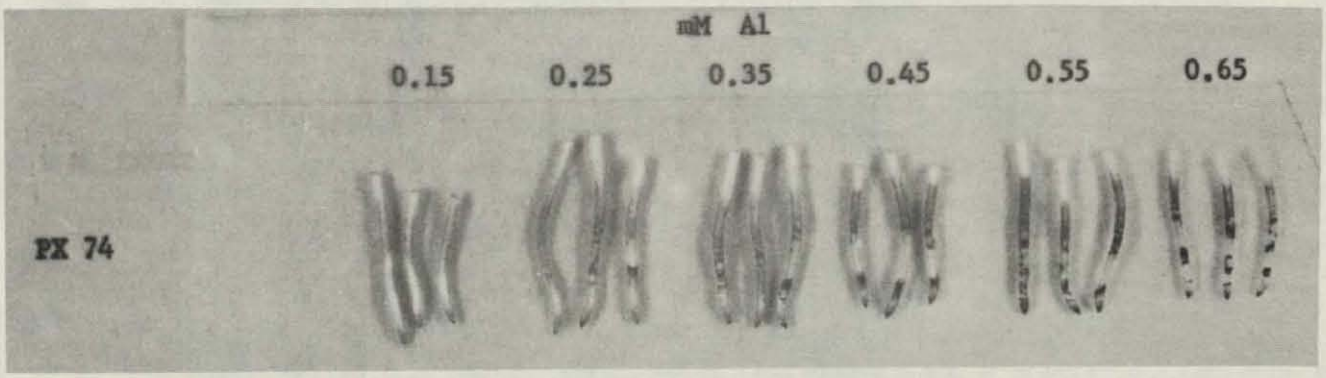


Fig. 10 b

*U.S. GOVERNMENT PRINTING OFFICE : 1979 0-280-931/FAS-55