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RAPID SCREENING OF WHEAT FOR TOLERANCE TO ALUMINUM IN BREEDING VARIETIES BETTER ADAPTED TO ACID SOILS[1]

E. Polle, C.F. Konzak, and J.A. Kittrick[2]

INTRODUCTION

Most acid soils of the world produce food at levels far below their potential. These include three billion acres in the tropics representing the world's greatest potential for increased food production. The worst nutrient problems of acid soils are aluminum and manganese toxicity and a deficiency of available phosphorus. In many countries,

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the costs of adding large amounts of lime and P fertilizers to at least partially correct these problems are prohibitive. Instead of altering the soil, a much less expensive strategy is to select those crop varieties that do well on the soil as it is.

It is fortunate that in cereals and most grain legumes, a wide range in genetic variability for tolerance to the toxic factors of acid soils occurs. Differences in P use efficiency also may be present among the germ plasm resources available to the breeder. Tolerances to Al and Mn toxicities appear to be inherited independently, are combinable, and evidently do not affect adaptation to alkaline soils.

Al^{3+} is usually the major toxic component in acid soils and this bulletin describes two simple methods developed to permit efficient screening of wheat lines or populations for tolerance to Al. The two methods for screening wheat have been designed to meet the practical needs of plant breeders. They are simple and rapid enough to be used for eliminating Al-intolerant lines prior to field tests. The materials and equipment required are usually found at experiment stations with facilities adequate to support plant breeding research.

The two methods described herein use different ways to assess the response to Al by wheat seedling plants. The recommended method is the simplest. It involves observing the pattern of root tip staining with hematoxylin after germinating seedlings are exposed to Al for a few hours. It requires a laboratory with a reasonably constant temperature of about 25 C and a set of calibrating cultivars. The alternate method involves measurement of the reduction in root elongation when seeds soaked in water 24 hours are cultured for 4 days in a nutrient solution

containing Al. This requires a germinator with controlled temperature to obtain relatively uniform seedling root growth. Dimensions of the equipment for growing wheat seedlings can be altered to fit other sizes of containers that may be available elsewhere and are presented here only as a guide. However, the user should not depart substantially from the number of seedlings/volume of solution used.

STAIN METHOD

Planting Trays

The following materials are required for building planting trays sufficient for testing 60 varieties at three levels of Al or 180 varieties at one level of Al:

- 1 piece 30 x 30 cm of 1/16 inch (1.6 mm) Lucite
- 1 piece 10 x 30 cm of 1/8 inch (3.2 mm) Lucite
- 3 pieces 24 x 40 cm Nylon screen
- 1 piece 12 x 30 cm of 1 inch (25.4 mm) Styrofoam
- Ethylene dichloride (EDC)

From a clean piece of scrap Lucite (without lining paper) obtain shavings with a drill. Dissolve the shavings in EDC until a thick glue is obtained. This glue can be applied using a strip of polyethylene.

Cut 33 strips 30 x 0.65 cm from the 1/16 inch Lucite and six strips 30 x 1.27 cm from the 1/8 inch Lucite. Tape together two strips 30 x 1.27 cm and 1/8 inch thick and three strips 30 x 0.65 and 1/16 inch thick. Cut 16 notches in the taped strips 0.4 cm deep and 1/16 inch wide. Space the notches 1.71 cm apart (center to center) starting at 2.1 cm from one end as shown in Fig. 1a. Cutting all strips at once with a table saw insures a perfect correspondence between notches for a planting tray. Repeat the operation for the remaining two trays.

Cut the remaining 30 cm strips into 48 strips 15 cm long. Tape together three groups, 16 strips in each. Cut five notches simultaneously in each group. These notches should be 0.4 cm deep and 2.54 cm apart starting about 2 cm from one end. The first and the last of these notches are 1/8 inch (3.2 mm) wide and the three center ones are 1/16 inch (1.6 mm) wide. Mark all the strips at one end of each taped package.

The frame of one planting tray is constructed from one package of six 30 cm strips and one package of sixteen 15 cm strips. Start gluing together the four external strips on a flat surface as shown in Fig. 1b. Take care that each group of strips within a frame has the marked ends pointing in the same direction. In this way, small errors in spacing the notches will not affect the appropriate mounting of the frame. Allow 15 minutes for the frame to dry before gluing the rest of the strips. Apply the glue in each intersection. After gluing, make sure that all the strips have one edge on the same plane by adjusting each against a flat surface (Fig. 1c).

Allow 15 minutes for the structure to become rigid and cut all the excess strips (those of 1/16 inch thickness) from all sides of the frame Place the frame with the flat side up in a wooden board, stretch the nylon fabric over the frame and staple it to the board (Fig. 1d). Apply a thick EDC-Lucite glue along all the strips. Allow to dry 10 minutes and apply a second coat. After 4 hours at 25 C, cut the Nylon fabric flush with the border of the frame with a razor blade.

For each planting tray, cut two blocks of Styrofoam 2 x 10.6 x 4.0 cm and tie them to the edges of the tray with rubber bands as in Fig. 1e. When placed in water, the Nylon screen should be about 2.8 cm above the water level. After pushing down the tray until the screen contacts

the water surface, surface tension will keep the screen in contact with the water.

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 one 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 $MgCl_2 \cdot 6H_2O$; 0.1 mM of $(NH_4)_2SO_4$; and 0.4 mM of NH_4NO_3.

Solution B - 0.1 M Al: Dissolve 24.14 g of $AlCl_3 \cdot 6H_2O$ in about 900 ml of distilled water. Dilute to one liter.

Solution C - 0.25 M HCl: 20.83 ml of concentrated HCl diluted to one 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 one liter of volume.

Procedure

For testing 60 varieties at three levels of Al (0.18, 0.36, and 0.72 mM Al) use one plastic container 46 x 38 x 14 cm and three of the planting trays already described. Place the three planting trays in the container with 12 liters of water.. The screen of the planting trays should be in contact with the water. Plant the 60 varieties in the same order in each tray. One person can plant three trays with 60 varieties each in about 1 hour. For this, four seeds in each section are generally enough. Cover the three trays with Saran wrap or any thin clear plastic, leaving the borders of the film floating in the water. Plant in the morning hours. Bubble air in the water, taking care that the air does not accumulate under the screens. For this

purpose, the aeration tube can be inserted through the stem (of wider diameter than the aeration tube) of one inverted 2 to 3 inch (51 to 76 mm) diameter funnel. The funnel can be positioned in the solution by clamping its stem to an external support. The height of the funnel in the solution is adjusted to catch the air bubbles and at the same time to keep the floating trays out of the bubbling region.

The next morning change the water of the tray to 12 liters of nutrient solution. This is made by placing in a 1 liter graduated cylinder, 760 ml of water and 240 ml of stock nutrient solution. Add this solution to enough water to make 12 liters in the large tray. Then with a 10 ml serological pipet, add enough 0.25 M HCl to bring the pH to 4.0. Record the amount of 0.25 M HCl added and use the same amount in subsequent work. After transferring the planting trays to this solution, cover and aerate as before.

About 4 p.m. the next day, transfer each planting tray to a separate nutrient solution containing Al. These three solutions are made up each with 60 ml of stock nutrient solution and 5.4, 10.8 or 21.6 ml of 0.1 M Al solution; the final volume of each is diluted to 3 liters in a plastic container 13 x 31 x 11 cm. The planting trays are then placed in the Al treatments with the screens touching the water. Bubble air in the solution, but omit the Saran wrap henceforth.

At about 9 a.m. the next morning, transfer the planting trays to the large container with 12 liters of distilled water for about 1/2 to 1 hour. Then discard the water and put the trays (without the styrofoam blocks) in the bottom of the container. Pour a shallow layer of hematoxylin solution, enough to cover the roots, into the tray. Move the tray occasionally to allow the hematoxylin solution to be in contact

with all the roots for 15 minutes. Next, wash the trays directly with flowing distilled water, replace the styrofoam blocks, and float the trays with the screen in contact with the aerated distilled water in the large container for about 1/2 hour.

After the washing period, score the plants by pulling one plant (with roots > 1.5 cm) of each variety and each Al treatment. Separate the tip of the root from the plant with tweezers and place it on a moist filter paper. By labeling the filter paper, roots, varieties and treatments can be identified. One person can sample three trays with 60 varieties each in about 30 minutes.

After sampling, estimate the degree of Al tolerance. Fig. 2 shows the pattern of root staining of five varieties with increasing Al concentrations. Fig. 3 displays 50 varieties, each root treated with 0.18, 0.36 and 0.72 mM Al, left to right. The evaluation can be made immediately after separating the roots, or a copier can produce an adequate reproduction. For this purpose and before sampling the roots, put the moist filter paper on top of a 22 x 28 cm (1/4 inch thick) Lucite plate. After sampling, cover the filter paper carrying the roots with a 22 x 28 cm (1/16 inch thick) Lucite plate whose borders are taped to the supporting Lucite plate. In this way, the root appearance can be recorded with a copy machine. IBM Copier III and Xerox are adequate when set at the higher contrast. If desired, the roots can be kept in the covered filter paper for several hours, but some fading of the stain color is observable after 12 hours.

For the stain method, the inbibition period is carried out under the ordinary light and dark conditions of the laboratory. After the germinating seeds are placed in the nutrient solution and until the test is completed, alternate periods of 12 hours

per day of light and dark are provided. Incandescent light 85 u Einsteins sec^{-1} m^{-2} (400 - 700 nm waveband) or any other suitable greenhouse lamp can be used for this purpose.

Varieties for Calibration

Brevor, Druchamp, Chinese Spring and Atlas 66 can be used as standard varieties. Including these in each test will allow a consistent classification of test materials, even if small variations in ambient conditions occur from one test to another. Other varieties in Fig. 3 can also be used as standards.

ALTERNATE METHOD

Materials

Lucite holders: Units for testing 20 wheat varieties require the following materials:

165 x 110 cm of Lucite 1/16 inch (1.6 mm) thick

28 x 50 cm of Lucite 1/8 inch (3.2 mm) thick

12 1 quart (or 1 liter) polyethylene food freezer containers

12 1 pint (or 500 ml) polyethylene containers

Ethylene dichloride (EDC)

From the 1/16 inch Lucite, cut (with a table saw) 120 plates 8.5 x 14 cm, 120 strips 9 x 1 cm and 24 strips 7 x 2.6 cm. From the 1/8 inch Lucite cut 240 strips 3 x 0.45 cm and 24 strips 8.6 x 2.6 cm.

Place the plates on a smooth surface. Use the glue described in the Stain Method to glue the 3 x 0.45 cm strips to the plates as shown in Fig. 4a. After 2 or 3 minutes, glue the 9 x 1 cm strips to the top of the 3 x 0.45 cm strips, as shown in Fig. 4b. Glue two strips 8.6 x 2.6 cm to two strips 7 x 2.6 cm for the support collar, as shown in Fig. 4c. Cut a rectangle 6.3 x 8.6 cm in the center of the lid of a

1 quart container so that the support collar can be placed as shown in Fig. 4d, with 10 plates accommodated as in Fig. 4e. This unit can be covered with a 1 pint container as in Fig. 4f. The dimensions of the collar and the containers are such that when the latter contains 450 ml of nutrient solution, only about 2 cm of the lower part of the plate will be immersed in the liquid. Commercial type containers (Republic-Freezette, Chicago, IL 60648) are adequate. For other types of containers, the height of the collar can be modified to obtain the conditions described.

Filter paper: Cut 120 pieces 8.2 x 14 cm of Whatman Chromatography Paper 3 MM, 0.33 mm thickness, medium flow rate (W & R Balston Ltd., England).

Germinating bags: Cut, fold and staple 20 nylon bags 5 x 8 cm; attach a tag to each.

Fungicide: Terrachlor, 75% in p-chloro nitrobenzene (PCNB).

Solution A - Buffer-nutrient Solution: Dissolve 163.4 g of potassium biphthalate ($KHC_8H_4O_4$) in about 1.8 liters of distilled or deionized water. Then dissolve in this solution, 1.2 g of magnesium sulfate anhydrous ($MgSO_4$), 1 g of potassium nitrate (KNO_3), 1.2 g ammonium nitrate (NH_4NO_3) and adjust the volume to 2 liters. When 20 ml of this solution are diluted to 1 liter, the final concentrations are: 0.1 mM of $MgSO_4$; 0.1 mM of KNO_3; 0.15 mM of NH_4NO_3; and 8 mM of $KHC_8H_4O_4$.

Solution B - 0.1 M Calcium Chloride ($CaCl_2$): Dissolve 11.1 g of $CaCl_2$ in about 800 ml of distilled water and bring volume to 1 liter.

Solution C - 0.1 M $AlCl_3$: Dissolve 24.14 g of $AlCl_3 \cdot 6H_2O$ in about 800 ml of distilled water and bring volume to 1 liter.

Solution D - Zero Al Treatment: 20 ml of solution A and 40 ml of

solution B per liter. For 20 varieties prepare 6 liters.

Solution E - 1.6 mM Al Treatment: 20 ml of solution A, 16 ml of solution B, and 16 ml of solution C per liter. Prepare 6 liters for 20 varieties.

Procedure

Place about 100 seeds of each variety in a tagged nylon bag (Fig. 5a). Immerse the bags of seeds in distilled water by hanging them from the border of the container or by means of suitable bars across the container. A large tray with about 12 liters of water is suitable for 50 bags. Bubble air through the water. Carry out this inbibition-germination period at room temperature (about 22 C).

After 24 hours, replace the water with the same volume of a suspension of 0.1 g/l of Terrachlor fungicide. Treat the seeds with this solution for 30 minutes. Maintain aeration during this period to keep the Terrachlor in suspension. Discard the Terrachlor suspension and allow the seeds to drain for a few minutes.

Then plant the seeds in the Lucite holders with one sheet of Whatman Chromatography Paper. Handle the seeds with tweezers and plant with the "crease" towards the lucite plate and the embryo pointing down (Fig. 5b). Plant 10 seeds of a variety or line in one Lucite holder for each replicate. Three replicates per Al treatment are desirable. During the planting procedure, keep the two test solutions (0 and 1.6 mM Al) in plastic trays where holders and filter papers can be readily immersed. Air bubbles trapped behind the papers are usually avoided if both Lucite holders and filter paper are immersed as a single unit in the test solution. Take care to avoid contamination of the control treatment filter paper by the Al solution and vice versa. To avoid this

problem, it is advisable to plant on two 12 x 15 x 1/4 inch sheets of Lucite, one for each test solution. Use ordinary number 2 lead pencil for marking the number or name of each variety on the filter paper.

After planting, place the appropriately labeled replicates in groups of 10 in polyethylene containers. To each container, add 450 ml of the appropriate test solution. Then cover the containers and holders with the 1 pint polyethylene containers and place them in a dark chamber at 25 C.

Two days after planting, remove the container covers and return the containers with the holders to the dark chamber at 25 C. Four days after planting, determine the estimated average root length in each holder. Calculate an average of the three replicates of each treatment.

The top, back, and front view of the lucite holders are shown in Figs. 6a, 6b, and 6c.

With this procedure, one person can plant 300 Lucite holders in about 4 hours. This number could include 50 varieties with two Al treatments and three replicates per treatment.

Calibration and Evaluation

To evaluate the results, two approaches can be taken. If all varieties tested show equal root growth in the control treatment, the value of the average root length in the 1.6 mM Al treatment can be taken as an index of the relative Al tolerance of each variety. If the root growth in the control treatment varies substantially from one variety to another, the ratio of the length of the root grown in 1.6 mM Al to the control length can be used as a relative index of Al tolerance.

Whatever the method of evaluation used, it is advisable to include some reference standards for calibration. The varieties Brevor, Druchamp,

Chinese Spring and Atlas 66 can be used for this purpose. Typical values of the ratio of the root length at 1.6 mM of Al to the length of the root in the control treatment for these varieties are 0.24, 0.38, 0.54 and 0.83 respectively.

LEGEND FOR FIGURES

Fig. 1a. Strips for one planting tray taped together, showing the notches made with the table saw.

Fig. 1b. Four external strips for one planting tray glued together.

Fig. 1c. The transverse strips are being glued to the planting tray.

Fig. 1d. The nylon fabric stretched over the tray and stapled to the wooden board, and then glued to the planting tray.

Fig. 1e. The planting tray is shown finished with the two blocks of styrofoam attached to each end.

Fig. 2. Pattern of staining of five varieties pertaining to different levels of Al tolerance. The root of each variety has been treated with the Al concentrations indicated at the top of the figure. All varieties show a stainable region that corresponds approximately to the growth of the tip during the Al treatment. This region is only partially stained below a concentration that is characteristic of each variety. Thus, in Al-susceptible Brevor, the stainable region is completely stained above 0.2 mM Al. In the less susceptible Druchamp, the stainable region is covered up above 0.4 mM. Moderately tolerant Chinese Spring is completely stained above 0.8 mM. Al-tolerant Atlas 66 shows a complete staining of the growing region at 1.2 mM. Cotipora, a highly Al-tolerant variety, shows some small unstained region at this concentration of Al.

Fig. 3. Groups of three roots of 50 wheat varieties. Each root in a group, from left to right has been treated with 0.18, 0.36, and 0.72 mM Al. In the top row from left to right are the most sensitive of the varieties tested: Akadaruma, Thatcher, Brevor, Burt, Stephens, PI344195, Kharkov, Gaines, Nugaines, Luke, and Thatcher 4D (chromosome substitution in Chinese Spring). They show a complete staining at the three levels of Al. The second row from the top shows Druchamp, Inia 66, Monon, and PI344180. In these varieties, the 0.18 mM Al treatment leaves a region largely unstained. The third row from the top (left to right) shows Alba (Redmond), PI344179, Chinese Spring, Yamhill, and Blue Boy. In these varieties, the 0.18 and the 0.36 mM Al treatment leave a large clear region. The more tolerant varieties are Seneca, Thorne, PI344182, Fielder, Trintani, Azteca F67, PI344176, Toropi, PI344197, PI193916, Pitic, Sonora 64 (fourth row from the top, left to right); Atlas 66, Sawtell, PI353388, PI344162, Lagoa Vermelha, Erechim, PI344188, PI353389, PI344160, IAS20, Carazinho (fifth row from the top, left to right); and BH1146, Cotipora, Cinquentenario, Preludio, Albatroz, PI344192, and PI344198 (sixth row from the top, left to right). These varieties show an unstained region at the 0.18, 0.36, and 0.72 mM Al.

Fig. 4a. The lateral strips 3 x 0.45 cm are glued to the plate in such a way that the front face corresponds to the 1/8 inch thickness of the Lucite material.

Fig. 4b. The transverse strips 9 x 1 cm are glued to the lateral strips.

Fig. 4c. Support collar made by gluing two strips 8.6 x 2.6 cm with two strips 7 x 2.6 cm.

Fig. 4d. The support collar is placed on the lid of the 1 quart container.

Fig. 4e. Ten plates can be fitted in the support collar.

Fig. 4f. The polyethylene container with the plates is now covered with a 1 pint container.

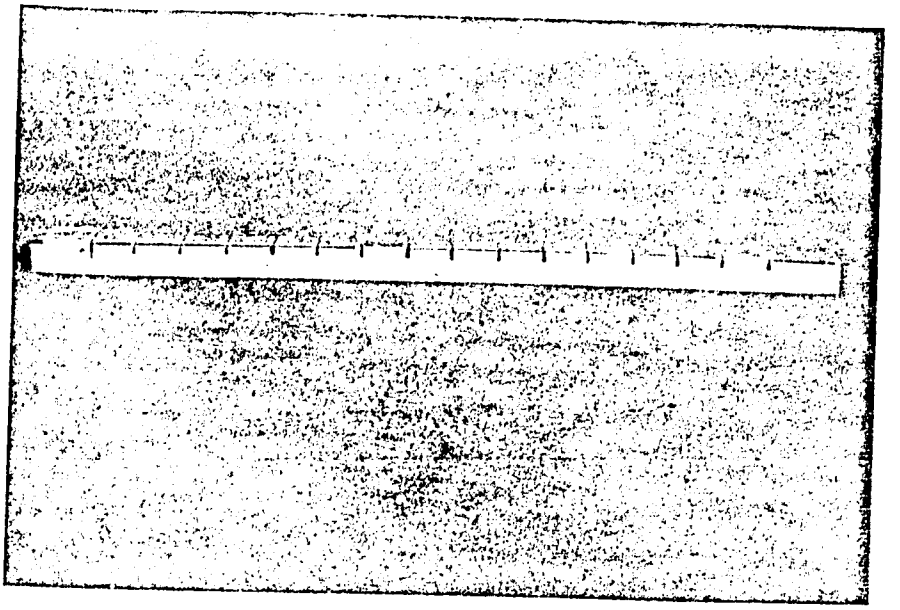
Fig. 5a. The germinating bag is made by folding and stapling two of the sides. The final dimensions of the bag are 5 x 8 cm.

Fig. 5b. After placing the chromatography paper under the transverse strip of the plate, the seeds are planted "crease" down as shown.

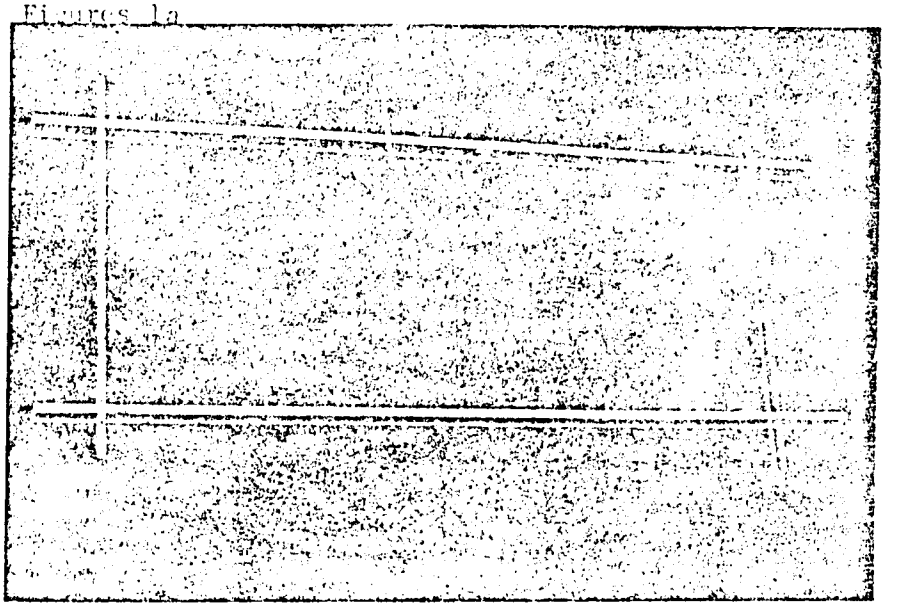
Fig. 6a. After planting, the filter paper is kept covering and in contact with the seeds with the help of a rubber band.

Fig. 6b. The fourth day after planting, the seeds are germinated as shown in this front view of the plate.

Fig. 6c. Back view of the same plate as in Fig. 6b, showing the root development through the Lucite plate.



Figures 1a



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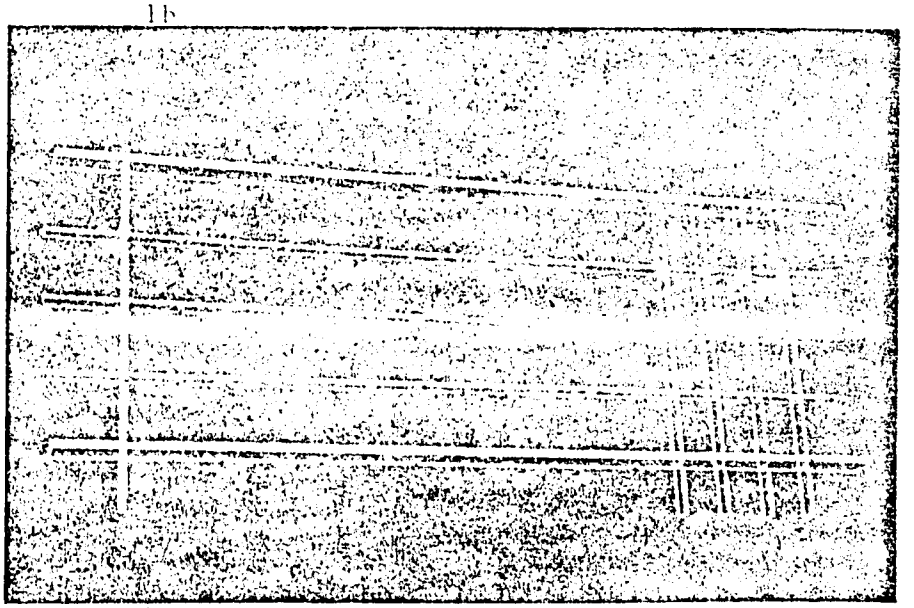

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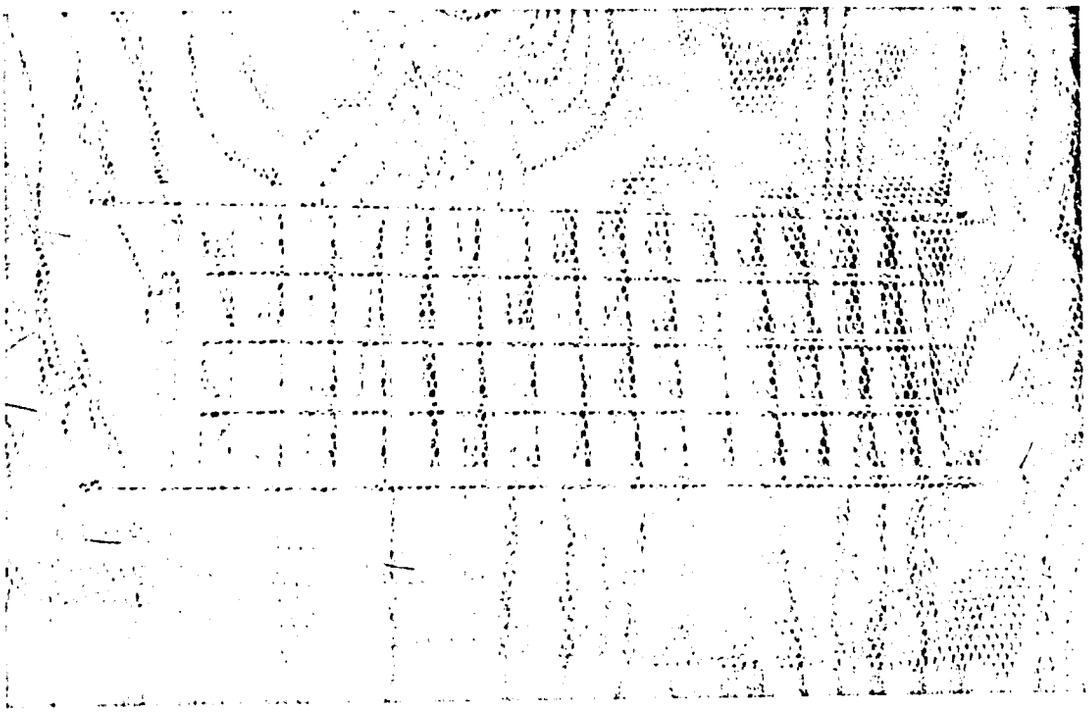
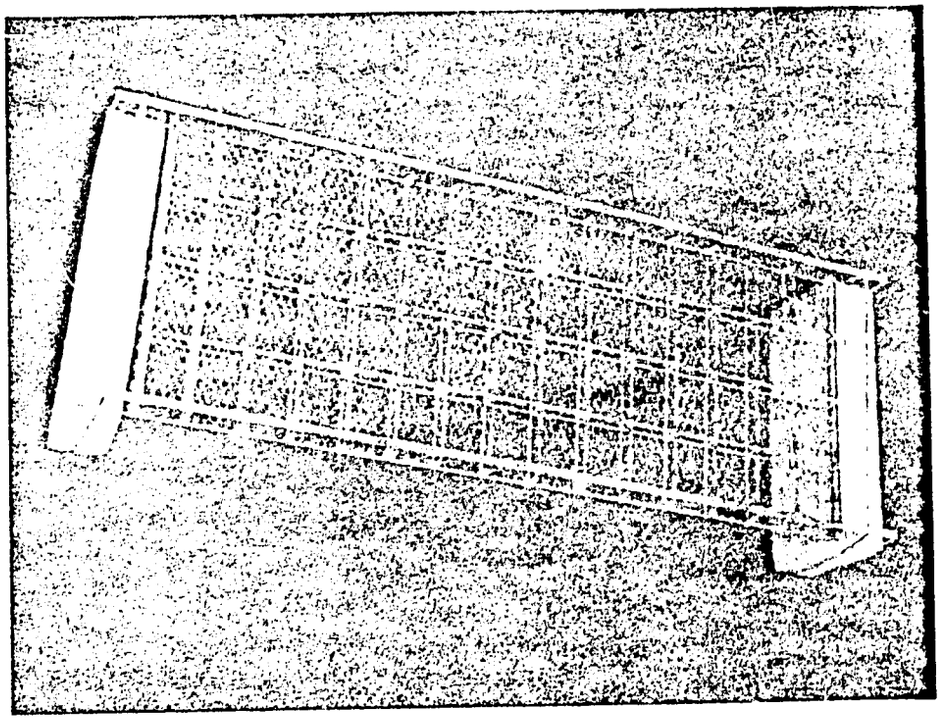


Figures 1d



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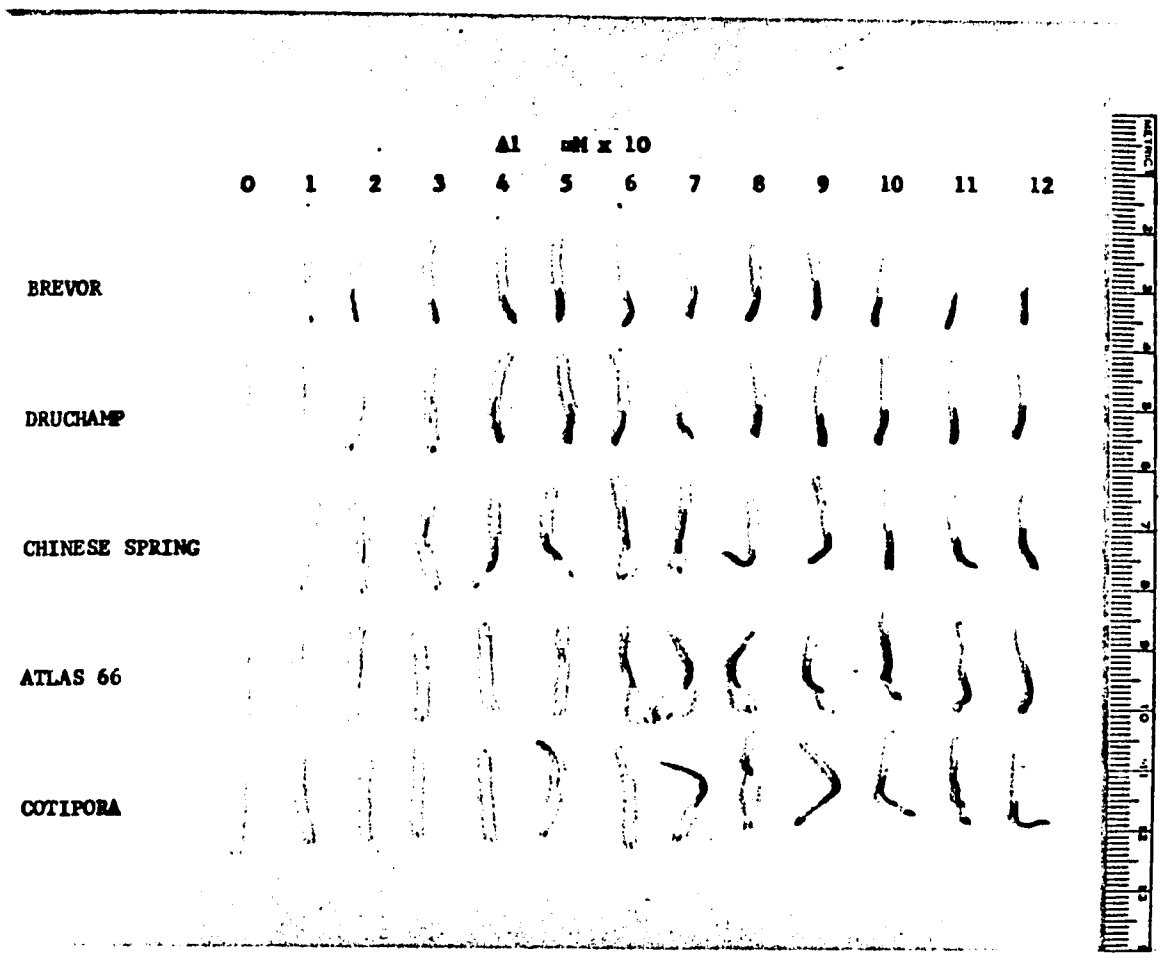
Al mM x 10
0 1 2 3 4 5 6 7 8 9 10 11 12
BREVOR
DRUCHAMP
CHINESE SPRING
ATLAS 66
COTIPORA

Figure 2

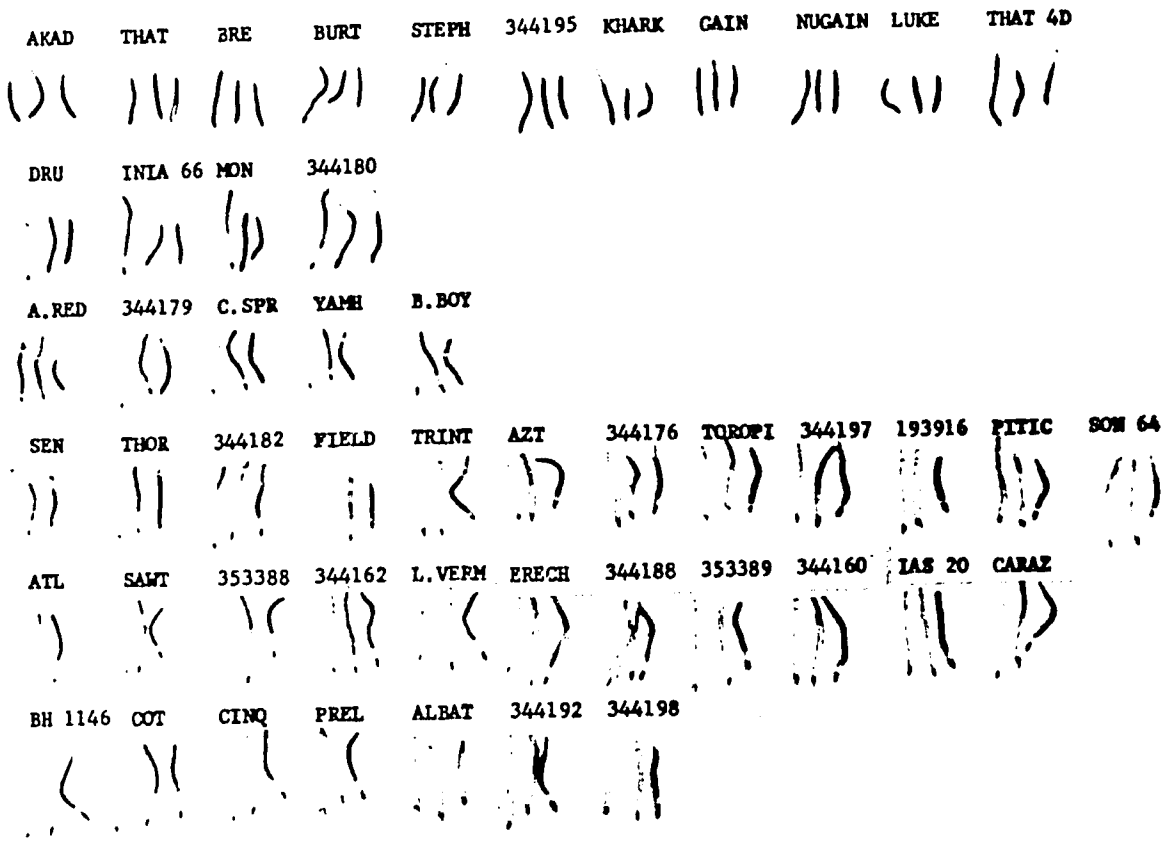
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Figure 3

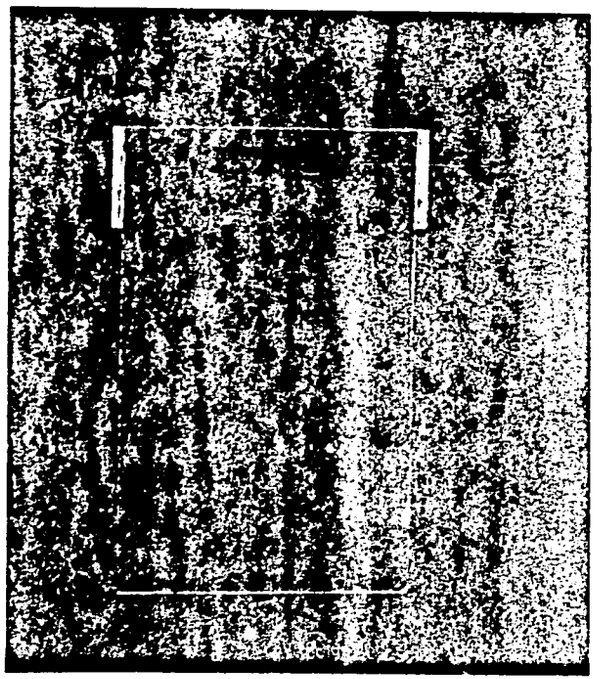
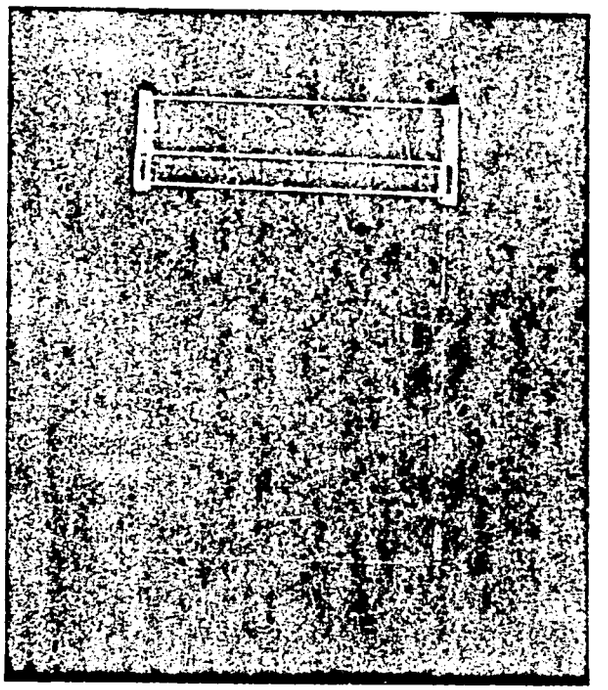


Figure 4a



4b

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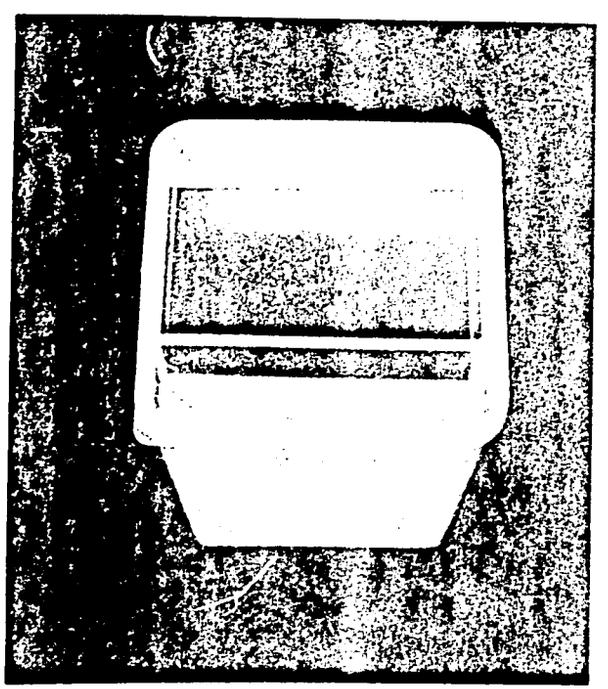

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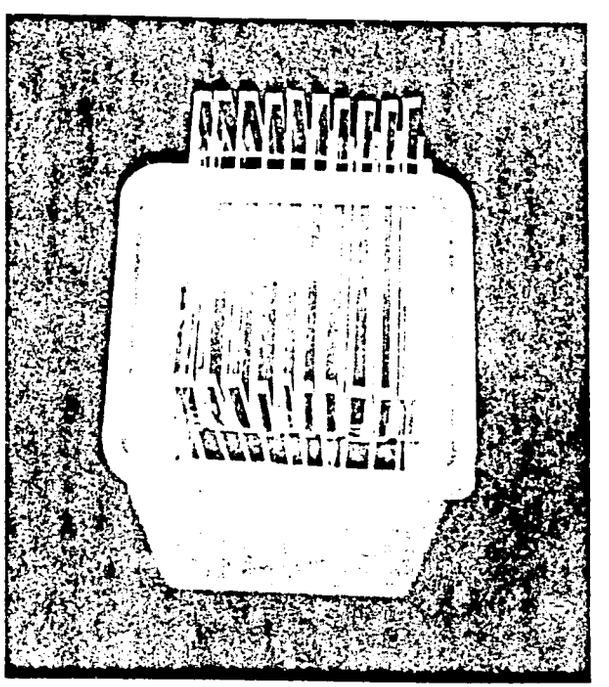

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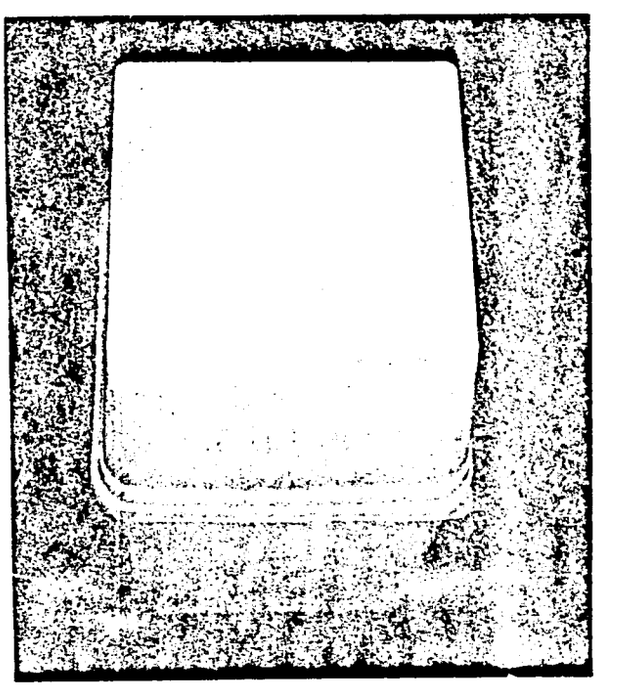
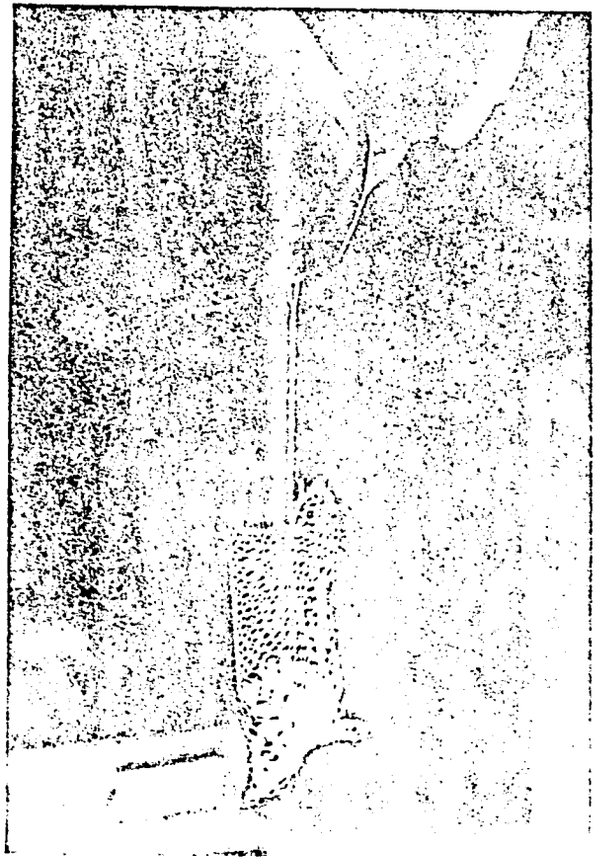
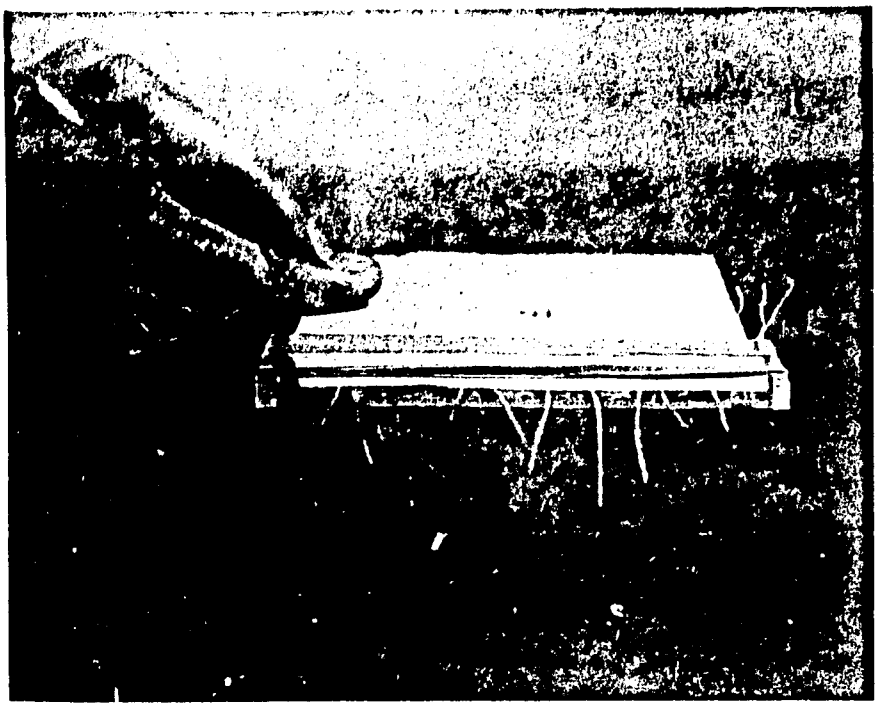


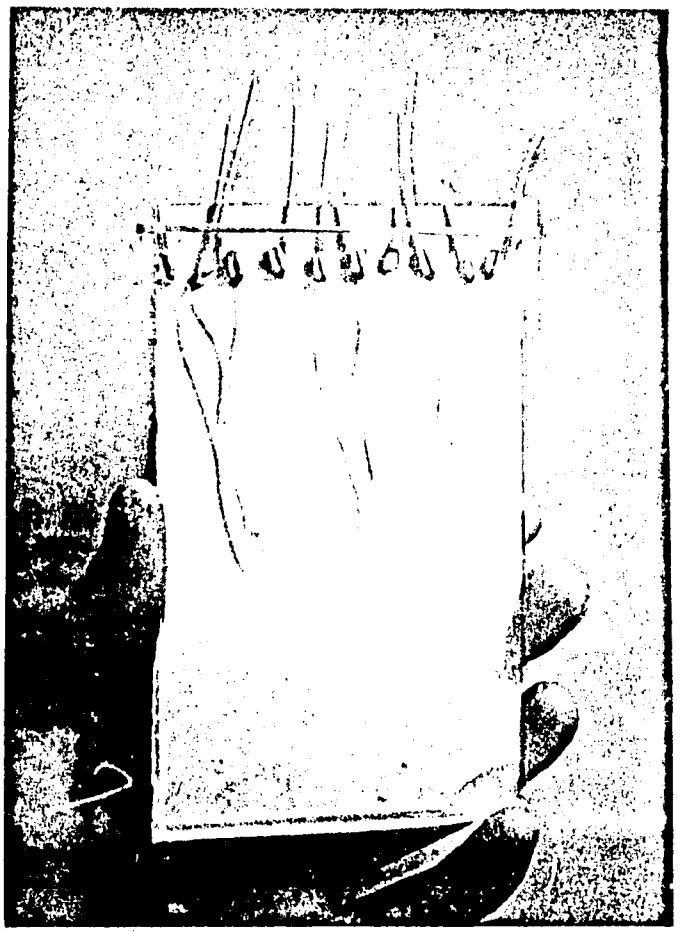


Figures 5a



Figures 6a

6b



6c