No. 48

REPORT ON THE VEGETABLE BREEDING WORKS AT CITRUS AND VEGETABLE SEED RESEARCH CENTRE UNDER BANGLADESH AGRICULTURAL RESEARCH INSTITUTE, JOYDEBPUR, DACCA.

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REPORT ON THE VEGETABLE BREEDING WORKS AT CITRUS AND VEGETABLE SEED RESEARCH CENTRE UNDER BANGLADESH AGRICULTURAL RESEARCH INSTITUTE, JOYDEBPUR, DACCA.

September 23rd to November 28th, 1980

Submitted by

(MR. TATSUYA MOCHIZUKI)

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1. **Introduction:**

I was assigned by the JICA to see the problems of vegetable breeding research in Bangladesh and I came here on September 23rd, 1980 and stayed upto November 28th, 1980. During my 67 days stay, I had three main dutys, these are (1) rearrangement of laboratory equipments to facilitate research work, especially vegetable breeding research, (2) Collection and screening of local germplasm of eggplant including wild relatives for future breeding programme, and (3) to transfer microscopic techniques how to observe the chromosomes to local researchers. By this time we set some experiments and taught the procedures to our local researchers, Mr. A. Ahad Miah (S.S.O. Vegetable), Mr. A. Rashid, S.O (Vegetable) and Mr. Asit Sarker, S.O. (Vegetable) in order to make progress in their research fields.

I would like to express my sincere gratitude to Japanese team leader Mr. S. Aihara, Vegetable expert Mr. S. Tasaki and other Japanese experts for helping me to complete my work and to Dr. Kazi M.Badruddoza, Director, BARI and Mr. Abdur Razzaque, P.S.O, Citrus and Vegetable Seed Research Centre for their helpful co-operation. Lastly, I express my gratitude to Mr. A. Ahad Miah, S.S.O (Vegetable) and other staffs of Citrus and Vegetable Seed research centre for their help during my stay in Bangladesh.

2. Screening of eggplant germplasm for Bacterial wilt resistance.

In Bangladesh, eggplant is one of the most important vegetable for both in Kharif and Rabi season. But every year, bacterial wilt causes heavy loss to eggplant production. This disease is very severe and common in eggplant, but there is not any breeding work in this country to screen out the resistant varieties from local and exotic materials.

(1) Isolation of causal bacteria, Pseudomonas Solanacearum.

(a) Collection of the infected plants;

On 30th September and 2nd October we visited at Kashimpur A.D.E, B.A.D.C. to collect the infected plant and got about ten number of plants which seems to infected by bacterial wilt. And the distinction was done in order to determine whether these plants were infected or not. Generally, we distinct the plant which infected by bacterial wilt, to cut the stem of the plant and to observe inner symptom, if the plant was attacked by bacterial wilt, the vescular bundle will be rotten as shown brownish colour and also can observe if the cut stem be kept into a beaker with clear water, the whitish bacteria come out from the cut portion to the water.

(b) Preparation of media for isolation;

In order to isolate the causal bacteria from the infected plant, we usual use Potato-Dextrose-Agar media.
The following I will mention on process and order regarding with preparation of P.D.A media.

First of all, 300g of peeled Potato be out into small pieces and boiled in one liter of distilled water for about 30 minuties. After boiling, 20g of Dextrose (if Dextrose is not available can be used substitute Saccharose for Dextrose) is mixed to the potato extract, then 15g of Agar powder is mixed under the temperature of the extract is in between 50-60°C, the media should be sterilized in the autoclave for at least 20 minutes by the following pressure and temperature.

Pressure	2.1 kg/cm^2
Temperature	121°C

(c) Isolation of the causal bacteria;

The stem of the infected plant is out the several pieces from the diseased portion by the length of 2-3cm, within the several stem pieces, we will choise the most fresh and most top portion which can observe the diseased symptom, because the lower and old diseased portion, it is happen usually contaminated by other fungus and bacteria which habit in rotten organ, it is difficult to taken up only bacterial wilt causal bacteria.

The stem is sterilized by ethly alcohol in a few records and mashed by sterlized water, and crushed the vessel part in a few drops of sterilized water on sterilized petridish by the sterilized needle or pincette to get the original bacterial suspension, For these series of work, if possible it had better use the clean bench and as much as possible endeauor to keep clean on your environment.

Due to get the isolated colonies of the bacteria, the original suspension is diluted with sterilized water, at first the original suspension is transfered to other petridish which contain a few drops of sterilized water by round topped needle, and againtr transfered from the 1st petoridish to the 2nd, and the 2nd to the 3rd and so on, Because the original suspension contain huge number of bacteria, therefore due to deduct the popuration of bacteria to propagate on the media as a proper number of colonies which can easily picked up and can isolate as a individually.

Then the liquid P.D.A should be poured to those petoridishes when the temperature of it is at about 50°C, and mixed uniformly with the bacteria suspension. After 2-3 days inculation, various colored bacterial colonies are appeared on the media, and we should choise the ivory coloured ones for our purpose. In order to pick up the isolated bacteria, each colonies is transplant into other petoridish which contains a few drops of sterilized water and pour the liquid P.D.A in the petoridish.

(d) Test of pathogenecity of isolated bacteria;

After the isolation, we have got 8 numbers of isolates, 55, B_3, 75, 65, 22, B_1, 93 and 21, But all of these isolates might not be equally pathogenic to the eggplant, therefor for testing pathogenecity on eggplant, we had to inoculate the pathogens into the eggplant to identify their pathogenicity to cause bacterial wilt of eggplant. For the inoculation of pathogen to the eggplant seedlings, we made the suspension of bacteria, at first added 10ml of sterilized

water to test-tube P.D.A. slant media on which the individual bacterias were incubated already.

we used two methods at same time, as follows:

Injection method	- Inject the bacteria suspension into the stem use by bounded needles.
Dipping method	- To cut the root ($\frac{1}{3}$) and soak into the bacteria suspension, then after transplant to the polyethelen pot with sterilized soil and pour the remained suspension to the soil near by plant.

Finally, we found that isolate No. 21 and 93 were very highly pathogenic to eggplant which called "Baromashi".

(e) Mentainance of Bacteria isolates.

Pseudomonas solanacearum is easily to lose its pathogenecity on the Agar media under the room temperature, so, in order to keep its pathogenicity so long, it'd better to take the methods as follows:

Cold preservation	- put the suspension in the test-tube and covered with sterilized liquid paraffine then keep under low temperature (5°C)
Soil preservation	- Keep the bacteria in the infected soil with the chips of infected plant under the room condition.

(2) Screening of eggplant varieties for bacterial wilt resistance

(a) Materials and method;

The varieties which we used in this experiment are shown in the Table No. 1 (page No.9-10). Seeds were sown on 27th, September, and seedlings were transplanted into the polyethylene pot (9cm diameter) which fill up the seterilized soil. After about a month to wait raise the seedling on the stage of 3-4 foliage leave, It was on 30th, October, inoculation was done by dipping method to use isolate No. 21 with 10 ml suspension per plant. (Because this method is most suitable to clarify the difference of resistance among the varieties. If we choose the injection method, the symptoms will be too severe so it is difficult to clarify the difference of resistance between medium resistant with rather strong resistant.)

And for this experiment, inoculation beds (1m x 4m x 10cm, with sterilized sandy soil) were prepared in the glasshouse. Observation was done on 7, 9, and 11 days after inoculation, and the symptoms of each plants were recorded by means of symptom classified method as shows in the table No. 1 (page No. 9-10). After observation, disease index and percentage of infected plants were calculated by the formulas as shows in the table.

(b) Result;

The result of the screening test is shown in the table No. 1 (page No. 9-10). Although "Chusei-Shinkuro" which is susceptible crontrol variety was severely damaged, "D. M. P" and "Taiwan Naga" which are resistant control varieties were slighly infected by the Bangladeshi bacterial wilt caused bacteria.

These results were very similar to the experiment which we had done in Japan, so that, it is clear that inhere is not any qualitative difference between the isolaties of this experiment and in Japan. Among the indigenus varieties, "Khatkatia long" shown higher resistance as like "D. M. P", and "Nayankazal" was rather resistant to the bacteria. But the other hand, "Baromashi" and some other varieties were very susceptible rather than Japanese susceptible long fruit varieties. Then, it was cleared that there is a great variation of bacterial wilt resistance of eggplant varieties in Bangladesh. In case of wild relatives, S. mammosum showed very highly resistance, but S.integrifolium and S. pumelo which had been collected in Sylhet and widely ranged in this country, were completely susceptibel.

(c) Discussion;

Although every scientist know that the Indian subcontinental is the "gene-center" of several crops including the eggplant, there was'nt any effective breeding work to screen out on resistant materials to the bacterial wilt in this country. I had thought that there must be resistant materials in this country, and I could find out a promising material, " Khatkhatia long".

The heredity of the bacterial wilt resistance is usually polygenic, so it is a little difficult to improve the variety which has enough resistance. But it is not impossible to improve the resistant variety with carful mass-selection. The selection should be done under severely infected condition.

For that purpose, breeders use both infected field and inoculation bed. Although we can observe both resistance and economical characters in the infected field, but it is very difficult to maintain the field. So, usually we use the inoculation bed, and I've made the bed in the glasshouse at C.V.R.C. Although "Khatkhatia long" is resistant to bacterial wilt, but it is not enough to use only one variety as breeding material. Because we don't know whether possible combination of characters will be suitable for this country or not. Sometimes resistance varieties have inferior characters. In order to solve such a kind of problem, we should search more resistant materials, " D. M. P " and " Taiwan Naga " are resistant and originated from tropical or sub-tropical area, so if these economical characters will be suitable, these varieties will be promising S. mammosam is completely resistant, so we can use this wild relative for root-stock if it has the glafting compatibility with cultiver.

In future, resistant varieties will be improved in this country, but resistant varieties are not allmighty to solve the disease problem. Resistant varieties should be use with other effective control method, otherwise these varieties will be damaged within a few years.

Table No. 1

Serial No.	Name of Variety	7 days after inoculation						9 days						11 days						
		Symptom Class				D.I.	P.I.	Symptom Class				D.I.	P.I.	Symptom Class				D.I.	.I.	
		0	1	2	3		%	0	1	2	3		%	0	1	2	3		%	
1.	Islampuri	12	14	0	1	0.63	56	2	16	3	6	1.48	93	4	9	3	11	1.78	85	Local
2.	Nayankazal	25	1	1	0	0.14	7	9	15	0	3	0.89	67	9	11	0	7	1.19	67	-do-
3.	Bhanger	22	3	2	0	0.26	19	2	14	4	7	1.59	93	4	2	0	21	2.41	85	-do-
4.	Sadha Jumki	7	7	3	10	1.59	74	0	4	3	20	2.59	100	1	0	0	26	2.89	96	-do-
5.	Khatkhatia long	23	2	0	2	0.30	15	20	4	0	3	0.48	26	18	2	0	7	0.85	33	-do-
6.	-Do- round	25	2	0	0	0.07	7	16	8	1	2	0.59	41	12	3	0	12	1.44	56	-do-
7.	Muktakeshi long	16	8	1	2	0.59	41	9	3	0	15	1.77	67	6	0	0	21	2.33	78	-do-
8.	-Do- round	13	6	1	7	1.07	52	3	9	1	14	1.96	89	4	1	0	22	2.48	85	-do-
9.	Baromashi	11	6	0	10	1.33	59	2	6	3	16	2.22	93	0	0	0	27	3.00	100	-do-
10.	D.R.Chowdhury	17	5	2	3	0.67	37	2	9	0	16	2.11	93	4	5	0	18	2.19	85	-do-
11.	Longla	17	7	2	1	0.52	37	9	1	5	12	1.74	67	4	2	1	20	2.37	85	-do-
12.	Shingnath	14	7	2	4	0.85	48	2	13	1	11	1.78	93	3	1	0	23	2.59	89	-do-
13.	Baraft Kuli	4	5	0	7	1.63	75	0	0	0	16	3.00	100	0	0	0	16	3.00	100	-do-
14.	Malaysia	12	9	2	4	0.93	56	3	3	1	20	2.41	89	5	1	0	21	2.37	81	Malaysia.
15.	Okitsu No.1	21	2	1	3	0.48	22	8	3	5	11	1.70	70	3	0	1	23	2.63	89	Japan
16.	Dingars Multiple purple	25	1	1	0	0.11	7	15	11	0	1	0.52	44	20	0	4	3	0.63	26	-do-
17.	Taiwan Naga	25	1	1	0	0.11	7	16	8	0	3	0.63	41	14	2	0	11	1.30	48	-do-

Cont. Table No. 1

Serial No.	Name of Variety	7 days after inoculation Symptom Class				D.I.	P.I	9 days Symptom Class				D.I.	P.I.	11 days Symptom Class				D.I.	P.I	
		0	1	2	3			0	1	2	3			0	1	2	3			
18.	Kabe Naga	9	10	3	5	1.15	67	2	3	0	22	2.56	93	0	2	0	25	2.85	100	Japan
19.	Nagasaki Naga	26	0	0	1	0.11	4	13	12	0	2	0.67	52	12	3	0	12	1.44	56	-do-
20.	Kumamoto Naga	27	0	0	0	0.00	0	8	11	0	8	1.30	70	5	0	0	22	2.44	81	-do-
21.	Kurume Naga	24	2	0	1	0.19	11	16	6	0	5	0.78	41	13	1	1	12	1.44	52	-do-
22.	Sadohara Naga	17	2	3	5	0.85	37	7	6	1	13	1.74	74	4	1	0	22	2.48	85	-do-
23.	Chusei Shinkuro	10	4	1	12	1.56	63	3	2	0	22	2.52	89	0	0	0	27	3.00	100	-do-
24.	S. integri folium	14	9	3	1	0.67	48	1	8	7	11	2.04	96	1	3	2	21	2.59	96	Wild relatives
25.	S. mammosum	27	0	0	0	0.00	0	25	1	0	1	0.15	7	22	3	1	1	0.30	19	-do-
26.	S. pumilo	4	10	0	13	1.81	85	2	0	0	25	2.78	93	2	0	0	25	2.78	93	-do-

inoculation date : 30.10.1980

Symptom Class
- 0 no symptom
- 1 Slightly wilting
- 2. rather wilting
- 3 sevierly wilting or dead

D.I. (disease index) $$D.I. = \frac{1 \times n_1 + 2 \times n_2 + 3 \times n_3}{n_o + n_1 + n_2 + n_3}$$

P.I. (Percentage of plant infected) $$P.I. = \frac{n_1 + n_2 + n_3}{n_o + n_1 + n_2 + n_3} \times 100$$

n_o, n_1, n_2, n_3 No. of plants in each symptom class.

3. Isolation of Fusarium oxysporium from the infected plants;

Watermelon is one of the most favrite fruit vegetable in Bangladesh. But sometimes, Fusarium wilt causes heavy loss to watermelon production in every year. Unfortunately there is no research work on Fusarium wilt of cucurbitaceae crops in this country. In order to make progress in this field, we taught to the Bangladeshi researchers how to isolate the *Fusarium oxysporum* from the infected plants at first.

(1) Collection of infected plants;

At first, we tried to collect the infected plants of cucurbitaceae crops, but it was too early to find the materials. Then instead of the cucurbitaceae crops, we collected the infected tomato and cauliflower crop from Kashimpur A.D.E, B.A.D.C, on 25th October.

(2) Isolation of Fusarium oxysporium;

In order to isolate the causal fungi, *Fusarium oxysporium* we used Komada's media. Composition of the Komada's media is as follows:

Basal medium	K_2HPO_4	1.0g
	KCl	500.0mg
	$MgSO_4 \cdot 7H_2O$	500.0mg
	Fe-EDTA	10.0g
	L-asparagine	2.0g
	D-galactose	20.0g
	Agar	15.0g
	Distilled water	1.0 liter

Antimicrobial supplement	PCNB (25% wp)	3.0 g
	Oxgall	500.0 mg
	$Na_2B_4O_7 \cdot 10H_2O$	1.0 g
	Streptomycine sulfate	1.0 g

The basal medium was cooled to about 50°c, then supplied with antimicrobial agents and ajusted the acidity to PH 3.8 with diluted phosphoric acid, immediately before pouring to petridish.

Surface of the infected stems were sterilized by ethyl alcohol, and the stems were crushed on a few drops of sterilized water in sterilised petridish to get the suspension of microrgans. The suspension was diluted with sterilized water and poured on the Komada's media in petridish.

After 7 days inculation under room temperature, we found many colonies of fungi and a few colonies of bacteria on the media. But in case of Komada's media, these fungal colonies are almost Fusarium species, and we can easily identify the species of Fusarium or form (pathogenic types) of F. oxysporum by the color of the colonies.

Thus we have several numbers of isolates which were isolated on the Komada's media. The characteristics of these isolates are as follows:

Sl.No.	Isolate No.	Colour of colony on the Komada's media	Host
1.	T 3-1	Pink	Tomato
2.	T 1-3	-do-	-do-
3.	C 1-1-1	-do-	Cauliflower
4.	C 3-1-1	-do-	-do-
5.	C 3-2-2	-do-	-do-
6.	C 3-2-2	-do-	-do-
7.	C 4-1-1	-do-	-do-
8.	C 4-2-1	-do-	-do-
9.	C 4-3-1	-do-	-do-
10.	C 3-1-3	White	-do-
11.	C 4-2-2	-do-	-do-

Although these were isolated on the Komada's media, but in order to identify them exactly, we must check the other characteristics such as pathogenicity to various kinds of crops, shapes of hypha or spore and so on. But we can isolate the causal fungi of Fusarium wilt of watermelon from infected plants with same procedure.

4. Study and observation of eggplant chromosomes under microscope

In order to make progress in the field of interspecific hybridization, it is necessary to observe the chromosomes. But our local researchers don't have enough experience to observe the chromosomes even in their university days. So we set some experiment to show them how to observe the chromosomes of eggplant.

(1) Acetocarmine method;

The seeds of Islampri, Bhangar and Baromashi were treated with G.A solution and sowed into petridish with filter paper, After germination , root tips (5-10 mm) were collected for observation.

The procedure to observe the chromosomes is as follows:

(a) Pre-treatment:

Colchicine method - dip the fresh materials into 0.05% colchicine water solution for 2-3 hours.

Cold water method - dip the fresh materials into cold water (5°c) for 10-24 hours.

Oxyquinoline method - dip the fresh materials into 0.02M 8 hydroxyquinoline water solution for 2-3 hours.

After pre-treatment, materials are sometimes kept in 50-75% ethyl alchol water solution until observation.

(b) Observation:

Root tips are put on a few drops of acetocarmine with 10% 1N HCl on the slide glass, and heated by the spirit lamp for maceration. Then covered with cover glass and smashed with finger smoothly to spread the cells. The technique of smashing is a little difficult for beginners, but it is impossible to get a clear figure of chromosomes without it. Observation is done under microscope and usually we use 500~1000 magnifications to count the number of chromosomes of each cells.

Finally we could abserve the chromosomes of eggplant, but there weren't enough practices for local reseachers. So they should continue to observe untill they can observe the chromosomes and count the numbers of chromosomes by themselves.

(2) Feulgen's method

Feulgen's method is one of the most effective method to observe the chromosomes. Although we don't have enough chemicals for this method, but we can get them in the near future. So I taught the procedure of this method to local researchers. The procedure is as follows:

(a) dip the materials (fresh or fixed) in 1N HCl water solution in test-tube, and keep the test-tube at 60°c for 5-15 minutes by waterbath.

(b) Keep the materials in the Schiff's reagent for 2-3 hours.

- procedure to make the Schiff's reagent -

1 g. of fuchsin (basic) is added to 200 cc of boiling water and melted. The solution is filtered when the temperature is at 50°c, and 20cc of 1N HCl water solution is added. 1 g. of $Na_2S_2O_5$ is added when the temperature is at 25°c. Then keep the solution in a refrigerator (below 5°c) for more than one day.

(c) Wash the materials by the $Na_2S_2O_5$ solution.

- composition of the $Na_2S_2O_5$ solution -

$Na_2S_2O_5$	0.5 g
1N HCl	5 cc
pure water	100 cc

(d) Preparation is made by means of smashing and observed under microscope.

Screening of eggplant germplasm for bacterial wilt resistance

Fig. 1 Eggplant cultivation naer Kashimpur A.D.E.

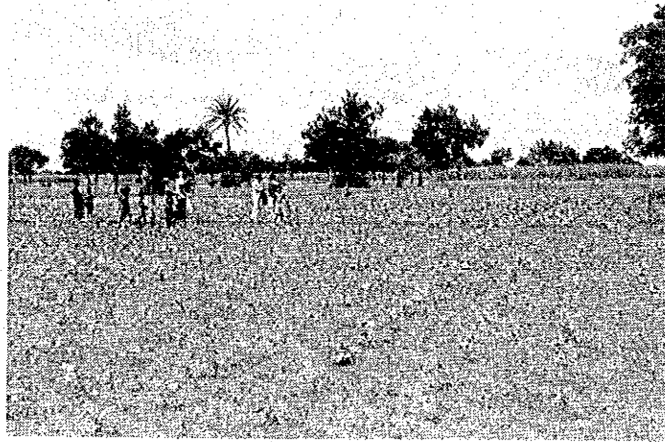
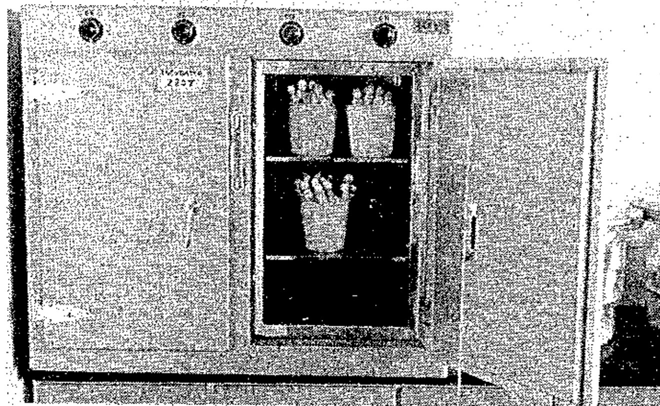


Fig. 2 - do -



Fig, 3 Incubation of the bacteria, Pseudomonas solanacearum

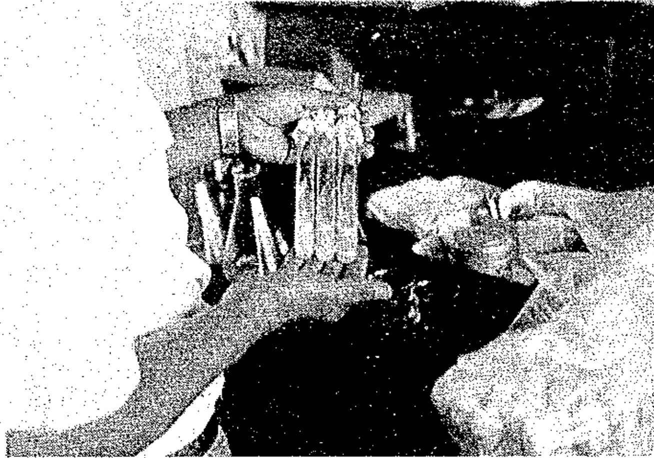


Fig. 4 Bacteria cultivation on PDA slant medium

Fig. 5 Preparation of bacteria suspension from testtube cultuer

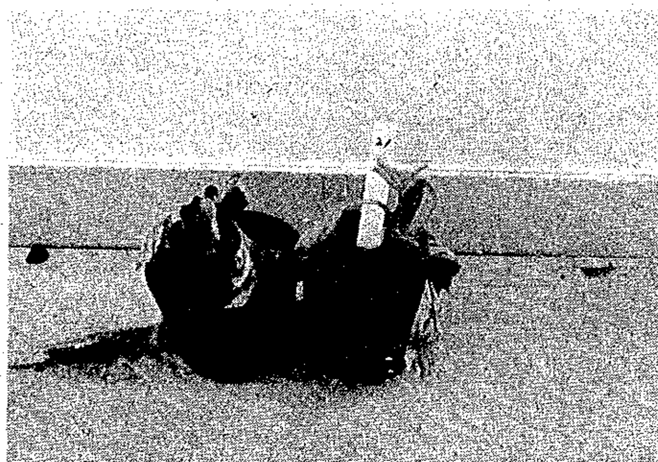


Fig. 6 Symptom of bacteria wilt inoculated with injection method

Fig. 7 Pathogenesity test of the bacteria isolates

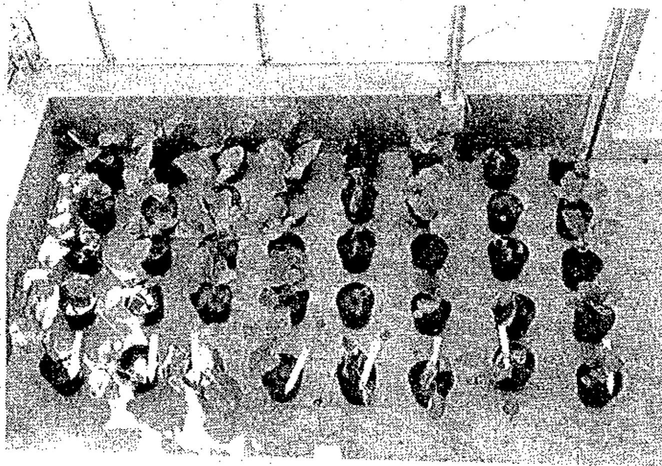


Fig. 8 - do -

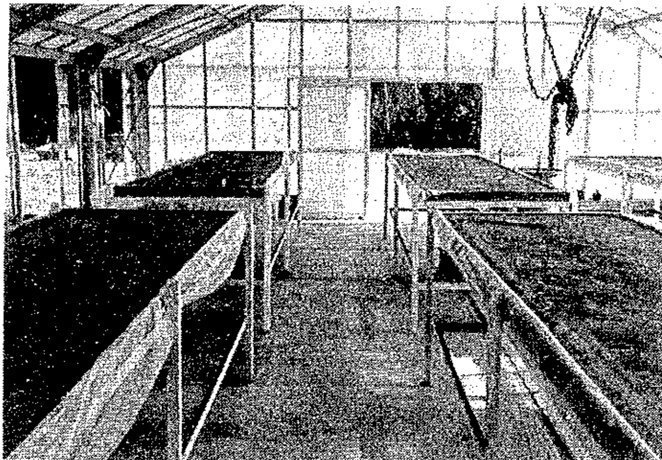


Fig. 9 Inoculation bed in glasshouse

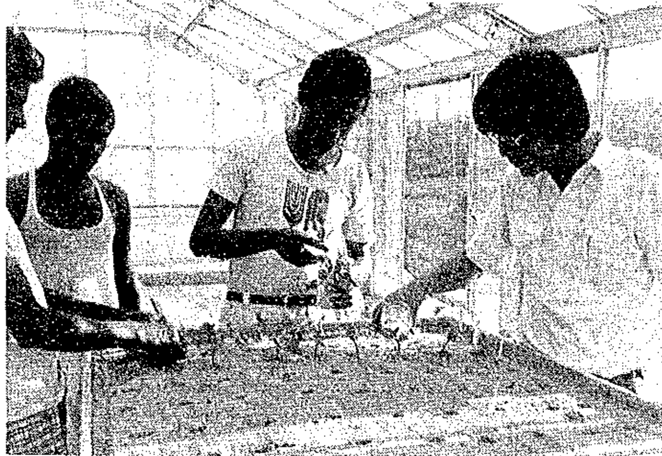


Fig. 10 Inoculation by dipping method

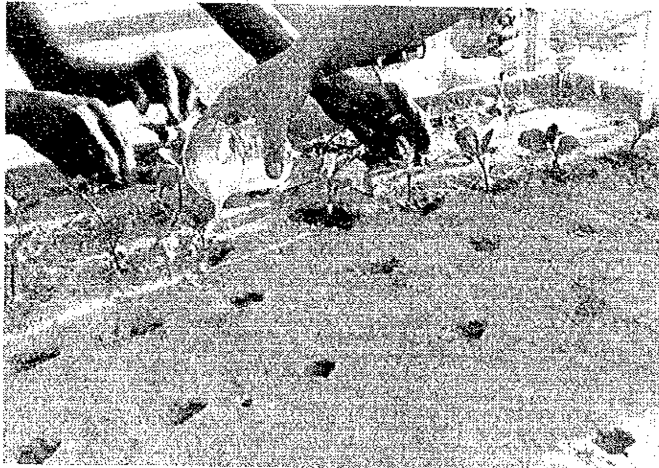


Fig. 11 - do -

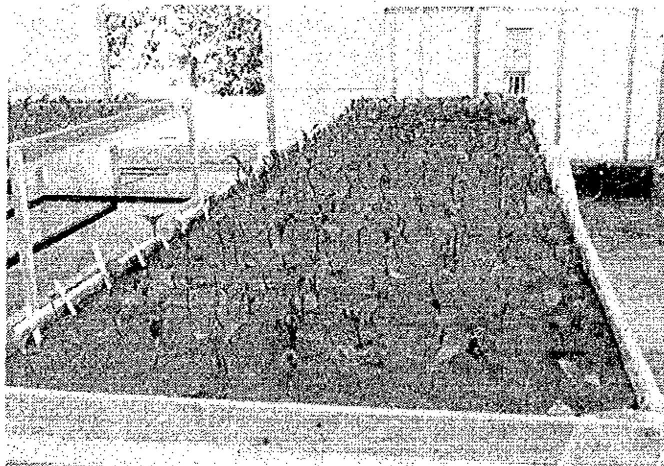


Fig. 12 Variety trial for bacterial wilt resistance

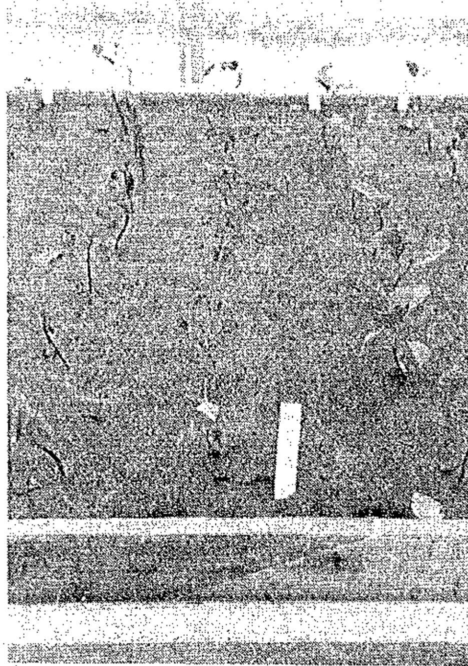


Fig. 13 - do - center:susceptible variety
right:resistant variety

Fig. 14 Preservation of the bacteria in soil

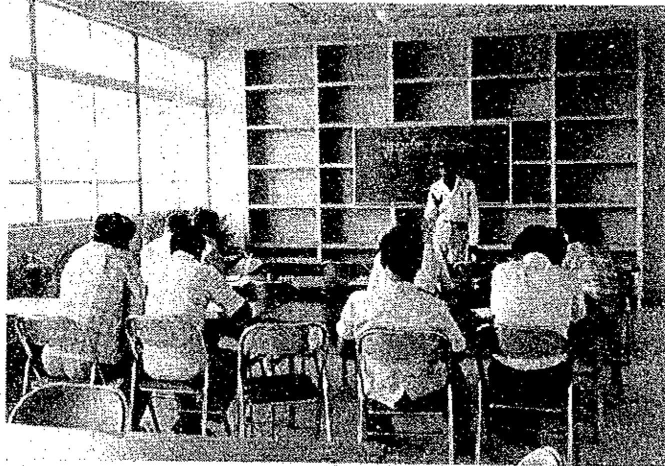


Fig. 15 Seminar on this experiment by Mr.Roshid

Isolation of Fusarium oxysporum from infected plants

Fig. 16 Transplant of the fungi,Fusarium oxysporum from petridish cultuer to testtube slant medium

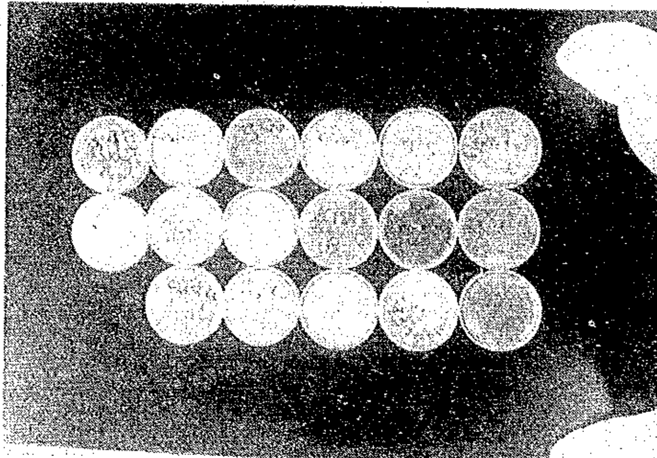


Fig. 17 Isolates of fungi from infected tomato and cauliflower

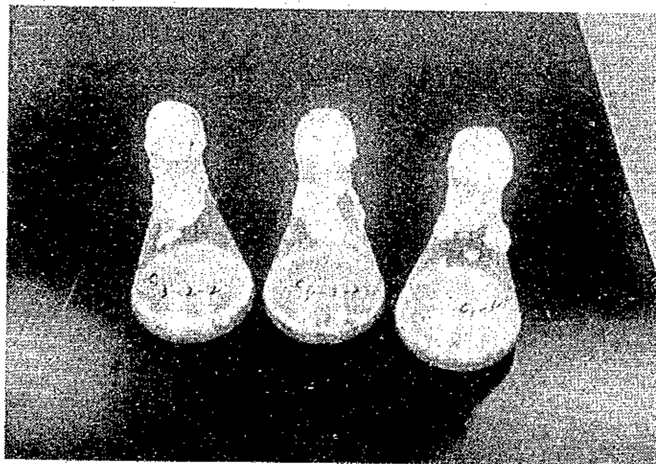


Fig. 18 Multiplication of the fungi for inoculation

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