OBSERVATIONS ON THE WILT DISEASE OF THE BANANA

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Received March 3, 1956

The wilt disease of banana is prevalent in most of the countries where banana is grown, being caused by *Fusarium oxysporum* var. *cubense*. It affects several varieties and is known to be particularly severe on Gros Michel. The Cavendish group contains varieties known to be immune or highly resistant to wilt.

In India the disease has been gaining in importance in recent years. It was first reported from Chinsurah in 1911 (Basu, 1911). Since then reports of its occurrence in Bombay, Madras and Hyderabad are increasing. In Bombay the Kali and Basrai varieties are known to be resistant (Uppal, 1940). In Madras the varieties Monthan, Rasthali, Pachanadan and Tirunelveli Peyan suffer more from wilt, than Poovan. Park (in Wardlaw, 1935) has recorded that Mondan, Sonamondan, Kattumondan and Kolikkuttu varieties were found to be susceptible in Ceylon. Owing to the increased incidence of the disease in Madras State in recent years a detailed study of the malady was undertaken and the results obtained are described in this paper.

In the Madras State over a hundred varieties of bananas are under cultivation. Some varieties have a restricted region of distribution while others are more widely grown. Sundararaman (1928–29 to 1934–35) found that the disease was confined to a few varieties in each district. In Tiruchirapalli District, Rasthali was affected while in Madura, Chinnamondan and Vellai were involved. He has also reported that Monthan was infected in more than one district. Pachanadan, Kullan and Anaikomban did not become affected even on artificial inoculation. It was also reported that the pathogen from the wilted plants had been identified by Wollenweber to whom the cultures had been sent as *F. cubense* var. *inodoratum*. Recently the disease was found to be prevalent in other districts also. The variety Neypoovan was severely infected at the Agricultural Research Station, Anakapalle, in 1949. On the Lower Pulneys the incidence of wilt is increasing in the variety Sirumalai. In Tirunelveli the variety Peyan is being severely damaged year after year. Monthan and Pachanadan are being destroyed in large numbers in Coimbatore District. The disease is observed to be more in light

soils and garden land conditions than in the wet lands. The incidence of wilt is widespread in the State of Madras.

MATERIALS AND METHODS

Detailed investigations were carried out on the reaction of over twenty vaieties of bananas common in the State and the cultural characters of the isolates of the pathogen were studied. The banana plants were raised from suckers carefully selected from an area where wilt had not been noticed at any time. They were planted in big mud pots filled with garden soil. The pathogen was isolated from single spores and maintained on Brown's agar. In testing the reaction of the varieties the isolate from Monthan was used. The agar media were adjusted to pH 6 except where otherwise stated. The cultures were maintained at the laboratory temperature (26–31° C.). Measurements of spores were always made from cultures 21 days old.

EXPERIMENTAL RESULTS

In order to standardise the inoculation technique and select the most effective one for general adoption Monthan plants were inoculated in different ways. Cultures of the pathogen grown on sterilised bits of the rhizome or sand-oats medium were mixed with the soil in the pots after the plants had established; suspensions of the spores were poured into the soil at the base of the plants; the roots of the plants were exposed in four places and the culture of the fungus grown on oats agar was placed in contact with them and covered up with soil. Successful and consistent infection was obtained with the first two methods and these were adopted in all later infection experiments. The plants wilted completely in sixteen weeks.

Varietal Reaction.—Eighteen varieties were included in these trials to assess their susceptibility to this disease. The plants were inoculated after they had established in the pots and had begun to put forth fresh leaves. The results are given in Table I.

The control plants in all the varieties remained healthy and grew normally. The infected plants were invariably stunted and had thinner pseudostems. The wilting of the plants and the splitting of the outer sheaths were characteristic. On removal from the pots it was found that in many cases the roots were discoloured and had been destroyed to a large extent. When the rhizome was cut open the characteristic violet streaks and patches were visible. In all cases the pathogen was reisolated from the infected plants. Five varieties were not infected at all. Nor did they exhibit any stunting of the growth. These were Moongil, Poovan, Peyladen, Rajabale and Vamanakeli. In the field also the disease is very rare in the last variety.

TABLE I

Varietal Reaction

Variety	No. inoculated	No. infected	Incubation period	Remarks
			Weeks	
Chakkarakeli	5	3	16	Infected plants wilted. tissues of rhizome discoloured
Gros Michel ..	5	5	8	,,
Kali ..	5	5	12	,,
Kapur ..	5	5	12	,,
Kullan ..	4	2	14	Two wilted, others stunted.
Mindoli ..	5	2	16	,,
Monthan ..	5	5	16	All wilted
Moongil ..	5	Nil	..	All healthy
Neypoovan ..	5	5	9	All wilted
Pachanadan ..	5	5	12	,,
Peyan ..	5	5	12	,,
Peyladen ..	5	Nil	..	All healthy
Poovan ..	5	,,	..	,,
Rajabale ..	5	,,	..	,,
Rasthali ..	5	5	9	All wilted
Sirumalai ..	5	5	12	,,
Vamanakeli ..	5	Nil	..	All healthy
Vellaivazhai ..	5	5	16	All wilted

Vamanakeli belongs to the Cavendish group which is reputed to include several resistant and immune varieties. But Kullan which also belongs to the same group was partially infected. It is satisfying to note that there are a number of varieties which exhibit resistance to this disease. But attempts are being made to popularise the variety Gros Michel. A note of warning must be sounded here. This is a highly susceptible variety and its expansion will only result in the spread of this wilt.

In addition to the trials in the pots, infection experiments were conducted under field conditions, in wet lands. Healthy suckers of 28 varieties were planted in March after ploughing in a green manure crop of sunnhemp (*Crotalaria juncea*). The varieties were planted in two blocks, one for inoculation and the other to serve as control. After the plants had established the plants in one block were inoculated by mixing the contents of one 1000 c.c. flask of sand-oats culture of the pathogen with the soil, round each plant. At the end of sixteen weeks it was found that most of the plants excepting those of Poovan, Rajabale, Moongil, Peyladen and Vamanakeli

were much stunted and had thinner pseudostems. Young plants growing in inoculated soils have been observed to be severely stunted almost from the start in other countries also (Wardlaw, 1935). However all the inoculated plants of Kunnan, and Thattillakunnan wilted. In Karivazhai half the number of plants died while in Anaikomban, Ayiramkai Rasthali and Chenkadali a third of the plants wilted. The pathogen was reisolated from the affected plants in all cases. The control plants which were in a separate block were all robust and normal. The results also showed that infection does not spread rapidly under wet land conditions as in the pots. Therefore such conditions do not appear to be ideal for testing the susceptibility of the varieties.

The spread of infection from the mother plant to the suckers could be readily traced in many on cutting through the rhizome. There was significant difference between the root system of the infected plants and the controls. In the latter many long whitish roots could be seen while in the wilted plants the roots were few and most of them were dead and brown in colour. In a few all the roots had been destroyed.

The pathogen readily infects some varieties of banana but not others. In order to find out whether the resistance of some of the varieties is due to any inhibitory influence of the sap, the fungus was grown on media to which the sap of particular varieties had been added. Extracts of the rhizome and roots of Monthan, Gros Michel, Vellaivazhai, Poovan, Peyladen and Vamanakeli were prepared and filtered through Chamberland's filter. These were added to Brown's agar at the rate of 10 c.c. to every 100 c.c. of the medium. The pathogen grew well on all these media and no difference could be made out in the rate of growth of the fungus on Brown's agar and on those to which the extracts had been added. Hence it is concluded that the sap does not exert any inhibitory influence (at the concentration used). The resistance must be attributed to some other factor.

Host Range.—To determine whether any other local economic crop plants served as collateral host for the pathogen, inoculation experiments were conducted on plants allied to banana and others known to be parasitised by other species of *Fusarium*. These included *Canna indica*, *Curcuma longa*, *Maranta arundinacea*, *Zingiber officinale*, and three-weeks-old plants of *Allium cepa*, *Cajanus cajan*, *Capsicum annuum*, *Crotalaria juncea*, *Cyamopsis tetragonoloba*, *Gossypium arboreum*, *Lycopersicum esculentum* and *Nicotiana tabacum*. The inoculations were made by adding the culture of the fungus grown on sand-oats medium to the soil at the base of the plants. In no case was there any evidence of infection. The inoculated and the control

plants were alike healthy. The pathogen appears to have a restricted host range.

Studies of the Pathogen

Cultural Characters.—To begin with, the pathogen was isolated from diseased Monthan plants and the characters of this isolate were observed. It grew well on the common laboratory media filling the Petri-dishes (10 cm.) in 8 days. Some differences were however noticed in the nature of growth, pigmentation and sporulation on the different media and these are described in Table II.

Table II

Characters of the Isolate from Monthan

Particulars		Potato dextrose agar	Oats agar	Brown's agar	Cooked rice
Growth		Mycelium submerged	Luxuriant aerial	Little aerial	White, loose
Pigmentation of medium		Shell pink with patches of indigo blue	Purple vinaceous to claret red turning pompeian red	Not appreciably pigmented	Starting with hermosa pink turns to violet purple
Odour		No odour	No odour	No odour	Lilac odour
Sporulation		++	+++	+	+++
Chlamydospores		Single or in pairs, 5–6×5–6μ	Single or paired 6–9×5–9 μ	Mostly on conidia, single or paired 7–12×5–9 μ	Very rare
Septation groups and size of conidia in μ	0–	5·5–12·5×2·5–3·5 6×3	5·5–12×2·5–3·5 8×3	5–11×3–3·5 10×3·5	5–12×2·5–3·5 8×3
	1–	5·5–14×2·5–4 9·5×3	8–12·5×2·5–3·5 11×3	8–15·5×3–5 12×4	7–20×2·5–3·5 12×3
	2–	8·5–18·5×3 13×3	15–28×3–4·5 20×4	15·5–31×3–5 24×4	10–20×3–5 13×3·5
	3–	22–43×3–5 30×3·5	25–45×3·5–4·5 31×4	18·5–50×4–5 33·5×4·5	..
	4–	28–47×4–5 36·5×4·5	28–43×3·5–4·5 33·5×4	31–43×4–5 36×4·5	..
	5–	28–43×4–5 37×4·5	28–46×4–5 33×4·5	31–53×4–5 43×4·5	..

[*Note.*—The intensity of sporulation is indicated by + mark, the increase being denoted by the increase in the number of marks. The measurements are shown in two lines in each septation group, the upper figures denoting the range and the lower ones the average.]

The pigmentation varied with the medium, no pigment being formed in Brown's agar. Maximum sporulation was obtained on oats agar and cooked rice. Lilac odour was conspicuous in the culture on cooked rice. On the other media no odour could be detected. Chlamydospores and sickle-shaped macroconidia were very rare on cooked rice.

Temperature and Growth of the Isolate.—The temperature relations of the isolate were determined by growing it under different constant temperatures. The choice of the range of temperature was limited by the available equipment. The diameter of the growths was measured at 24-hour intervals and are recorded in Table III. The inoculum was cut out with a sterilised cork-borer giving 8-mm. discs.

TABLE III

Temperature and Growth of the Monthan Isolate

No. of days	Diameter of the growth in millimetres at					
	5° C.	15° C.	30° C.	33° C.	35° C.	37° C.
1	8	8	14	8	8	8
2	8	14	27	12	8	8
3	8	20	42	18	8	8
4	8	28	54	26	8	8
5	8	32	69	30	8	8
6	8	38	82	36	8	8
7	8	44	Dish full	42	8	8

The maximum growth was at 30° C. among the temperatures included in the trials. The growth is slowed down considerably at 15° and 33° C. practically to the same extent. The dishes kept at 5°, 35° and 37° C. were taken out and left at the laboratory temperature (27°–29° C.). In 24 hours, growth was evident in the first two sets but not in the dishes exposed to 37° C., indicating thereby that the fungus had lost its viability by such long exposure to this temperature.

Other Cultural Characters.—The pathogen was able to tolerate a wide range of pH, *viz.*, from 3 to 7·5. The best growth was at pH 4 to 6. The capacity of the organism to utilise different sources of nitrogen was tested by adding peptone, asparagin, urea, potassium nitrate and sodium nitrate, to a basic medium lacking in nitrogen. When these substances were added in such quantities as to contain equivalent amounts of nitrogen no differences could be made out between them either in the rate of growth or in the intensity of sporulation (up to one gram of nitrogen per litre of the medium).

Meridith (1941) has reported that monthly applications of two ounces of sodium nitrate to the soil per plant was beneficial. The spores are stated to be inactivated by adding one part of sodium nitrate to five parts of soil culture. But in our studies it was found that there was no effect at the concentration used. However the addition of 0·5 and 1 per cent. of urea to the medium had an adverse effect on the growth and sporulation of the fungus. This substance was added to the normal oats agar and the growths were compared. At the 0·1 per cent. level there was no appreciable difference between the growths and sporulation on oats agar and the urea added one. But as the amount of urea increased to 0·5 and 1 per cent. levels the diameter of the growths was very much reduced and sporulation became scanty.

The isolate was grown in dishes kept in complete darkness, in dishes exposed to diffuse light during the day and darkness during the night and in dishes exposed to continuous light with the help of two 100 watt bulbs, to note the effect of light on growth. There was no difference between them either in the rate of growth or in the intensity of sporulation.

Enzyme Production.—The enzymes produced by the fungus were determined. The capacity of the organism to grow on certain media was taken as an index of the formation of particular enzymes in the mycelium of the fungus (Uppal and Kulkarni, 1937). A basic medium containing $MgSO_4$ 0·5 gm., K_2HPO_4 1·0 gm., KCl 1·5 gm., ferrous sulphate 0·1 gm., agar 20 gm. and distilled water 1,000 c.c. was prepared. To this were added different substances for testing particular enzymes.

Amidase.—This was determined by growing the fungus on the medium after adding 0·5 per cent. asparagin and 5 c.c. of 2 per cent. rosolic acid to a litre of the medium. The medium had a light yellow tint to start with. When the fungus grew the colour changed to bright red indicating that the asparagin was being acted upon by amidase liberating ammonia which was responsible for the reddening.

Diastase.—Two per cent. soluble starch was added to the basic medium. The fungus produced a good growth and when the dish was half covered it was flooded with a solution of iodine. The medium under the growth and a margin of half a centimetre round it remained white while the rest was coloured dark blue. This indicated that the starch in the unstained portions had been utilised by the fungus by the production of diastase.

Emulsin.—Amygdalin was added to the basic medium. There was good growth on this medium showing thereby that the fungus is able to utilise this substance.

Erepsin.—Finely divided casein (one per cent.) was added to the basic medium. The medium was cleared of the bits of casein as the fungus grew, from below the growth and to a small distance in advance of the growth due to the digestion of casein by the production of erepsin.

Lipase.—Litmus cream agar was used to test the presence of this enzyme. The medium under the fungal mat and to some distance all round it assumed a bright red colour indicating the production of acid. Lipase was being produced which acted upon the fat resulting in the formation of acid.

Oxidase.—This was determined by growing the fungus in potato dextrose agar to which 0·25 per cent. of tannic acid had been added (Bavendam's method, in Ramakrishnan, 1941). A deep brown halo developed round the growth indicating the utilisation of tannin by the production of oxidase.

Trypsin.—Egg albumen agar was used. The growth of the fungus was good and the medium underneath the fungus was cleared as the albumen had been utilised, with the formation of trypsin.

Survival of the Fungus in the Soil.—The pathogen survives in the soil. Wardlaw (1941) has recorded that in land subjected to inundation, wilt is negligible. Stover (1954) reports that in soils under one and a half to three feet of water the fungus survived for 25 to 80 days. Under one inch of water in the laboratory the fungus survived from 45 to 165 days. The survival was more at lower temperatures than at 23–24° C. The length of time for which the fungus remained viable in the soil under local conditions was determined. Bits of the rhizome, infected with the fungus, were buried in the soil six inches below the surface in pots under different treatments. In one series the soil was thoroughly mixed with farmyard manure added at the rate of 5 tons per acre. In a second series lime was added to the soil at the rate of one ton per acre. In a third set of pots the soil moisture was maintained at 20 per cent. of its water-holding capacity. The soil moisture was maintained at 100 per cent. of its water-holding capacity in a fourth set and water was allowed to stagnate to a depth of two inches in the fifth set. At intervals of a fortnight isolations were made from the buried bits. The inability to isolate the pathogen was taken as a sign of the loss of viability of the fungus. It was found that the fungus could be isolated from the fifth series of pots (with two inches of stagnant water above the soil level) only for two fortnights after the treatment. In the limed soil the fungus retained its viability for four fortnights. From the other pots it could be isolated even after four months (after which the experiment was discontinued). This indicates that flooding the soil and application of lime to the soil will be useful in eradicating the fungus from the soil. The low incide ce of wilt

under wetland conditions may be due to the inability of the fungus to survive under those conditions in the soil.

Strains of the Pathogen.—During the course of these studies isolations were made from other naturally infected varieties of bananas. Two of these isolates, one from Pachanadan from Coimbatore and another from Kali from Lower Pulneys, were compared with the original isolate from Monthan. All of them were pathogenic to Monthan. But the two former isolates exhibited differences in other characters.

On potato dextrose agar the Monthan strain produced submerged growth while the other two gave rise to woolly aerial growths. On the different media, there were differences between the three in the depth of colour imparted to the medium. No odour was developed on cooked rice unlike in the Monthan isolate. The two new strains produced three to four or three to seven septate macroconidia respectively on cooked rice. Differences were evident in the size of the spores also. Thus more than one strain of the pathogen is present in the country. The pathogenic range of these strains and their distribution have to be taken into consideration before deciding on the suitability of different varieties of bananas to different tracts with reference to the wilt problem.

Summary

A detailed study of the wilt of banana was made. The assessment of the reaction of over twenty common varieties of the host showed that Poovan, Moongil, Peyladen, Rajabale and Vamanakeli were not infected when artificially inoculated. Infection studies conducted under wet-land conditions do not always give a correct picture of the reaction of the varieties but plants grown in big pots react satisfactorily. Susceptible varieties growing in infected soil are stunted in growth even though they may not always be killed. The pathogen does not infect any allied host-plants included in the studies. The resistant varieties do not have any pathogen-inhibiting substance in the sap.

The cultural characters of the fungus are described. The pathogen thrives best at about 30° C. and can tolerate a wide range of pH in the medium. The enzymes produced by the fungus have been determined. The pathogen does not survive in the soil for 45 days when two inches of water are maintained over it. When lime is added to the soil the fungus is inactivated in about two months. Under other conditions it remains viable for over four months. Strains of the pathogen isolated from Pachanadan and Kali exhibited differences in various characters from the isolate from Monthan.

We wish to express our gratitude to the Horticulturist and Professor of Horticulture, Agricultural College, Coimbatore, for arranging the supply of the suckers of different varieties of bananas and to the Superintendent, Central Farm, Coimbatore, for all the help in conducting the field trials.

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EXPLANATION OF PLATES

PLATE VIII

FIG. 1. Wilted Pachanadan in the field.

FIG. 2. Wilted Monthan showing splitting of sheaths.

FIG. 3. Culture of the pathogen in starch agar to which iodine solution was added to show the complete utilisation of starch under the fungus mat.

FIG. 4. Peyan infected; to the left is the control.

FIG. 5. Rajabale not infected; control is in the left extreme.

FIG. 6. Sirumalai infected, control to the left.

FIG. 7. Vamanakeli not infected; control left extreme.

FIG. 8. Vellaivazhai infected plants stunted; control left extreme.

FIG. 9. Kullan infected; control left.

FIG. 10. Kali infected; control left extreme.

FIG. 11. Chakkarakeli infected; control left.

PLATE IX

FIGS. 12, 13, and 17. Monthan, Chakkarakeli and Kullan respectively, showing the reduction and damage to the roots in the diseased plants; control left and diseased to the right.

FIG. 14. Infection spreading to the sucker from the parent rhizome.

FIG. 15. Mindoli infected; control left.

FIG. 16. Pachanadan infected; control left.

FIG. 18. Rasthali infected; control left.

FIG. 19. Kapur infected; control left.

FIG. 20. Neypoovan infected; control left.


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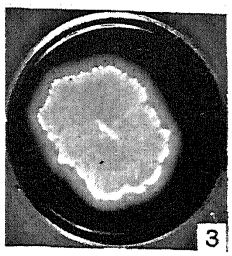
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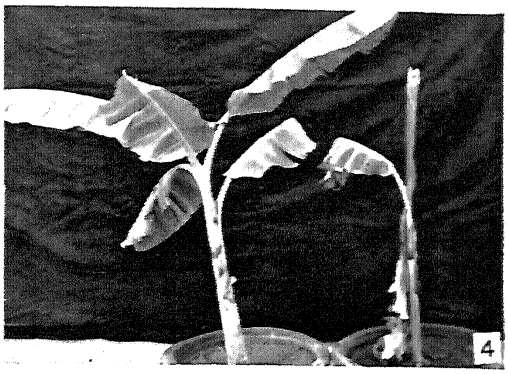
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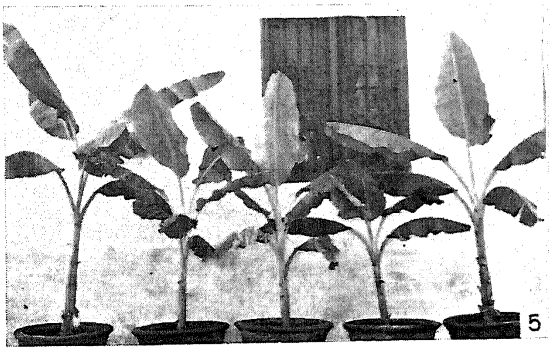
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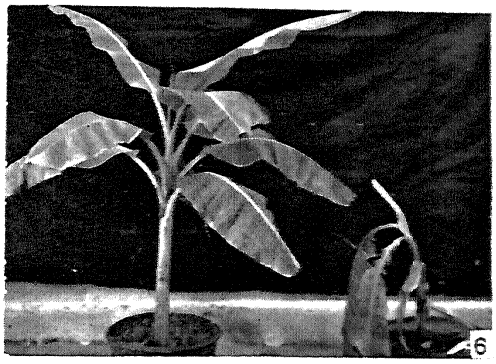
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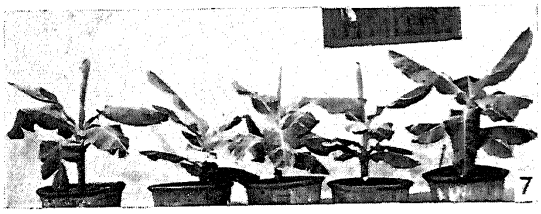
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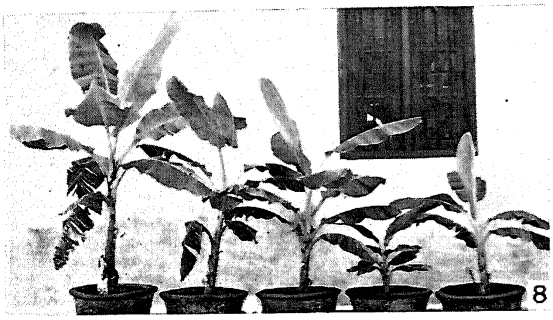
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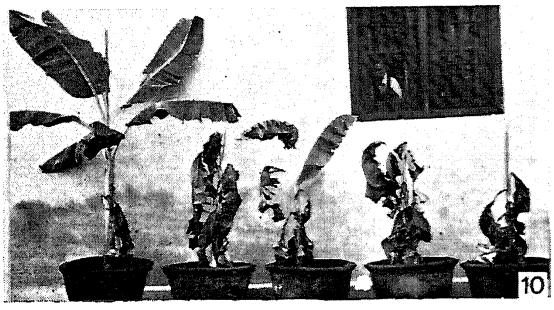
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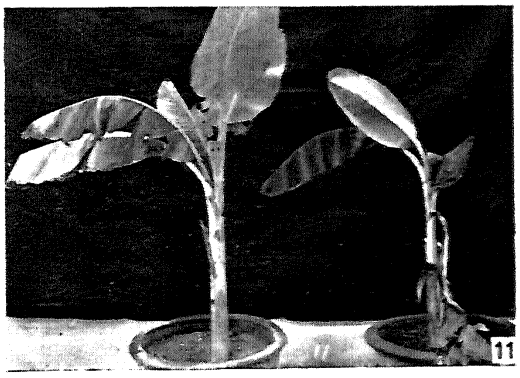
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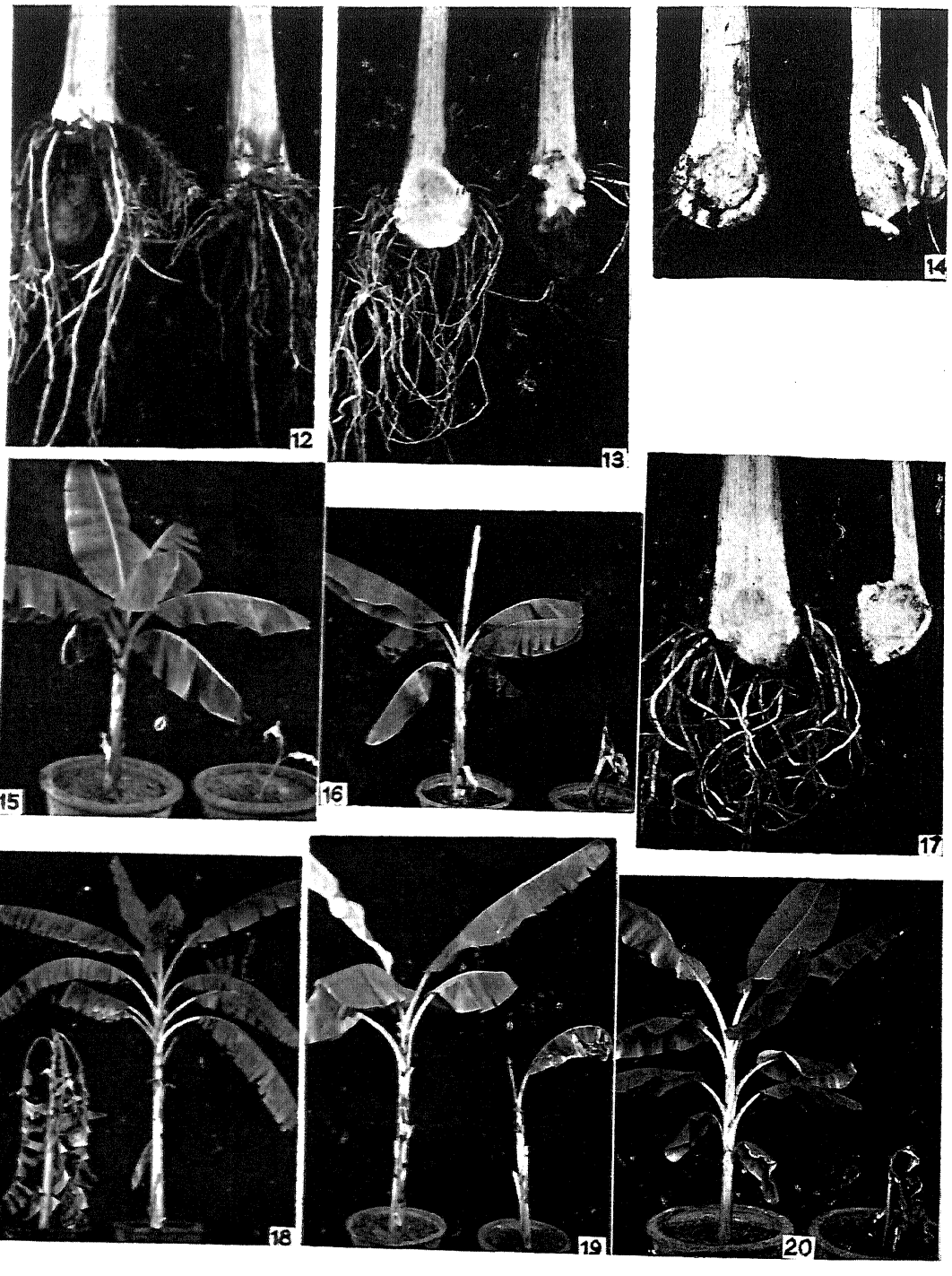
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