TONG METHOD: AN EFFECTIVE SCREENING TECHNIQUE FOR YOUNG CASSAVA SEEDLINGS FOR DETECTING RESISTANCE AGAINST CASSAVA BACTERIAL BLIGHT DISEASE IN ZAIRE

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Abstract

The artificial inoculation of *Xanthomonas manihotis* by the tong method is recommended for detecting resistance in young cassava seedlings, 3 to 8 weeks old, against cassava bacterial blight (CBB) disease, but is not suitable for cassava plants 3 months or older. The technique could use either CBB gum exudates or cut pieces of angular, water-soaked, leaf spots immersed in water for 12 to 24 hours or water solution of pure culture of X. manihotis as inoculum. Tiny, angular, leaf spots developed on inoculated cassava leaves after 24 to 48 hours.

Introduction

One of the problems mostly encountered in screening cassava seedlings for detecting resistance against cassava bacterial blight (CBB) disease is the identification of a technique which is fast, simple, relatively easy to use and results in infection. Although methods of pathogenecity of *Xanthomonas manihotis* had been developed, namely, 1) spraying of inoculum, 2) leaf rubbing 3) stem puncture, 4) petiole puncture and 5) leaf clipping (Anon., 1975; Terry, 1976), for large scale screening work, these methods are time-consuming and inefficient.

The purpose of this investigation was to determine whether the tong method can be developed for efficient resistance screening of very young cassava seedlings against CBB disease in the field. The paper describes the results obtained.

Materials and Methods

The technique employs the use of a tong (a kitchen utensil commonly used for frying bacon) and a scrable (a kitchen aid used for cleaning pots and pans) (Fig. 1).

The sources of inoculum used were obtained either from gum exudates or cut pieces, 2 mm from the edges of angular, water-soaked, leaf spots immersed in sterile water for 12 to 24 hours, or a bacterial suspension of 36 to 48 hours old culture of *X. manihotis*.

Pure culture of *X. manihotis* was obtained from angular leaf spots, previously surface sterilized with 95% ethyl alcohol, cut into small pieces with sterile scalpel and aseptically soaked in sterile water for 1 to 3 hours. Then 0.1 ml dilution of 10^3 to 10^4 bacterial suspension was pipetted into a test tube containing half the concentration of warm nutrient agar, mixed, and poured directly on the surface of a hard nutrient agar plate. After 24 to 48 hours, small colonies of *X. manihotis*, originating from a single bacterial cell greyish white in color, shiny, convex surface, with round, smooth edges, appeared on the plates. A single colony was scraped with sterile loop and streaked over the surface of the agar plate. Serial transfers were made, 2 to 3 days after, to several nutrient agar plates.

Thirty-six 02864 and 30 M'pelolongi seedlings, 3 weeks old respectively, were grown and inoculated in the greenhouse.

Inoculation was made by pressing the leaves and stems between the rough surfaces of the scrabble previously dipped in bacterial suspension (Fig. 2). The untreated check plants were treated in a similar manner using sterile water instead of bacterial suspension.

Cuttings of 541 selected cassava seedlings originating from the cassava stump collection, preliminary and advanced yield trials of 1978, and from the cassava seedling nursery introduced at M'vuazi, Zaire from South America and IITA, were planted in two rows, 5 plants per row, in the field. One row of seedlings, 3 to 8 weeks old, was inoculated by the tong method for screening against CBB.

Results and Discussion

Inoculation of CBB organism in the greenhouse and in the field.

Inoculated seedlings of 02864 and M'pelolongi, using different sources of inoculum in the greenhouse, all developed tiny, angular, water-soaked leaf spots, 24 to 48 hours after introduction of the bacteria. Gum exudation on inoculated stems and die-back were observed 7 to 12 and 20 to 25 days after inoculation, respectively. No symptoms of CBB was observed on the control plants (Table 1).

Inoculated seedlings in the field, using the same sources of inoculum, likewise developed tiny, angular leaf spots after 24 to 48 hours. On susceptible plants, gum exudation and die-back were observed 10 to 14 and 20 to 25 days after inoculation, respectively. No CBB symptoms were observed on highly resistant plants. On resistant plants, only the inoculated leaves developed angular leaf spots which later abscised from the stems and on moderately resistant plants, angular leaf spots likewise developed on inoculated leaves, but the stems showed tiny dark-brown, water-soaked, lesions and no sign of gum exudation. A summary of the results are presented in Table 2.

The technique has the following advantages: 1) the method uses inoculum of either gum exudates or cut pieces of angular leaf spots in water, or a water solution of pure *X. manihotis* isolate, 2) rapid development of angular leaf spots on inoculated seedlings was observed after 24 to 48 hours, thus, identification of the organism or of the disease in question is facilitated. Other methods, namely, spraying of inoculum and leaf clipping, developed angular leaf spots from 5 to 7 days after inoculation (Anon. 1975; Terry, 1976). The rapid development of angular leaf spots may be due to tiny leaf lesions made with the scrabble without oozing of cassava latex from the lesion, thereby, the bacterial cells gain immediate penetration and invasion of the surrounding plant cells at the point

of inoculation. All other methods, except spraying the inoculum, involve oozing of cassava latex at the point of injury. This aspect may have delayed the entry of the bacteria into the plant cells. The advantage of the tong method is that cassava as young as 3 to 8 weeks old can be inoculated in the field.

In conclusion, the tong method is an effective screening tool, for young cassava seedlings against CBB disease.

References

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Table 1. Greenhouse inoculation with *X. manihotis* by the tong method.

Inoculated Test Plants	CBB SYMPTOMS DEVELOPMENT		
	Angular leaf spots (hours)	Gum exudation on the stem (days)	Plant die-back (days)
02864[a]	24 to 48	7 to 12	20 to 25
02864 (control)	–	–	–
M'pelolongi[b]	24 to 48	7 to 12	20 to 25
M'pelolongi (control)	–	–	–

[a]Thirty-six 02864 and a set of 2 of 6 test plants per inoculum used.

[b]Thirty M'pelolongi and a set of 2 of 5 test plants per inoculum used.

Table 2. Field inoculation with *X. manihotis* by the tong method.

Inoculated Cassava Clones in Field	No. of Plants inoculated	No. of Plants selected	Disease categories[b]		
			1	2	3
Cassava stump collection	133	33	85	15	–
PYT and AYT, 1977	88	37	44	39	17
AYT, 15 best INERA clones, 1977	15	7	14	71	14
AYT, 1978	44	36	25	64	11
Cassava seedling nursery introduced from South America and IITA	241	32	84	16	–

[a]Plants selected are showing multiple resistance against the 3 major cassava diseases.

[b]Disease categories are expressed as percentage of the total plants selected in each case, 1) highly resistant, no infection of CBB, 2) resistant, angular leaf spots are present only on inoculated leaves, but no infection on the stem, 3) moderate resistant, angular leaf spots are present on inoculated leaves, stem showed tiny dark brown, water-soaked lesion, but no gum exudation and die-back.

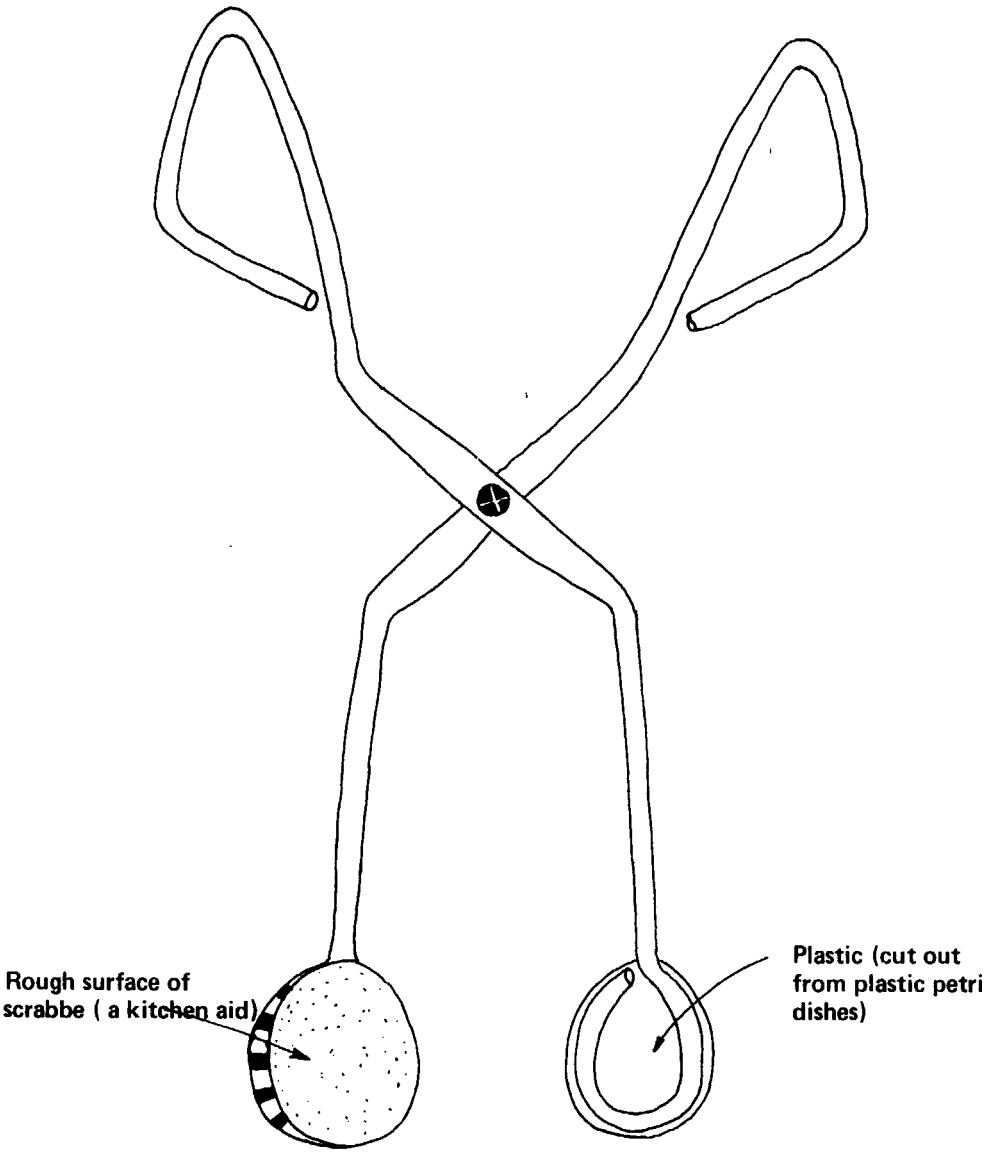

Fig. 1. Tong (a kitchen utensil commonly used for frying bacon) used for rapid inoculation of CBB organism

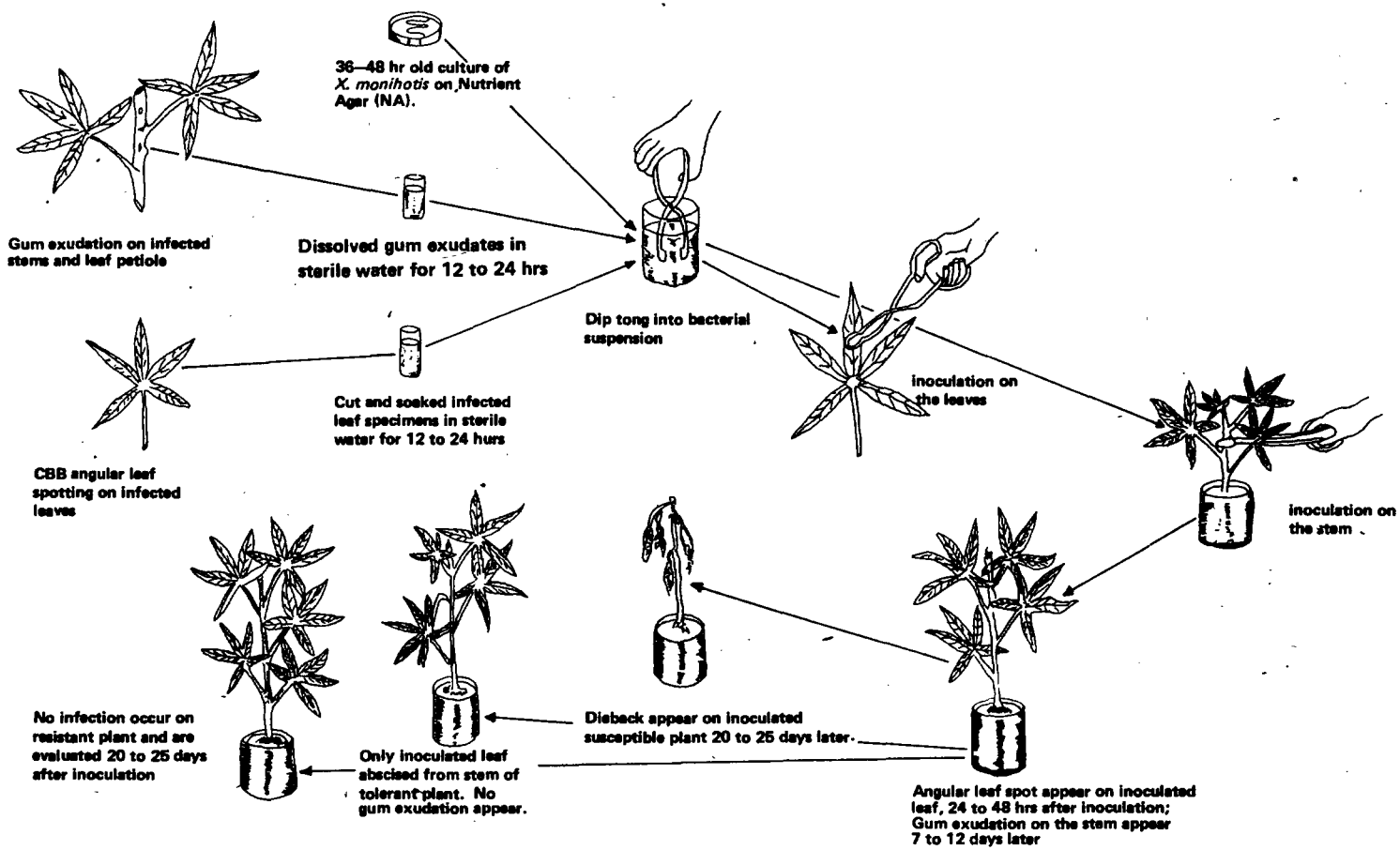

Fig. 2. Tong method for screening resistance against CBB.

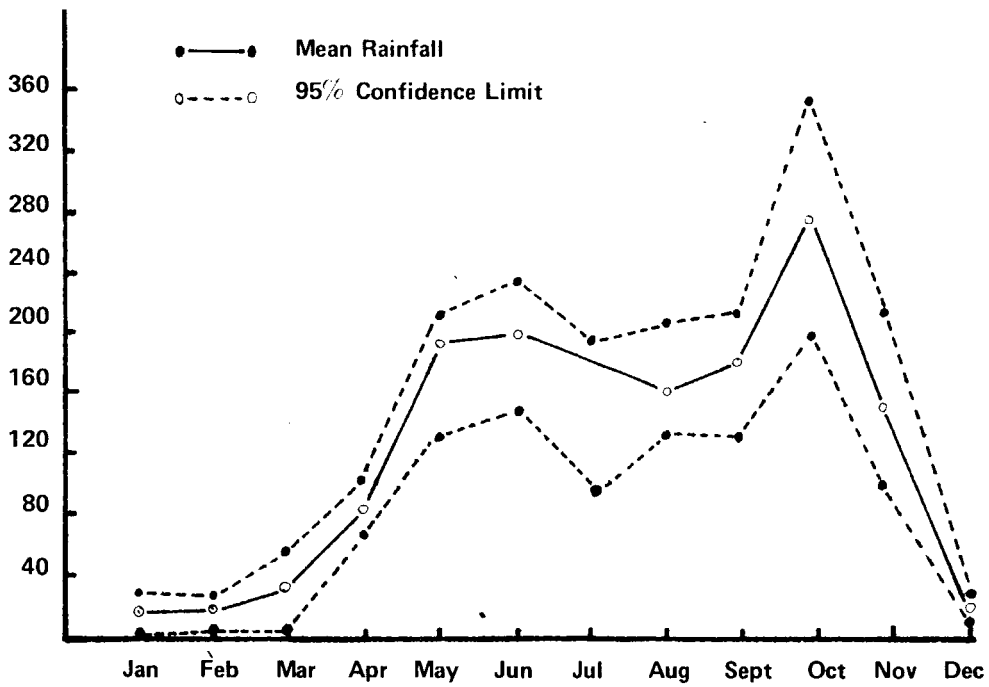

Fig. 3. Rainfall Distribution and 95% Confidence Limits, Media Luna