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SCREENING TECHNIQUES FOR BACTERIAL LEAF STREAK RESISTANCE IN MALAYSIA

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Ringkasan

Saringan secara besar-besaran bagi mencari jenis-jenis padi yang dapat menahan dari penyakit jelalur daun bakteria telah dijalankan dengan cepat dan berhasil dengan menggunakan alat penyembur bermata yang boleh diubah-ubah dan mempunyai kuasa tekanan yang kuat. Semai-semai berumur dua puluh lapan hari yang ditanam di dalam pasu (5" garis pusat x 4") disembur sebanyak dua kali dalam masa yang sama dari pangkal perdu. Catitan dibuat 12 hari selepas disembur.

Satu perubahan dalam teknik ini di mana tapak semaian digunakan menggantikan pasu-pasu dengan menanam semai-semai berbaris-baris antara 3 inci jaraknya dari satu sama lain disyorkan bagi saringan besar-besaran untuk selalu digunakan. Adalah disyorkan yang semai-semai itu disembur dua puluh satu hari selepas ditabur.

Suntikan dengan menggunakan "copper gauge technique" ke atas pokok yang berumur dua puluh lapan hari didapati mudah dan baik untuk mendatangkan kesan jangkitan, tetapi terlalu sangat memakan masa dan sukar dibuat jika banyak jenis-jenis padi dan "genetic lines" hendak disaring. Teknik yang menggunakan "sand-paper dan poly-spray" didapati tidak begitu memuaskan bagi saringan besar-besaran untuk selalu digunakan. Teknik menenggelamkan pangkal perdu anak-anak padi tidak memberi kesan serangan.

Bacterial leaf streak caused by *Xanthomonas translucens* f. sp. *oryzicola* (Fang *et al*) comb. nov., has been found to occur in West Malaysia only recently (Singh, 1971).

Severe outbreaks are common in the Kedah and Province Wellesley regions affecting susceptible varieties. The disease could occur as early as the active tillering stage. The severity of the disease increases until it assumes its most severe form at the panicle initiation stage. It gradually disappears at the heading stage and the plant finally recovers from the attack. For highly susceptible varieties, the disease continues even after the heading stage, in which case, the yield may be greatly reduced.

The disease has been reported to occur in the Philippines, South-China, Japan, U.S.S.R., India, Thailand, Vietnam, Indonesia, Cambodia and Malaysia. (Pordisimo 1958; Ou, 1972; Ou, *et al*, 1970; Goto, 1971; Singh, 1971).

Development of resistant varieties has been recognised as an economical and promising method of controlling diseases (Athwal, 1972). Current researchers in major rice growing countries are focussing on breeding for resistance to major diseases, including bacterial leaf streak (Exconde & Lipis, 1972).

New varieties and numerous genetic lines that are constantly produced have to be screened rapidly and efficiently in order to identify those which are resistant and moderately resistant to bacterial leaf streak.

Many inoculation techniques have been tested and proven effective (Pordesimo, 1958; IRRI, 1967, Ou, *et al*, 1970; Ou and Jennings, 1969; Patel and Shekhawat, 1971; Goto, 1971). But the majority are not suitable for mass screening purposes. Hence this study is aimed at developing an inoculation technique which is rapid and efficient for mass screening purposes.

Materials and Methods

Bacterial isolate: Streak isolate (S7/3C-3), from Bumbong Lima, was cultured on Wakimoto's Potato Semi-synthetic Agar (PSA): 300g potato decoction, 0.5g $Ca(NO_3)_2$ $4H_2O$, 2.0g Na_2HPO_4. $12H_2O$, 5.0g peptone,

Preparation of inoculum: Bacterial culture (48-hour-old) grown on PSA plates was used for the inoculation studies. Sufficient plates of bacterial cultures were prepared and incubated for 48 hours.

The culture was suspended in distilled water and passed through muslin cloth to remove bits of agar in it. The bacterial suspension was diluted until the final inoculum had an optical density of 0.4 at a wave-length of 625 mu.

Test plant: A susceptible variety of rice, Bahagia was used in the inoculation studies. Two seedlings were grown in each pot (5" diameter x 4"). Basal dressing of 40 lbs N/ac, 40 lbs P/ac and 30 lbs K/ac was applied. Top dressing was done on the 3rd week with 40 lbs N/ac. The seedlings were inoculated 28 days after sowing.

Treatment: The experiment was conducted in randomised complete block design in three replicates. Each treatment consisted of 10 pots per replicate. The seven treatments are: -

(i) power-spray, (ii) copper-gauze, (iii) sand-paper, (iv) poly-spray, (v) flooding seedling base, (vi) power-spray control, (vii) copper-gauze control.

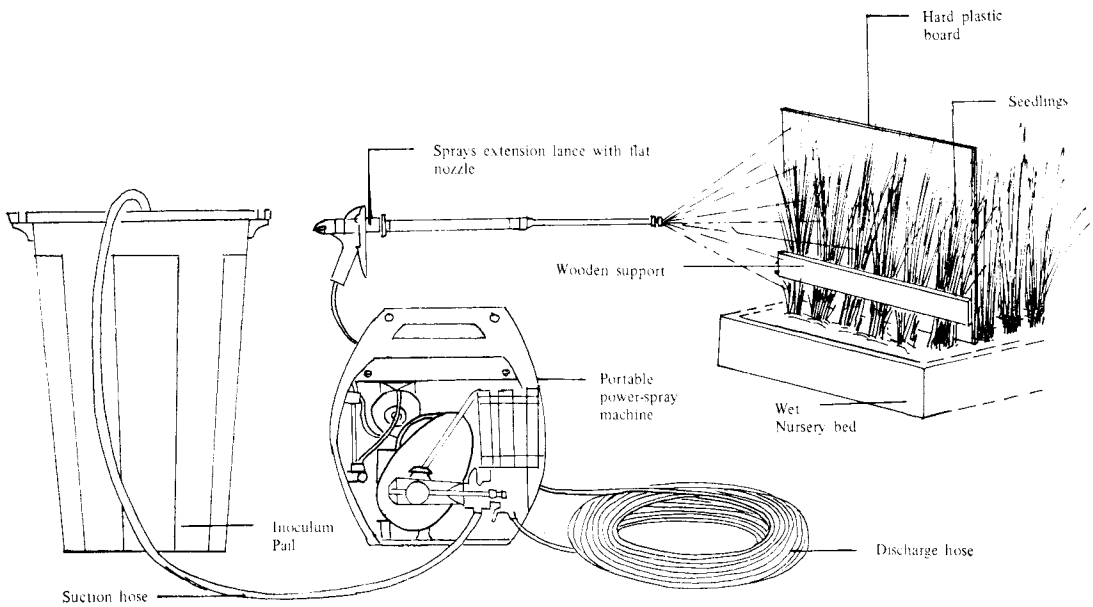

Fig. 1: The Power-spray Technique

The seedlings were predisposed to disease infection by spraying the foliage lightly with water by means of a poly-spray before inoculation.

(i) *Power-spray* (Super portable Hi-pressure pump, Tokyo, Maruyama Mfg. Co. Ltd. Japan) was used to spray each pot of seedlings twice from the base. The seedlings were clamped at the base onto a thick plastic board ($1\frac{1}{2}$' x $1\frac{1}{2}$') by means of a small piece of thin plank and tilted at an angle for easy spraying.

A slight modification of the technique for mass screening purposes is shown in Fig. 1. This included the use of wet seedling beds rather than pots. Test varieties could be planted in rows of 1' — 1½' long and spaced 3" apart.

(ii) Small pieces of copper gauze of mesh size 0.5 mm x 1 mm were wrapped around a piece of circular iron projection of dimension 7 mm long and 2.5 mm in diameter. The whole piece was mounted on the upper half of a 5-inch-long forceps by means of molten lead (Fig. 2). The lower half of the forceps was wrapped with cotton wool which served to hold the inoculum during inoculation. All expanded leaves were inoculated in the middle region on either side of the midrib. The forceps were dipped into the inoculum after inoculating the plants in each pot.

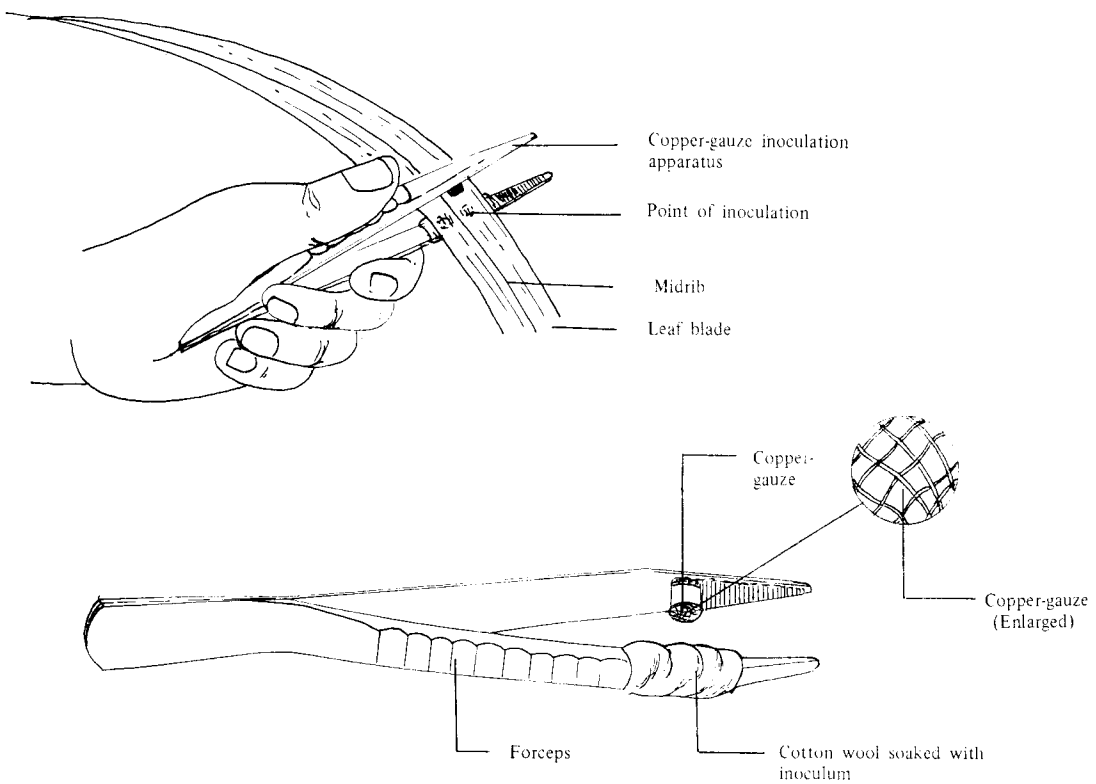

Fig. 2: The Copper-gauze technique

(iii) Strips of fine sand paper, 5 mm x 5 cm, were wrapped around the tip of the upper half of the forceps (5 inches long). It was held in place by means of a rubber band The tip of the lower half was wrapped with cotton wool which served to hold the inoculum. All expanded leaves were inoculated in the middle region. The forceps were dipped into the inoculum after inoculating the plants in each pot.

(iv) *Poly-spray* (Super poly-spray, Associated Sprayers Ltd., Birmingham, 7 England) was used to spray the inoculum onto the foliage for about 5 seconds.

(v) *Flooding of seedling base* was done by pouring 50 mls of the prepared inoculum into each pot of seedlings. Water level in the pots was raised to the brim by adding distilled water.

(vi) *Power-spray control* was done by means of the power-spray as described in (i) above, using distilled water only.

(vii) *Copper-gauze control* was done by means of a pair of copper-gauze apparatus as described in (ii) above, using distilled water only.

The experiment was carried out in an insect-proof cage. Observations were made daily and scoring was done 12 days after inoculation. All inoculated leaves were scored based on IRRI Scales for bacterial leaf streak (IRRI, 1968):-

Scale	Greenhouse (Average length of lesion)	Field observations	
0	No lesions	No lesions	
1	Less than 1 mm	Sporadically a few lesions	Field looks green
2	1 - 2 mm	Very few lesions	
3	3 - 5 mm	Few lesions per plant but all over the field	
4	6 - 10 mm	Generally few lesions but some plants heavily infected	
5	11 - 20 mm	Many lesions per plant all over the field	
6	21 - 30 mm	Many lesions, some leaf tips yellow	Field looks yellow
7	31 - 40 mm	Leaf tips yellow	
8	41 - 60 mm	Whole leaves appear yellow	
9	More than 60 mm	Leaves drying	

Results

Initially water-soaked, dark-green interveinal, narrow translucent streaks developed on the second day after inoculation for the power-spray, copper-gauze and sand-paper treatments. Similar symptoms were seen on seedlings inoculated by means of the poly-spray technique 4 days after inoculation. The lesions later turned yellow to yellow-orange in colour and were clearly translucent when held against a light source. Small, round, waxy-yellow bacterial ooze were seen on the surface of the lesions. There was no infection in the flooding treatment, copper-gauze control and power-spray control. Average scores for all treatments are given in Table 1.

Duncan's Multiple Range Test indicates that the power-spray treatment differed significantly from the copper-gauze, sand-paper and the flooding treatments. However, there was no significant difference between the copper-gauze and the sand-paper treatments.

Table 1: Average score for each treatment

TREATMENT	SCORE		
	R1	R2	R3
Power-spray	6.4	6.0	6.3
Copper-gauze	3.5	3.3	3.3
Sand-paper	3.3	3.7	4.0
Poly-spray	2.3	2.0	2.0
Flooding seeding base	0	0	0
Power-spray control	0	0	0
Copper-gauze control	0	0	0

The analysis of variance (Table 2) shows that there was no significant difference between the replicates but a highly significant difference between the treatments.

Table 2: Analysis of Variance Table on Transformed Data+

Source	D.F.	S.S.	M.S.	Variance ratio
Replicate	2	0.0002	0.0001	0.03243 N.S.
Treatment	6	2.4774	0.4129	1339.1496**
Error	12	0.0037	0.0003	
Total	20	2.4813		

+ = Transformed Data = Log (Av. observed data +1)

N.S. = Non-significant

** = Significant at 1% level

Discussions

Flooding the seedling base with bacterial suspension did not induce any infection. This may be due to the fact that no injury or wound was inflicted on the test plants. Although infection could take place through stomata, seedlings could escape stomatal infection because of variations in environmental conditions (Goto, 1971).

As the poly-spray technique induced infection only on the fourth day after inoculation and the score on the twelveth day after inoculation was very low, the technique was considered unsuitable for mass screening purposes.

Although the sand-paper technique gave good infection, lesion formation and lesion spread, the technique was not recommended because the sand particles tended to drop off from the paper base through repeated inoculation (Goto, 1971). The technique was also time-consuming as each leaf blade had to be inoculated individually. Inoculated leaf blades tended to break at the point of inoculation.

The copper-gauze technique was simple and required very light pressure for inoculation. It induced good infection, lesion formation and lesion spread. As each leaf had to be inoculated individually, the technique was rather time-consuming. In view of limited labour force available, and numerous varieties and genetic lines to be screened constantly, the technique is considered not practical for mass screening purposes. However, because of its efficiency in inducing good symptoms, it is recommended for testing the virulence of various streak isolates and for detailed screening of a few varieties.

The power-spray technique has been shown to be definitely superior to the copper-gauze and sand-paper techniques in terms of infection, lesion formation and lesion spread. The technique was simple and inoculum could be introduced directly and uniformly into the leaf blades by the powerful sprays, through wounds thus created and also through natural openings. In situations where there is a shortage of labour force and a large number of varieties and genetic lines have to be screened constantly and efficiently, the power-spray technique is strongly recommended. Also, the large volume of inoculum required for mass screening of large number of varieties and genetic lines at one time is in this case, of little problem, as the streak pathogen is a fast-growing organism (IRRI 1967) and culturing of the organism is fairly easy.

Mass screening in the nursery using the power-spray technique could be done by sowing seeds in rows of 1' — 1½' long with 3" spacing between rows. Three standard check varieties which are resistant (score 1 – 2) e.g. T(N)1, moderately susceptible (score 5 – 6) e.g. TKM 6 and susceptible (score 7 – 8) e.g. Bahagia, are planted at intervals of every 10 test lines. The 21-day-old seedlings are sprayed three times from the base upwards with the help of a thin plank and a thick plastic board to tilt the seedlings at an angle (Fig. 1). Scoring, based on IRRI scales may be done 10 – 14 days after inoculation depending on the scores of the three standard check varieties.

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Summary

Mass screening for varietal resistance to bacterial leaf streak was rapidly and efficiently carried out by using a portable motorised high pressure power-spray. Twenty eight-day-old seedlings, planted in pots (5" diameter x 4") were sprayed with inoculum twice from the base. Readings were taken 12 days after inoculation.

A modification of the technique which included the use of wet nursery bed instead of pots, with seedlings planted in rows and spaced three inches apart, was recommended for routine mass screening. It was recommended that seedlings be inoculated twenty-one days after sowing.

Inoculation by the copper-gauze technique on twenty-eight-day-old seedlings was found to be simple and efficient in inducing symptoms, but it was time-consuming and laborious if large number of varieties and genetic lines were to be screened. The sand-paper and the poly-spray techniques were found to be inefficient for routine mass screening. The technique of flooding seedling base did not induce any infection.

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