was stained with silver nitrate as described by Petit *et al.*[20].

In the preliminary experiments, young incompletely expanded leaves from node numbers 3 to 5 (from the apex) of 35-day-old seedlings were tried for genetic transformation. Fully expanded leaves were not found suitable for cocultivation because of excessive leaching of the phenolics. Cocultivation in the hormone-free MS medium led to only callus formation at the proximal cut end of the leaves. The emergence of roots did not occur even after callusing. However, when the explants were cocultured on the MS medium in the presence of 1 mg/l NAA, 2–5 roots per explants were observed after 17 ± 2 days of bacterial inoculation (Figure 1 *a*). Emergence of hairy roots was observed in about 40% of the plated leaf explants (25 explants were used in three different experiments, out of which 10 responded positively). These results indicate that presence of auxin during cocultivation plays a critical role for the induction of hairy roots in *C. sinensis*. Similar observations have already been made in case of carrot, pea and tobacco[3,21]. Emerging roots were white at the beginning but under continuous light they turned green. For root biomass production, both liquid MS basal and MS supplemented with 1 mg/l NAA media were used. Branching was not observed in hormone-free MS medium although the elongation occurred to some extent. Addition of 1 mg/l NAA in the medium showed marked difference in branching pattern as well as in the growth rate (Figure 1 *b, c*). The biomass production was approximately 3 times higher (i.e. 1.34 ± 0.655 g) in auxin-supplemented medium as compared to basal MS medium (i.e. 0.42 ± 0.177 g) after 35 days of culture. Presence of mannopine in the hairy roots and its absence in nontransformed control roots, as evident from paper electrophoresis (Figure 1 *d*), confirmed the transformed nature of roots.

The results summarized above clearly indicate that *A. rhizogenes*-mediated genetic transformation in *C. sinensis* is a possibility and fast-growing hairy root lines can be induced in tea. Based on these preliminary findings efforts are now under way to produce hairy roots in vegetative stem cuttings and *in vitro*-regenerated microshoots of tea so that an efficient mass propagation system can be deviced for this difficult-to-root cash crop of India.

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A study on mechanism of phyllody disease resistance in *Sesamum alatum* Thonn.

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Phyllody disease is a highly destructive mycoplasma disease of *Sesamum* transmitted by leafhopper. Resistance to the disease has been recently reported in *Sesamum alatum* and hybrids between *S. alatum* and *S. indicum* were produced. Screening of the F_2 generation under controlled condition using insect vector showed that the resistance may be controlled by a single recessive gene. Screening of F_3 generation under natural condition using infector-row-technique could not confirm the single recessive gene inheritance of phyllody resistance. Further screening by grafting method of inoculation required information on survival rate of seedlings after grafting and mechanism of resistance present in *S. alatum*. Experimental results showed 40% survival rate after grafting. Graft inoculation of mycoplasma-like organisms (MLOs) followed

by visual screening and vector inoculation followed by molecular screening using MLO DNA probe revealed that the resistance to phyllody disease in *S. alatum* was only due to insect resistance and its implication in screening methodology is discussed.

SESAMUM (*Sesamum indicum* L. $2n = 26$), is an important oilseed crop in India particularly in the southern states. Phyllody in *Sesamum* is caused by a mycoplasma-like organism (MLO)[1] and is transmitted by a leafhopper, *Orosius albicinctus* Distant[2]. The infected plant is characterized by transformation of all floral parts, except stamens, to leaf-like structures and the anthers rarely contain functional pollen leading to nearly total sterility[3]. The disease is particularly severe in summer leading often to total loss of the crop. And, though the disease has been reported as early as in 1928 with 80 to 90% infection[4], until now no variety with appreciable degree of resistance has been developed for want of source of resistance. However, recently, screening by vector insect under controlled condition showed that *S. alatum* Thonn. ($2n = 26$), a wild relative of the cultivated species was resistant (93.37%) to the disease[5].

With the objective of transferring the phyllody resistance to the cultivated varieties, *S. alatum* × *S. indicum* hybrid was successfully produced[6] and its hybrid nature has been established[7]. By screening the F_2 generation of this cross, phyllody resistance had been reported to be controlled by a single recessive gene[8]. When the progeny lines of twenty F_2 plants called F_3 families were screened by infector-row-technique under natural condition, more number of plants than expected were observed without phyllody symptom. Therefore, the possibility of utilizing the grafting method of inoculation for screening was explored and the results are discussed.

For screening by infector-row-technique, twenty seeds each from twenty F_2 plants were collected and raised in progeny rows called F_3 families. A field trial was laid out at the Oilseed Breeding Station of the Tamil Nadu Agricultural University, Coimbatore, India, in the summer season. Five plots of each 4 × 4 m were laid out in a place around which different varieties of sesamum and *Crotolaria juncea* (alternative host) were grown for other field trials. Each plot was comprised of four F_3 families (each two rows and ten plants in each row), *S. alatum*, *S. indicum* and *C. juncea* in the following order. First row was *S. indicum* (infector row) followed by two rows of one F_3 family. Then, a row of *S. alatum* was included which was followed by two rows of another F_3 family. Again, an infector row was planted so that 1 : 5 ratio of infector row and test row was maintained. The other two F_3 families were also accommodated in the same way. Further, in each plot an inner border row of *S. indicum* at a distance of 30 cm and an outer border row of *C. juncea* at a distance of 40 cm around were also raised (Figure 1). Observations were recorded 45 days after sowing till maturity.

For screening by graft inoculation, twenty plants each of *S. indicum* and *S. alatum* were raised in pots in an insect-proof glass house. Direct transmission of phyllody MLO was carried out by side wedge grafting. Small young twigs of phyllody-infected sesamum plants were cut in a wedge shape at the bottom and were inserted into the cleft made on 15–20-day old seedlings. The grafted portion was wrapped with polythene stripes. The whole plants were covered with perforated polythene bags and kept in mist chamber. After the grafts were established, the polythene bags were removed and the plants were transferred to an insect-proof glasshouse.

Inoculation of *S. indicum* and *S. alatum* by vector insects was also done by using wooden box technique[9]. When the plants were 75 days old (usually phyllody symptom appears 45 days after sowing which coincides with flowering), leaves were collected from (i) *S. indicum* with phyllody symptom, (ii) *S. indicum* without phyllody symptom, and (iii) *S. alatum* in which all the plants were without phyllody symptom. From the leaf samples total genomic DNA was extracted with slight modifica-

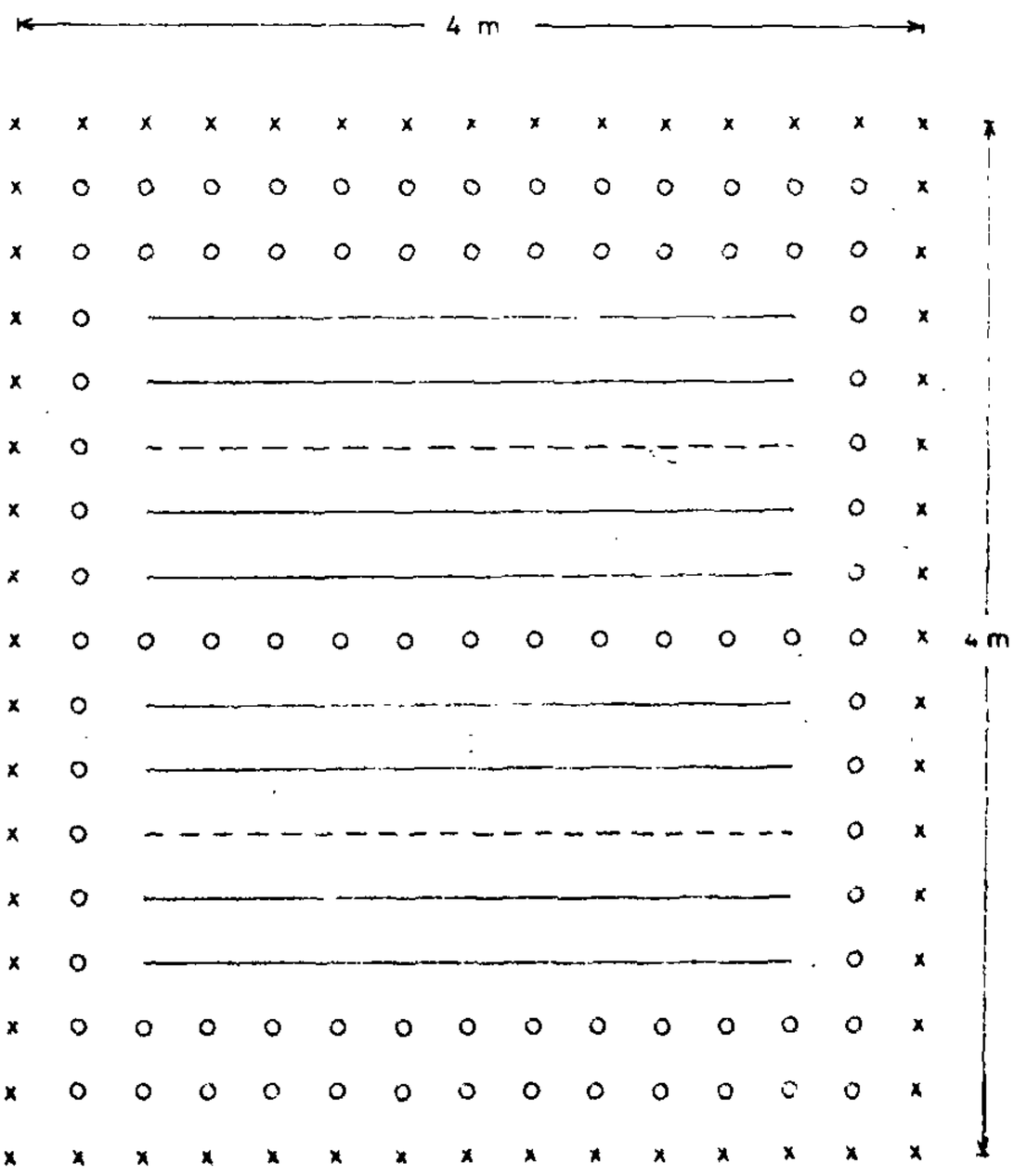

Figure 1. Field screenings of F_3 families of *S. alatum* × *S. indicum* infector-row-technique.

tion as described by Kuske and Kirkpatrick[10] using the PS buffer[11]. The DNA was probed with WBDL (Witches' Broom Disease of Lime) MLO DNA probe (provided by J. M. Bove, INRA, France) in dot blot hybridization.

Dot blot hybridization was performed using Bio-rad dot blot apparatus. The total genomic DNA was denatured at 98°C for 5 min and immediately chilled on ice. The DNA at different concentrations (30, 50 and 120 ng) was loaded on Hybond-N$^+$ membrane with dot blot apparatus and fixed by baking at 80°C for 2 h. The probe was made by random prime labelling of 25 ng of WBDL MLO DNA at 25°C for 1 h. Prehybridization was performed in 5 × SSC containing 5 × Denhardts' solution (0.1% Ficoll, 1% PVP and 0.1% BSA), 50 mM sodium phosphate buffer (pH 6.5), 50% formamide and 200 µg/ml sonicated Salmon sperm DNA. Hybridization was done overnight at 42°C essentially in the same buffer with radioactively (^{32}P-dCTP) labelled probe DNA. After proper washing with SSC and SDS, the filter was air dried and exposed to X-ray film with intensifying screen at –70°C for three days. Then the film was developed and observed for radioactive signal.

In the field screening by infector-row technique, 92 and 65.5% plants with phyllody symptoms were observed in *S. indicum* and *C. juncea* respectively while *S. alatum* showed 100% resistance. All the F_3 families segregated for resistance and susceptibility. Resistance to phyllody disease being reported to be controlled by single recessive gene, theoretically, five plants in each family were expected to be resistant. But, in individual families, the number of plants without phyllody symptom varied from six to eleven. The χ^2 analysis of the data revealed that only nine families (family number 6, 12, 14, 15, 16, 17, 18, 19 and 20) fit into the 3 : 1 ratio of susceptibility and resistance reported earlier. And, in total, as against the theoretical expectation of 100 plants, 179 plants were healthy (Table 1). This may be because the screening was done under field conditions relying on the natural incidence of the disease.

Artificial screening using viruliferous insect vector in cages is generally considered to be a reliable method. However, difficulties and tediousness in collection and maintenance of the insects, making them virulent considering the inoculum acquisition period and incubation period in the vector, confirming the virulence before inoculation, uniform inoculation according to the number of insects per plant, inoculation feeding period, and incubation period in the host, effect of forced feeding, etc. prompted us to look for alternative methods of screening. Since the causal organism was not culturable and the organism proliferates only in the phloem cells, transmission from live infected plants by grafting was the only choice. The problem in this method was severe seedling mortality after grafting. Following the standardized procedure described before, we were able to get 40% survival rate in *S. indicum*. All the plants surviving after grafting showed phyllody symptom indicating assured inoculation in one step which otherwise is complicated in vector screening. However, to apply this technique for screening, information on the mechanism of phyllody resistance in *S. alatum* was crucial.

Table 1. Disease reaction of individual F_3 families in screening using infector-row-technique

Family no.	No. of plants screened	Disease reaction		Observed χ^2 value†
		Healthy	Infected	
1	20	10	10	6.67
2	20	11	9	9.60
3	20	10	10	6.67
4	20	11	9	9.60
5	20	9	11	4.26
6	20	7	13	1.06*
7	20	9	11	4.26
8	20	14	6	21.6
9	20	11	9	9.60
10	20	13	7	18.40
11	20	11	9	9.60
12	20	8	12	2.40*
13	20	10	10	6.67
14	20	7	13	1.06*
15	20	6	14	0.26*
16	20	6	14	0.26*
17	20	7	13	1.06*
18	20	6	14	0.26*
19	20	7	13	1.06*
20	20	6	14	0.26*
Total	400	179	221	

†Expected χ^2 value at one degree of freedom was 3.84 at 5%.
*Significant at $p < 0.05$.

When 20 plants each of *S. indicum* and *S. alatum* were inoculated by grafting, eight plants in each survived after grafting. *S. alatum* which was resistant to phyllody disease (93.37% resistance under controlled condition as reported earlier and 100% resistance under natural condition, in the present study) when inoculated with vector insects expressed phyllody symptom in all the plants. This may be due to more inoculum load in graft transmission than in vector transmission or the species may not be resistant to MLO. Earlier, *Abelmoschus manihot* var, *pungens*, *A. crinatus*, *Hibiscus panduraeformis* and *H. vitifolius* which were resistant to vector transmission of yellow mosaic virus were also resistant to graft transmission[12]. In the same experiment, *H. subderifa* which was resistant to vector transmission became susceptible to graft transmission. This showed that the increased inoculum load could not be the only reason that made *S. alatum* susceptible to the disease. Alternatively, resistance to phyllody in *S. alatum* may be due to insect (vector) resistance not MLO resistance. To verify this, both *S. indicum* and *S. alatum* were inoculated with vector insect and the DNA from the plant system were probed with MLO DNA for the

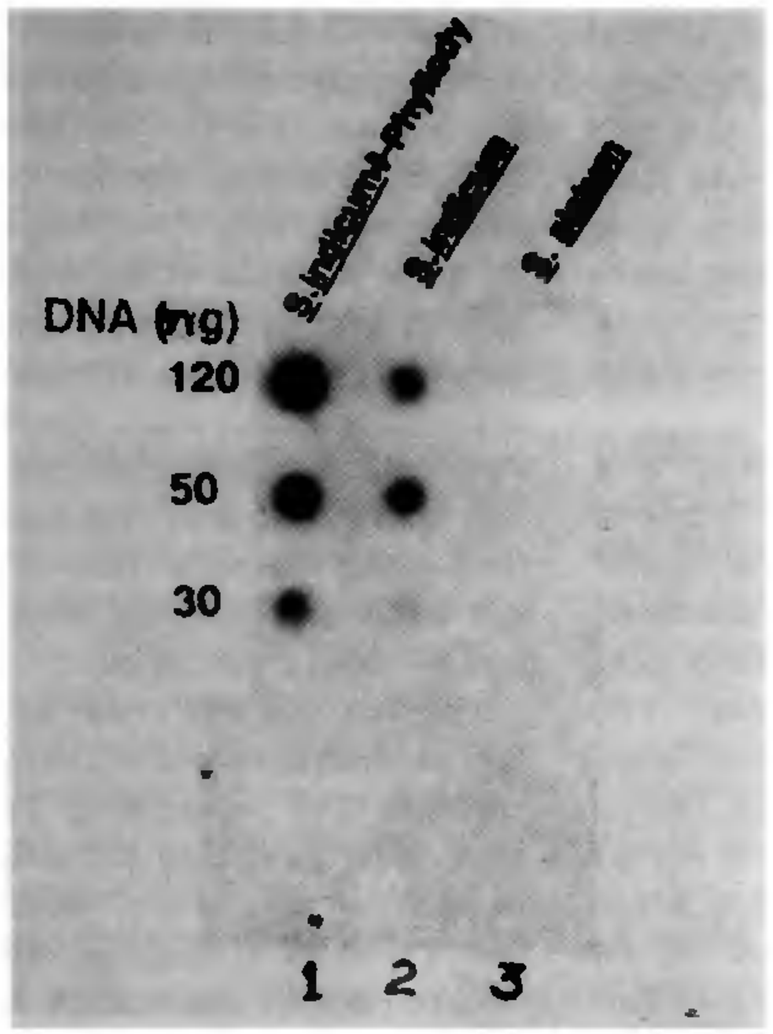

Figure 2. Dot blot hybridization of WBDL MLO DNA probe to the total genomic DNA of (1) *S. indicum* with phyllody symptom, (2) *S. indicum* without phyllody symptom, and (3) *S. alatum* without phyllody symptom after vector inoculation.

presence of the causal organism. The MLOs were detected both in *S. indicum* with phyllody symptom and without the symptom indicating its sensitivity to detect MLO even before the symptom appears. The same probe did not detect any MLO in *S. alatum* (Figure 2). This showed that phyllody resistance in *S. alatum* was due to insect resistance which did not allow the entry of the MLOs into the plant system. Consequently, when the MLOs were deliberately introduced by graft inoculation phyllody symptoms were expressed as it was not resistant to the causal organism. Therefore it can be concluded that *S. alatum* is resistant to phyllody disease through insect resistance only.

This finding has two implications in screening method: (i) since the species is not resistant to the causal organism, graft inoculation or any other form of direct delivery of MLOs should not be used for phyllody resistance screening in *Sesamum*, and (ii) since the species is resistant to the disease-transmitting vector, insect inoculation can be followed by optical, immunological or molecular detection of MLO for rapid and reliable screening as the presence of the causal organism is an early indication of susceptibility. And, infector-row-technique may be used at preliminary stage of screening to reduce the number of entries for vector screening which is tedious and time consuming.

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