BREEDING BRINJAL RESISTANT TO LITTLE LEAF DISEASE

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In an attempt to standardise the screening technique to locate sources of resistance against little leaf disease of brinjal, several transmission tests were carried out. Out of different methods tested, grafting was found to be most efficient and a period of seven to nine days for the infected scion to remain united with the healthy stock was found to give the optimum inoculation. Though the disease was transmissible through the leaf hopper, *Cestius phycitis*, percentage of successful transfers was very less. Out of 164 brinjal cultivars tested three were found to produce symptoms late when artificially inoculated and to remain free from the disease under field conditions. Out of 11 wild species tested *Solanum integrifolium* and *S. gilo* showed resistance due to hypersensitive reaction to the pathogen. Standard susceptible cultivar, Pusa Purple Long was crossed with these two species. The F_1 progenies of these crosses behaved like their resistant parents in disease reaction. Further investigations in this line are in progress. Studies on possible preformed resistance factors revealed that both the resistant wild species were very rich in total phenolics and the field-resistant cultivar S.212–1 was very rich in ascorbic acid content.

INTRODUCTION

The little leaf is one of the most important diseases of brinjal in India. Although no definite knowledge about the losses in India owing to this disease is available, it is estimated that this disease causes considerable damage to the brinjal crop. It is nearly becoming a limiting factor for brinjal cultivation in many parts of this country.

For handling such a problem a two pronged approach is necessary. As the disease is virulent in many parts of the country an acceptable resistant variety should be bred which will help to control the disease at a minimum cost. Another approach to the problem is the partial control of the disease with the use of some selected chemicals.

Keeping these in view investigations since 1967 are being carried out at the Division of Vegetable Crops and Floriculture, Indian Agricultural Research Institute, New Delhi.

MATERIALS AND METHODS

Brinjal cultivars and allied species of *Solanum* maintained at the Division of Vegetable Crops and Floriculture were used in these studies. The experiments requiring insect-free conditions were conduced in an insect proof nylon net-house.

Transmission

Four methods of transmission were tried to assess their relative efficiency in screening.

(*i*) *Transmission through roots*—The experiment consisted of five different media and four time durations given for absorption through the roots.

Media—(*i*) Distilled water, (*ii*) Phosphatic buffer, (*iii*) Buffer +0.5 per cent ascorbic acid, (*iv*) Buffer +0.5 per cent sodium sulphite, and (*v*) Buffer + 0.1 per cent thioglycolic acid.

Duration of root-dipping (*i*) 1 hr, (*ii*) 6 hr, (*iii*) 12 hr, and (*iv*) 24 hr.

Sorensen's orthophosphate buffers were used in this experiment. For collecting the infectious exudate in the different media two sources of inoculum were used and duplicate sets of each of the media were employed. In one set, ten grammes of finely chopped material of young, severely infected shoots were added to each of the media, while in the other set only the cut ends of infected shoots were immersed in the media. One hundred ml of each of the media were taken in each case. Observations on the appearance of disease symptoms were taken 8 weeks after transplanting.

(*ii*) *Transmission by root-knot nematodes* (*Meloidogyne* spp.)—Paired disease and healthy plants method (Raski and Hewitt 1967) was used in this experiment. It consisted of growing of one diseased and one healthy plant in the same pot containing sterilised soil. About 1000 larvae of the nematodes were placed in the root zone of the plants of each pot.

(*iii*) *Transmission by leaf hopper* (*Cestius phycitis*)—A colony of this vector was built up on little leaf affected brinjal plants in the insect rearing cages. Healthy, young, 15–20 cm tall plants grown in earthen pots were enclosed in glass chimneys covered with muslin tops. Adult insects reared on little leaf infected plants were transferred into glass chimneys. Ten to twelve leaf-hoppers were kept per plant. The insects were allowed to feed on the plants for 15 days.

(*iv*) *By grafting*—Young, healthy seedlings of 15-20 cm in height and grown in pots, were inoculated by side wedge grafting. Young shoots from diseased plants were used as scions and healthy plants as stocks.

Standardisation of graft transmission

The standardisation of this method consisted of removal of scions at different intervals after grafting e.g., 3, 5, 7, 9, 11, 13 and 15 days along with control in which scions were not removed.

Scoring of disease symptoms

For the purpose of screening under controlled conditions and in other experiments, the disease intensity on every plant was measured by grading the symptoms into four different classes according to its severity as given below:

Symptom category	*Score*
Severe	1
Less severe	2
Mild	3
No symptom	4

Metabolic changes and preformed resistance factors

For studies on metabolic changes one susceptible variety, Pusa Purple Long (PPL) and one resistant wild species *Solanum integrifolium* were taken. For studying preformed resistance factors four lines were taken—susceptible variety PPL, resistant wild species *S. integrifolium* and *S. gilo* and field-resistant variety S. 212-1. For metabolic changes samples with uniform stem and leaf portions were collected both from uninoculated and inoculated plants four weeks after grafting. For preformed resistance factors samples were collected four weeks after transplanting from the healthy plants.

Ascorbic acid was determined by the indo-phenol-xylene extraction method (Association of Vitamin Chemists, 1966). The sugars and starch were estimated by the colorimetric method of Somogyi (1952) using Somogyi's and Nelson's reagents. The nitrogen was estimated by the modified Kjeldahl's method (Jackson 1962). Estimation of phosphorus was made by colorimetric method. The phenolic content was estimated according to the procedure described in A.O.A.C. (1960).

Result and Discussion

Transmission

(*i*) *Transmission through roots*—This method was not found to be successful in transmitting the disease. It had deleterious effect on the survival of the plants particularly with the increasing duration of root-dipping. The injurious effect was more pronounced when chopped diseased—shoots were used as source of inoculum.

(*ii*) *Transmission by root-knot nematodes* (*Meloidogyne* spp.)—This method was also not effective in transmitting the disease.

(*iii*) *Transmission by leaf hopper* (*Cestius phycitis*)—Transmission by leaf hoppers was not uniform. It varied from no transmission to the maximum of 13.3 per cent transmission. In majority of the plants symptoms were clear and fully developed six weeks after appearance of first symptoms.

(*iv*) *Transmission by grafting*—There were cent per cent transmission in all the cultivars tested. In cultivar PPL the symptoms appeared 21 days after grafting.

Standardisation of graft transmission—From the foregoing experiments, grafting was found to be the only suitable method of transmission to meet largely the requirements of screening. However, in this method, the quantity of the inoculum passing through the graft-joint cannot be controlled and hence there is every possibility of the test plants receiving a higher concentration of the pathogen from the scion. Another possible variation is the continuous supply of inoculum from the diseased scion if the same is allowed to grow for longer time. On the contrary, if the scion dies soon after grafting, transmission has to take place within a short period. Hence this experiment was conducted to meet the above requirements.

On the basis of the appearance of the plants under different treatments it was observed that there was no development of symptoms in plants in which scions were allowed to remain united for three and five days, respectively. Plants under all other treatments produced symptoms four weeks after inoculation. It was also noticed that when the diseased scion was allowed to remain united with the stock

TABLE I

Effect of removal of diseased scion at various intervals on disease incidence in 'Pusa Purple Long

Scions removed after		Average score of 5 plants recorded after				
		2	4	6	8	10
3 days	T_1	4.0	4.0	4.0	4.0	4.0
5 ,,	T_2	4.0	4.0	4.0	4.0	4.0
7 ,,	T_3	4.0	2.4	2.2	2.0	1.8
9 ,,	T_4	4.0	2.4	2.2	1.6	1.6
11 ,,	T_5	4 0	2 0	2 0	1 8	1 2
13 ,,	T_6	4.0	2.4	2.0	1.4	1.0
15 ,,	T_7	4.0	2.2	1.4	1.0	1.0
Not removed	T_8	4.0	2.0	1.2	1.0	1.0

TABLE II

Testing of brinjal cultivars against little leaf disease under controlled conditions

Sl. No.	Accession no. or name	Average no. of days after inoculation after which symptoms first appeared
1.	S. 195-2	65
2.	S. 195-5	60
3.	S. 195-6	60
4.	S. 212-1	70
5.	S. 238-4-11	70
6.	S. 252-1-1	56
7.	S. 252-2-1	50
8.	S. 433	65
9.	Pusa Purple Long	21

for longer duration the disease symptoms were more pronounced (Table I). From these observations it may be concluded that a minimum period of seven to nine days should be given for the diseased scion to remain united with the stock for optimum disease expression.

Screening for resistance

Screening of cultivars of brinjal and related wild species of *Solanum* was done both under field and controlled conditions. For screening under field conditions the percentage of infection under each cultivar and allied species was recorded.

For screening under controlled conditions 15 plants of each were inoculated by grafting. The disease intensity was measured by grading the symptom into different classes according to its severity as produced six weeks after the appearance of first symptoms.

Screening of cultivars—One hundred and sixty four cultivars and lines were tested under field condition. The little leaf disease was found to be very sporadic in nature. Out of 164 cultivars 66 were found to be susceptible.

Under controlled conditions 120 cultivars were screened by grafting. It was observed that all the cultivars tested were susceptible though a few exhibited symptoms late. Most of the cultivars produced symptoms between three to five weeks after grafting. However, seven of them produced symptoms eight weeks after grafting.

Though there was no cultivar found to be absolutely resistant, three lines which produced symptoms late under controlled conditions and which remained free from the disease under field conditions as recorded for a number of years were selected as the field-resistant lines. These were S. 212-1, S. 252-1-1 and S. 252-2-1.

Screening of wild species—Eleven wild species were tested both under field and controlled conditions. All the wild species except *S. integrifolium* and *S. gilo* were found, to be susceptible. These two species showed necrotic reaction at the graft union which might be due to hypersensitive reaction of the host to the pathogen and remains free from any visual symptom. Anjaneyulu and Ramakrishnan (1968) also reported similar results.

Breeding for resistance

An attempt was made to transfer resistance from *S. integrifolium* and *S. gilo* to *S. melongena* by crossing these wild species with cultivar PPL. PPL crossed successfully only as the male parent with *S. integrifolium* and as the female parent with *S. gilo*. The F_1 plants reacted similarly as their resistant parents. This indicated that in F_1 the resistance was a dominant character. Recently natural fruit setting has been observed in some of the F_1 plants of both the combinations under open pollination. A few fruits have also set in the F_1 plants which were backcrossed to the susceptible parent and also selfed for the production of F_2 progenies. Nasrallah and Hopp (1963) reported sterility in the F_1 hybrids obtained from the crosses between *S. melongena* and *S. gilo*. Similar sterility in F_1 hybrids between *S. integrifolium* and *S. melongena* was reported by Hagiwara and Iida (1939).

Metabolic changes

The little leaf disease increased the dry matter and decreased the moisture content in the susceptible variety but decreased the former and increased the latter in the resistant wild species. There was a decrease in ascorbic acid and phosphorus content in both the species following infection but the percentage of decrease was more in the resistant species. Following inoculation total nitrogen content decreased in the susceptible variety but increased in the resistant wild species. Conversely, the disease increased the reducing and non-reducing sugars, starch and total carbohydrate in the susceptible cultivar and decreased the same in the resistant wild species (Table IV).

TABLE III

Testing of related wild species of Solanum *against little leaf disease under controlled conditions*

S. No.	Name of the species	Average no. of days after inoculation after which symptoms first appeared	Remarks
1.	*S. xanthocarpum*	21	
2.	*S. nigrum*	35	
3.	*S. sisymbrifolium*	35	
4.	*S. incanum*	30	
5.	*S. khasianum*	40	
6.	*S. melongena* var. *insonum*	45	
7.	*S. indicum* (from Annamalai)	30	
8.	*S. indicum* (from Orissa)	30	
9.	*S. torvum*	30	
10.	*S. elaeagnifolium*	40	
11.	*S. integrifolium*	—	Showed necrosis at the graft joint and remained free from the disease.
12.	*S. gilo*	—	

TABLE IV

Metabolic changes in uninoculated and inoculated plants of susceptible variety and resistant wild species

Constituents	Pusa Purple Long			*S. integrifolium*		
	Uninoculated	Inoculated	Per cent increase or decrease	Uninoculated	Inoculated	Per cent increase or decrease
Dry matter (in percentage)	15.110	15.560	+2.980	15.700	15.480	—1.400
Moisture (in percentage)	84.890	84.440	—0.530	84.300	84.520	+0.260
Ascorbic acid (in mg/100 g fresh wt.)	1.655	1.258	—23.987	4.700	2.449	—47.893
Reducing sugar (in per cent dry wt.)	0.450	0.550	+22.222	0.550	0.333	—39.454
Non-reducing sugar (in per cent dry wt.)	1.000	1.117	+11.700	0.950	0.604	—36.421
Starch (in per cent dry wt.)	7.100	7.750	+9.155	7.500	7.350	—2.000
Total carbohydrate (in per cent dry wt.)	8.550	9.417	+10.140	9.000	8.287	—7.922
Total nitrogen (in per cent dry wt.)	2.812	1.787	—36.451	1.210	2.216	+83.140
Total phosphorus (in per cent dry wt.)	0.175	0.160	—8.571	0.250	0.210	—16.000

TABLE V

Preformed resistance factors in cultivars and wild species of brinjal

Cultivars and wild species	Total phenolic content in mg/100 g of fresh material	Ascorbic acid content in mg/100 g of fresh material
Pusa Purple Long	81.25	4.498
S. 212-1	78.75	15.557
S. integrifolium	145.00	5.296
S. gilo	111.25	5.767

Performed resistance factors

The resistant wild species *S. integrifolium* and *S. gilo* were found to contain more amount of phenolic compounds than the susceptible (PPL) and field-resistant (S. 212-1) cultivar (Table V). It has been found by many workers that in virus infections there is a stimulation in the production of phenolic substances particularly around the necrotic lesions in incompatible host-virus combinations (Goodman *et al.* 1967). In the present case high amount of phenolic compounds in the resistant wild species might be responsible for their hypersensitive reaction to the pathogen.

The ascorbic acid content in the field-resistant variety, S. 212-1, was found to be very high as compared to the susceptible cultivar and resistant wild species (Table V). It appears that high amount of ascorbic acid present in the field-resistant variety may be one of the factors contributing towards field-resistance.

There was no qualitative difference in free amino acid content among different lines.

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