

# Selection of Resistant Parents in a Tomato Breeding Program for Late Blight Resistance in Cameron Highlands, Malaysia

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## Introduction

Tomato is one of the crops in which the breeding for disease resistance has been successfully conducted. Most of the cultivars released recently have some kinds of disease and pest resistance. The resistance to Fusarium wilt, Fusarium root rot, Tobacco mosaic virus, Verticillium wilt, leaf mold, or root-knot nematode is possessed singly or in combination by a cultivar. Though late blight caused by *Phytophthora infestans* is a serious problem in tomato production, no commercial variety resistant to late blight has been developed yet.

Tomato breeding for late blight resistance was started about forty years ago in USA,<sup>4,8)</sup> and followed by other countries such as Japan<sup>5,6)</sup> and the Netherlands.<sup>7)</sup> Since then the breeding has been faced to a great difficulty. One reason is the frequent appearance of virulent races which can overcome the resistance. Since there exist various races of *P. infestans*,<sup>2,9)</sup> horizontal resistance, which is commonly controlled by multiple genes, is needed to be incorporated into tomato cultivars. Another reason is that inoculation test is not easy because of the instability in the pathogenicity of the causal fungus. As the fungus easily loses its pathogenicity under various cultural conditions, it is difficult to get a

pathogenic strain for inoculation test.

Cameron Highlands, situated at an altitude of 900 to 1500 m in lat. 4°N, is the biggest area of vegetable production in Malaysia. The climate there is cool and humid, so that late blight of tomato is prevalent throughout the year. Outlook of the tomato field changes nearly brown at the later stage of growth, though fungicides are sprayed frequently. The late blight resistant variety is demanded urgently.

The tomato lines including the accessions in West Virginia University and the breeding lines of Japan were assessed for the resistance to late blight by artificial inoculation with *P. infestans* and by natural infection in the open field of MARDI (Malaysian Agricultural Research and Development Institute) Station, Cameron Highlands. Some economic characters of these lines were also investigated for the evaluation of suitability as parental materials in a tomato breeding programme.

## Materials and methods

**Materials:** Twenty-eight lines, listed in Table 4, were used. These include the lines developed at Nagano Chushin Agricultural Experimental Station in Japan, the accessions of West Virginia University, some cultivars so far reported as resistant, and two susceptible cultivars, Ponde-

rosa and a local one.

**Inoculation test:** Seeds were sown in plastic trays on August 29. The seedlings with 1 or 2 leaves were transplanted into 9 cm plastic pots in a glasshouse. The plants were inoculated by spraying the suspension of zoosporangia of *P. infestans* at 7 to 8 leaf stage in a plastic tunnel. The suspension was prepared by washing off zoosporangia from infected tomato leaves collected in Cameron Highlands, and then diluted with water to the concentration at ca. 15 sporangia per one view of microscope ( $\times 100$ ). After 3 days, each plant was observed and classified into one of the following disease indices: 1) no symptom; 2) necrotic small lesions; 3) larger lesions without zoosporangia formation; 4) larger lesions with zoosporangia formation; 5) severe and active stem and leaf lesions.

**Field test:** In the field test, the resistance of tomato lines was assessed by two experiments. Tomato plants were grown without spraying

fungicide in one experiment, while the fungicide Mancozeb was sprayed once a week in other experiment. Seeds were sown in plastic tray on Aug. 8. The seedlings were transplanted into 9 cm pots on Aug. 27, and then to the open field at  $1 \times 0.5$  m spacing on September 28. Each plot, containing five plants inside, was arranged at random, and duplicated. The ratio of the infected leaf area to the whole leaf area on each plant was recorded at weekly intervals. Some economic characters were also investigated.

## Results

### 1) Inoculation test

The varietal difference of disease severity was conspicuous (Table 1). The most resistant line, Fla P-14, showed no or slight symptom, while the susceptible cultivar, Ponderosa, was severely infected. Since the distribution of indices was

Table 1. Disease severity of tomato lines after inoculation of *P. infestans*

Lines*	Number of plants in disease index class					Average of disease index
	0	1	2	3	4	
Fla. P-14	10	5	0	0	0	0.33
W.V. 700	2	6	3	1	0	1.25
W.V. 36	2	2	7	0	0	1.45
Nagano-6	0	10	6	1	0	1.47
PI166365	2	2	11	0	0	1.60
Nagano-7	0	5	11	0	0	1.69
Fla. LBR-2	0	4	14	1	0	1.84
Nagano-3	0	3	14	1	0	1.89
Nagano-2	0	0	14	0	0	2.00
Nagano-9	0	5	6	6	0	2.06
Red cherry	0	0	25	2	0	2.07
Nagano-1	1	0	11	3	0	2.07
Lux. cherry	1	0	7	4	0	2.17
Nagano-4	0	0	12	1	1	2.21
W.V. 64	0	0	6	0	1	2.29
Nagano-10	0	0	10	5	0	2.33
Nagano-5	0	1	4	8	0	2.54
Nagano-8	0	0	1	10	0	2.91
PI204776	0	0	1	5	1	3.00
Fla. P-1	0	0	0	10	3	3.23
W.V. 19	0	0	0	7	4	3.36
Local	0	1	1	0	17	3.73
Fla.LBR-1	0	0	0	4	18	3.84
W.V. 63	0	0	1	0	12	3.85
Nova	0	0	0	1	14	3.93
Atom	0	0	0	0	14	4.00
Ponderosa	0	0	0	0	11	4.00

\* Lines are arranged in the order of increasing disease severity.

continuous in every tomato line, the average of indices can be regarded as the representative value of disease severity on each line. W.V. 700 and W.V. 36 were highly resistant, and Nagano-6 and Nagano-7 were fairly resistant. Other Nagano lines and W.V. 64 showed moderate resistance. Atom, Nova, W.V. 63, and Fla LBR-1 did not show any resistance. W.V. 19 and Fla P-1 were severely infected by *P. infestans* collected in Cameron Highlands, which can be classified as highly virulent race 0 by Wilson's classification.<sup>9)</sup> UCPA1169 was not tested in this experiment.

## 2) Field test

Tomato plants grew well in every plot, though some lines, W.V. 700, PI166365, PI204776 and Local were not enough in number of plants to be assessed because of poor germination of the seeds. The first symptom of late blight was observed on the lower leaves of UCPA-169 in

the field without spraying on Oct. 24. The symptom spread rapidly in the unsprayed field, where the chance of infection seemed to be almost equal. Other disease and pest were not observed. The scoring of disease severity was started on Oct. 31, and continued till Dec. 9 in the unsprayed test (Table 2), and till Jan. 20 in the sprayed test (Table 3). The score represented in Table 2 and Table 3 are the average of 5 to 10 plants. The development of symptom was much slower in the sprayed test than in the unsprayed one. This shows the remarkable effect of the fungicide to control late blight.

In the unsprayed test, the varietal difference of symptom severity was apparent during the former half period of investigation, though most lines became heavily infected on the day of last scoring, Dec. 9. The spread of infected leaf area on resistant lines was slower than that on susceptible lines. The mean value of seven times scorings was used as the representative values of

Table 2. Percentage of infested leaf area of tomato lines in the unsprayed field test

Lines*	Date of scoring							Average
	Oct. 31	Nov. 7	Nov. 11	Nov. 18	Nov. 25	Dec. 2	Dec. 9	
	%	%	%	%	%	%	%	%
Fla. P-14	0.0	0.0	5.0	11.0	48.0	66.0	76.0	29.43 a**
W.V. 700	0.0	2.0	5.0	16.0	71.0	83.0	88.0	37.86 b
W.V. 36	3.0	5.5	19.0	31.0	71.5	82.5	88.0	42.93 bc
Nagano-6	5.0	5.0	8.0	48.0	75.0	81.0	88.0	44.29 cd
UCPA1169	10.7	7.1	16.4	32.4	76.4	81.4	85.7	44.29 cd
Red cherry	5.5	10.0	22.5	32.5	75.5	81.5	84.5	44.57 cd
Fla. LBR-2	5.0	7.2	19.4	37.2	81.7	82.2	85.6	45.48 cd
Nagano-3	2.5	5.0	19.0	49.0	75.0	81.0	95.5	46.00 cd
Nagano-7	4.5	5.0	15.5	43.0	80.0	84.5	93.5	46.57 cd
PI204776	10.0	7.5	17.5	37.5	82.5	87.5	88.8	47.32 cde
Nagano-5	1.3	5.0	10.0	31.3	90.0	95.0	98.8	47.32 cde
Nagano-10	6.0	6.5	25.0	61.5	75.0	80.0	93.0	49.57 de
Nagano-2	3.9	7.8	12.8	58.3	80.6	87.8	96.1	49.60 de
Nagano-9	4.5	6.0	18.0	68.0	78.5	84.5	93.0	50.36 de
W.V. 64	6.3	5.0	22.5	60.0	83.8	87.5	92.5	51.09 def
Nagano-1	6.7	7.8	30.0	60.6	83.3	82.2	93.9	52.06 ef
Nagano-4	5.5	6.5	18.0	65.0	85.5	89.0	97.5	52.43 ef
Nagano-8	9.0	9.5	25.0	62.0	86.0	87.0	97.0	53.64 ef
Lux. cherry	11.0	30.0	42.0	55.0	79.0	86.0	95.0	56.86 f
W.V. 19	23.0	41.0	53.0	67.5	84.0	89.0	98.5	65.14 g
Fla. P-1	22.0	54.0	62.0	78.5	90.5	83.5	98.0	71.21 h
Ponderosa	33.3	81.1	84.4	88.3	90.6	93.3	97.8	81.27 i
W.V. 63	27.8	75.6	82.8	95.0	96.1	97.2	98.9	81.90 i
Nova	55.0	80.0	91.0	100.0	100.0	100.0	100.0	89.43 j
Fla. LBR-1	72.0	92.0	92.0	93.0	96.0	95.0	98.0	91.14 j
Atom	91.0	100.0	100.0	100.0	100.0	100.0	100.0	98.71 k

\* Lines are arranged in the order of increasing disease severity.

\*\* Duncan's multiple range test at 5% level.

Table 3. Percentage of infested leaf area of tomato lines in the sprayed field test

Lines*	Date of scoring													Average
	Oct. 31	Nov. 7	Nov. 11	Nov. 18	Nov. 25	Dec. 2	Dec. 9	Dec. 16	Dec. 23	Dec. 30	Jan. 6	Jan. 13	Jan. 20	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
UCPA1169	0.6	2.2	3.3	7.8	12.8	19.4	26.1	26.7	27.8	33.3	37.2	47.2	59.4	23.38 a**
Fla. P-14	0.0	0.0	5.0	12.0	12.0	14.0	23.0	30.0	41.0	50.0	54.0	58.0	59.0	27.54 ab
Red cherry	0.6	5.0	9.4	16.3	24.4	28.8	35.0	35.0	36.9	41.3	44.4	53.1	58.1	29.86 ab
Nagano-7	0.0	0.0	0.0	11.5	23.0	25.0	29.5	36.0	45.5	45.5	49.5	68.5	75.0	31.46 abc
Fla. P-1	0.5	6.0	8.5	17.5	22.5	29.5	34.0	36.0	45.5	46.5	48.5	64.0	71.5	33.12 bcd
W.V. 19	0.0	5.0	8.5	15.0	24.5	29.5	34.5	37.5	43.0	49.0	51.5	64.5	71.5	33.38 bcd
Nagano-9	0.0	0.5	0.5	13.0	24.5	29.0	39.0	43.0	46.5	51.0	53.5	66.0	72.0	33.73 bcd
Fla. LBR-2	0.5	4.0	6.5	17.5	28.0	37.5	41.0	43.5	45.5	49.5	56.0	59.0	63.0	34.73 bcd
Nagano-4	0.0	0.0	1.1	12.8	23.3	25.6	35.6	42.2	46.7	52.8	63.9	81.1	89.4	36.50 bcd
Nagano-10	0.0	0.0	0.6	13.1	26.3	31.3	41.3	42.5	49.4	60.0	64.4	73.1	79.4	37.02 bcd
Nagano-2	0.0	0.6	2.2	12.8	27.2	32.2	39.4	45.0	51.1	56.1	61.1	72.8	81.7	37.09 bcd
Lux. cherry	3.8	8.8	8.8	18.3	40.0	33.8	46.3	52.5	56.3	56.3	56.3	57.5	58.8	38.27 bcde
W.V. 36	0.0	3.8	13.8	33.1	36.3	38.1	45.6	48.8	53.1	55.6	58.1	58.8	61.3	38.94 bcde
Nagano-3	0.0	1.5	4.5	12.5	27.0	35.0	44.0	48.0	50.5	59.5	66.0	79.0	88.0	39.65 cde
Nagano-8	0.5	2.0	4.0	23.0	35.0	41.0	47.0	49.0	57.5	62.0	64.5	72.5	80.5	41.42 def
Nagano-1	0.0	1.0	2.0	8.5	29.5	34.5	41.5	45.0	51.5	71.5	76.0	85.0	95.0	41.62 def
Nagano-5	0.0	0.0	1.7	13.3	28.3	35.0	43.3	50.0	55.0	75.0	80.0	93.3	95.0	43.85 defg
Nagano-6	0.0	1.0	3.0	30.0	41.0	52.0	55.0	59.0	70.0	79.0	79.0	87.0	87.0	49.46 efg
W.V. 64	0.0	0.0	1.0	16.0	41.0	54.0	56.0	60.0	66.0	75.0	86.0	98.0	100.0	50.23 efg
Fla. LBR-1	1.3	25.0	46.3	65.0	67.5	62.5	56.3	58.8	60.0	63.8	58.8	60.0	60.0	52.69 fg
Ponderosa	0.5	9.0	15.5	52.0	54.0	56.0	57.5	64.0	67.5	76.5	77.5	83.0	86.0	53.77 g
W.V. 63	3.0	12.0	25.0	75.0	82.0	88.5	90.5	93.5	94.5	95.0	93.5	94.0	95.5	72.46 h
Nova	1.0	9.0	28.0	96.0	95.0	96.0	99.0	100.0	100.0	100.0	100.0	100.0	100.0	78.77 hi
Atom	0.6	8.8	56.3	99.4	100.0	99.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	81.88 i

\* Lines are arranged in the order of increasing disease severity.

\*\* Duncan's multiple range test at 5% level.

disease severity during whole period of investigation. Fla P-14 showed the highest resistance, followed by W.V. 700, W.V. 36 and Nagano-6. Other Nagano lines and W.V. 64 showed moderate resistance. The resistance of W.V. 19 and Fla P-1 was relatively lower. Atom, Nova and W.V. 63 were more susceptible than Ponderosa, probably due to their denser arrangement of leaves. The rank of resistance of lines was quite parallel to that in the inoculation test. There is a high correlation between the average score in this test and the mean value of indices in the inoculation test ( $r=0.915$ ).

In the sprayed test, the varietal difference of disease severity was conspicuous in the latter half period of investigation. UCPA1169 showed the highest resistance, followed by Fla P-14, Red cherry and Nagano-7. W.V. 19 and Fla P-1 also showed relatively higher resistance, while W.V. 36 and Nagano-6 were ranked lower in resistance. The correlation between the mean values of the sprayed test and those of the

unsprayed test is not so high ( $r=0.796$ ).

### 3) Investigation of economic characters

The measurement of economic characters are summarized in Table 4. W.V. 63, W.V. 64, Atom and Nova showed determinate growth habit. In Nagano-5, both determinate and indeterminate types were observed. The growth of Atom and Nova was compact, and leaves were crowded. UCPA1169 had long internodes and sparse leaves. Since the percentage of fruit set in the unsprayed test was low, the numerical data of fruit characters presented in Table 4 were obtained only from the sprayed test. W.V. 19 and Fla P-1 set small and plum shaped fruit, and W.V. 700 and Fla P-14 set small fruits as found in *Lycopersicon pimpinellifolium*. According to Gallegly and Marvel (1955), Fla P-1 is the same as W.V. 19. From the observation of disease

Table 4. The economic characters of late blight resistant lines

Name	Abbreviation	Plant height*	Plant type**	Percent of compound flower cluster	Number of flowers in a flower cluster	Fruit set percent in unsprayed test	Fruit set percent in sprayed test	Fruit shape***	Fruit color	Fruit height	Fruit diameter	Fruit weight
		cm		%		%	%			cm	cm	g
West Virginia-19	W.V. 19	96.3	I	5.1	12.0	2.0	61.3	Pl	Red	1.9	3.0	15.5
West Virginia-36	W.V. 36	107.3	I	1.9	7.6	23.0	64.3	R	Red	1.5	1.4	5.5
West Virginia-63	W.V. 63	67.5	D	0.0	5.7	1.2	22.7	SF	Red	4.9	4.0	83.1
West Virginia-19	W.V. 64	69.5	D	0.0	5.9	0.0	35.4	SF	Red	5.3	4.1	90.8
West Virginia-700	W.V. 700	117.0	I	20.0	12.0	14.5	—	R	Red	—	—	—
Fla LBR-1		101.5	I	0.0	12.5	7.5	33.7	R	Red	2.1	2.3	13.6
Fla LBR-2		114.3	I	0.0	9.7	22.6	88.4	R	Red	1.7	1.5	8.5
Fla P-1		89.5	I	1.8	10.3	0.7	55.7	Pl	Red	1.9	3.0	16.0
Fla P-14		109.5	I	6.9	10.9	70.7	61.7	R	Red	1.4	1.4	3.4
305-52-1-2-3	Nagano-1	87.8	I	1.8	6.4	2.7	49.2	SF	Pink	5.1	3.6	91.0
306-4-24-2-1	Nagano-2	93.5	I	0.0	6.4	3.1	46.5	R	Pink	5.3	5.1	126.8
FL-24-Se-3	Nagano-3	124.0	I	8.6	7.0	9.6	45.0	R	Pink	5.2	4.6	111.8
FL-24-Se-4	Nagano-4	104.3	I	3.7	6.6	0.5	46.2	SF	Pink	6.3	5.0	164.8
PB <sub>1</sub> -3-2-Se-3	Nagano-5	85.0	I & D	19.0	6.6	0.0	28.9	R	Pink	5.2	4.7	107.6
FLR-24 F <sub>1</sub>	Nagano-6	87.5	I	17.9	7.6	0.0	27.1	SF	Pink	5.2	4.3	79.8
FLR-64 F <sub>1</sub>	Nagano-7	92.0	I	3.4	6.5	2.5	43.8	R	Pink	5.1	4.8	112.8
FLR-65 F <sub>1</sub>	Nagano-8	110.5	I	7.1	7.5	6.6	29.5	R	Pink	4.8	4.5	103.8
FLR-66 F <sub>1</sub>	Nagano-9	109.3	I	11.7	7.1	4.1	56.8	SF	Pink	5.6	4.3	121.5
FLR-51 F <sub>1</sub>	Nagano-10	114.3	I	11.3	6.6	2.4	43.1	R	Pink	5.3	4.9	122.0
Atom		35.0	D	3.2	8.4	2.0	40.6	R	Red	2.5	2.7	22.4
Nova		54.0	D	0.0	5.9	0.9	37.7	LC	Red	2.8	5.5	40.4
Red cherry		118.5	I	0.0	8.1	46.4	73.8	R	Red	2.0	1.9	8.0
Luxuriant cherry	Lux. cherry	102.5	I	0.0	6.7	44.8	56.0	R	Red	1.6	1.5	5.5
PI166365												
PI204776		123.0	I	9.1	11.0	20.5	—	R	Red	2.4	2.3	8.0
UCPA1169		124.0	I	2.1	6.2	18.6	60.8	F, I	Red	4.9	3.1	70.6
Ponderosa		107.8	I	80.0	10.9	0.0	21.6	F	Pink	5.7	3.9	113.5
Local variety	Local											

\* investigated on Nov. 16

\*\* I: Indeterminate, D: Determinate

\*\*\* Pl: Plum, R: Round, SF: Slightly flattened, F: Flattened, LC: Lengthened cylindrical, I: Irregular



resistance and economic characters, Fla P-14 seems to be the same as W.V. 700 or a selected line from W.V. 700. All fruits of Nagano lines were pink colored, round and big. Ponderosa set small fruit probably due to severe infection of late blight.

## Discussion

In order to assess the late blight resistance of 28 tomato lines, an inoculation test with *P. infestans* and field tests with and without spraying fungicide were carried out. The similar results were attained from the inoculation test and the unsprayed field test, but the result of the sprayed field test was a little different from them. In the inoculation test and the unsprayed field test, W.V. 36 and Nagano-6 ranked third and forth in descending order of resistance respectively, while 13th and 18th in the sprayed field test. Conversely W.V. 19 and Fla P-1 were ranked 20th and 21st in the unsprayed field test, but sixth and fifth in the sprayed field test. From these results it seems probable that the mechanism of resistance of W.V. 36 and Nagano-6 are different from that of W.V. 19 and Fla P-1.

For the selection of late blight resistance, the field test will be more beneficial under certain circumstances than the inoculation test, because many plants can be tested easily in the former. The inoculation test needs highly concentrated suspension of zoospores. The collection of zoospores from infected tomato leaf is laborious. The mycelia of *P. infestans* did not grow well on the slice of potato tuber purchased in Cameron Highlands, resulting in unsuccessful propagation of inoculum. The soil of fields in Cameron Highlands seems to be heavily infected by *P. infestans*, so that the uniform infection of *P. infestans* is expected in the experimental fields. In the field test, spraying fungicide seems to be inevitable. Without spraying fungicide, it is difficult to evaluate the fruit characters and to obtain offsprings even in a resistant line. The selection without spraying may facilitate the outbreak of a new virulent race.

Fla P-14 can be recommended as a resistant parent for late blight, though the fruit of this

line is very small. UCPA1169, which was evaluated as intermediate resistance by Ko (1975),<sup>3)</sup> also seems to be a promising resistant source, though the shape of the fruit of this line is unfavorable. The repeated backcrossings to the parent which has big and good shaped fruit are necessary to improve their fruit characters. The breeding using these parents requires many years, but a highly resistant variety is expected to be developed. Some of Nagano lines showed tolerable resistance and set fairly big fruit. Nagano-7, which showed high resistance and good fruit characters, is an  $F_1$  hybrid. Though it is not known whether the favorable characters of Nagano-7 are due to hybrid vigor or complementary gene action, it would be expected to select a good pure line from offsprings by selfing of the line. In Malaysia, red colored tomato is preferred in market. From a cross between the Nagano line such as Nagano-2 and W.V. 64, it may be possible within a few years to raise a resistant line with red fruit for commercial cultivation in Cameron Highlands.

As tomato can be grown in the field all year round in Cameron Highlands, repeated selection of two or three generations can be made in a year without any facilities. Cameron Highlands is considered to be one of the most suitable places for breeding tomato resistant to late blight.

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