# Testing Methods for Breeding Disease Resistant Vegetables in Japan

By TAKASHI KURIYAMA

Department of Plant Breeding, Vegetable and Ornamental Crops Research Station

Intensive studies on the breeding of disease resistant vegetables began in the 1950's in Japan. Important techniques for testing disease resistance have been established mainly in the Horticultural Research Station and have been used by seed companies to produce many commercial varieties.

After the establishment of the Vegetable and Ornamental Crops Research Station in January 1973, the Division of Breeding and that of Phytopathology of the Station placed a research priority on the development of screening techniques for disease resistance, and good results are expected.

At present, the tests are conducted by means of natural disease development or artificial infected field (soil-borne disease), and the seedling-test method is now under study with an aim of increasing efficiency of tests.

This report presents these testing methods with an emphasis on the seedling test.

## Fusarium wilt of tomato, *Fusarium oxysporum* f. *lycoperici*

*1) Preparation of inoculum*

A solution of 10 g of peptone, 20 g of maltose, 0.5 g of $NH_4H_2PO_4$ and 0.25 g of $MgSO_4 \cdot 7H_2O$ disolved in 1,000 ml of water is sterilized for 30 minutes under a pressure of 15 lbs, and 100 ml each of this solution is poured into 300 ml flasks. The thallus of *Fusarium oxysporum* f. *lycopersici* is transplanted to the solution and incubated at 27°C for seven to ten days. The flask is shaken 2~3 times during the incubation. A mat of fungi appeared on the surface of the solution is separated by filtration, washed with water, crushed into small pieces and kept in a thermostat, after adding 25 ml of sterilized water, at 27°C for one day. Then the fungal suspension which contains the fungi obtained from one flask is diluted with two liters of water. This diluted suspension of fungi can be used for 200 to 400 seedlings of tomato.

*2) Raising tomato seedlings*

Seedlings are raised for 30 to 40 days in a bench or pots containing bed soil sterilized by steam. Inoculation to the seedlings is done at the five-leaf stage.

*3) Preparation of seed bed for inoculation test*

A mixture of sand (more than 60%), bed soil (10–20%) and farmyard manure (10–20%) is sterilized by steam and deposited in a layer of about 15 cm high on electric heating-wires arranged under the bench in a glasshouse (or a plastic greenhouse) to keep the temperature of the soil at the range of 25 to 32°C. An automatic thermo-regulator can be utilized conveniently for this purpose.

*4) Inoculation and assessment of disease resistance*

Seedlings grown for the test are taken out from the soil. Roots of the seedlings are washed in water to remove soil almost completely and then placed into the inoculum. Then the seedlings are transplanted in the test bed with a spacing of $20 \times 12$ cm. The rest of the inoculum is diluted with water and injected into the test bed. The test beds are

watered sufficiently and shaded in daytime for several days to prevent withering.

Seedlings of the susceptible variety are also mix-planted to check the time of disease appearance. Thus, the seedlings which show no symptoms of disease at the time when the seedlings of the susceptible variety have shown apparent disease symptoms accompanied by withering can be selected as the resistant plants (Plate 1).

Plate 1. Seedling test of tomato Fusarium wilt

## Bacterial wilt of tomato and eggplant, *Pseudomonas solanacearum* E. F. Smith

As to this disease, seedling-test method is still under study, so that the adult-plant test in infected fields is applied at present.

*1) Establishment of infected field*

Tomato or eggplant is planted in a good moisture-retentive field. When the soil temperature has risen over 25°C, 100 ml of the inoculum prepared as described below is injected several times into the soil near each plant at intervals of one month. In the next year and afterwards, soil injection of the pathogen once a year results in the occurrence of disease in nearly all plants of the susceptible variety. The field, therefore, can be used as the test field for disease resistance every year.

*2) Preparation of inoculum*

A solution of 1 g of $NH_4H_2PO_4$ and 5 g of of sucrose in 1 liter of distilled water is autoclaved (30 min 15 lbs). One loopful quantity of the primary culture of *Pseudomonas solanacearum* is placed into 100 ml of the prepared solution, and after five-day incubation at 30°C, it is diluted with water to 500 times the original volume to get the inoculum.

*3) Assessment of disease resistance and selection*

To breed a completely resistant strain of the vegetables against the bacterial wilt might be difficult because polygene is involved. Therefore, plants which suffered to a least extent are regarded as resistant.

Since the disease does not appear until a fairly late stage of growth of the resistant plants, ripened fruits are taken from the plants which are not yet affected by disease at that time and they are reserved as the parent fruits to get seeds. At the end of growth, the plants which scarcely showed the disease symptoms are regarded as resistant plants, and the fruits obtained from such plants are used for seed preparation.

The epidermis of the stem is sampled at the part just above the ground to examine the color of vessels. The plants showing no browning in vessels are regarded as superior resistant plants.

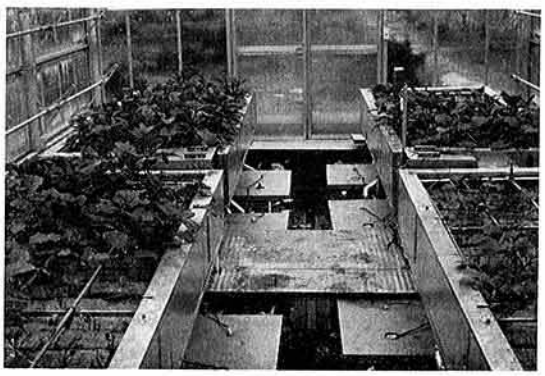


Plate 2. Seedling test of eggplant bacterial wilt

*4) Seedling test method*

As the above test requires four-month period at least, seedling test has been studied.

The seed bed of the seedling test consists of sand principally, and culture solution which is kept at a temperature of 25 to 30°C is sprinkled and circulated in the soil. Therefore, this method may be regarded as a kind of sand culture (Plate 2).

From results of the tests on the eggplant, it can be concluded that disease resistance can be detected at the end of three to six weeks when five-leaf seedlings with the roots washed in the water and submerged in the inoculum are planted in the seed bed which is kept at a temperature of 30°C.

## Bacterial canker of tomato, *Corynebacterium michiganense* (E. F. Smith) H. L. Jensen

*1) Preparation of inoculum*

The bacteria stocked on artificial medium is inoculated once to tomato plant and re-isolated for use in the test because virulence of the pathogen declines owing to successive transfer on artificial medium.

To prepare the medium, 300 g of potato broth, 2.0 g of $Na_2HPO_4 \cdot 12H_2O$, 0.5 g of $Ca(NO_3)_2\ 4H_2O$, 5.0 g of peptone, 20.0 g of sucrose and 15.0 g of agar are dissolved in 1,000 ml of distilled water.

After the sterilization of the medium *Corynebacterium michiganense* is inoculated to the medium to run the slant culture for 4 days at 24°C, and 0.7% solution of NaCl is added to adjust the concentration of the medium at $10^8$ cells/ml.

*2) Raising tomato seedlings*

Seedlings raised in an ordinary bed soil are planted to pots of 9 cm in diameter and inoculated at the four-leaf stage. Increased rate of fertilizer application causes accelerated disease. Seedlings may be raised in any season provided that no trouble occurs in the growth of plants.

*3) Inoculation*

Inoculation is made by the needle-prick stem inoculation method. A sewing needle fixed on the needle of 2 ml injection tube is used to prick the base of petiole of the second leaf.

*4) Assessment of disease resistance and selection*

Disease symptoms begin to appear on susceptible plants about one week after the inoculation. The assessment is carried out at a regular interval for about eight weeks, and diseased plants are eliminated each time. Furthermore, plants showing latent-disease are removed at the stage of seed collection. Plants showing disease symptoms only on the first to third lower leaves are selected as resistant ones, while plants showing disease symptoms on upper leaves are regarded as susceptible.

## Fusarium wilt of cucumber, *Fusarium oxysporum* f. *cucumerium* Owen

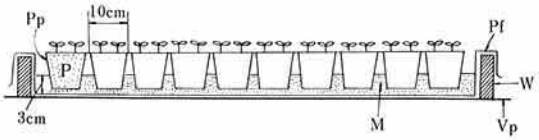
Seedling test method for this disease is still under study but it seems to be fairly promising.

*1) Preparation of inoculum*

Into 1,000 ml of water, 1.0 g of $K_2HPO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 10 mg of FeEDTA,, 1.0 g of yeast extract, 2.0 g of L-asparagin and 30 g of glucose are added, and after sterilization inoculum is added. Then, 250 ml of the solution is placed into 500 ml culture flasks. After the shaked-culture at 28 to 30°C for seven to ten days, the mixture is filtered twice with gauze and kept in a refrigerator at 3°C to make the thallus precipitate. After the removal of the supernatant solution with a syphon, the precipitate is stirred and transferred to graduated centrifuge tubes for centrifugation at 300 rpm for five minutes. Again after the removal of the supernatant, the volume of the precipitate is determined. Then the precipitate is diluted 1 : 1,000 (v/v) with water and stirred well to prepare the inoculum.

*2) Raising seedlings of cucumber and test bed*

Two cucumber seeds are sown in perlite of fairly large size contained in a square pot (10 × 10 cm), and each pot is arranged in a tank to raise the seedlings with the culture solution as shown in Fig. 1.




P : Perlite
$P_p$ : Plastic pot
W : Wooden frame
$P_f$ : Polyethylene film
$V_p$ : Hard polyvinyl
M : Culture solution

Fig. 1. Raising seedlings of cucumber for Fusarium wilt test

*3) Inoculation and investigation*

At the two-leaf stage of the seedlings, 100 ml of the inoculum described above is injected into the soil in each pot kept in a room at 28 to 30°C (it may decrease to 20°C at night). Resistant plants are selected after three to five weeks when all seedlings of the susceptible variety, planted as the control of the test, have died.

## Powdery mildew of melon, *Sphaerotheca fuliginea*

*1) Preparation of inoculum*

Since powdery mildew of melon is caused by an obligate parasite, melons containing *Sphaerotheca fuliginea* are grown by an isolated culture for the purpose of preservation of the fungi. Susceptible varieties are used to increase the inoculum to be used for the test; Spores taken from the leaf surface of the fungi-preserving plants are inoculated to susceptible plants to multiply the pathogen 15 days before the inoculation test (spores taken from diseased leaves at around the 15th day after inoculation reveal usually the strongest virulence).

*2) Prepaartion of test plants and inoculation*

Pots of 12 cm in diameter, containing normal bed soil, are arranged in a glasshouse. One seed of the test plant is sown in each pot. The plants are inoculated with the pathogen at the stage of cotyledon or the first true leaf development. For the breeding of resistant varieties to be grown only in spring season (from February to July), the inoculation to the developing cotyledon is preferable, and in the breeding for all year round resistance, the inoculant should be applied to the first true leaf.

The grain of spores (0.1–1.0 mm in diameter) obtained from diseased leaves prepared as above is applied to the surface of the cotyledon or the first true leaf by using a writing brush at a density of more than one grain per 1 $cm^2$. After inoculation, no particular environment is needed; temperature is of little influence. But watering on the leaf surface must be avoided.

*3) Investigation*

Non-diseased indivduals are selected 15 days after inoculation as resistant plants.

## References

1) Komada, H. & Ezuka, A.: Varietal resistance to Fusarium wilt in cucumber. I. relation between the resistance reaction of adult plants in field and that of seedlings in greenhouse. *Bull. Veg. & Ornam. Crops Res. Sta. Japan, Ser. A*, 1, 233–245 (1974).

2) Kuniyasu, K. & Kuriyama, T.: Studies of practical method for selecting the tomatoes resistant to bacterial canker caused by *Corynebacterium michiganense*. I. Seedling inoculation method and factors affecting disease development. *Bull. Hort. Res. Sta. Japan Ser. B*, 12, 119–132 (1972) [In Japanese with English summary].

3) Suzuki, I., Sugahara, Y. & Todaka, S.: On six promising selections of tomatoes bred for high resistance to Fusarium wilt, and proposal on breeding system for wilt resistance. *Bull. Hort. Res. Sta. Japan, Ser. B*, 1, 57–73 (1962) [In Japanese with English summary].

4) Suzuki, I. et al.: Studies on breeding eggplants and tomatoes for resistance to bacterial

wilt. I. Investigations on method of evaluating the resistance and on the source of resistance in eggplants and tomatoes. *Bull. Hort. Res. Sta. Japan Ser. A*, 3, 77–106 (1964) [In Japanese with English Summary].

5) Takada, K., Kanazawa, K. & Takatsuka, K.: Studies on the breeding of melon for resistance to powdery mildew. I. Difference of resistance among varieties and the breeding of the resistant variety 'Sunrise'. *Bull. Veg. & Ornam. Crops Res. Sta. Japan, Ser. A*, 1, 59–91 (1974) [In Japanese with English summary].