Breeding Methods for Disease-Resistance of Melon, and Development of New Lines with Combined Resistance

By KATUYA TAKADA

Crop Research Division, Chugoku National Agricultural Experiment Station
(Fukuyama, Hiroshima-ken, 450 Japan)

The breeding works presented in this paper were carried out by the author during the period when he belonged to the Breeding Division of the Vegetable and Ornamental Crops Research Station.

Breeding methods

1) Breeding method for CMV-resistance

Among 4 kinds of virus attacking melon in Japan, CMV (Cucumber mosaic virus) is widely distributed, causing severe crop damages. In Japan, studies on breeding for CMV resistance were mainly carried out by the author and his co-workers. At first, by searching for sources of the resistance, it was found that Oriental melon and Oriental pickling melon both widely distributed in East Asia have a high resistance[11]. Then, in order to conduct the breeding for the resistance by utilizing these resistance sources, method of seedling test was established[2]. For this test, tabacco plants were used as a virus multiplication plant, and changes of CMV concentration in tabacco leaves was made clear. At the time when the virus concentration reached the highest (1 week after inoculation), the juice of fresh leaves was taken and diluted with 5 times volume of phosphate buffer solution (0.05 M) for the use (Fig. 1). This procedure was found to be most suitable. As there is no change in the resistance for different CMV strains, the use of a highly virulent strain selected makes it possible to get always stable tests.

The relation of the resistance to seedling age, temperature, light, and nutritional conditions was examined. The resistance appeared differently between cotyledon stage and true leaf stage of seedlings: the resistance specific to each variety is expressed at the stage after the true leaf expansion. Expression of the resistance is influenced only by the temperature after the inoculation; it is desirable to carry out the test at average temperature of 25°C, but not at low temperature which inhibits the appearance of the resistance (Fig. 2). Light hardly effects the resistance. As to the nutritional conditions, it was found that an increased application of nitrogen and phosphate promoted the disease occurrence, while that of potassium suppressed it. Therefore, it is effective to grow seedlings with plenty of nitrogen and phosphate, and less potassium (Fig. 3).

Comparison of the virus concentration in plants of resistant varieties and susceptible varieties showed that it is low for resistant varieties and high for susceptible ones, due to virus multiplication in the latter.

Mode of inheritance of CMV resistance was also investigated. Phenotype of F_1 hybrids is intermediate between both parents, and 2–3 genes (different by varieties) are involved. Relation of the resistance to other characters indicated that a cultivar "Mi tang ting" showed no definite correlation, so that it can be used as a resistant material in breeding for CMV resistance, but "Shiro-uri No. 2" has correlation between the resistance and flesh quality, and hence it is difficult to breed resistant melon by using this variety[13].

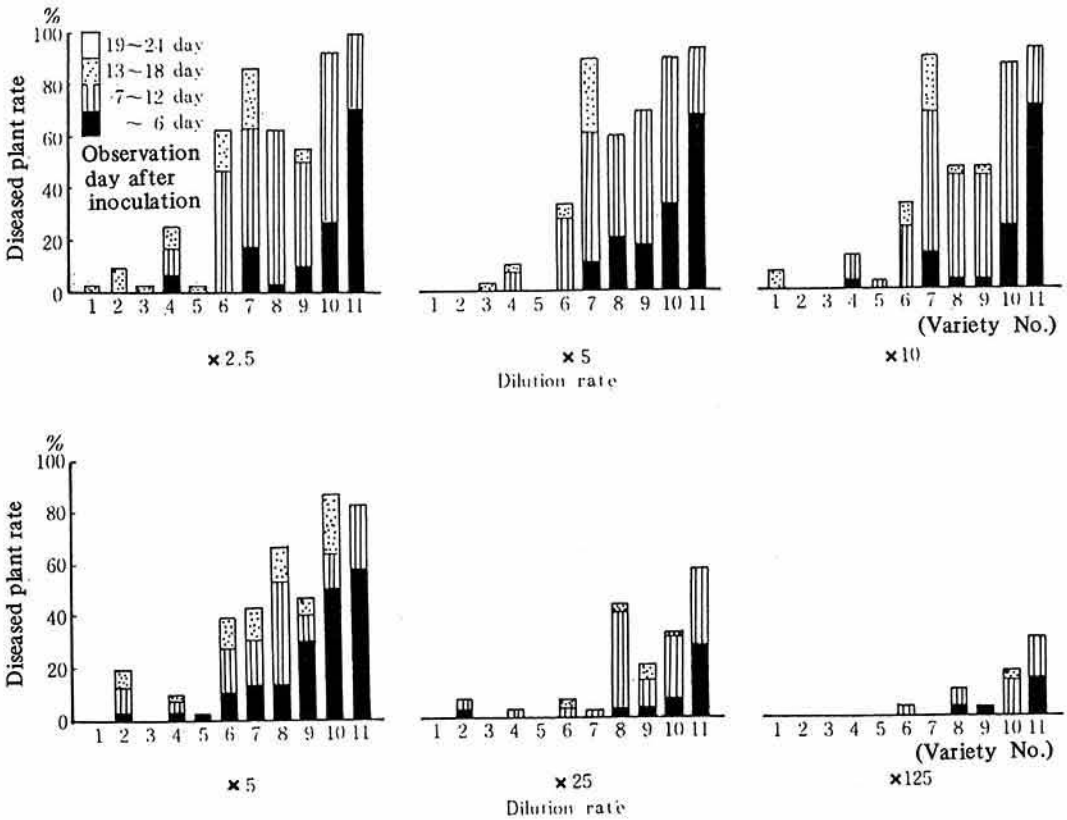

Fig. 1. Relationship between diseased plant rate of melon and dilution rate of CMV inoculum

Variety No. (1) Tōkyō-wase (2) Kankoku No. 1
(3) Shirouri-nigō (4) Mi tang ting
(5) Kankoku No. 5 (6) Sanuki-wase
(7) Shōwa-ōgon (8) Wescan
(9) Honey Dew (10) Pearl
(11) Earl's Favorite

2) Breeding method for resistance to gummy stem blight

Gummy stem blight occurs seriously under a hot and humid climate of Japan. As it is difficult to control this disease, breeding for resistance to this disease is an important task.

There are only a few studies on this disease in Japan, so that method of testing for breeding was studied by the author. Similar to the CMV resistant materials, sources of the resistance were found in melon and Oriental melon of East Asia, and they are very promising as breeding materials, (Table 1) showing always stable resistance, irrespective of cropping types[14]. Then, the method of seedling test was established. At first, effectiveness of spray, needle prickling, and spotted inoculation was compared. At a humid condition (R. H. 95%), any method of inoculation can be employed (except petiole inoculation), but at a medium (80%) and low humidity (60%), only the needle prickling was effective (Fig. 4). For the breeding work which deals with a large number of plants, the needle inoculation by which the testing can be done irrespective of humidity changes

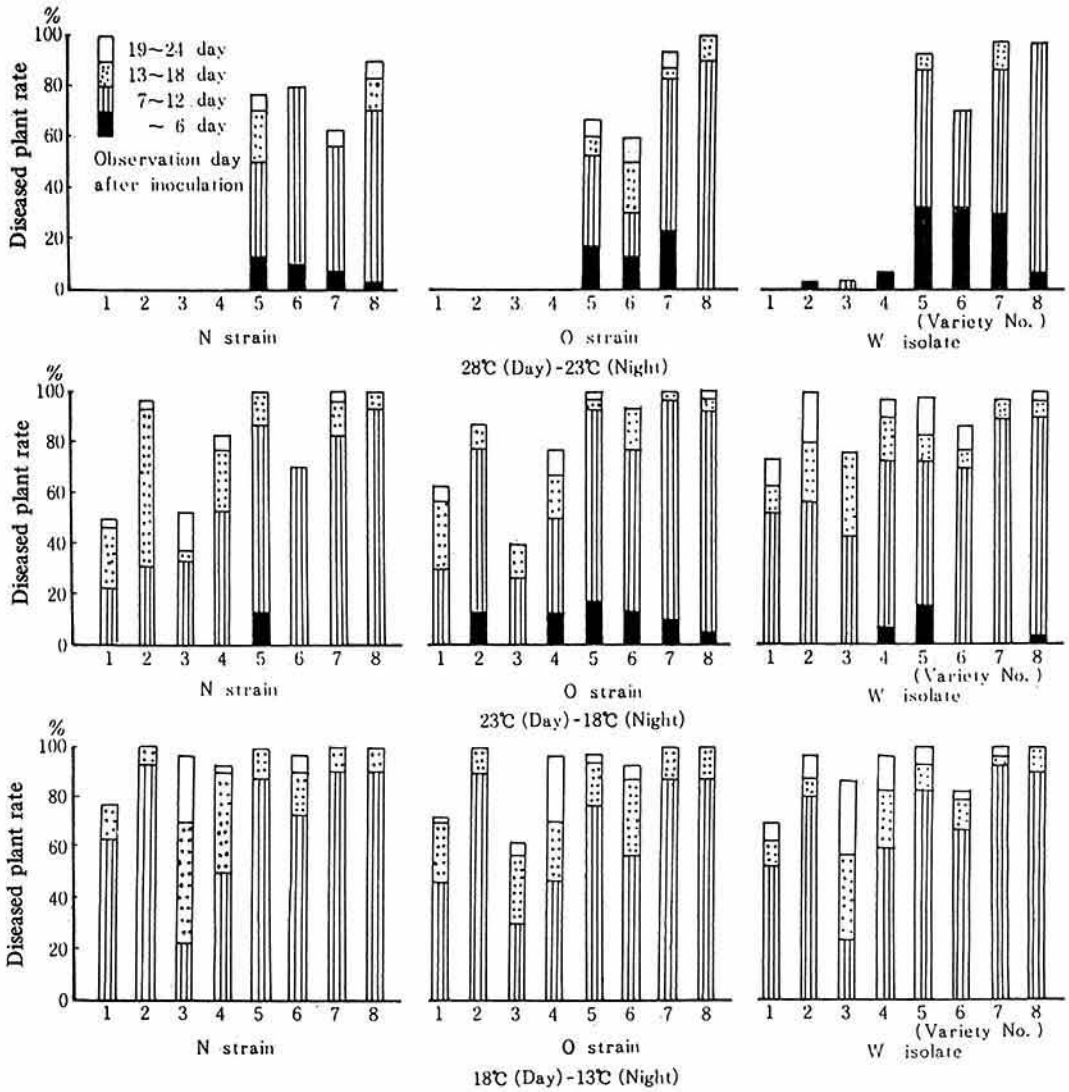

Fig. 2. Relationship between diseased plant rate of melon and various CMV isolates under different temperature

Variety No. (1) Tōkyō-wase (2) Kankoku No. 1
(3) Shirouri-nigō (4) Mi tang ting
(5) Shōwa-ōgon (6) Wescan
(7) Honey Dew (8) Pearl

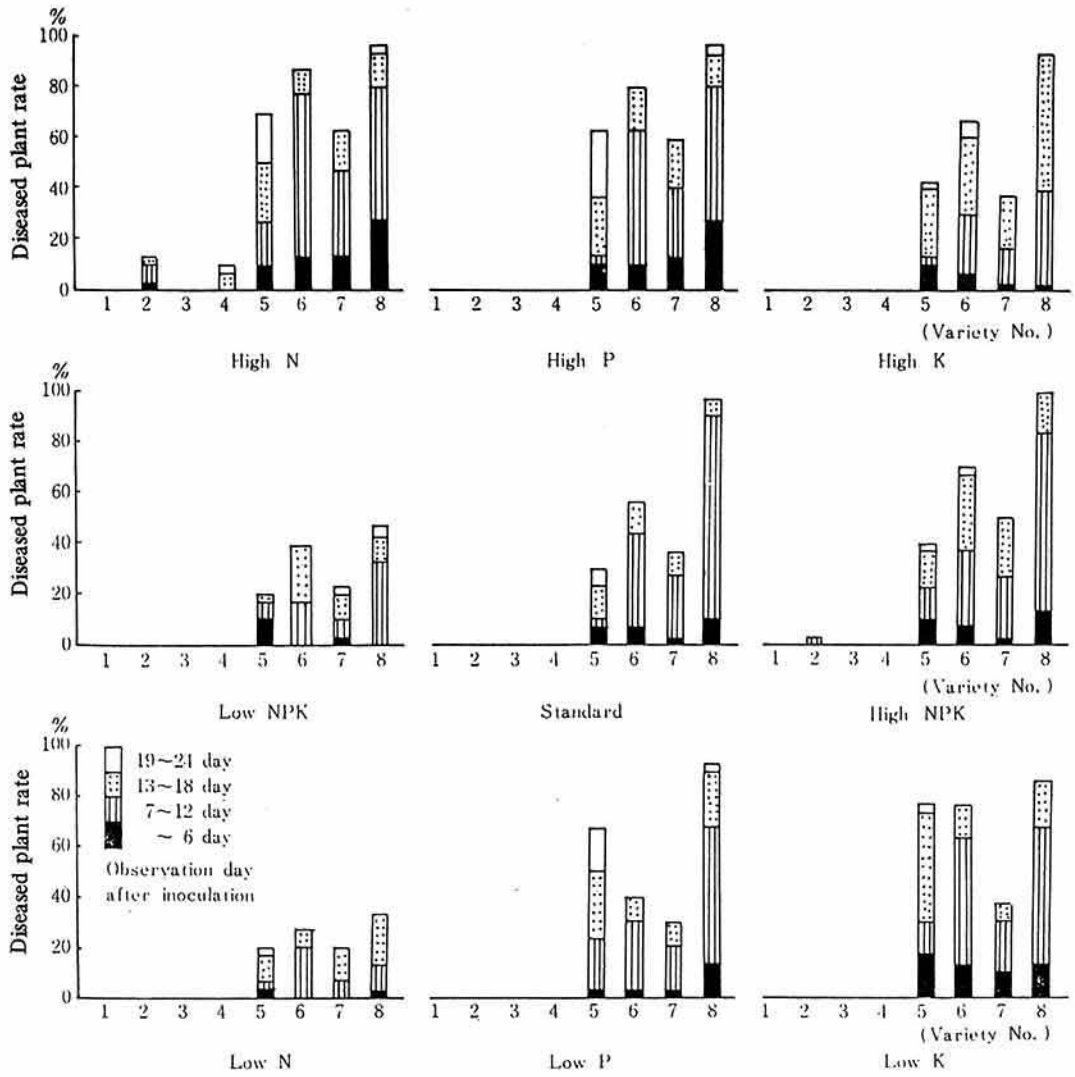

Fig. 3. Relationship between nutritious condition and diseased plant rate of melon by CMV

Variety No. (1) Tōkyō-wase (2) Kankoku No. 1
(3) Shiro-uri No. 2 (4) Mi tang ting
(5) Shōwa-ōgon (6) Wescan
(7) Honey Dew (8) Pearl

Table 1. Distribution of disease index to gummy stem blight in melon varieties in the world (1966)

Area	Disease index											
	1～10	11～20	21～30	31～40	41～50	51～60	61～70	71～80	81～90	91～100	Total	Average
Japan (Muskmelon)						1	1			1	3	70.1
Japan (Oriental melon)		13	15	11	5						44	26.7
Japan (Oriental pickling melon)	4	5	8	4	3						24	24.5
South Korea		14	12	3							29	28.9
P. R. China		1						1	1		3	59.0
Formosa*						3		1	1		5	64.2
U.S.A.					2	3	3	3			11	63.5
South Asia				1				2	1		4	63.9
Middle and Near East						1	2	2	2	6	13	84.5
North Africa				1		1		1		2	5	70.0
U.S.S.R.										2	2	94.4
Other				1			1	3	6	11	22	86.0
Total or Average	4	33	35	21	10	8	6	13	11	21	165	44.2

[a] Presumably bred in U.S.A.

is most advantageous. Effect on disease occurrence of needle prickling (with wounding) and spotted inoculation (without wounding) was compared, and it was found that there is no difference in disease occurrence and resistance expression between the two methods, and the resistance expressed under natural infection appeared by the needle inoculation[15].

After the inoculation, it was found that lesions enlarge at higher temperature (28°C), and varietal differences of the resistance are expressed more evidently under a high light intensity. At the seedling age, just at the time of cotyledon expansion, the varietal differences in the resistance are not clear, while at the stage after the true leaf expansion, the resistance specific to each variety is apparently shown. Nutritional conditions effect the resistance of stems: the lesions are apt to enlarge with high concentration of either nitrogen, phosphate or potassium. But, for the test, any concentration can be used, except high concentration of phosphate, which causes extremely large lesions for resistant varieties[16].

Growing condition for seedlings prior to the inoculation also influences the resistance. Growth under the condition of high temperature, low light intensity, and high humidity reduces the resistance of resistant varieties, so that it is desirable to grow seedlings to be used for the tests at relatively low humidity and low temperature (below 28°C) with a good exposure to light[20].

Appropriate density of the spore suspension to be used for the test was $100 \times 10^3/ml$: at this density the varietal differences of the resistance became rapidly to be evident. No difference in pathogenicity was observed with different isolates, but only the difference in aggressiveness was recognized. Accordingly, it is enough to select and use isolates with high aggressiveness[17,18].

Mode of inheritance of the resistance to Gummy stem blight is that the phenotype of F_1 hybrids is intermediate between both parents, and 2–3 resistance genes (different by varieties) are presumed (Takada, unpublished). As to genetic correlation to other characters, correlation between the resistance and flesh quality was observed in "Shiro-uri No. 2", and hence the use of this variety as a breeding material seems to be difficult. However, no correlation with inferior characters was found in "Mi tang ting", which was regarded to be a quite promising breeding material.

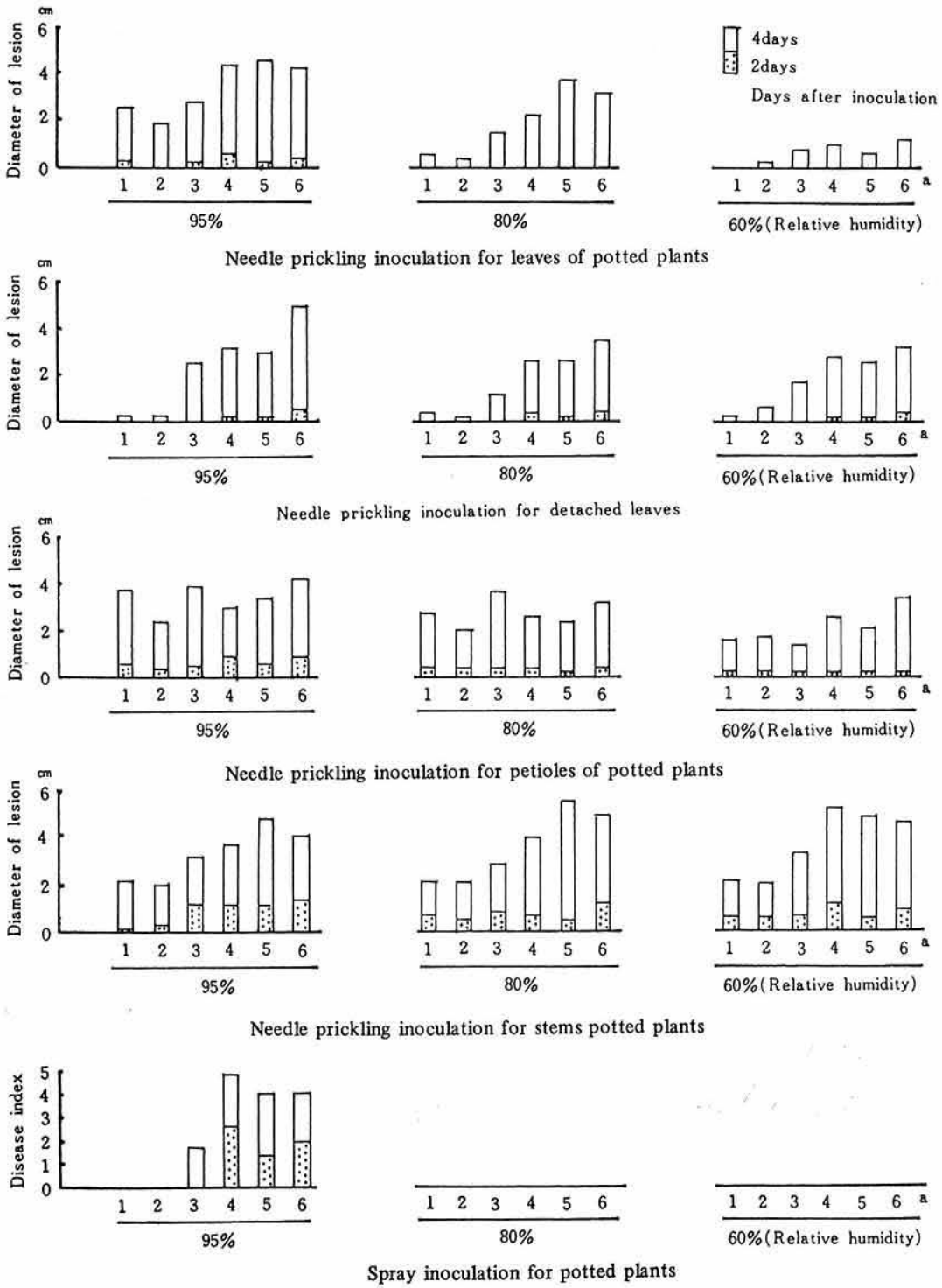

Fig. 4. Relationship between diameter of lesion of gummy stem blight and inoculation method and different relative humidity after inoculation

a. Variety No. (1) Shiro-uri No. 2 (2) Mi tang ting
(3) Kaga-shiromaru-nashiuri (4) Rio Gold
(5) Honey Dew (6) Pearl

3) Breeding method for resistance to powdery mildew

Severe damages to melon occur in both of the culture under structure and the open culture in Japan. Breeding for the resistance has progressed, by the use of resistant varieties developed in U.S.A. as the main breeding material. Tamai[24] made clear for the first time that the resistant varieties in U.S.A. express their resistance in Japan. To get more precise classification of resistant varieties, the author collected varieties of other countries, in addition to the U.S. varieties, and all of them were tested by natural infection in an open culture. The F_1 hybrids between the resistant varieties selected in the above test and susceptible varieties were grown in a plastic greenhouse in the spring and the autumn to examine the phenotype and its change by culture seasons. It was found that there are two groups of varieties: the one which showed stable resistance in both seasons (Table 2) and the other which showed high resistance in the spring but not in the autumn. The former is suitable for the use as breeding material, and as the resistance appears as dominant in F_1, they can be used as parents of F_1 hybrids. At the seedling stage, the group with the stable resistance expressed its resistance only after the true leaf expansion, but cotyledons were diseased, while the group with unstable resistance showed the resistance in cotyledons. Therefore, the resistance of the former group can be identified by true leaf inoculation, while the latter group by cotyledon inoculation[8].

Furthermore, the author analized the mode of inheritance of the resistance. In the group with unstable resistance, one gene is involved, and in the group with stable resistance 2 genes are involved[9]. Later, the author developed resistant lines, "Hiratsuka No. 2" to "Hiratsuka No. 5", by the fixation of hybridization progeny between resistant parents, "Georgia 47" and "C 68 selection", and "Pearl".

4) Breeding method for resistance to Fusarium wilt

In the melon culture in Japan, with plenty of culture under structure and difficulty of adopting cropping system effective to control diseases, Fusarium wilt is one of the diseases requiring the breeding of resistant varieties. Suzuki[7], Shiina et al.[5], Sugawara[6], and Takada[22] studied resistant breeding materials: Suzuki studied mainly on melon culture under greenhouse, Shiina et al. and Sugawara found out promising materials by examining mainly Cantalope introduced from U.S.A., and Takada examined the resistance to Fusarium wilt strains using 370 varieties of many countries. Furthermore, the response of resistant materials to *Fusarium oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *luffae*, and *F. oxysporum* f. sp. *lagenariae* was examined. *F. oxysporum* f. sp. *cucumerinum* showed a strong pathogenicity to melon: the melon varieties resistant to other Fusariums were infected considerably by it. Varieties resistant to all of them are "Baik Bo Aegi", and "Hiratsuka No. 3"[21,22].

Testing method for Fusarium wilt resistance has not been sufficiently studied in Japan. However, Sugawara[6] proved that the application of root dipping inoculation, developed by Wellman[25] as a testing method of Fusarium wilt resistance for tomato, was effective to melon. Komada[4] reported that perlite medium is useful as the culture medium for testing of Fusarium in general[6,25].

5) Breeding method for resistance to downy mildew

As crop damage by this disease is severe under humid condition of Japan, the breeding for resistance is important. Kanazawa et al.[1] tested the resistance to this disease by natural infection in the field, and found that "Chosen melon", "Mi tang ting", "Rio Gold", and "Wescan" were very resistant. Kanazawa[2,3] tried seedling test by using the above-listed resistant materials but the varietal difference was small and not enough. Later, the author (unpublished) found that the secondary infection, occurring after the infection by artificial inoculation, causes quite clear varietal difference in the resistance.

Table 2. Varietal difference of *C. melo* L. in the resistance to powdery mildew under plastic greenhouse in autumn

Class	Multiplication of colony	Variety	F_1 hybrids[a]		
			Multiplication of colony	Class	Inheritance of resistance
Highly resistant (RR)	none (0)	Georgia 47, C 68 P. M. R. No. 5, P. M. R. No. 6 C-389 A, Homegarden 41013	Very slight	Resistant (R)	Incompletely dominant (Ⅰ)
		Okinawa, 41014 41015, 90125 90126	Not checked		
Resistant (R)	Very slight (1～10)	C-108	Slight	Medium (M)	Incompletely dominant (Ⅱ)
		Rio Gold, Edisto # 58-21, Tal Hagilboa Chin ri hae	Severe	Highly susceptible (SS)	Incompletely dominant (Ⅲ)
		India No. 2	Not checked		
Moderately resistant (MR)	Slight (11～20)	Casca de cavalho	Severe	Highly susceptible (SS)	Incompletely dominant (Ⅲ)
Medium (M)	Moderate (21～50)				
Moderately susceptible (MS)	Moderately severe (15～70)				
Susceptible (S)	Severe (71～85)	Resistant, Wescan C. B. R. No. 1, Imperial 45 P. M. R. No. 45, P. M. R. No. 450 Manilla	Severe	Highly susceptible (SS)	Incompletely dominant (Ⅲ)
		Nepal No. 1	Not checked		
Highly susceptible (SS)	Very severe (86～100)	Mi tang ting, Portugal No. 3494 Life, Pearl			

[a] Between varieties with different degrees of resistance and 'Pearl'

Development of varieties with combined resistance

By utilizing resistant breeding materials and methods of seedling test described above, the author succeeded in developing promising selections, "Anō No. 1", "Anō No. 2" and "Anō No. 3", which have combined resistance to CMV, gummy stem blight, powdery mildew, Fusarium wilt and downy mildew.

1) Breeding process

The breeding process is shown in Fig. 5. Firstly in 1964, "Pearl" (excellent flesh quality and flavor) was crossed by "Georgia 47" (resistant to powdery mildew and Fusarium wilt), and selection of the progeny for the resistance to powdery mildew and Fusarium wilt as well as major characters was made by combining seedling test and mature plant test in the spring and winter under a plastic house. In 1971, "Hiratsuka No. 3", which has the resistance to powdery mildew and Fusarium wilt, and high quality, was obtained. This selection was crossed by "Mi tang ting" (resistant to CMV, gummy stem blight, and downy mildew) in 1971, and the progeny was subjected to the selection for the resistance to these 5 diseases and major characters by using mainly the seedling tests, supplemented by mature plant tests in the open field. To improve the quality, the raised line was crossed by "Maruike No. 3" (a selection of Earl's Favourite, good quality and keeping quality), and the similar selection as above was applied. As a result, "Anō No. 1", "No. 2" and "No. 3" which have combined resistance and high quality were raised.

2) Characteristics of "Anō No. 1, No. 2, and No. 3"

(1) Disease resistance of them is shown in Table 3. In the field trial, Hiratsuka No. 3

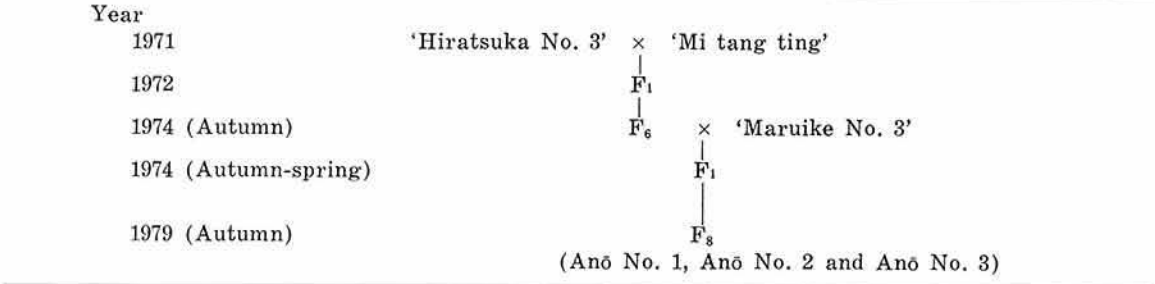

Fig. 5. Breeding process of melon cultivars with combined resistance, Anō No. 1, Anō No. 2 and Anō No. 3

Table 3. Resistance to diseases in the raised lines

Line	Seedling stage				Mature plant				
	CMV[a]	Gummy[c] stem blight	Powdery[b] mildew	Fusarium[a] wilt	CMV	Gummy[b] stem blight	Powdery[b] mildew	Fusarium[a] wilt	Downy[b] mildew
Anō No. 1	10%	0.5 cm	0	0%	0%	15	36	0%	20
Anō No. 2	7	0.5	0	0	0	10	0	0	20
Anō No. 3	4	0.5	0	0	0	10	30	0	20
Hiratsuka No. 3	95	2.4	0	0	—	100	—	—	—
Mi tang ting	6	0.4	100	100	0	15	80	56	20
F_1 Hiratsuka No. 3 × Mi tang ting	64	1.4	0	0	21	50	0	0	38

a: Disease plant-rate, b: Susceptible index, c: Diameter of lesion

Table 4. Fruit characters of the raised lines

	Days from flowering to harvest	Days of keeping quality	Flesh weight	Skin[a] color	Flesh thickness	Soluble solid (Bx)	Flesh[a] color	Fragrance	Flesh quality
			g		cm				
Anō No. 1	40	8	770	LGY	3.0	12.0	LG	High	Excellent
Anō No. 2	41	8	1110	LGY	3.3	12.5	LG	High	Excellent
Anō No. 3	45	12	1280	LGY	3.0	13.0	LG	High	Excellent

a: L=Light G=Green Y=Yellow

(susceptible to gummy stem blight, CMV, and downy mildew), used as a control, was killed by 100% by gummy stem blight. Although the degree of occurrence of the other diseases in this line was not clear, all of these raised lines showed a strong resistance to all the diseases, with only a few infection or without any infection. They showed only a few lesions on lower leaves against powdery mildew, while susceptible varieties showed an index of disease of 8.0. They also showed 0% in death rate against Fusarium wilt, in contrast to 56% of susceptible varieties[23].

(2) Other characteristics are given in Table 4. Differences in these three lines were observed with number of days from flowering to harvest (40–45 days), keeping quality (8–12 days) and fruit weight (770–1280 g) but other characters are similar: beautiful net, light greenish yellow in skin color, light green flesh, and excellent flesh quality with slightly weak fragrance, and moderate sweetness[23]

References

1) Kanazawa, K., Takada, K. & Takatsuka, K.: Studies on the melon resistance to disease. I Experiment of the melon resistant to disease *Ann. Rept. Veg. Div. Hort. Exp. Sta.*, 20–28 (1965) [In Japanese].
2) Kanazawa, K., Takada, K. & Takatsuka, K.: Studies on the melon resistance to disease. II. Evaluation of inoculation methods. *Ann. Rept. Veg. Div. Hort. Exp. Sta.*, 8–13 (1966) [In Japanese].
3) Kanazawa, K., Takada, K. & Takatsuka, K.: Studies on the melon resistance to disease. III. Evaluation of inoculation methods. *Ann. Rept. Veg. Div. Hort. Exp. Sta.*, 38–41 (1968) [In Japanese].
4) Komada, H.: Studies on the vegetable resistance to fusarium wilt I. Cucumber varieties resistance to fusarium wilt and screening methods for breeding material. Summary on the meeting of Jap. Phytopath. Soc. 39(3) (1973) [In Japanese].
5) Shiina, T., Umemoto, T. & Oguuda. F.: Studies on the resistance of cantaloupe varieties to infection of fusarium wilt. *Bull. Yamagata Agr. Exp. Sta.*, (3) 105–114 (1968) [In Japanese with English summary].
6) Sugahara, H.: Applying the root dipping technique to screening of fusarium with resistance and studying on sources of resistance to Melon and cucumber. *Bull. Veg. & Ornam. Crops. Res. Sta.* Ser. C(1). 15–27 (1974) [In Japanese with English summary].
7) Suzuki, H.: Melon varieties resistant to fusarium wilt and strain of the causal fungus *Bull. Shizuoka. Agr. Exp. Sta.*, 11, 65–72 (1966) [In Japanese with English summary].
8) 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.* Ser. A(1) 59–91 (1974) [In Japanese with English summary].
9) Takada, K., Kanazawa, K. & Takatsuka, K.: Studies on the breeding of melon for resistance to powdery mildew. II. Inheritance of resistance to powdery mlidew and correlation of resistance with other characters. *Bull. Veg. & Ornam. Crops. Res. Sta.*, Ser. A(2) 11–31 (1975) [In Japanese with English summary].
10) Takada, K. et al.: On the promising selection melon 'Hiratsuka No. 2, No. 3, No. 4, and No. 5'. Summary on the autumn meeting of Japan Hort. Soc., 180–181 (1974) [In Japanese].
11) Takada, K. et al.: Studies on the breeding of

the melon resistance to cucumber mosaic virus. I. Difference in resistance among Melon varieties and the regional differences in their distribution. *Bull. Veg. & Ornam. Crops Res. Sta.*, Ser. A(5) 1–21 (1979) [In Japanese with English summary].

12) Takada, K.: Studies on the breeding of the melon resistance to Cucumber mosaic virus. II. Testing method of resistance of Melon at the stage of seedling. *Bull. Veg. & Ornam. Crops Res. Sta.* Ser. A(5) 23–69 (1979) [In Japanese with English summary].

13) Takada, K.: Studies on the breeding of the melon resistance to Cucumber mosaic virus. III. Inheritance of resistance of melon to cucumber mosaic virus and other characteristics. *Bull. Veg. & Ornam. Crops Res. Sta.* Ser. A(5) 71–79 (1979) [In Japanese with English summary].

14) Takada, K Kanazawa, K. & Takatsuka, K.: Studies on the breeding of Melon resistant to gummy stem blight. I. Dicerence and regional distribution of varietal resistance. *Bull. Veg. & Ornam. Crops. Res. Sta.*, Ser. A(7) 1–10 (1980) [In Japanese with English summary].

15) Takada, K.: Studies on the breeding of Melon resistant to gummy stem blight. II. Evaluation of inoculation methods and post-inoculation environments for screening breeding materials. *Bull. Veg. & Ornam. Crops. Res. Sta.*, Ser. A(7) 11–12 (1980) [In Japanese with English summary].

16) Takada, K.: Studies on the breeding of melon resistant to gummy stem blight. III. Evaluation of inoculation methods and growth stage and nutritional status of test plants used for screening breeding materials. *Bull. Veg. & Ornam. Crops Res. Sta.*, Ser. A(7) 23–33 (1980) [In Japanese with English summary].

17) Takada, K.: Studies on the breeding of melon for resistance to gummy stem blight. III. Density of spore suspension for screening breeding material. Summary on the spring meeting of Japan Hort. Soc. 162, (1977) [In Japanese].

18) Takada, K.: Studies on the breeding of melon for resistance to gummy stem blight. V. Pathogenicity of the strain for cucurbits and resistance of melon for the strain. Summary on the spring meeting of Jap. Hort. Soc., 214–215 (1978) [In Japanese].

19) Takada, K.: Studies on the breeding of Melon for resistance to gummy stem blight. VI. Phenotypes of the resistance in F_1 hybrids. Summary on the spring meeting of Japan Hort. Soc., 140–141 (1979) [In Japanese].

20) Takada, K.: Studies on the breeding of melon for resistance to Gummy stem blight. VII. Pre-inoculation environments for screening breeding materials. Summary on the autumn meeting of Jap. Hort. Soc. 172–173 (1980) [In Japanese].

21) Takada, K.: Studies on the breeding of Melon for resistance to Fusarium wilt. I. Melon varieties resistant to Fusarium wilt. *Ann. Rept. Plant breeding Div. Veg. & Ornam. Res. Sta.*, 55–66 (1979) [In Japanese].

22) Takada, K.: Studies on the breeding of Melon for resistance to Fusarium wilt. II. Melon varieties resistant to Fusarium wilt and strains of the causal fungs. *Ann. Rept. Plant breeding Div. Veg. & Ornam. Res. Sta.*, 66–73 (1979) [In Japanese].

23) Takada, K.: On the promising selection of melon, Anō No. 1, No. 2 and No. 3, Combined resistance to CMV, gummy stem blight, fusarium wilt, powdery mildew and downy mildew. Summary on the spring meeting of Jap. Hort. Soc. (1980) [In Japanese].

24) Tamai, T.: Resistanc e melon to powdery mildew, No. 5 and No. 45. *Agr. & Hort.*, 27, 65–66 (1952) [In Japanese].

25) Wellman, F. L.: A technique for studying host resistance and pathogenicity of fusarium wilt. *Phytopath.*, 29, 945–956 (1939).

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