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Screening and Breeding for Resistance to Viruses in Cucurbits

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ABSTRACT

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Screening and breeding for resistance to virus diseases in cucurbits have been conducted at TARI for more than 10 years. Research work focused on three important species, *i.e.* cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.) and sponge gourd (*Luffa* spp.). In cucumber, 54 varieties collected from different countries were tested for their resistance to zucchini yellow mosaic virus (ZYMV), type W isolate of papaya ringsopt virus (PRV-W), cucumber mosaic virus (CMV) and cucumber green mottle mosaic virus (CGMMV). The results showed that 19 commercial varieties were resistant to CMV but tolerant or susceptible to ZYMV, PRV-W and CGMMV. All others reacted variably to different viruses except Hei-Pei-Ta-Kua and Chang-Ching which were highly resistant to all 4 viruses. Analysis of inheritance showed that resistance to ZYMV is controlled by a recessive gene and to PRV-W a dominant gene. To search for a dominant gene for resistance to ZYMV, 72 cucumber varieties and 60 inbred lines from other sources were tested again in 1991, and several resistant plants were obtained. The ZYMV resistant variety Tai-Chung-Mu-Kua was used as a breeding parent in crossing with commercial or local varieties. Several lines resistant to ZYMV were selected from the hybridization program. In muskmelon, 60 varieties/lines were screened for resistance to CMV, PRV-W and ZYMV through inoculation tests. Screening was based on visual symptoms as well as ELISA test results. Preliminary data showed that Hei-Sa-Mi and New Melon were resistant to CMV, while line B-63-3, PI 180280 and PI 414723 were resistant to PRV-W. All tested varieties/lines except PI 414723 were susceptible to ZYMV. The breeding program of melon for resistance to major viruses is in progress. In sponge gourd, 33 varieties of *Luffa cylindrica* and 27 of *L. acutangula* were tested for resistance to two major viruses, *i.e.* CMV and ZYMV. The results indicated that most varieties of *L. cylindrica* were resistant to CMV, but susceptible to ZYMV. In contrast, *L. acutangula* varieties were susceptible to CMV, but resistant to ZYMV. Thus interspecific hybridizations of *Luffa* spp. were proceeded. Some promising lines with resistance to both ZYMV and CMV, and with good horticultural characters have been selected from BC_4F_4 population. They will be released to the farmers in the near future.

Key words: Cucurbits, Viruses, Resistance, Breeding.

INTRODUCTION

The major cucurbitaceous plants grown in Taiwan include *Citrullus lanatus* (watermelon), *Cucumis melo* (muskmelon, oriental pickling melon), *Cucumis sativus* (cucumber), *Momordica charantia* (balsam pear), *Benincasa hispida* (wax gourd), *Cucurbita* spp. (squash, pumpkin), *Luffa* spp. (vegetable sponge, angled loofah), *Lagenaria siceraria* (bottle gourd), *Sechium edule* (chayote), and *Trichosanthes anguina* (snake gourd), with a total acreage of 45,000–50,000 ha. (1). The high temperature and humidity in summer favor the occurrence of diseases on cucurbits such as downy mildew, powdery mildew, gummy stem blight, anthracnose, angular leafspot, Phytophthora and Fusarium wilts. Diseases caused by viruses are also important and difficult to control. In dealing with virus diseases, the use of resistant varieties is emphasized.

Breeding program for resistance to viruses in cucurbits has been installed at TARI with the participation of breeders and pathologists. In 1985, diseased samples collected from main cucurbit production areas were assayed for the presence of zucchini yellow mosaic virus (ZYMV), type W isolate of papaya ringspot virus (PRV-W, formerly watermelon mosaic virus-1 = WMV-1), watermelon mosaic virus-2 (WMV-2), cucumber mosaic virus (CMV), and cucumber green mottle mosaic virus (CGMMV) by ELISA (3,4). ZYMV was identified as the most wide spread virus in all cucurbits except bottle gourd which was most frequently infected by CGMMV (3,4,5,14). This paper summarizes the results from 10 years of screening and breeding for virus resistance in cucurbits in Taiwan Agricultural Research Institute (TARI). Three important crops, *i. e.* cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melon* L.) and sponge gourd (*Luffa* spp.) were the focus of this work.

CUCUMBER

Screening for resistance to CMV, CGMMV, PRV-W and ZYMV

Fifty-four varieties/lines planted in spring or summer in 1983 were separately inoculated with CMV, CGMMV, PRV-W and ZYMV at the seedling stage. The screening results were summarized in Table 1 and briefly described as follow:

TABLE 1. Reaction of *Cucumis sativus* to viruses

Reaction	No. of varieties/lines reacted to			
	CMV	CGMMV	PRV-W	ZYMV
Highly resistant	38	15	13	5
Resistant	2	10	10	9
Susceptible	5	13	6	11
Highly susceptible	9	16	25	29
Total	54	54	54	54

TABLE 2. Reaction of cucumbers to virus combinations

Reaction	No. of varieties/lines reacted to				
	PRV-W + CMV	PRV-W + CGMMV	ZYMV + CMV	ZYMV + CGMMV	ZYMV + PRV-W
Highly resistant	0	0	0	0	0
Resistant	1	0	1	0	0
Susceptible	5	4	9	0	10
Highly susceptible	8	10	4	14	4
Total	14	14	14	14	14

To CMV: All Taiwan commercial varieties were found to be highly resistant to CMV, all local landraces except Shuang-Shi and Improved Ching-Pei were resistant, all American and South-East Asian varieties were susceptible. All Japanese commercial varieties and Nong-Fa Nos. 18 and 19 were also highly resistant to CMV.

To CGMMV: All Taiwan commercial varieties were resistant to CGMMV, except V. O. 262, China No. 1, Joy, and Kim-Chung 258 which developed serious mosaic and stunting symptoms. All local landraces except Tung-Kua-Ching and Hei-Pei-Ta-Kua were susceptible to CGMMV. All introduced varieties and lines except PI 188807, PI 227208 and Chang-Ching were also susceptible.

To PRV-W: All but 4 commercial varieties showed resistance. All except Hai-Pei-Ta-Kua and Cucumber No.2 were susceptible. All TARI inbred lines and introduced varieties were susceptible, except Chang-Ching and CITC-70-186 which were resistant.

To ZYMV: All commercial varieties were susceptible with the exception of V. O. 262 and Joy which were tolerant. All the landraces, TARI inbreds and introductions were also susceptible except for Hei-Pei-Ta-Kua, Cucumber No. 2, and Charng-Ching.

Generally speaking, all varieties/lines were susceptible to one or more viruses except Hei-Pei-Ta-Kua and Chang-Ching which were highly resistant to all four viruses when separately inoculated at the seedling stage.

Screening for resistance to virus complex

Cucumbers in the field are frequently infected by more than one kind of viruses and yields are significantly reduced (14). To screen for resistance to virus complex, 14 varieties were inoculated with combinations of two different viruses as shown in Table 2. Only Hei-Pei-Ta-Kua was resistant to complexes of PRV-W and CMV, ZYMV and CMV, but it was susceptible to other 3 combinations of viruses. Other varieties were all susceptible to such virus complex. The yellow spotting symptom appeared on susceptible plants as early as 4 days after inoculation. Vein banding and severe mosaic symptoms developed progressively on plant growth. Hei-Pei-Ta-Kua was resistant to all 4 viruses individually tested, but it was partially susceptible to combinations of viruses with mottling and mosaic symptoms. All other varieties developed more severe symptoms and had shorter incubation period when inoculated with virus combinations instead of individual virus.

Inheritance of virus resistance in cucumber

Inheritance of ZYMV resistance: Cucumber No. 2 was resistant to ZYMV and developed no symptoms when inoculated, while Shuang-Shi was susceptible to

TABLE 5. Reaction of cucumber inbreds at F_5, F_6 and F_7 generations to ZYMV[1]

Generation	Total plants tested	No. of plants with		No. of symptomless plants
		Mosaic	Mottle	
F_5	3,209	1,858 (57.9%)	839 (26.1%)	512 (16.0%)
F_6	2,196	1,184 (53.9%)	578 (26.3%)	434 (19.8%)
F_7	2,759	491 (17.8%)	69 (2.5%)	2,199 (79.7%)

[1] There were 142 inbred lines for F_5 generation, 136 for F_6 and 108 for F_7. seedlings were inoculated with ZYMV for each generation. Symptomless F_5 plants were selected and inbred. Symptomless and low ELISA value plants were selected and inbred at F_6 generation.

TABLE 6. Results of ELISA tests on cucumber inbred lines inoculated with ZYMV

Line/combination	Generation	ELISA value	Generation	ELISA value[1]
Tai-Chung-Mu-Kua (TCMK)	S_{11}	0.017	S_{12}	0.008
Hai-Pei-Ta-Kua (HPTK)	S_9	0.020	S_{10}	0.016
TCMK × Shuang-Tou-Chi	F_6	0.178	F_7	0.046
TCMK × China No 1	F_6	0.126	F_7	0.021
TCMK × Green Bowl	F_6	0.062	F_7	0.015
HPTK × Chu-Shan	F_6	0.511	F_7	0.048

[1] Average of ELISA values from 10 test plants.

TABLE 7. Reaction of different cucumber varieties and TARI inbred lines to ZYMV

Reaction	No. of varieties	No. of TARI inbreds
Highly resistant	4	9
Resistant	4	11
Susceptible	18	12
Highly susceptible	46	28
Total	72	60

Since the resistance of Hai-Pei-Ta-Kua and Tai-Chung-Mu-Kua to ZYMV is controlled by a single recessive gene, it is not easy to transfer their resistance to commercial varieties. Therefore, another set of materials including 60 TARI inbred lines and 72 varieties were tested again to search for dominant resistance genes to ZYMV. It was found that 4 varieties and 9 TARI inbred lines were highly resistant to ZYMV (Table 7). These 4 varieties were Vantage, Cucumber 249, Chung-Nong No. 4, and Bountiful No. 2. All resistant plants were maintained by inbreeding to promote homozygosity with the hope of finding donimant resistance genes for breeding use. At present, studies on the inheritance of these new resistance sources are in progress.

Breeding of new cucumber lines

All of the collected cucumber varieties/lines were first investigated for their horticultural characteristics. Selected varieties were grown for 6–7 generations by inbreeding. Eight valuable lines were obtained which were early and high yielding (6). Diallel cross analyses of these 8 lines indicated that there were 12 combinations with a short growing period, high yielding, good general and specific combining abilities. Based on the results from regional tests, 2 lines i. e., No. 183 and No. 132, were selected. They produced a yield of 6.04 and 6.21 t/ha., respectively. The first 10 days of harvest was as high as 1.8 t/ha. The dark green fruits were uniform in size, white spined, with thick flesh and small seed cavity. The shelf life is long and the skin color does not change under high temperature conditions. These two lines also had better resistance to ZYMV, CMV, and PRV-W than commercial varieties did (Table 8).

MUSKMELON

Screening for resistance to CMV, ZYMV and PRV-W

The results of screening for resistance to CMV, ZYMV and PRV-W were summarized in Table 9.

To CMV: Sixty-eight varieties/lines were inoculated with CMV at seedling stage. The preliminary results showed that only one variety was highly resistant, 2 resistant, 11 susceptible and 54 were highly susceptible. The varieties resistant or highly resistant to CMV included Jade-Beauty, Fung-Shin No. 2 and Sky-Rocket.

To ZYMV: Sixty varieties/lines were inoculated with ZYMV at seedling stage, no varieties were found

TABLE 8. Yield performance of new cucumber hybrids tested in four regional trails[1]

Hybrids	Early yield[2]		Total yield[3]		Disease reaction[4]		
	Weight (t/ha)	Fruits (No./ha)	Weight (t/ha)	Fruits (No./ha)	CMV	PRV-W	ZYMV
TN 132	1.84	2,512	6.04	8,589	R	R	S
TN 183	1.86	2,498	6.21	8,676	HR	HR	R
Green Bowl (CK)	1.82	2,236	6.16	8,556	S	T	HS
Li Feng (CK)	1.80	2,330	6.03	8,556	R	T	HS

[1] The four regional trails were located in Taichung, Tounan, Tapei and Yenpu.
[2] Early yield was the average of first 10 days of harvest from 4 trails.
[3] Total yield was the average from 4 trails.
[4] HR = highly resistant, R = resistant, T = tolerant, S = susceptible, HS = highly susceptible.

TABLE 9. Reaction of *Cucumis melo* to different viruses in the greenhouse inoculation tests

Reaction	No. of varieties/lines reacted to		
	CMV	ZYMV	PRV-W
Highly resistant	1	0	5
Resistant	2	0	1
Susceptible	11	1	0
Highly susceptible	54	59	8
Total	68	60	14

to be resistant to ZYMV. The PI 414723 was reported as a resistant source in the United States (2), it also showed susceptible to ZYMV in this test (5). Screening results indicated that only one variety was susceptible, while the remaining 59 varieties were all highly susceptible to ZYMV.

To PRV-W: Fourteen varieties/lines were inoculated with PRV-W at seedling stage, the variety Delicate and some PI introductions were found resistant, however most commercial varieties were susceptible.

In summary, there were varieties or lines with resistance to CMV or PRV-W in separate inoculation tests, but there was no variety/line with resistance to ZYMV. Even PI 414723 which was reported as resistant to ZYMV in USA was found to be susceptible in our tests (2,9). However, a few plants of PI 414723 showed some degrees of tolerance to ZYMV. Thus, through generations of inbreeding from those selected plants, lines resistant to ZYMV are expected to be available.

Relationship between temperature and virus resistance

Muskmelon varieties/lines were inoculated in both spring and summer seasons. The average maximum and minimum temperatures were 30.3 C and 22.9 C in the spring, 34.1 C and 25.1 C in summer. It was found that when the test plants were inoculated with CMV at the seedling stage, both the disease incidence and the disease index were higher in summer than in spring. On the other hand, when the seedlings were inoculated with ZYMV, the disease incidence was about same for the two seasons, but the disease index was quite different (Table 10). In general, viruses cause more serious infection to muskmelon in the warm season.

VEGETABLE SPONGE

Screening for resistance to CMV and ZYMV

Sixty varieties of vegetable sponge including 33 *Luffa cylindrica* and 27 *L. acutangula* varieties/lines were screened for resistance to CMV and ZYMV in spring 1984. The results were showed in Table 11. For CMV study, of the 33 *L. cylindrica* varieties under test, 32 were resistant or highly resistant, and only one variety was susceptible. In *L. acutangula*, 19 varieties were susceptible, 5 varieties resistant, while other three varieties, *i.e.*, SLK-V-036, SLK-V-022 and IDA-V-166 were highly resistant. Symptoms on susceptible plants included mild mosaic or mottling. Screening for resistance to ZYMV, the results showed that all 33 *L. cylindrica* varieties except Seven-beauty were susceptible. The main symptoms on susceptible plants were rugose and mosaic. All introduced varieties of *L. acutangula* from China were susceptible, but local varieties and varieties introduced from South East Asia were all resistant. The symptoms on the susceptible plants mainly consisted of chlorotic spots (13).

Interspecific hybrids of *Luffa* spp.

As most of *L. cylindrica* varieties are susceptible to ZYMV, but resistant to CMV, and most of *L. acutangula* varieties susceptible to CMV, but resistant to ZYMV, the two species should probably compensate each other for disease resistance (13). Because they also differ in many morphological and other characters, such as floral structure, time of anthesis, fruit shape,

TABLE 10. Infection of *Cucumis melo* varieties by CMV and ZYMV at different seasons

Virus	Plant variety	Summer, 1990		Spring, 1991	
		No. of plants tested	Disease incidence (%)	No. of plants tested	Disease incidence (%)
CMV	Sunrise	11	100	20	15
	Delicate	12	100	30	57
	Sky-rocket	10	50	30	23
ZYMV	New-century	11	100	32	87
	Sunrise	11	100	30	96
	Silver-light	11	100	30	100

TABLE 11. Reactions of *Luffa* spp. to CMV and ZYMV in greenhouse

Reaction	*L. cylindrica*		*L. acutangula*	
	CMV	ZYMV	CMV	ZYMV
Highly resistant	24	1	3	23[1]
Resistant	8	1	5	3
Susceptible	1	1	6	0
Highly susceptible	0	30	13	1
Total	33	33	27	27

[1] No. of *Luffa* spp.

TABLE 12. Results of selection for ZYMV resistance in different backcross generations of interspecific hybrid of *Luffa* species

Generations	No. of combinations	Total plants	Reaction		
			R	T	S[1]
BC_1	25	2,518	572	357	1,589
BC_2	18	693	111	184	398
BC_3	16	871	109	198	564
BC_4F_1	16	4,442	738	14	3,564
BC_4F_2	12	1,219	64	84	1,071
BC_4F_3	10	622	66	16	540
BC_4F_4	10	480	355	32	93

[1] R: resistant, T: tolerant, S: susceptible

and fruit quality (8,10,11,12). Hybridization between these 2 *Luffa* species as conducted in this study was the objective of improving the fruit quality and disease resistance. The results from F_1 generation plants obtained showed good plant vigor and satisfactory fruit setting with many horticultural charateristics such as number of corolla, number of style, fruit surface and seed coat close to *L. acutangula* (11,12,13), but the F_1 fruits tasted bitter (10,11,13). The male flower of F_2 plants contained little amount of pollens and these pollens were underdeveloped, so it was difficult to obtain F_3 progenies. Successive backcross of the F_1's to the non-recurrent parent was carried out to BC_4 generation. At each backcross generation, seedlings were inoculated with ZYMV. Then, all susceptible plants were discarded and resistant plants transplanted to field. As the resistant plants set fruit, the immature fruits were picked up and tasted to screen out bitter ones. Only plants bearing no bitter fruit were kept until maturity. Harvested fruits were also cooked to see whether they turned brown. If they turned brown, they were discarded. All plants selected by these criteria had satisfactory level of disease resistance were with early maturity, and produced good quality fruit which retained green color after cooking. Therefore, the breeding cycle was carried out to the BC_4F_4 generation, and the fertility, seed germination and resistance to ZYMV of interspecific hybrids of *Luffa* species increased through backcrossings. In BC_4F_4 generation, 387 plants (80.6%) were found resistant or tolerant to ZYMV. However the disease incidence and resistance to ZYMV still varied among different combinations (Table 12). In 1992, regional trials were under taken at TARI and in Pingtung prefecture. The anthesis and node of the first female flower of TARI breeding lines were earlier and lower than the commercial or local check varieties. The yield of TARI breeding lines was also significantly higher than that of commercial, local check varieties, especially the harvests of the first 10 and 20 days (Table 13,14).

FUTURE PROSPECTS

The occurrence and distribution of virus diseases in cucurbits in Taiwan have been disclosed. The use of resistant cultivars is an effective and economic method for controlling plant viruses. Therefore, breeding programs for virus resistance in cucumber, melon and vegetable sponge have been conducted at TARI during the past decade. At present, some virus resistant lines are developed as potential parents for future use, and one cucumber and one vegetable sponge lines which are resistant to major viruses and possess with good

TABLE 13. Average yield and horticultural characters of *Luffa* breeding lines in TARI

Variety/line	Days of 1st ♀ open	Node of 1st ♀ located	Early yield[1] I		Early yield[2] II		Total yield	
			weight (t/ha)	Fruit (No./ha)	weight (t/ha)	Fruit (No./ha)	weight (t/ha)	Fruit (No./ha)
TARI No.1	63.75 b[4]	11.5 a	3.96 c	6,563 c	9.20 b	15,925 bc	27.59 ab	46,463 ab
TARI No.3	61.50 a	9.8 a	3.32 bc	6,563 c	10.19 b	19,688 c	30.54 b	54,513 b
TARI No.5	67.75 c	16.8 b	2.24 b	3,500 b	7.45 b	12,163 b	23.01 ab	36,225 a
Check[3]	85.00 d	24.8 c	0.15 a	263 a	0.42 a	788 a	19.71 a	33,950 a

[1] Early yield includes fruits from the first 10 days of harvest.
[2] Early yield includes fruits from the first 20 days of harvest.
[3] Variety used for check was Seven-beauty from Known-you Seed Co.
[4] Means within columns separated by LSD test at 5% level.

TABLE 14. Average yield performance of *Luffa* breeding lines in Ping-tung

Variety/line	Early yield[1] I		Early yield[2] II		Total yield	
	weight (t/ha)	Fruit (No./ha)	weight (t/ha)	Fruit (No./ha)	weight (t/ha)	Fruit (No./ha)
TARI No.1	11.25 b[4]	17,840 b	29.76 b	44.64 b	34.83 b	79,200 b
TARI No.3	13.83 c	22,260 c	31.62 b	48.30 b	40.62 c	83,160 c
Check[3]	1.98 a	13,020 a	3.66 a	24.48 a	29.76 a	55,080 a

[1] Early yield includes fruits from the first 10 days of harvest.
[2] Early yield includes fruits from the first 20 days of harvest.
[3] Variety used for check was a Ping-tung local variety.
[4] Means within columns separated by LSD test at 5% level.

horticultural characteristics have been selected for regional trials, with the promise of being registered and released to the farmers soon.

Since Taiwan is in the tropical and subtropical areas, the environmental conditions, different cropping systems and the varieties changing affect the incidence and severity of different cucurbits by various virus infections. For the possibilities of the occurrence of new viruses or strains and the development of resistant cultivars, it is still need to make a systematic virus survey in the cucurbit fields in the island-wide again, locate more sources of resistance, and study the inheritance from both domestic and foreign germplasms. In the future, other important cucurbits such as watermelon, bitter gourd, bottle gourd, wax gourd and squash/pumpkim are likely to be included in the breeding program. Recently, approaches using gene transfer technologies indicate some promise for obtaining plants which are resistant or tolerant to viral diseases. Thus, cooperation with different scientists as a team work should urgently be made. We hope more cucurbit varieties with multiple viral resistance will be available in the near future.

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摘　　要

蕭吉雄[1]、楊偉正[1]、張有明[1]、王三太[1]、黃秋雄[2]．1993．瓜類抗病毒選育．植病會刊 2:241-248．(1. 台中縣　台灣省農業試驗所園藝系，2. 台中縣　台灣省農業試驗所植物病理系)

本文敘述有關胡瓜、甜瓜及絲瓜之抗病毒篩選、抗病性遺傳及抗病品種選育等之試驗結果。胡瓜：54個胡瓜品種分別對胡瓜嵌紋病毒(CMV)，木瓜輪點病毒—— 西瓜系統(PRV-W)，胡瓜綠斑嵌紋病毒(CGMMV)，和矮南瓜黃化嵌紋病毒(ZYMV)等四種病毒之抗病性篩選結果，得知19種胡瓜商業品種對CMV具有抗病性，但對CGMMV，PRV-W或ZYMV則分別爲耐病性或罹病性。除黑皮大瓜(Hei-Pei-Ta-Kua)和長青(Chang-Ching)對上述四種病毒具有強抗病性外，其餘品種都有不同程度的感病性。由胡瓜抗病遺傳分析，得知胡瓜對ZYMV之抗病性係受一對隱性遺傳基因所控制，而對PRV-W之抗病性則係受一對顯性遺傳基因所控制。爲求獲得顯性之ZYMV抗病基因，於民國80年，另進行72個胡瓜品種和60個農試所自交系之ZYMV抗病性篩選，並獲得一些抗病單株。以抗ZYMV之台中目瓜(Tai-Chung-Mu-Kua)品種分別和地方品種或商業品種雜交，並由雜交後代篩選到一些抗ZYMV之單株。甜瓜：60個甜瓜品種(系)分別對CMV、PRV-W和ZYMV三種病毒之抗病性篩選結果，得知黑沙蜜甜瓜(Hei-Sa-Mi)和New Melon兩品種對CMV病毒具有抗病性，B-63-3, PI 180280，PI 414723等對PRV-W病毒具有抗病性，至於對ZYMV病毒除PI 414723略具抗性外，其餘品種都不抗病，有關甜瓜對病毒之抗病性轉移工作，仍在繼續進行中。絲瓜：進行60個絲瓜品種，其中包括圓筒絲瓜(*Luffa cylindrica*)33種，稜角絲瓜(*Luffa acutangula*)27種，對CMV和ZYMV病毒之抗病性篩選，得知圓筒絲瓜抗CMV而對ZYMV感病，稜角絲瓜對CMV罹病但抗ZYMV；以圓筒絲瓜爲輪迴親，稜角絲瓜爲非輪迴親進行種間雜交，在BC_4F_4世代獲得一些抗ZYMV和CMV，且園藝性狀良好之品系，目前正進行區域試作，未來擬提請命名登記，供農民種植。

關鍵詞：瓜類、病毒、抗病篩選、育種。