Hybrid Breeding of Red Pepper (*Capsicum annum* L.) Using Cytoplasmic Male Sterile Lines Combined with *Phytophythora* Resistance in Korea

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ABSTRACT

We characterized the resistances such as to waterlogging, *Phytophythora* and viruses in hybrids between Italian and Korean mother lines and screened them for complex resistances and agronomic traits to select elite multi-resistant lines for hybrid breeding. Resistance to waterlogging was selectable due to diversity of the resistance. *Phytophythora* resistance introduced from Italian lines could also be combined with resistance to other diseases and restoration abilities from cytoplasmic male sterility that has been maintained in Korean varieties.

Key words : Red pepper, Kim Chi cultivar, *Phytophythora* resistance, complex resistance, hybrid breeding, Cytoplasmic male sterility

INTRODUCTION

Red pepper (*Capsicum annuum* and *C. frutescens*) is widely used as an important spice, pickles and fresh vegetable today in Asia. This situation has been established within a short term after the introduction of red pepper from South America (NAJ, 1992). During the spread around the world, many variations and cultivars were generated. (Haiser, 1995; Terpo, 1966). Tropical, sub-tropical and many other types have been diversified with the development of breeding activities. Today, the vegetable pepper is the most important spice vegetable in the world and even in Asia.

In Korea, we have produced special types for Kim Chi pickles type. We have established a hybrid seed production system for Kim Chi pepper breeding. However, an urgent subject for breeding is to establish high yield, disease, resistant (Daskalov and Poulos, 1994) and waterlogging-resistant cultivars. To overcome the difficulty of pepper breeding, we have introduced useful genetic resources from the world and developed a resistance breeding program (Kim et al.,

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1989).

We started this breeding program to combine cytoplasmic male sterility (CMS) with *Phytophythora* resistance. In the present paper, we describe the possibility of combining CMS, *Phytophythora* and virus resistances with other desired commercial characteristics for hybrid breeding of Korean pepper.

MATERIALS AND METHODS

We used several typical types of cultivars including special Kim Chi type for hybrid breeding (Fig. 1 and 2). In addition to *Phytophythora* disease resistance, wet tolerance and other important agricultural characteristics were investigated using Italian lines,

Table 1 Characteristics of resistant lines for TMV, PVY, nematodes and P. capsici used in crossing programs

Line	Resistance	Fruit character					
		Length (cm)	Width (cm)	Thickness (mm)	Weight (g)	Color	Pungency
T3		10.3	1.6	2.0	12.5	Red	Hot
T4*		12.5	3.5	5.0	30.6	Red	Sweet
T8		14.5	2.0	2.0	15.0	Red	Hot
TT10		9.4	2.0	2.0	16.1	Red	Sweet
T17	*P. capsici*	9.4	1.3	2.0	5.8	Dark red	Hot
T18		12.5	1.4	2.0	8.0	Red	Hot
T112-1		8.1	1.0	1.0	6.4	Red	Hot
T112-2		6.5	1.0	1.0	4.7	Red	Hot
T116-1		8.6	1.5	2.0	6.2	Light red	Hot
T116-2		10.0	1.4	2.0	6.2	Light red	Hot
T25-1		8.5	2.0	2.5	8.3	Red	Sweet
T25-2		11.6	2.1	2.5	16.0	Red	Sweet
T25-3	TMV	9.3	2.4	4.0	9.4	Red	Sweet
T26-1		6.0	2.0	2.0	8.3	Red	Sweet
T26-2		5.0	2.0	2.0	7.2	Red	Sweet
T30-1		9.6	2.0	3.0	14.2	Red	Hot
T30-2		8.3	1.5	2.0	5.5	Red	Hot
T30-3		7.3	1.1	2.0	4.5	Red	Hot
T31-1	PVY	12.9	4.0	3.0	54.5	Red	Sweet
T34-1*		9.5	5.0	2.0	48.3	Red	Sweet
T34-2		15.0	5.0	2.0	92.5	Red	Sweet
T34-3		13.2	4.0	3.0	98.2	Red	Sweet
T37-1*		16.7	2.7	5.0	55.0	Red	Sweet
T37-2*	Nematodes	15.0	3.0	3.0	39.5	Red	Sweet
T37-3*		14.0	2.5	3.0	30.0	Red	Sweet

Resistance to *P. capsici* was reconfirmed in all 10 lines by the inoculation test at Tohoku Seed Co. (Japan). Asterisks show lines excellent for commercial use with resistance.

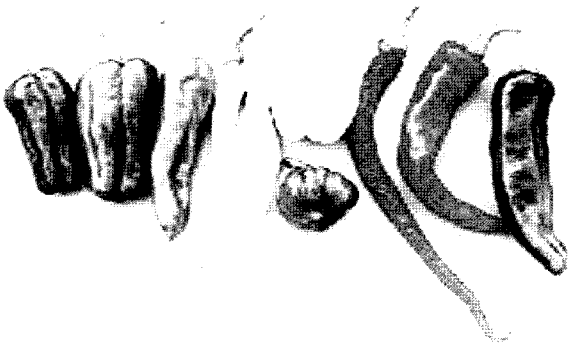

Fig. 1. Various fruits used for red pepper breeding. For Korea Kim Chi cultivars, the right three types are used as parental lines. The left three types are used for sweet pimento breeding. The middle four types are rarely used for Kim Chi breeding, but may be important in the near future.

Fig. 2. A typical Kim Chi line that has long, slender and pointed fruits with dark green color at young stage but bright red color at ripened stage.

Korean lines and their hybrid lines in the present study. Through the disease-resistance tests, we selected suitable mother lines and used them to breed new lines (Table 1).

For the watertlogging test, water was filled up to 15cm above the soil in the test box at the 8 to 10 leaf stage. After 15 days of water logging, the plant growth was examined. To obtain multi-resistant lines with restoration ability for CMS lines for hybrid breeding, we performed crosses between CMS-derived Korean elites and Italian disease-resistant lines (Table 2 and 3)

Resistance to *Phytophythora* was tested by the ordinal inoculation test at Tohoku Seed Company in Japan (Fig. 3). After selecting 241 stable resistant lines and other useful materials we performed for Fl breeding. Using 25 Italian *Phytophythora*-resistant lines, 25 TMV and PVY-virus resistant and nematode-resistant lines, new restorer lines, and 61 other desirable lines with high quality and 13 pure lines (Table 1 and 2).

The Italian lines resistant to TMV, PVY and nematode were selected at Napes University in Italy. The hybrid lines obtained by using Italian lines were screened for the above resistances at Dong Won Nong San Seed Co. in Korea. We tested 330 segregants for *Phytophythora* resistance were tested for the genetic behavior. The level of resistance to *Phytophythora* was evaluated by the survival rate at 20 days after inoculation and classified into five ranks: ±; (0~10%), +; (10~20%), ++(20~30%), +++; (30~50%) and ++++; higher than 50% in survival percentage). According to the resistance criterion, the resistance to *Phytophythora* in hybrid lines was evaluated using 20 individuals for each line (Table 4).

RESULTS AND DISCUSSION

1. Commercial characteristics of the cultivars

We examined the correlation of the fruit productivity with other vegetative characteristics to find a convenient selection indicator for some fruit characters. Leaf size and leaf shape index showed positive correlatios, but the character of cotyledon did not (Hirose, 1965). Practically, leaf size is useful for the selection of fruit size and productivity. The 'cluster type' plants flowered the earliest, 70 to 75 days after sowing, and had strong pungency. Pungency evaluation at 20 to 25 days after flowering is adequate to judge the stability of pungency as reported by Ohta (1962). A long-style flower is generally better to set fruit

Table 2 Resistance of hybrid lines from Korea hybrid varieties crossed with Italian disease resistant lines and superior lines

Hybrid N0	Combination	Resistance	Hybrid No	Combination	Resistance
PC1	TO1 x T13	*P. capsici*	38	TO5 x T116-2	*P. capsici*
2	TO1 x T17	ditto	39	TO6 x T25-3	TMV
3	TO1 x T18	ditto	40	TO7 x T3	*P. capsici*
4	TO1 x T26-1	TMV	41	TO7 x T17	ditto
5	TO1 x T26-2	ditto	42	TO7 x T26-2	TMV
6	TO1 xT112-1	*P. capsici*	43	TO7 x T30-3	PVY
7	TO1 x T112-2		44	TO7 x T34-2	ditto
8	TO3 x T4	*P. capsici*	45	TO7 x T112-1	*P. capsici*
9	TO3 x T8	ditto	46	TO7 x T116-1	ditto
10	TO3 x T17	ditto	47	TO8 x T3	*P. capsici*
11	TO3 x T25-3	TMV	48	TO8 x T4	ditto
12	TO3 x T30-3	PVY	49	TO8 x T25-2	TMV
13	TO4 x T8	*P. capsici*	50	TO8 x T31-1	PVY
14	TO4 x T17	ditto	51	TO9 x T3	*P. capsici*
15	TO4 x T18	ditto	52	TO9 x T10	ditto
16	TO4 x T25-2	TMV	53	TO9 x T17	ditto
17	TO4 x T25-3	ditto	54	TO10 x T17	*P. capsici*
18	TO4 x T26-2	ditto	55	TO10 x T26-1	TMV
19	TO4 x T30-3	PVY	56	TO10 x T31-1	PVY
20	TO4 x T34-1	ditto	57	TO10 x T112-1	*P. capsici*
21	TO4 x T37-1	Nematodes	58	TO10 x T116-1	ditto
22	TO4 x T112-2	*P. capsici*	59	TO11 x T8	*P. capsici*
23	TO4 x T116-1	ditto	60	TO11 x T10	ditto
24	TO5 x T3	*P. capsici*	61	TO11 x T25-1	TMV
25	TO5 x T4	ditto	62	TO11 x T26-1	ditto
26	TO5 x T68	ditto	63	TO11 x T30-1	PVY
27	TO5 x T10	ditto	64	TO11 x T31-1	ditto
28	TO5 x T17	ditto	65	TO11 x T34-2	ditto
29	TO5 x T18	ditto	66	TO11 x T112-2	*P. capsici*
30	TO5 x T25-1	TMV	67	TO13 x T3	*P. capsici*
31	TO5 x T25-2	ditto	68	TO13 x T17	ditto
32	TO5 x T25-3	ditto	69	TO13 x T25-1	TMV
33	TO5 x T26-1	ditto	70	TO13 x T25-2	ditto
34	TO5 x T26-2	ditto	71	TO13 x T25-3	ditto
35	TO5 x T30-2	PVY	72	TO13 x T30-1	PVY
36	TO5 x T112-2	*P. capsici*	73	TO13 x T112-1	*P. capsici*
37	TO5 x T116-1	ditto	74	TO13 x T116-2	ditto
88	TO13 x R*		99	T189-1 x T116-1	*P. capsici*

Table 2 continued

Hybrid No	Combination	Resistance	Hybrid No	Combination	Resistance
89	T83-2 x T10	*P. capsici*	100	T189-2 x T112-2	ditto
90	T83-2 x T17	ditto	101	T191-1 x T-18	*P. capsici*
91	T83-2 x T25-1	TMV	102	T191-1 x T25-1	TMV
92	T103-1 x T10	*P. capsici*	103	T191-1 x T26-2	ditto
93	T179-2 x T4	*P. capsici*	104	T191-1 x T112-2	*P. capsici*
94	T184-2 x T116-2	ditto	105	T191-2 x T18	*P. capsici*
95	T184-3 x T25-1	TMV	106	T191-2 x T112-2	ditto
96	T187-2 x T116-2	*P. capsici*	107	T191-2 x T116-1	ditto
97	T188-3 x T25-1	TMV	108	T191-2 x T116-2	ditto
98	T188-3 x T116-2	*P. capsici*			

* The F_1 is for restorer (C line), which has normal fertility

Table 3 Fruit characters of superior lines selected from the crossings with P.capsici resistant lines

Bred line	Mother original line or cultivars	Fruit characters						Remarks Usefulness
		Length (cm)	Width (cm)	Thickness (mm)	Weight (g)	Color	Pungency	
T53-2	LT-16	8.7	1.3	2.0	8.2	Dark red	Hot	For Rf(Asian type)
T149-2	F25056 x CM33 P4APT	10.8	3.0	3.0	28.3	Red	Sweet	For Rf(Piment type)
T179-1	IT-ENEA-496-1	20.9	1.7	4.0	32.3	Dark red	Hot	For CMS(Asian type)
T235-1	Hong Il Pum	8.8	1.3	2.0	8.2	Red	Hot	For Rf
T238-1	Wang Jung Wang	11.2	1.8	3.0	11.0	Red	Hot	For Rf
T241-1	Mu Kung Hwa-1	9.8	1.4	2.0	5.2	Red	Hot	For Rf
T241-2	Mu Kung Hwa-2	9.0	1.3	2.0	4.4	Red	Hot	For Rf

Rf means restoration of fertility.

compared with a short -style flower whose fruit easily falls off after fruit setting.

The common plant type is the half-open type, but 'Yatsubusa' and many pimento cultivars belong to the slender bushy type, and cultivars in the 'cluster sky' group are of the semi-dwarf type. Erect fruit and short internode are fittable to dense-cultivation in spite of open plant shape. Tolerance to waterlogging increased as vigor of the root system increased, especially as the fine root system develoed. For example, 'Mu Kung Hwa' and 'Muckgeri' are highly tolerant to water-logging, plant type 50% survival percentage (Fig. 3a, b and c).

2. Disease resistance test in cross combinations °°

(1) Disease resistance test in Italy and Japan

Five TMV-resistant lines marked with asterisk* in Table 1, 7 PVY-resistant and 3 nematode-resistant lines were selected by screening tests (Table 1).

Furthermore, 10 *Phytophythora*-resistant lines were also selected to test for resistance to *ph1* and *ph2* races by inoculation test on the soil. (Fig. 4)

These selected lines are very useful sources for breeding. In addition, 36 lines, excellent agricultural and commercial traits, were selected as restorer C lines with good characters through the screenings of 241 lines and 108 hybrid lines in the field test. To select

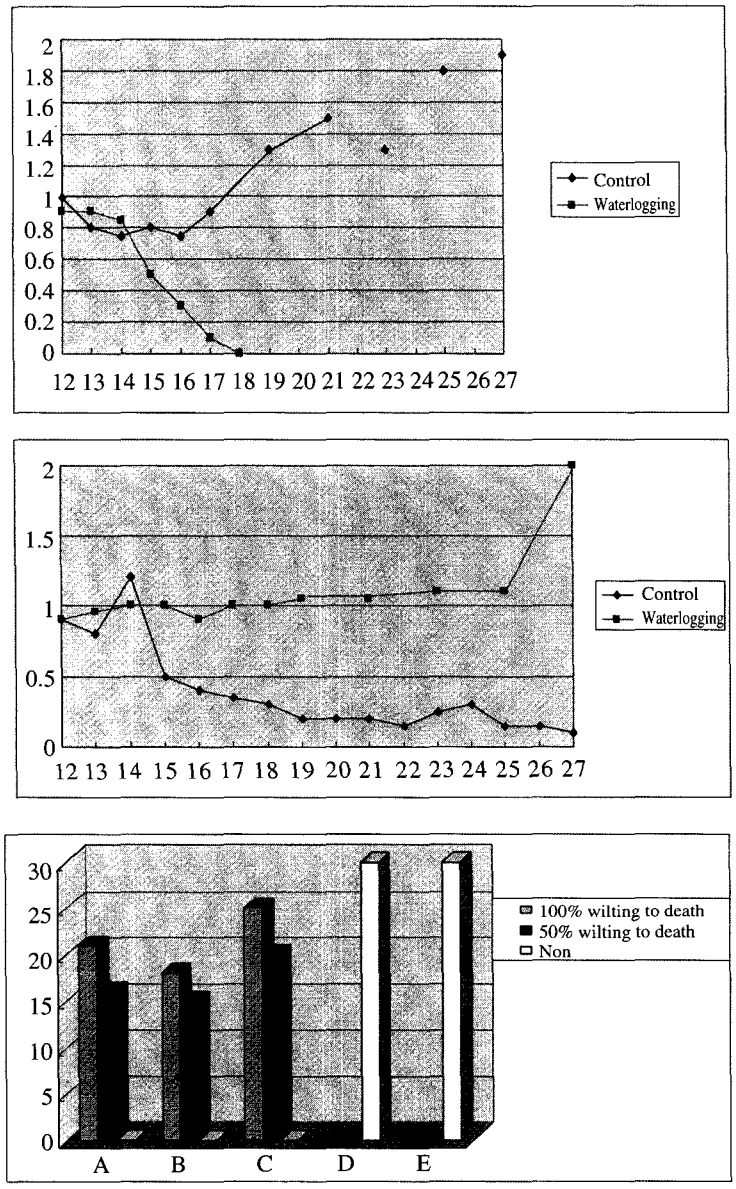

Fig. 3. Water logging tolerance test of Korean cultivars. a: Usual non-tolerant cultivar 'Kim Chi', b: Considerably tolerant cultivar 'Mu Kung Hwa'. c: Comparison of water logging tolerance. Data show the days until reaching 50% and 100% death after water logging.

complex disease resistance and high yield we selected, 22 F_1 cultivars/lines and 11 other lines as female lines, and 22 Italian cultivars/lines and 14 C lines as male parents. They were used for 108 cross combinations (Table 2).

Thirty six selected lines in Table 3 have various good characters, contributing to higher breeding performance. Among them, T53-2 and T179-1 produced dark green fruit and stronger pungency, which will be appropriate for Southeast Asian type. T149-2 is a sweet pimento type, and is useful for sweet pepper breeding. Some useful materials for breeding were furthermore found in

Table 4 Degree of resistance evaluated by the inoculation with *P. capsici*

Individual	Line	No. of inoculated plants	Response at 20 days after inoc. Resitance		
			Diseased	Non-diseased	Ranks
PC2	T17	20	11	9	+
3	T18				
4		20	15	5	++(m)
9*	T8	20	8	12	++++
13*	T8	20	11	9	+++
26*	T8	20	10	10	++++
28*	T10	20	12	8	+++
30	T17	20	16	4	+ (m)
31		20	15	5	++(m)
32		20	17	3	+(m)
33		20	14	6	++(m)
34		20	18	2	+(m)
39		20	16	4	+(m)
42		20	16	4	+
52*	T10	20	8	12	++++
53*	T17	20	11	9	+++
54*	T17	20	12	8	+++
55*		20	14	6	++(m)
59*	T8	20	12	8	+++
61		20	18	2	∓ (m)
62		20	16	4	+ (m)
68*	T17	20	12	8	+++
90*	T17	20	10	10	+++
97*		20	13	7	+++(m)
101	T18	20	10	12	++++
105	T18	20	10	10	+++

The resistance wa shown by the percentageof survivals at 20 days after inoculation and was classified into five ranks: evaluated as ±(0-10%) , + (10-20%), ++ (20-30%), +++ (30-50%) and ++++(higher than 50%), respectively. (m) means multiple - resistance to *P. capsici* and TMV. Asterisks show the complex resistance against *ph1* and *ph2* races of P. capsici

these materials.

F_1 lines obtained by crossing the Korean lines with *Phytophythora* resistant lines were resistant to *ph1* and *ph2* races, but the standard Korean ard cultivars were susceptible to them under the same test conditions (Fig. 5). These results showed that Korean CMS lines and C lines were not uniform, and that fixation for homozygosity was not enough due to the shortness after the introduction of pepper into Korea.

Fig. 4. Soil test for phytophythora resistance at 3 to 4 leaf stage.

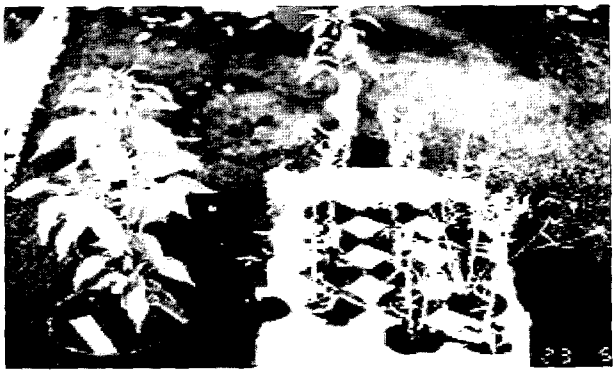

Fig. 5. Line susceptible line to phytophythora, where none of 20 plants tested survived after two weeks.

Fig. 6. Lesion showing hypersensitive resistant symptom for phythophytora.

Fig. 7. Strongly resistant selected line. A all plants survived and grew normally for two weeks after inoculation.

(2) Resistance test in Japan

In the next year, 241 cultivars/lines were tested for the resistance to *Phytophythora* isolated at Tohoku Seed Company (Miyagi, Japan). Eighty-two cultivars /lines were 50% infectious or completely resistant, and 149 lines were susceptible and wilted. Among the 10 Italian lines, for example, T17, T18, T112-1, T112-1, T116-1 and T116-2 expressed strong resistance to other races of *Phytophythora*, indicating that it is dominant. The 25 lines that were resistant to TMV, PVY and nematode had also various desirable agricultural characteristics, qualitative and quantitative characteristics such as fruit size, fruit color and pungency (Table 3).

Especially, T4 was resistant to *Phytophythora*, T34 to PVY, and T37-1, T37-2 and T37-3 lines to nematodes having good commercial qualities such as sweetness. In addition, T10 and T25-1 had the same characteristics, such as TMV resistance and sweet taste as those of Korean cultivars, confirming the usefulness as pepper breeding materials.

(3) Resistance test on Korean lines

We screened 330 lines for *Phytophythora* resistance with high quality of commercial characters, to find out excellent cultivars (Table 3). Although lines with 100 % resistance could not be selected, one line that all survived through developing symptoms (Fig. 6) could be selected. Thus, the resistance is thought to controlled by more than one gene.

Since the lines completely susceptible to

Phytophythora but resistant to TMV, PVY and nematodes were obtained, these resistances are supposed to be independently controlled. Although most of the hybrid lines crossed with TMV- resistant male parents were susceptible to *Phytophythora* diseases, 12 combinations, pc4, 30, 31, 32, 33, 34, 39, 42, 55, 61, 62 and 97 had 10 to 35 % resistance. This shows the possibility of breeding complex resistance to TMV and *Phytophythora*.

Pc9, 13, 26 and 59 lines from T8 line derived from *ph1* and *ph2* resistant type, pc27 and 52 from T10 and pc2, 28, 53, 54, 68 and 90 from T17 and pc3, 101 and 105 from T18 line did not express full resistance, but expressed resistance higher than 40%. T8, T17 and T18 lines have excellent Korean type characteristics; especially T10 is good breeding material for sweet pepper (Fig. 7). Lines 112-1 and 116-1 which were justified as *Phytophythora* resistant in Italy and Japan expressed weak *Phytophythora* resistance than others in Korea. They seemed to be horizontal resistance. This type of difference in the resistance was sometimes observed between the responses to the ordinal soil inoculation and drop inoculation to absorbent cotton attached to an excised stem. For example, S7-7 for Kim Chi was selected firstly by soil treatment as a resistant line from the cross between resistant CM331 and PI201234, but was susceptible in the field test. In another case, 'Ao-togarashi' was susceptible to *Phytophythora* by the ordinal field test, but was oppositely resistant to *Phytophythora* by the inoculation method. However, the reason for the weak resistance different of 112-1 and 116-1in Korea is not clear.

Selection for complex resistance is most important. However, full resistant line may not be selected at the first step. In the actual to breeding, resistance to TMV, PVY and nematode must be selected following the selection for strong *Phytophythora* resistance.

The present experiment showed the possibility of hybrid breeding of commercial cultivars, having multiple resistance with desirable agricultural characteristics and CMS-restorer.

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