Sources of Resistance to Bacterial Wilt Found in Vietnam Collections of Pepper (*Capsicum annuum*) and Their Nuclear Fertility Restorer Genotypes for Cytoplasmic Male Sterility

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Eleven hot pepper accessions collected in Vietnam showed stable resistance to bacterial wilt as well-known resistance sources, MC4 and MC5, in repeated inoculation tests with different *Ralstonia solanacearum* isolates conducted from 2004 to 2010. Seven of these accessions (specifically KC981, KC1006, KC1021, KC1027, KC1045, KC1050, and KC1055) resulted in stable male sterile F₁ plants in the crosses with a cytoplasmically male sterile (CMS) Chilseong (CMS-A, *Srfrf*), and therefore, they were maintainers (CMS-B) with a genotype of *Nrfrf*. The rest (KC980, KC995, KC999, and KC1009) produced stable male fertile F₁ plants in the crosses, and therefore, were restorers (CMS-C) with a genotype of *N(S)RfRf*. Therefore, the maintainer and restorer sources of resistance may be used in preference in breeding maternal (CMS and their maintainers) and paternal parents (restorers) for resistance to bacterial wilt, respectively, in the hybrid breeding system utilizing cytoplasmic male sterility.

Keywords : breeding, *Capsicum annuum*, cytoplasmic male sterility (CMS), *Ralstonia solanacearum*, resistance

The Chili pepper (*Capsicum annuum*) is widely grown in Vietnam with a diversity of climatic conditions and different local consumer's preferences. Local pepper collections have been carried out in Vietnam, and maintained in the Fruit and Vegetable Research Institute (Hanoi) since 1996. However, their reactions to main diseases attacking pepper were still poorly understood. Bacterial wilt caused by *Ralstonia solanacearum* affects more than 200 plant species, and occurs in most tropical, subtropical and warm temperate areas (Hayward, 1994). *R. solanacearum* enters plant roots through wounds or natural openings. Once it gets inside the xylem, the bacterium multiplies and spreads rapidly throughout the plant's vascular system. Susceptible

plants respond by wilting, yellowing and dying. The disease occurs mainly in Asian countries (Kennet et al., 1970). In Vietnam, the disease damages 20–40%, 25–45% and 20–30% of potato, tomato and groundnut production areas, respectively (Doan et al., 2006). Pepper cultivated in Southeast Asia is often confronted by the different biovars and strains of the bacterium, ranking this pathogen as the fourth major disease of the crop. The disease is also widely observed in Japan (Horita and Tsuchiya, 2001). The causal bacterium is soil-borne with an extremely wide host range and persistence in the environment (Hayward, 2000). Although there are several methods to control the disease such as field sanitation, crop rotation and use of bactericides, host plant resistance has been a major strategy for managing bacterial wilt in pepper. Some resistant pepper accessions have already been found such as MC5, PBC631, PI322727, PI358812, PBC066, Kerting, PI322726, PI369994, PI369998, PI377688, KC00382, PI322728 (Kim et al., 1998; Matos et al., 1990), and LS2341 (Mimura and Yoshikawa, 2009). However, *R. solanacearum* is highly heterogeneous. So, the resistance to bacterial wilt can be location-specific (Hanson et al., 1996). In fact, damage has been observed in the pepper cultivars Fushimi-amanaga and Manganji which were previously known to be resistant (Hashimoto et al., 2001). Thus, it is necessary to find more diverse resistant sources to genetically cope with the highly variable strains of the pathogen.

Cytoplasmic male sterility (CMS) resulting from interaction between the cytoplasmic and nuclear genes was found in *Capsicum* by Peterson in 1958 and its morphology was investigated in 1974 by Horner and Rogers. CMS is a maternally inherited character, causing plants to fail to produce functional anthers, pollen grains or male gametes. The advantage of a CMS system is that male sterile plants can generate homozygous offspring in sterility, being an economically important trait in F₁ hybrid seed production by avoiding hand emasculation steps. However, unstable sterility can happen under cool conditions (Novak et al., 1971; Shifriss and Frankel, 1971; Shifriss and Guri, 1979).

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In Korea, most hybrid seeds of hot peppers are produced by means of CMS, containing 3 lines: line A (*Srfrf*), line B (*Nrfrf*) and line C (*N(S)RfRf*) (Yu, 1990). Depending on their nuclear genotype for the CMS-restoring gene (*Rf*), pepper lines can be classed as either maintainers (*Nrfrf*) or restorers (*N(S)RfRf*). With the aim of breeding F_1 hybrid pepper resistant to bacterial wilt, we identified the nuclear genotype of the new sources of resistance to *R. solanacearum* with respect to the fertility-restoring gene (*Rf*) for male sterile cytoplasm.

Seeds of 116 pepper accessions from Vietnam and 29 others from Korea and Nepal were sown in September 2004 in 128-cell trays filled in with 'Wonjo Mix' medium (Nongkyung Co., Ltd., Korea). Sixteen seedlings of each accession were raised to 2–3 foliage leaf stage in 32-cell trays and inoculated artificially with *R. solanacearum* suspension at about one month after sowing. After disease evaluation, 3 healthy plants of each resistant accession were selected and grown in big pots containing 2 kg of 'Wonjo Mix' medium for seed production. Seeds of 41 Vietnamese accessions harvested from the first selection were sown for the confirmative evaluation in 2005 with some other known resistant and susceptible controls. Sixteen plants of each line of one-month-old seedlings were inoculated by dipping the roots in the bacterial cell suspension in 3 replications of a completely randomized design. Disease-free plants in the

resistant accessions were continuously selected for further research. In 2010, the 2 different isolate inoculations were implemented with 36 materials of which 7 Vietnamese accessions were involved.

A highly virulent isolate of *R. solanacearum* from Miryang (Korea) was selected and used in the inoculations in 2004 and 2005. New fresh isolates from Miryang and Sangju, Korea were used in the 2010 evaluation. Seedlings were inoculated at around 30 days after sowing. Inoculum was prepared by harvesting the 48-hour culture of the pathogen on TTC medium (Schaad et al., 2001). Seedlings were pulled out from 128-cell trays and dipped in bacterial suspension of 10^7 cfu/ml (OD = 0.14 at 600 nm), then transplanted to 32-cell trays. Inoculated seedlings were maintained in the greenhouse at around 25–35°C, and routinely watered. Disease evaluations were done from 20–30 days after inoculation. The severity of wilting symptoms of individual inoculated plants was rated on a scale of 1 to 5, where: 1 = no visible symptoms; 2 = one to less than half of the foliage wilting; 3 = about half of the foliage wilting; 4 = nearly all of the foliage wilting; 5 = the whole plant wilting and dead. Data was statistically analyzed by SAS program.

On the basis of preliminary screening in 2004, confirmative evaluation in 2005, and evaluation to two different strains in 2010, the 11 best Vietnam accessions, which are

Table 1. Reaction to *Ralstonia solanacearum* of selected Capsicum pepper accessions collected in Vietnam

Accession	Origin	Disease index ^a to strain of				Previous report
		Miryang (2004)	Miryang (2005)	Miryang (2010)	Sangju (2010)	
KC980	Vietnam	1.1	1.4 ab ^b	1.5 a-d	1.4 a-d	
KC981	Vietnam	1.0	1.9 a-h	— ^c	—	
KC995	Vietnam	1.0	1.6 a-e	1.3 ab	1.1 ab	
KC999	Vietnam	1.1	1.7 a-f	1.1 a	1.1 ab	
KC1006	Vietnam	1.0	1.7 a-f	1.9 a-e	1.0 a	
KC1009	Vietnam	1.1	2.2 a-l	1.1 a	1.1 ab	
KC1021	Vietnam	1.0	1.6 a-c	—	—	
KC1027	Vietnam	1.0	2.0 a-i	—	—	
KC1045	Vietnam	1.1	2.2 a-l	—	—	
KC1050	Vietnam	1.0	1.6 a-d	1.9 a-e	1.1 ab	
KC1055	Vietnam	1.0	2.1 a-k	1.5 a-d	1.3 a-c	
KC126	PI358812	1.0	2.1 a-o	2.4 c-g	1.0 a	Kim et al., 1998
KC350	MC4	1.2	2.0 a-j	1.0 a	1.0 a	Perera et al., 1983
KC351	MC5	1.2	1.3 a	1.2 a	1.1 ab	Matos et al., 1990
KC1262	ANN647	—	4.3 v-y	4.5 j	3.6 fg	
Cheonhatongil	F_1 hybrid	—	4.8 y	—	—	

^a 1 = no visible symptom; 2 = one leaf to less than half of the foliage wilting; 3 = about half of the foliage wilting; 4 = nearly all of the foliage wilting; 5 = the whole plant wilting or dead.

^b Mean separation within columns by Duncan multiple range test at 5% level.

^c Data not available.

comparable to the known sources of resistance to bacterial wilt such as PI358812, MC4 and MC5 (Kim et al., 1998; Matos et al., 1990), were selected and the results are given in Table 1. Because of the high temperature in the summer inoculation in 2005, many resistant accessions found in 2004 tended to susceptibility. In the 2010 test, KC1006, KC1050, and KC126 (PI358812) showed some differential reaction to the two different strains of the bacterium, but all the others performed consistently. The Vietnamese accessions were collected throughout the country from 1996 and maintained at the Fruit and Vegetable Research Institute (Hanoi, Vietnam) by periodic increase of seed in the net house. Although pepper is predominantly self-pollinated, a degree of out-crossing may occur (Tanksley, 1984). The homozygosity level of the accessions used in this study at the beginning was not known. Therefore, the resistant plants of each accession were continuously self-pollinated and selected until 2010 to obtain homozygosity in the selections.

Inheritance of resistance to this pathogen had been studied in some of these accessions, and the results showed that resistance to *R. solanacearum* in pepper was a quantitative character, and was governed by oligogenic or polygenic inheritance. The resistance was inherited in partial dominance to recessive mode (Tran and Kim, 2010). Although a hypersensitive type of response to *R. solanacearum* has been reported in some species (Arlat et al., 1994; Carney and Denny, 1990), none of these *Capsicum* pepper accessions was found hypersensitive or immune. Rahman et al. (1999) found that restriction of the spread of the pathogen was associated with resistance to bacterial wilt in *C. annuum* and was mainly attributed to some induced, morphological and physical barriers. In addition, KC995, KC1006, and

KC1027 of them were also resistant to bacterial spot caused by *Xanthomonas euvesicatoria* (Tran and Kim, 2007). Thus, they may be good materials for breeding multiple resistances to bacterial wilt and bacterial spot.

The resistant accessions were crossed to a cytoplasmically male sterile Chilseong (CMS-A, *Srfrf*) in 2009. Seeds of the F_1 's were sown in 128-cell trays filled with 'Wonjo Mix' medium. One month after sowing, seedlings were transplanted to 32-cell trays. Fertility of the F_1 plants was examined by visual observation of anthers for production of pollens with the aid of a magnifier as the first flowers on the plants began to bloom. Pollen parents were classified into restorer (*N(S)RfRf*) or maintainer (*Nrfrf*) of cytoplasmic male sterility on the basis of their ability to produce male fertile F_1 plants in the crosses with a cytoplasmic male sterile Chilseong with *Srfrf* plasma-genotype.

The results of fertility examination of F_1 's between the CMS Chilseong (*Srfrf*) and bacterial wilt resistant selections showed that KC350, KC980, KC995, KC999, and KC1009 were producing male fertile F_1 plants, and therefore, were restorers carrying the *N(S)RfRf* genotype (Table 2). The others, KC351, KC981, KC1006, KC1021, KC1027, KC1045, KC1050, and KC1055, produced male sterile F_1 plants and were identified as maintainers carrying the *Nrfrf* genotype (Table 2). The results agreed with the previous report of Kim et al. (2004) that KC350 and KC351 were a restorer and a maintainer, respectively. In cytoplasmic male sterility system, instability in maintenance and restoration of cytoplasmic male sterility was often observed in sweet or fresh fruit type pepper (Lee, 2001; Shiffriss, 1997). The accessions used in this study were all pungent types and instability was not observed in any stage of the study.

The bacterial wilt resistant accessions of *C. annuum* from

Table 2. Male fertility of F_1 's between a cytoplasmic male sterile line, Chilseong-A (*Srfrf*) and sources of resistance to *Ralstonia solanacearum* found in Vietnam peppers, their nuclear restorer genotypes for male sterile cytoplasm, and fruit characters

Accession	Male fertility of F_1	Nuclear genotype	Fruit length (cm)	Fruit dia. (mm)	Fruit weight (g)	Pericarp thickness (mm)
KC980	Fertile	<i>N(S)RfRf</i>	11.8	16.1	12.5	2.7
KC981	Sterile	<i>Nrfrf</i>	10.1	14.5	9.4	—
KC995	Fertile	<i>N(S)RfRf</i>	8.0	9.7	4.3	1.3
KC999	Fertile	<i>N(S)RfRf</i>	7.9	13.6	6.9	1.5
KC1006	Sterile	<i>Nrfrf</i>	11.5	13.0	9.6	1.7
KC1009	Fertile	<i>N(S)RfRf</i>	7.7	8.4	2.5	1.3
KC1021	Sterile	<i>Nrfrf</i>	9.4	21.6	21.0	2.4
KC1027	Sterile	<i>Nrfrf</i>	3.5	10.9	1.9	1.3
KC1045	Sterile	<i>Nrfrf</i>	9.5	13.0	8.4	1.7
KC1050	Sterile	<i>Nrfrf</i>	7.6	11.9	5.1	1.3
KC1055	Sterile	<i>Nrfrf</i>	7.4	11.7	3.0	1.1
KC350	Fertile	<i>N(S)RfRf</i>	9.6	19.4	10.6	1.7
KC351	Sterile	<i>Nrfrf</i>	10.6	15.5	11.9	1.7

the Vietnam collection were evaluated for detailed fruit morphology. All newly introduced accessions resistant to *R. solanacearum* produce red, hot fruits and were very uniform, indicating that they are homozygous. Most of them bear pendent fruits, except KC999 producing erect fruits. In comparison with KC350 and KC351, fruit sizes of lines originating from Vietnam seem smaller with exception of KC980 and KC1021 (Table 2). These additional materials will be valuable resources in breeding for resistance and nuclear restorer genotype information would be useful in planning the breeding programs for resistant F_1 hybrids using cytoplasmic male sterility. Thus, the accessions with restorer genotype, $N(S)R/rf$, may be preferentially used in breeding lines for bacterial wilt resistant paternal parents and those with maintainer genotype, Nrf/rf , for cytoplasmically male sterile maternal parents in hybrid breeding system.

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