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Testing Bacterial Spot Resistant Lines of Capsicum Pepper for Nuclear Genotype Interacting with Male Sterile Cytoplasm

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고추 더뎅이병 저항성 계통의 세포질웅성불임 관련 핵내유전자형 검정

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ABSTRACT : Capsicum pepper selections from PI163192, PI241670, PI244670, PI271322, PI308787, PI 322719, and PI369994 were confirmed to be non-hypersensitively resistant to race 3 of *Xanthomonas campestris* pv. *vesicatoria*. A resistant cultivar, 'SR', was shown to be hypersensitive. Four Korean local cultivars, a cytoplasmic male sterile line (A-line) and its maintainer (B-line) were highly susceptible. The resistant selections and cultivars were crossed with a male sterile A-line (*Smsms*) and fertility of their F_1 hybrids was examined by observing the pollen production, testing pollen germination, and quantifying the amount of pollen produced per anther to identify the genotype interacting with the male sterile cytoplasm. The seven resistant PI selections turned out to be restorers (*N(S)MsMs*) and 'SR' to be a maintainer (*Nmsms*).

***Key words*: *Capsicum annuum, Xanthomonas campestris* pv. *vesicatoria*, resistance, cytoplasmic male sterility, breeding.**

Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge)Dye is one of the major diseases on pepper in Korea. The disease starts from infected seeds or debris of previous crops and spreads in rainy wet weather, often during typhoons, resulting in an epidemic. Resistance to the disease was found in many chile type peppers (14, 27, 28). The resistant lines were introduced into Korea and their resistance to a Korean isolate of the pathogen was confirmed (16). It is generally accepted that two types of resistance are involved in resistance to bacterial spot in pepper, namely hypersensitive resistance and general resistance (11, 13, 14, 23). Hypersensitive type of resistance was found in PI163192, PI260435 and PI271322, and the hypersensitive reaction in the lines has been reported to be controlled by independent single dominant genes (4, 5, 9, 14, 15). The genes controlling the hypersensitive reaction in PI163192, PI260435, and PI271322 are designated as Bs_1, Bs_2, and Bs_3, respectively (9, 15, 20). Non-hypersensitive general resistance, presumably controlled by a polygene, was found in another selection of PI 163192 (14, 18, 19), and in PI241670, PI244670, PI 369994 and some others (14).

Differentiation of pathotype in *X. campestris.* pv. *vesicatoria* was first reported by Cook and Stall (6) in 1969 on the basis of ability of the pathogenic bacterium to infect the pepper cultivars containing the B_{s1} gene. Six races have been identified so far in the world on the basis of infection specificity to the cultivars with the hypersensitive resistance genes, B_{s1}, B_{s2}, and B_{s3} (1, 2, 6, 7, 10, 20, 21, 24, 25). Distribution of race 1 and race 3 has been reported in Korea (17).

Almost all of the commercial cultivars planted in Korea are chile type F_1 hybrids. Male sterility is generally utilized to save labor for hand emasculation in hybridization (22, 29). Both cytoplasmic and genic male sterility systems are used, but the cytoplasmic system is more convenient and more widely used. Sterility in the cytoplasmic system in pepper depends upon the interaction of male sterile cytoplasm with a nuclear gene (8, 26). Sterility occurs only when male sterile cytoplasm is combined with homozygous recessive genotype. All the other combinations result in fertility. The male sterile line, which is often referred to as A-line and the cyto-genotype of which is symbolically expressed as *Smsms*, is reproduced by pollinating with pollen

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from a maintainer, which is also referred to as B-line and the cyto-genotype of which is expressed as *Nmsms*. The A-line (*Smsms*) is pollinated with pollen from a restorer, which is also referred to as C-line and the cyto-genotype of which is expressed as *N(S)MsMs*, to produce male fertile F_1 hybrid seed. Therefore, it would be worthwhile to breed resistant maintainer and restorer for development of diverse resistant F_1 hybrid cultivars. Given this background, identification of the genotype interacting with male sterile cytoplasm of bacterial spot resistant lines would be indispensible information for successful planning of the long term breeding program.

In this study, selected resistant sources were tested first for resistance to race 3 of the bacterial spot pathogen and the resistant selections were crossed with a cytoplasmically male sterile line (A-line) to identify the genotype interacting with the male sterile cytoplasm by examining the fertility of the resultant F_1 hybrid plants.

MATERIALS AND METHODS

Male sterile lines. Cytoplasmic male sterile line (CMS-A) and its maintainer (CMS-B) were received from Dr. Y. S. Lee in Nong-Woo Seed Co. Ltd. The CMS-A and CMS-B were derived from 'Shinhong', an F_1 hybrid cultivar which was released in 1986 by the Horticultural Experiment Station in Suwon (22). The male sterile cytoplasm 'Shinhong' originated from PI164835.

Bacterial spot resistant selections. Bacterial spot resistant selections from U.S. PI lines in the experiment have been selected by single plant selection with self-pollination from U.S. PI lines for at least 4-5 generations since 1979-1980 (14, 16). SR, a breeding line containing all three hypersensitive resistance genes (B_{s1}, B_{s2} and B_{s3}), was received from A.M. Hibberd in Australia.

Testing for resistance to bacterial spot. The bacterial spot resistant lines, male sterile line and its maintainer, and 4 Korean local cultivars were sown in Biomix (Hung-Nong Seed Product) in 128-hole-tray (Sam-Jeong Co.) and the germinated seedlings were transplanted to a 32-hole-tray. One month old seedlings were inoculated by atomizing the bacterial suspension of approximately 10^8 cells per ml at one point of the underside of a fresh open leaf on a plant until a spot about 5~7 mm in diameter of water-soaking appeared. Hypersensitive reaction was observed 72 hours after the infiltration and the characteristic necrotic lesions were confirmed one week after inoculation. The lesions on non-hypersensitively resistant plants were evaluated on a scale of 1 to 5 based on the type of lesion and satellite spots around the lesion, i.e. 1=dry lesion at infiltration point without any satellite spots, 2=oil-soaked lesion with some satellite spots around the arrested lesion, 3=lesion with water-soaked edge and water-soaked satellite spots, spotted area about 25% of the total leaf area, 4=the same type of lesion as 3 but the spotted area about 50% or more of the total leaf area, 5=yellowing on whole leaf or defoliated.

Individual plants selected from the resistant lines, male sterile line, its maintainer, and Korean local cultivars on the basis of resistance and general performance including viral symptoms during the test period were transplanted to a soil-compost mixture in a 30 cm diameter pot for growing and making crosses. Crosses were made between the male sterile line (A-line) and bacterial spot resistant selections by routine bud pollination method. The F_1 hybrid seeds were secured from the fruits set by the crosses.

Identification of the genotype interacting with male sterile cytoplasm. The F_1 hybrid seedlings were grown as before. The seeds were sown in Biomix in a 128-hole-tray and transplanted to a 32-hole-tray. About one month old plants were transplanted to soil in pots 13 cm in diameter. As the plants began to bloom, flowers were observed for pollen production with aid of a magnifier when necessary. Once fertility was confirmed, viability of the pollen was tested. Pollen grains were scattered on a 1.5×2 cm sheet of agar medium on a slide glass. The agar medium for pollen germination was 1% agar supplemented with 10% sucrose and 100 ppm boric acid. The slide glass was placed on 2 sheets of wet filter paper in a Petri dish. The Petri dishes were incubated at 25°C for germination for 3 to 4 hours. Then pollen grains were observed under a microscope at 200× magnification for germination. The pollen grains that had pollen tubes longer than the diameter of the pollen grain were considered germinated. Three eye fields were counted per F_1 hybrid plant and the average was taken.

Quantity of pollen per anther was measured following Yu's method (29). An anther that was about to dehisce pollen was picked and put in a test tube. The tubes with anther were incubated at 25°C for 24 hours so that anthers split open and dehisced pollen. The tubes were lightly shaken on a Vortex mixer to dehisce pollen from the anther. Then 1 m*l* of aceto carmin solution was dropped into the test tubes and mixed with

the pollen by shaking. Five microliters of the pollen suspension in acetocarmin solution was taken with micropipet, dropped onto a slide glass and covered with an 18×18 mm cover glass. The cover glass exactly covered the surface area of the 5 μl solution. Total number of pollen grains was counted under the microscope. The mean of three coverglass area counts was taken and the number of pollen per anther was obtained by multiplying the dilution factor.

RESULTS AND DISCUSSION

Resistance to bacterial spot of resistant selections and male sterile lines. All the selections from the PI 163192, PI241670, PI244670, PI271322, PI308787, PI 322719, PI369994 were resistant as previously reported (14, 16) and SR was hypersensitive to race 3 of *X. campestris* pv. *vesicatoria* as expected (Table 1). In the degree of disease, PI163192 was the least diseased among the non-hypersensitively resistant selections and PI322719 was the most diseased. Cytoplasmic male sterile A-line and its maintainer (B-line) were highly susceptible, suggesting that introduction of resistance into the male sterile lines is desirable for development of diverse F_1 hybrid cultivars.

Table 1. Resistance to race 3 of *Xanthomonas campestris* pv. *vesicatoria* of resistant selections, Korean local cultivars and male sterile lines of pepper 7 days after infiltration of the bacterial suspension by a sprayer connected to a compressor

Original source or cultivar name (HR gene)	KC and selection No.	Frequency at disease index						Mean disease index
		HR[z]	NHR[y]					
			1	2	3	4	5	
PI241670	43-4-1		4	12				1.75
PI244670	47-14-1		11	5				1.31
PI271322 (*Bs*$_3$)	79-1-5		2	14				1.88
PI308787	112-7-2			7				2.00
PI322719	119-2-1			1	11	4		3.19
PI369994	127-2		9	6	1			1.50
PI163192	177-7-1		15	1				1.06
SR (*Bs*$_1$, *Bs*$_2$, *Bs*$_3$)	298	16						–
Chilsung	201				15	2		3.12
Subi	202					8	8	4.50
Kalmi	205				11	5		3.31
Punggak	207				4	10	1	3.69
CMS-A	381			1		7	25	4.78
CMS-B	382					7	9	4.56

[z]HR=Hypersensitive reaction.

[y]NHR=Non-Hypersensitive reaction, 1=dry lesion at infiltration point without any satellite spots, 2=oil-soaked lesion with some satellite spots around the arrested lesion, 3=lesion with water-soaked edge and water-soaked satellite spots, spotted area about 25% of the total leaf area, 4=the same type of lesion as 3 but the spotted area about 50% or more of the total leaf area, 5=yellowing on whole leaf or defoliated.

Although the PI selections and SR were tested for resistance to only race 3 of *X. campestris* pv. *vesicatoria*. in this study, all of the six PI selections except PI271322 are non-hypersensitively resistant to both race 1 and race 3, the pathotypes found so far in Korea (16, 17, 18, 19). 'SR', that contains *Bs*$_1$, *Bs*$_2$, and *Bs*$_3$, was hypersensitively resistant to race 3 as expected. PI271322, that contains *Bs*$_3$ gene only, was expected to be hypersensitive to race 1 only, so it was non-hypersensitive to race 3. The other resistant PI selections were reported to be multigenic and race-nonspecific (5, 14). It was also reported that PI271322 contained a race non-specific quantitative component of resistance in addition to *Bs*$_3$ gene (13). Hypersensitive resistance genes, *Bs*$_1$, *Bs*$_2$, and *Bs*$_3$ that were found in PI163192, PI260435, and PI 271322, respectively, were incorporated into bell type peppers (3, 20, 21). The hypersensitive resistance genes were, however, overcome by new pathotypes (6, 10, 20, 24). Already race 1 to race 6 are differentiated in the world and race 6 can overcome all of the three hypersensitive genes (1, 2, 6, 7, 10, 17, 20, 21, 24, 25). Thus race specific hypersensitive resistance was vulnerable to developments of new pathotypes. Non-hypersensitive resistance in the PI selections is high enough to be exploited in breeding for resistance. Potential of the quantitative component for more durable resistance were suggested by Hibberd and Gillespie (11) and Poulos et al. (23).

Identification of the genotype interacting with male sterile cytoplasm. All the bacterial spot resistant selections from the PI lines produced fertile F_1 plants in crosses with cytoplasmically male sterile A-line, indicating that they were restorers (C-line) (Table 2). However, SR, a bell-type breeding line received from Hibberd in Australia, produced male sterile F_1 plants, indicating that the line was a maintainer (B-line). Among the 4 Korean local cultivars, 'Chilsung', 'Subi' and 'Punggak' were maintainers and 'Kalmi' was a restorer as previously reported (12). Viability of the pollen on the fertile F_1 plants was confirmed. Some variation was also observed in the abundance of the pollen per anther. PI 271322 produced the most pollen-abundant F_1 plants in cross with the A-line, and PI322719 resulted in F_1 plants with the least amount of pollen.

Pepper cultivars have been classified into maintainers (*Nmsms*) or restorers (*N(S)MsMs*) depending on

Table 2. Fertility, pollen germination, and amount of pollen on the F_1 hybrid plants between a cytoplasmic male sterile line and bacterial spot resistant lines

Cross combination	Fertility		Pollen germination (%)	Quantity of pollen per anther	Genotype
	Fertile	Sterile			
CMS-A × PI241670	15		59.32	191,267	*N(S)MsMs*
CMS-A × PI244670	15		50.41	127,133	*N(S)MsMs*
CMS-A × PI271322	15		67.53	203,200	*N(S)MsMs*
CMS-A × PI308787	15		56.02	113,867	*N(S)MsMs*
CMS-A × PI322719	15		52.56	54,933	*N(S)MsMs*
CMS-A × PI369994	15		63.13	172,800	*N(S)MsMs*
CMS-A × PI163192	7		39.98	126,467	*N(S)MsMs*
CMS-A × SR		16	–	–	*Nmsms*
CMS-A × Chilsung		15	–	–	*Nmsms*
CMS-A × Subi		16	–	–	*Nmsms*
CMS-A × Kalmi	12		73.41	142,600	*N(S)MsMs*
CMS-A × Punggak		15	–	–	*Nmsms*

their ability to produce fertile F_1 hybrids in cross with male sterile A-lines (*Smsms*). Maintainers were generally more common among pepper cultivars and that restorers were often found among chile type pepper. Almost all the bell type peppers were maintainers (8, 26, 29). This study showed that all of the bacterial spot resistant selections from U.S. PI lines were restorers, whereas SR, which was a bell type cultivar, came out to be a maintainer as expected.

As a strategy for breeding cytoplasmic male sterile lines resistant to bacterial spot, the resistance may be introduced to the maintainer by backcross method. Diverse restorers may be bred by crosses between promising restorers and the resistant PI selections. Both resistant maintainers and restorers could be selected in the progeny of the crosses between any maintainers (CMS-B) and the resistant selections. 'SR' may be used as a donor parent to introduce Bs_1, Bs_2 and Bs_3 into Korean pepper type maintainers by backcross method.

Shifriss (26) suggested designating the genotypes, *Smsms, Nmsms* and *N(S)MsMs* as *Srfrf, Nrfrf,* and *N(S) RfRf,* respectively, to differentiate the ms gene that is interacting with male sterile cytoplasm from the genic male sterility gene, ms. However, we followed here the conventional expression that was generally used in Korea.

Although it was reported that the cytoplasmic male sterility derived from PI164835 is unstable and the utilization of the male sterility was hindered by this instability (8), the cytoplasmic male sterility was successfully used in producing F_1 hybrids and was reported to be fairly stable in Korea (29).

요 약

미국 식물도입국 계통 PI163192, PI241670, PI244670, PI271322, PI308787, PI322719, PI369994로부터 선발한 저항성 계통들은 더뎅이병균 race 3에 비과민형, 저항성 육성계통인 'SR'은 과민형으로 저항성을 나타내고, 4개의 한국 재래종과 세포질웅성불임계(A-line)와 그 유지계(B-line)는 매우 이병성임을 확인하였다. 저항성 선발계통을 세포질웅성불임계(*Smsms*)와 교배하여 그 F_1의 화분생산 관찰, 화분발아 검정, 약당 화분량을 조사하는 방법으로 임성을 조사하여 저항성 계통의 웅성불임관련 유전자형을 조사하였다. 그 결과 7개의 미국 식물도입국 계통(PI)은 모두 회복계(*N(S)MsMs*)로, 그리고 'SR'은 유지계(*Nmsms*)로 나타났다.

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