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Restorer Genotype for Male Sterile Cytoplasm of Genetic Resources Moderately Resistant to *Phytophthora capsici* in Capsicum Pepper

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Abstract. KC00256, KC00406, KC00462, KC00463, KC00820, and KC00821, the genetic resources that have previously been reported as moderately resistant to *Phytophthora capsici*, as well as the line KC01322, a new source of moderate resistance introduced from Laos, were tested against two strains (Pc003 and Pc005) of *P. capsici*. We also determined the nuclear restorer genotypes of these lines, in regards to their interaction with cytoplasmic male sterility, through crossing the resources with cytoplasmic male sterile Punggok-A (*Srfrf*) and determining the fertility of the F_1 hybrids. The studied lines exhibited a low level of resistance to both the strains of *P. capsici* compared to highly resistant CM334, but their response was fairly consistent for both *P. capsici* strains. KC00406, KC00462, KC00463, and KC01322 produced stable, male fertile F_1 plants indicating that they are restorers with genotype *N(S)RfRf*. KC00821 produced male sterile F_1 plants and was identified as a maintainer with genotype *Nrfrf*. The F_1 plants of the KC00820 cross, however, set a few male fertile flowers in the greenhouse at seedling stage, then became male sterile after being transplanted to the plastic greenhouse soil in May and remained so to the end of the growing season. Therefore, KC00820 is an unstable maintainer with genotype *Nrfrf*. The moderate resistance exhibited by these genetic resources may be integrated into breeding programs aimed at promoting higher levels resistance via recurrent selection or hybridization.

Additional key words: *Capsicum annuum*, cytoplasmic male sterility (CMS)

Introduction

Phytophthora blight, caused by *Phytophthora capsici*, is one of the most destructive diseases affecting pepper cultivation in Korea. The pathogen is soil-borne and spreads rapidly through water in the form of swimming zoospores that are released from sporangia (Erwin and Ribeiro, 1996; Katsura, 1972). As a result of this mode of spread the disease is most evident during the rainy season, especially in those fields where pepper has been grown continuously year after year (Kim et al., 2009). Accordingly, crop rotation is recommended and has been shown to alleviate the occurrence of the disease. However, the continuous cropping of pepper is a common practice for Korean farmers due to the lack of an alternative cash crop. Chemical control is rarely successful due to the soil-borne nature of the pathogen and the speed at which it spreads within a crop. Resistant cultivars have long been sought and, since 2005, resistant cultivars of acceptable quality are available from commercial seed companies. Among Korean pepper breeders, resistance to *P. capsici* is currently thought to be an essential prerequisite for any new dry red fruit crop cultivars. Originally, resistant cultivars were bred through cross-breeding with a well-known sources of resistance, such as CM334, PI201234, and AC2258 (= Mexican pepper 'Line 29') (Bosland and Lindsey, 1991; Gil Ortega et al., 1990; Hur et al., 1990; Kim, 1986, 1988; Kimble and Grogan, 1960). The pathogenic fungus, *P. capsici*, appears to evolve quickly against resistant cultivars as unusually virulent strains have been reported affecting resistant pepper cultivars (Kim et al., 2010). In addition, different resistant plant lines exhibit differing responses to the various *P. capsici* strains (Foster and Hausbeck, 2010; Glosier et al., 2008; Lee et al., 2010; Monroy-Barbosa and Bosland, 2008; Oelke and Bosland, 2003; Sy et al., 2008). *P. capsici* is heterothallic, with a mating system similar to that found in animals and higher plants, which allows it to

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rapidly adapt to adverse conditions through mutation and cross-fertilization. Continuous search for new sources of resistance to *P. capsici* is required in order to effectively manage the disease. We have reported a range of moderately resistant genetic resources (Kim et al., 2001) before and a diverse range of resistance sources was reported recently by Candole and Conner (2010).

Commercial cultivars in Korea are almost all F_1 hybrids. Either genic or cytoplasmic male sterility is usually incorporated into maternal parents for saving the labor for emasculation in hand pollination or for making insect pollination feasible. Cytoplasmic male sterility (CMS) is generally preferred to genic male sterility (GMS) as CMS allows the production of purely male sterile seeds while only 50% of seeds are male sterile in the GMS system. Although instability is often a hindrance in the utilization of CMS in sweet peppers, CMS is successfully used when breeding pungent types of pepper in Korea (Greenleaf, 1986; Lee, 2001; Shifriss, 1997; Yu, 1990). In regards to CMS, all genetic resources may be classified as either maintainers, which have a *Nrfrf* genotype, or restorers, which have a *N(S)RfRf* genotype (Greenleaf, 1986). The CMS-Rf genotypes of highly resistant genetic resources, such as PI123469, PI201232, PI201234, AC2258, and CM334, have been reported previously (Hwang and Kim, 1997). Herein, we report on the degree of resistance exhibited by the moderately resistant lines reported previously in Kim et al. (2001), as well as a new source of moderate resistance selected from among lines introduced from Laos, against two strains of *P. capsici*. We also determined the genotype regarding the restoration of cytoplasmic male sterility for these genetic resources.

Materials and Methods

Plant Materials

Most of the lines considered in this report have already been reported upon (Kim, 1988; Kim et al., 2001). However, KC01322 selected from collections in Laos has not been reported previously. KC00937 is a Phytophthora resistant breeding line received from the National Horticultural Research Institute in 2003. KC01260 and KC01261 were received from NongWoo Bio Co., Ltd. as bacterial wilt resistant materials in 2005.

Pathogen Materials

The plants lines were all tested against two strains of *P. capsci*,, Pc003, and Pc005. Pc003 was originally isolated from infected pepper plants in Youngyang, and Pc005 was isolated from infected roots of a rootstock variety, R-Safe, in Miryang, 2005. Pc005 has been observed as being unusually virulent (Kim et al., 2010).

Growing Seedlings, Inoculation, and Disease Rating

Seeds of the Phytophthora resistant lines, the CMS Punggok-A (*Srfrf*) line, and the F_1 hybrids between the two were sown in 128-cell-trays filled with 'Wonjo mix' medium (Nongkyung Agroindustrial Co., Ltd), and then transplanted to 50-cell-trays at 2- to 3-leaf foliage stage. Sixteen plants within each accession were inoculated with *P. capsci* about two months after sowing. For sporangia production, summer squash fruits were inoculated with the *P. capsici* isolates 5 days ahead of pepper inoculation and incubated in lab conditions at 20 to 25℃. The sporangia produced on the surface of the squash fruits were scraped into distilled water with scalpels. The sporangial suspensions were strained through two layers of gauze to remove the squash tissue. The numbers of sporangia per mL of suspension were counted with a haemocytometer, then adjusted to 2,000 sporangia per mL. The plants were inoculated by pouring 5 mL of the sporangial suspension on the base of stem, and were then watered everyday thereafter. The trays of the inoculated plants were placed on a greenhouse bench until the time of disease scoring, 14-20 days later.

The symptoms of the stems and above-ground sections were scored on a scale of 1 to 4, where 1 = no visible symptoms; 2 = dark lesion(s) visible on the base of stem, but surviving; 3 = wilting with dark lesion(s) on the base of stem; 4 = death. The root symptoms were scored on a scale of 1 to 5, where 1 = no visible symptom; 2 = root rot evident on 25% of the root system; 3 = 50% root rot; 4 = 75% root rot; 5 = complete root rot.

Identification of the Genotype Interacting with CMS

In 2009, the Phytophthora resistant lines were crossed with CMS Punggok-A plants to identify the nuclear restorer genotype of the resistant plant lines. Seeds of the F_1 hybrids 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 via visual observation of the pollen production by the anthers during bloom, with the aid of a magnifying glass as described previously (Hwang and Kim, 1997; Kim and Hwang, 1998). Pollen parents were classified as restorers (*N(S)RfRf*) or maintainers (*Nrfrf*) on the basis of their ability to produce male fertile F_1 plants when crossed with CMS Punggok-A lines, which had a genotype *Srfrf*.

Results and Discussion

Table 1 presents the reactions of moderately resistant

Table 1. Resistance to two strains of *Phytophthora capsici* (Pc) exhibited by the various genetic resources.

KC No	Pc003 (Youngyang)		Pc005 (Miryang)	
	Stem[z]	Root[y]	Stem	Root
KC00294 (CM334)	1.0 a[x]	1.0 a	1.0 a	1.0 a
KC00256 (P51)	1.0 a	3.6 cd	3.1 cd	4.0 cd
Tatan	1.0 a	3.7 c-e	1.0 a	4.3 de
KC00358-1	1.4 a-c	2.0 b	2.9 c	3.8 c
KC00263 (AC2258)	1.5 bc	3.5 c	1.0 a	3.0 b
Muhanjilju	1.5 bc	3.6 cd	2.7 c	4.5 e-g
KC00807	1.7 b-d	4.1 e-d	4.0 e	5.0 g
KC00358-3	1.8 cd	2.1 b	3.5 de	4.4 d-f
KC01322	2.0 d	3.4 c	3.8 e	5.0 g
KC00821	2.8 e	4.4 f-h	1.6 b	4.4 d-f
KC00937	3.0 e	4.0 c-f	3.1 cd	4.8 fg
KC00194	3.0 e	4.3 e-g	4.0 e	5.0 g
Subicho-1	3.0 e	4.8 gh	4.0 e	5.0 g
KC01261	3.0 e	5.0 h	3.6 de	4.8 fg
KC00463	3.0 e	5.0 h	3.9 e	5.0 g
KC01260	3.0 e	5.0 h	3.9 e	5.0 g
Cheongyang	3.0 e	5.0 h	4.0 e	5.0 g
KC00820	3.1 e	4.3 e-g	4.0 e	5.0 g
KC00406-2-2-1	3.1 e	4.8 gh	1.6 b	5.0 g
Nokgwang	3.1 e	4.8 gh	4.0 e	5.0 g
Geumtap	3.1 e	5.0 h	4.0 e	5.0 g
KC00462	3.2 e	4.4 f-h	3.7 e	4.8 fg
Subicho-2	3.2 e	5.0 h	4.0 e	5.0 g
PGA × KC01322	1.3 ab	4.8 gh	-	-
PGA × KC00821	2.8 e	4.3 e-g	-	-
PGA × KC00820	3.0 e	4.3 e-g	-	-
PGA × KC00406	3.0 e	4.5 f-h	-	-
PGA × KC00462	3.0 e	5.0 h	-	-
PGA × KC00463	3.0 e	5.0 h	-	-
PGA × Punggok1	3.0 e	5.0 h	-	-
PGA × Punggok2	3.1 e	4.9 gh	-	-
Punggok1	3.0 e	5.0 h	4.0 e	5.0 g
Punggok2	3.2 e	5.0 h	4.0 e	5.0 g

[z]1 = No disease symptom observed; 2 = necrotic lesion on the base of stem but still surviving; 3 = wilting; 4 = dead.
[y]1 = No root rot observed; 2 = about 25% root rot; 3 = about 50% root rot; 4 = about 75% root rot; 5 = complete root rot.
[x]Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

accessions and F_1 hybrids, produced from crosses of the moderately resistant lines with CMS Punggok-A plants, to two strains of *P. capsici*. The resistant accessions and the F_1's are listed primarily by severity of stem rot index followed by the root rot index resulting from inoculation with Pc003 (Youngyang). CM334 remained disease-free after inoculation with either strain. Tantan, a commercial rootstock cultivar, survived infection from both strains of *P. capsici* without presenting any above-ground symptoms but suffered a considerable degree of root rot. KC00256, originally known as P51 and a European breed that has been studied previously (Barksdale et al., 1984; Bartual et al., 1991, 1994), remained symptomless above ground after Pc003 inoculation, but Pc005 induced severe heavy stem and root rot in many plants. KC00358-1 was the next most resistant line, exhibiting relatively consistent reactions to both pathogen strains. Many KC00807 plants, originally selected for resistance to *P. capsici* among *Capsicum chinense* accessions, survived Pc003

but succumbed to Pc005. KC01322, the line from Laos, exhibited some tolerance to the disease. KC00820 and KC00821 are resources with bell-shaped fruits. KC00821 of them exhibited a higher resistance to both pathogen strains than KC00820. Few KC00937 and KC00194 plants survived. The sources of moderate resistance introduced here exhibited relatively low levels of resistance compared to those exhibited by the highly resistant lines such as CM334, AC2258, PI123469, PI201232, and PI201234 (Alcantara and Bosland, 1994; Bosland and Lindsey, 1991; Choe et al., 1985; Kimble and Grogan, 1960).

In general, Pc005 induced more severe symptoms than strain Pc003, indicating that *P. capsici* strain Pc005 was the more virulent of the two, as reported previously (Kim et al., 2010). However, the different plant lines exhibited fairly consistent reactions to the two pathogen strains, particularly in regards to root rot. Although differential responses of the root, stem and foliar tissues to *P. capsici* infection has been reported (Sy and Bosland, 2005; Walker and Bosland, 1999), root rot is the more important consideration for resistance as Korean farmers routinely spray chemicals to control anthracnose to the above-ground sections of their plants during the growing season. Inoculation of roots by drenching in a sporangial suspension, as was performed in this study, is, therefore, a dependable methodology for testing resistance to *P. capsici* in breeding.

Furthermore, considerable differences have been observed in the response of different resistant plant lines to the various *P. capsici* strains. *P. capsici* strains have been classified into 9 or 14 races based on the different reactions of different plant breeding lines, including PI201234 and CM334 (Glosier et al., 2008; Oelke and Bosland, 2003; Sy et al., 2008). Similarly, plants have shown to inherit race-specific *P. capsici* resistance (Monroy-Barbosa and Bosland, 2008). In Korea, variation in the virulence of *P. capsici* was first reported by Yang et al. (1989), and differences in the response of resistant lines to *P. capsici* strains were first reported by Hwang et al. (1996). Lee et al. (2010) classified the Korean strains of *P. capsici* into 11 races based on interactions between *P. capsici* strains and a selection of pepper cultivars. These race specific reactions should be considered when breeding for resistance, and the strains used in the selection procedures should be chosen carefully.

The moderately resistance genetic resources considered in this report can be used in breeding programs aimed at promoting *P. capsici* resistance via hybridization with other resistant lines or through recurrent selection, as has been demonstrated and recommended previously (Bartual et al., 1991, 1994; Palloix et al., 1990). Muhanjilju, a commercial hybrid cultivar, exhibited a reasonable level of resistance in this study. KC00406 and KC00462 exhibited similar symptoms to susceptible controls such as Nokgwang, Geumtap and Subicho selections, and in regards to disease severity, although the accessions exhibit resistance to certain strains of *P. capsici* (Kim, 1988; Kim et al., 2001). While it is generally accepted that *P. capsici* is evolving greater virulence in response to the prevalence of resistant cultivars, some of the moderately resistant lines have their own useful traits. KC00820, KC00821, and KC00256 (P51) produce bell shaped fruits, compared to the Chile-type fruit of other sources of resistance. KC00820, KC00821, and KC01322 have also been shown to be tolerant to bacterial wilt caused by *Ralstonia solanacearum* in an infested field observation, when compared to susceptible CM334 and AC2258. KC01322 was also tolerant to viral disease infection in a field where cucumber mosaic virus (CMV), pepper mottle virus (PepMoV), pepper mild mottle (PMMoV), and broad bean wilt virus (BBWV) were all endemic.

Among the F_1 generations of crosses with Punggok-A, KC01322 and KC00821 crosses were slightly more tolerant than the others, suggesting resistance of the hybrids was correlated with that of the respective pollen parents. Punggok is also a source of moderate resistance, which resulted in the F_1 generation exhibiting a similar level of resistance to their moderately resistant pollen parents. Thus, moderate resistance of Punggok-A served as a tolerant background to keep the moderate resistance of the pollen parents in resistance to *P. capsici*.

Fertility of the F_1 hybrids produced from crosses between CMS Punggok-A (*Srfrf*) plants and the moderately resistant lines are presented in Table 2. F_1 plants of the KC01322, KC00406, KC00462, and KC00463 crosses were all male fertile, indicating that the pollen parent lines are genetic restorers with a genotype *N(S)RfRf* or possibly *NRfRf*. The F_1 plants were abundant in pollen ranging from 1.61-2.07 $\times 10^4$ pollens per anther depending on the crosses. KC00821, in contrast, produced only male sterile F_1 plants and, therefore, was a maintainer with a genotype *Nrfrf*. The ability of KC00821 to successfully maintain CMS was further confirmed in the BC_2 plants produced in the following season in a backcross program. KC00821, therefore, may be bred into a CMS line, using the backcross method, for use as a maternal line for hybrid cultivars. Conversely, KC00820 occasionally set fertile male flowers resulting in a few fruits set in the greenhouse before transplanting to the plastic greenhouse soil in May, but never in the plastic greenhouse. BC_1 plants obtained by backcrossing with KC00820 showed a similar phenomenon but even in the plastic greenhouse after being transplanted, a few plants became male fertile during cool weather and reverted to male sterile during hot weather. Thus, the fertility

Table 2. Fertility status of the F_1 plants obtained from crosses between a cytoplasmic male sterile (CMS) line, Punggok-A (*Srfrf*, PGA), and sources of moderate resistance to *Phytophthora capsici*, and the CMS-Rf genotype of the pollen parents.

Cross	Date of 1st flower	Date of 50% Bloom	Days to flower[z]	Fertility	Rf genotype	Pollen /anther
PGA × KC01322	20-May	3-Jun	103	MF	*N(S)RfRf*	19,870
PGA × KC00820	5-May	12-May	81	MS[y]	*Nrfrf*	-
PGA × KC00821	4-May	9-May	78	MS	*Nrfrf*	-
PGA × KC00406	4-Jun	20-Jun	120	MF	*N(S)RfRf*	20,700
PGA × KC00462	7-May	8-May	77	MF	*N(S)RfRf*	16,080
PGA × KC00463	7-May	12-May	81	MF	*N(S)RfRf*	19,120
PGA × Punggok	11-May	13-May	82	MS	*Nrfrf*	-
PGA × Punggok	12-May	25-May	94	MS	*Nrfrf*	-

[z]From sowing on Feb. 20.
[y]Unstable and temperature sensitive.

of the plants was unstable, as expected in fresh fruit accessions (Lee, 2001), and appeared to be sensitive to temperature, as reported by Shifriss (1997). Shifriss (1997) reported that male sterility in *Srfrf* plants was stable at a mean temperature of 30℃, but became unstable when the temperature dropped to 24℃ by day and 17℃ by night in Israel. The critical temperature for stability of *Srfrf* plants derived from KC00820 still needs to be determined. Shifriss (1977) suggested that one could take an advantage of the temperature sensitivity of male sterility when producing male sterile parental lines during the cool season. Thus, KC00820 may be exploited through the careful management of the CMS-Rf genotype and by taking advantage of the bell-shaped fruit and its bacterial wilt tolerance after further tests and observation. The F_1 plants of the crosses of KC00820 and KC00821 in the maintainer group and KC00462 and KC00463 in the restorer group were earlier in blooming as compared to those of KC01322 and KC00406. Therefore, the accessions may be used in the breeding for resistance without delaying the maturity, a phenomenon often encountered in breeding for disease resistance.

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