

Screening of Sclerotinia Rot Resistant Korean Origin Perilla (*Perilla frutescens*) Germplasm Using a Detached Leaf Method

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Abstract - Sclerotinia rot, caused by *Sclerotinia sclerotiorum*, is a devastating disease that poses a serious threat to perilla production in Korea. Identifying effective sources of resistance offers long term prospects for improving management of this disease. Screening disease resistant genetic resources is important for development of disease-resistant, new cultivars and conduct related research. In the present study, perilla germplasm were screened *in vitro* against *S. sclerotiorum* using detached leaf method. Among 544 perilla accessions, two were highly resistant (IT226504, IT226533), five were resistant (IT226561, IT226532, IT226526, IT226441, and IT226589), five were moderately resistant (IT226525, IT226640, IT226568, IT220624, and IT178655), 16 were moderately susceptible, 31 were susceptible, and 485 were highly susceptible. The resistant accessions in this study could serve as resistance donor in the breeding of Sclerotinia rot resistance or subjected to selection procedure of varietal development for direct use by breeders, farmers, researchers, and end consumers.

Key words – Detached leaf, *Perilla frutescens*, Resistance, Sclerotinia rot

Introduction

Perilla (*Perilla frutescens* L.), originally from Southeast Asia, is an annual herbaceous plant under the family Lamiaceae, and the second-biggest upland crop in Korea. Nowadays, it is widely distributed in many regions of the world (Lee and Ohnishi, 2003). In 2017, perilla seed production was 50,738 metric tons with cultivation area covering approximately 43,352 ha (KOSIS, 2018). It has been used as an antioxidant, and a traditional herbal medicine for treating various diseases such as, anxiety, depression, cough, mild seasonal allergic rhino conjunctivities, intoxication, tumor, and some intestinal disorders (Assefa *et al.*, 2018; Makino *et al.*, 2003; Yang *et al.*, 2012). In Korea, perilla used as oil, as

an aromatic vegetable, in sushi, salads, pickles and mainly consumed with meat. Hence, the perilla cultivar has been developed with regard to either extracting oil or harvesting fresh leaves. Perilla oil is one of the richest omega-3 fatty acid sources among the edible seed oils (Asif, 2011; Eckert *et al.*, 2010). Linolenic acid occupies 63.1% of the overall fatty acid in perilla oil. Numerous functional components contained in perilla have been reported to show anti-inflammatory response, inhibition for α -glucosidase, and antioxidative activity (Banno *et al.*, 2014; Ha *et al.*, 2012; James *et al.*, 2000). Furthermore, α -linolenic acid, the main fatty acid in perilla seed, has been reported by various studies to reduce the risk of cardiovascular diseases and affect the change of brain lipid composition and recognition ability (Guixiang *et al.*, 2004; Kim *et al.*, 2010; Lee *et al.*, 2017; Zhang *et al.*, 2014).

In Korea, various diseases such as leaf spot, gray mold,

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anthracnose, Sclerotinia rot, rust, downy mildew, stem blight and Phytophthora blight have been found in perilla (Cho and Moon, 1994; Choi *et al.*, 2009; Kim *et al.*, 2001; Lee *et al.*, 2009; Maeng *et al.*, 2009; Moon *et al.*, 1998; The Korean Society of Plant Pathology, 2009; Yun *et al.*, 2007). Among them, Sclerotinia rot is one of the most devastating fungal diseases that decrease the yield of perilla significantly. Sclerotinia rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a ubiquitous necrotrophic fungal pathogen capable of infecting about 408 plant species among 75 families (Boland and Hall, 1994). The pathogen is recognized by the fluffy white mycelium and black sclerotia that develops on the surface of lesions (Bolton *et al.*, 2006).

Various authors have reported various inoculation techniques for screening of Sclerotinia rot in different crops such as pea, soybean, oilseed rape, dry bean and also perilla, by using cut stem, detached leaf methods, cut petiole inoculation, spray- mycelium & drop-mycelium, cotyledon inoculation, soil drenching (Afroz *et al.*, 2019; Chen and Wang 2005; Grau and Bissonette 1974; Kull, 2003; Miorini *et al.*, 2018; Del Rio *et al.*, 2001; Vuong *et al.*, 2004). Based on our previous study, the detached leaf method is a simple and rapid disease inoculation method for screening of Sclerotinia rot in perilla (Afroz *et al.*, 2019).

Plant genetic resources (PGR) represent a wealth of genetic diversity, part of which is of potential value for breeding better crop plants (Frankel, 1977). For instance, landraces or crop wild relatives, modern cultivars, breeding lines and close or distant relatives may bear valuable genes for disease resistance, yield-improving properties, or quality-related traits. Therefore, the success of breeding programs for disease resistance always depends on the availability of wide range of genetic resources. To understand the genetic basis of quantitative resistance, various researches have carried out for quantitative trait locus (QTL) mapping using monogenic and/or polygenic depending on the plant species, derived normally from crosses between a partially resistant parent and a susceptible parent (Wu *et al.*, 2016; Yin *et al.*, 2010; Zhao and Meng, 2003; Zhao *et al.*, 2006). Quantitative trait loci (QTL) mapping technique for polygenic resistance is used to identify loci related to *S. sclerotiorum* resistance in different crop species like soybean, common bean, sunflower

and *B. napus*, but there is no published data in its application in perilla so far (Chen, 2007; Godoy *et al.*, 2005; Hartman *et al.*, 2000; Kim and Diers, 2000). The objective of the present study was to determine the differential responses of perilla germplasm in Korea, by detached leaf method to identify the source of resistance and to evaluate the expression and relationship of resistances to Sclerotinia rot.

Materials and Methods

Plant material

Five hundred and forty four Korean origin perilla accessions (including 400 landraces, 29 cultivars, 24 breeding lines, and 1 relative wild type, and 90 unknown) were obtained from National Institute of Crop Science, and Jeonju, Republic of Korea, to identify the resistance of those perilla germplasms against Sclerotinia rot caused by *Sclerotinia sclerotiorum*. This experiment was conducted in a greenhouse and growth chamber of the National Agrobiodiversity Center (NAC), National Institute of Agricultural Science, and Jeonju, Republic of Korea. Experiments were done at the seedling stage when plant had five to six leaves. The characteristics of perilla accessions information were provided by Germplasm Management System of National Agrobiodiversity Center, National Institute of Agricultural Science, RDA, and Jeonju, Republic of Korea.

Fungal pathogen

Sclerotinia sclerotiorum isolate (KACC40457) was obtained from the Korean Agricultural Culture Collection (KACC) and confirmed the pathogenicity to perilla leaves using detached leaf method. *S. sclerotiorum* was sub-cultured and maintained at 25°C on Potato Dextrose Agar (PDA) in culture room.

Inoculation methods

Detached leaf method was used for this experiment. A single mycelial agar plug (size 7 mm² after growing at 25°C for 7 days) was cut from the margin of PDA with actively growing mycelial colony and was placed mycelial-side down at 1/3 point on the main leaf vein of detached leaf for *S. sclerotiorum*; at front side. For the sake of getting in touch

with the leaf surface, the plug was slightly compressed. The leaves were inoculated and kept in a plastic box with moistened paper towel to maintain humidity and incubated at 25°C in dark condition. Three replications, each consisting of fifteen leaves, were used in this experiment.

Disease rating & resistance response

Necrotic and water soaked lesions appeared after two days of inoculation. At 7 days after inoculation, lesions diameters were measured using a linear ruler. The resistance response was measured according to Naher *et al.*, 2018. In addition, the resistance levels were represented based on resistance ratio (%) based on the following categories: >90% = highly resistance (HR); 80.0 to 90% = resistance (R); 70.0 to 79.9% = moderately resistance (MR); 50.0 to 69.9% = moderately susceptible (MS); 30.0 to 49.9% = susceptible (S); <30% = highly susceptible (HS). Resistance ratio (%) was calculated by the following formula.

Resistance ratio (%) = (No. of leaves showed below 1 cm of lesion size/ No. of total evaluated leaves) × 100

Results

The results of resistance ratio (%) and resistant response of perilla accessions were as presented in Table 1, 2 and Fig. 1. Among the 544 accessions, two were highly resistant, five were resistant, five were moderately resistant, 16 were moderately susceptible, 31 were susceptible, and 485 were highly susceptible against *S. sclerotiorum*. Out of 400 landrace perilla accessions, only two were moderately resistant (IT

220624, IT178655) with a resistance ratio of 70.0%, eight were moderately susceptible, 15 were susceptible, and 375 were highly susceptible. All 24 breeding lines were highly sensitive. Out of 29 cultivars, two were susceptible, and 27 were highly susceptible. Also, one relative perilla accession was susceptible. Out of 90 unknown accessions, two were highly resistant (IT226504, IT226533) with a resistance ratio of 100%, five were resistant (IT226561, IT226532, IT226526, IT226441 and IT226589) with a resistance ratio of 80.0 to 86.7%, five were moderately resistant (IT226525, IT226640, IT226568, IT220624 and IT178655) with a resistance ratio of 70.0 to 76.9%, eight were moderately susceptible, 13 were susceptible, and 59 were highly susceptible.

The Morphological characteristics of seven accessions, which showed high resistance to sclerotinia rot, were as presented in Table 3. All accessions were planted on 30th May, 2017 and flowering occurred on 04 September, 2017 and all were green adaxial leaf color (except IT226526- pale green). Trichom density of all accessions was medium and leaf shapes were cordate. Four accessions (IT226533, IT226532, IT226526, IT226541) produced purplish green abaxial leaf color, whereas, IT226504, IT226561, and IT226589 produced green, purple, and pale green abaxial leaf color, respectively. The leaf length ranged from (13 to 17.25 cm) while the leaf width varied between (9.60 and 13.10 cm).

Discussion

In the present study, 544 perilla accessions were assessed *in vitro* against Sclerotinia rot caused by *S. sclerotiorum* using

Table 1. Resistant response of perilla accessions against Sclerotinia rot caused by *Sclerotinia sclerotiorum*

Type of Perilla accessions	Number of accessions	Resistant response ^z					
		HS	S	MS	MR	R	HR
Landrace	400	375	15	8	2		
Breeding line	24	24					
Cultivar	29	27	2				
Relative	1		1				
Unknown	90	59	13	8	3	5	2
Total	544	485	31	16	5	5	2

^zHS= highly susceptible; S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistance; HR = highly resistance.

Table 2. *Perilla* accessions that showed resistance ratio (%) & resistant response against *Sclerotinia sclerotiorum*

IT Number ^z	Accession name	Status	Resistance Ratio (%) ^y	Resistance Response ^x
226504	Population 8, Individual 15-1	Unknown	100	HR
226533	Population 23, Individual 45-2	Unknown	100	HR
226561	Population 33, Individual 65-1	Unknown	86.7	R
226532	Population 23, Individual 45-1	Unknown	80	R
226526	Population 20, Individual 39-3	Unknown	80	R
226541	Population 26, Individual 51-1	Unknown	80	R
226589	Population 28	Unknown	80	R
226525	Population 20, Individual 39-2	Unknown	76.9	MR
226640	P2006-62	Unknown	73.3	MR
226568	Population 37, Individual 73-3	Unknown	73.3	MR
220624	P2006-64	Landrace	70	MR
178655	Sujib	Landrace	70	MR
226506	Population 8, Individual 15-3	Unknown	66.7	MS
226505	Population 8, Individual 15-2	Unknown	66.7	MS
226584	Population 11	Unknown	66.7	MS
226514	Population 12, Individual 23-2	Unknown	60.0	MS
226563	Population 33, Individual 65-3	Unknown	60.0	MS
157513	10115	Landrace	60	MS
226593	Population 37	Unknown	53.3	MS
226515	Population 12, Individual 23-3	Unknown	53.3	MS
226507	Population 9, Individual 17-1	Unknown	53.3	MS
207957	Chonnam Hwasan-1997-47	Landrace	50	MS
157599	10205	Landrace	50	MS
157491	10092	Landrace	50	MS
113452	Chungnam Yeonggi-1985-13452	Landrace	50	MS
175927	Gyeongbuk Bonghwa-1992-2769	Landrace	50	MS
217571	kocf11	Landrace	50	MS
220629	P2006-81	Landrace	50	MS
229010	PF 08011	Landrace	46.7	S
220425	Yeongcheon-2	Unknown	46.7	S
226531	Population 22, Individual 43-3	Unknown	46.7	S
226582	Population 1	Unknown	46.7	S
226588	Population 26	Unknown	46.7	S
226594	Population 38	Unknown	46.7	S
247961	Population 31	Unknown	40	S
226503	Population 7, Individual 13-3	Unknown	40	S
226569	Population 38, Individual 75-1	Unknown	40	S
226571	Population 38, Individual 75-3	Unknown	40	S
105282	Gangwon Chungseong-1985-5282	Landrace	40	S
157436	10037	Landrace	40	S
157478	10079	Landrace	40	S

Table 2. Continued

IT Number ^z	Accession name	Status	Resistance Ratio (%) ^y	Resistance Response ^x
157509	10111	Landrace	40	S
157523	10128	Landrace	40	S
117074	Gyeongbuk Andong-1986-3333	Landrace	33.3	S
226464	Gyeongnum Namhae-2000-22	Relative	33.3	S
226500	Population 6, Individual 11-2	Unknown	33.3	S
226520	Population 15, Individual 29-3	Unknown	33.3	S
226530	Population 22, Individual 43-2	Unknown	33.3	S
226549	Population 29, Individual 57-1	Unknown	33.3	S
157567	10173	Landrace	30	S
157598	10204	Landrace	30	S
207975	Incheon Ganghwa-1997-17	Landrace	30	S
113291	Jeonbuk Gochang-1985-13291	Landrace	30	S
105274	Jeonbuk Okgu-1985-5274	Landrace	30	S
105347	Jeonbuk Imsil-1985-5347	Landrace	30	S
105674	Gyeonnam Miryang-1985-5674	Landrace	30	S
105801	Gyeongbuk Andong -1985-5801	Landrace	30	S
217414	Okdong Dolgae-1	Cultivar	30	S
261881	Joim	Cultivar	30	S
229027	PF 09012	Landrace	23.1	HS
220513	Ill-yeop	Cultivar	16.7	HS
274239	YPL5-2B-9-5-1-1	Breeding line	13.3	HS
226512	Population 11, Individual 21-3	unknown	0	HS
226540	Population 25, Individual 49-3	unknown	0	HS

** rest 480 are also highly susceptible (HS)

^zIntroduction number of National Agrobiodiversity Center (NAC), National Institute of Agricultural Science, Jeonju, Republic of Korea.

^y(No. of plants showed below 1cm of lesion size/No. of total evaluated plants) × 100.

^xHR=highly resistance (>90% of resistance ratio); R=resistance (80.0 to 90% of resistance ratio); MR=moderately resistance (70.0 to 79.9% of resistance ratio); MS=moderately susceptible (50.0 to 69.9% of resistance ratio), S=susceptible (30.0 to 49.9% resistance ratio); HS=highly susceptible (<30%=resistance ratio).

detached leaf method. Diseases due to *S. sclerotiorum* have traditionally been difficult to manage (Bolton *et al.*, 2006). Breeding for Sclerotinia stem rot resistance is complicated by polygenic resistance alleles, with several likely controlling structural disease avoidance phenotypes, like plant height, and others controlling physiological resistance mechanisms, and also complex genetic and environmental interactions. Breeding initiatives have mainly focused on increasing yield, then attempting to incorporate disease resistance traits. Therefore, molecular breeding is pursued as a significant approach for controlling sclerotinia diseases. Actually,

breeding for *S. sclerotiorum* resistant cultivars using conventional method is difficult since no immune or highly resistant germplasm is available from genetic resources like landrace, wild or relatives etc (Liu *et al.*, 2005). The present study found phenotypically two highly resistant (IT226504, IT226533), five were resistant (IT226561, IT226532, IT-226526, IT226441 and IT226589), five moderately resistant (IT226525, IT226640, IT226568, IT220624 and IT178655) perilla germplasm against *S. sclerotiorum* from unknown accessions. The results of this study with morphological characteristics of perilla flowering time, leaf shape cordate,

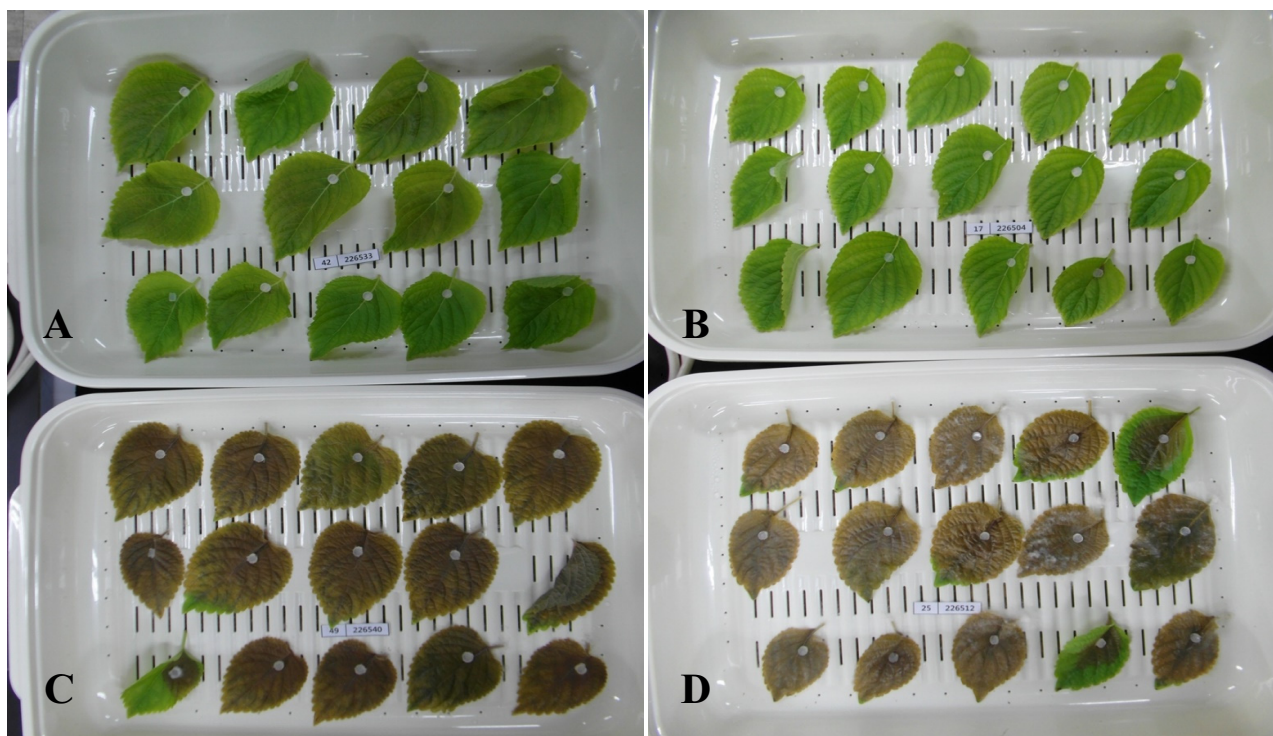


Fig. 1. *Perilla* accessions showing resistant response against *Sclerotinia sclerotiorum*. Highly resistant accessions (A: IT226533; B: IT226504); highly susceptible accessions (C: IT226540; D: IT226512).

Table 3. Morphological characteristics of 7 accessions which showed highly resistance to resistance of *Sclerotinia* rot provided by Germplasm Management System of National Agrobiodiversity Center, National Institute of Agricultural Science, RDA, Jeonju, Jellabuk-do, Rep. of Korea

IT Number ^z	Accession name	Planting date	Flowering date	Adaxial color of leaf	Abaxial color of leaf	Trichom density	Leaf shape	Length of leaves (cm)	Width of leaves (cm)
226504	Population 8 Individual 15-1	5/30/2017	9/04/2017	Green	Green	Medium	Cordate	16.35	12.60
226533	Population 23 Individual 45-2	5/30/2017	9/04/2017	Green	Purplish green	Medium	Cordate	16.75	13.10
226561	Population 33 Individual 65-1	5/30/2017	9/04/2017	Green	Purple	Medium	Cordate	13.00	10.75
226532	Population 23 Individual 45-1	5/30/2017	9/04/2017	Green	Purplish green	Medium	Cordate	15.50	12.00
226526	Population 20 Individual 39-3	5/30/2017	9/04/2017	Pale green	Purplish green	Medium	Cordate	17.00	12.25
226541	Population 26 Individual 51-1	5/30/2017	9/04/2017	Green	Purplish green	Medium	Cordate	17.25	13.10
226589	Population 28	5/30/2017	9/04/2017	Green	Pale green	Medium	Cordate	13.00	9.60

^zIntroduction number of National Agrobiodiversity Center (NAC), National Institute of Agricultural Science, Jeonju, Republic of Korea.

adaxial and abaxial leaf colors being pale green to purple are similar to the results of Kim *et al.*, 2011; Ma and Lee, 2017; Woo *et al.*, 2016. Identification of genetic variation is important for long-term achievements of breeding programs

and maximizes the use of germplasm resources (Mwangi *et al.*, 2019). The findings in this study can play a significant role to find out resistant breeding line & quantitative trait loci (QTL) against *S. sclerotiorum* for perilla.

Due to their purplish color, perilla leaves look attractive and containing high health beneficial anthocyanin content, most people like to consume. The present study highlighted *in vitro* screening of perilla germplasm resistant against *Sclerotinia sclerotiorum* that causes Sclerotinia rot using detached leaf method. The study also revealed that various levels of resistance to Sclerotinia rot exist in perilla germplasm collections. Out of 544 perilla accessions, two were highly resistant, five were resistant, five were moderately resistant, 16 were moderately susceptible, 31 were susceptible, and 485 were highly susceptible. As this study's experiment was conducted in seedling stage, it is recommended to conduct the experiment at different growth stage in experimental field agro-ecological conditions. Breeders could use the resistant germplasm as a source of resistance for the development of resistant cultivars.

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