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Screening for Resistance of Garlic Cultivars to White Rot Caused by *Sclerotium cepivorum*

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*Sclerotium cepivorum*에 대한 마늘 재배종의 저항성 검정

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ABSTRACT: The optimal quantity of inoculum was determined to screen resistance of garlic cultivars against *Sclerotium cepivorum*, and 30 cultivars was tested. The growth of the pathogen in detached roots, and stimulatory effect of the root extract on sclerotial germination of *S. cepivorum* were compared. Disease incidence was increased when the inoculum density was raised from 10 to 100 sclerotia. The optimal inoculum density to differentiate resistance or susceptibility of garlic cultivars was seemed to be 50 sclerotia. The cultivars collected from England, Japan, Nepal and Turkey, and cultivars such as common red, PI1356104 and PI135693 were less than the other cultivars in their disease incidence. The growth of *S. cepivorum* in detached roots varied from 23 to 33 mm according to garlic cultivars. There was no relationship between the disease incidence and the growth in detached roots. The sclerotial germination was increased significantly when root extract was added. The addition of only distilled water resulted in 13% germination, but the addition of 0.25 g of root extract in 100 ml distilled water resulted in more than 85% germination. There was no difference in the stimulation of sclerotial germination among cultivars which showed different resistance.

***Key words*: garlic, resistance, sclerotial germination, *Sclerotium cepivorum*, white rot.**

White rot of garlic caused by *Sclerotium cepivorum* has caused severe damages in major production areas (4, 5). *S. cepivorum* is a soilborne pathogen which is able to survive in the form of sclerotia in soil for long periods in the absence of their host plants (10). Sclerotia produced in axenic culture were reported to undergo a constitutive dormancy for 2 months (12). The constitutive dormancy was also present in sclerotia produced on onion bulbs under sterile conditions, and dormancy broke down after being buried for 1~3 months in unsterile soil, but not if the sclerotia were stored under sterile conditions (11).

Volatile degradation products of the alkyl and alkenyl sulphoxides of the genus *Allium* are responsible for triggering the germination of sclerotia of *S. cepivorum* under nonsterile conditions (2, 9). Only members of the genus *Allium* are host plants of *S. cepivorum* (8).

Many efforts have previously been carried out to select cultivar resistant to white rot in *Allium* species (3, 6-8, 12). There is evidence that garlic may be more susceptible than the other edible species of *Allium* (6). According to infection of onion, leek, garlic and *Allium fistulosum*, garlic was the most susceptible to the *S. cepivorum* (7).

In spite of severe incidence and damage, there is no report on screening method for assaying resistance and cultivars resistant to white rot of garlic in Korea

This study was conducted to determine optimal quantity of inoculum necessary for screening garlic cultivars reistant to white rot caused by *S. cepivorum*, to find the difference in the growth of *S. cepivorum* in detached roots and to check stimulatory effect of the root extract on sclerotial germination.

MATERIALS AND METHODS

Isolates and sclerotial production. The isolate of *S. cepivorum* was obtained from the diseased bulbs of

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garlic collected from Muan area, Chonnam, and maintained in potato dextrose agar (PDA) at 10°C, and transferred to PDA plates for preliminary culture as needed.

To produce sclerotia, mycelial disks from the margin of 5-day old colonies were placed on the center of PDA plates. The plates were incubated for 6 weeks at 20°C. The sclerotia were obtained by scraping the surface of the plates with spoon, and washing with distilled water through series of mesh size 500 and 400 μm sieve. To use similar sclerotia in size, the sclerotia on 400 μm sieve were used in all of this experiment. The harvested sclerotia were dried at room temperature and stored in gauze in unsterile, moistened soil for 10 weeks at 15°C.

Effect of inoculum density on disease incidence. Bulbs of five garlic cultivars were separated into individual cloves, and each healthy clove among them was sown in pot (4×4×4 cm) containing sterilized soil. There were 3 replications of 10 cloves. Immediately after sowing, 10, 25, 50, 100, 150 and 200 sclerotia were inoculated, respectively, on top of the soil, and covered with 1 cm of soil. The pots were placed at 20±2°C with relative humidity of 65~80%. Disease incidence was determined by counting infected plants with typical foliar symptoms 6 weeks after inoculation, and the roots were examined by uprooting them to ascertain infection by *S. cepivorum*.

Screening for resistance. Bulbs of 30 garlic cultivars (mostly collected cultivars) obtained from Mokpo experiment station, RDA, were separated into individual clove, sown in pot, and fifty sclerotia were inoculated, and cultivated as described above. There were 3 replications of 10 cloves.

Growth of *S. cepivorum* in detached roots. Roots were detached, washed with tap water, and cut into 7 cm long, and placed on moist filter paper in petridish.

A mycelial plug (1×0.5 cm) of *S. cepivorum* grown on PDA for one week was placed in one end of each detached root. The inoculated roots were incubated for one week at 15°C in darkness. The incubated roots were examined as used by Coley-Smith (11). The infected roots were cleared for 3 days in a mixture of chloroform, lactophenol and absolute ethanol (2:5:3 by volume) and stained for 2 days in phenylacetic aniline blue (phenol 50 g/*l*, aniline blue solution 10 g/*l*, glacial acetic acid. 15:1:4 by volume).

The maximum distance of mycelial growth in each root was scored. Twenty roots were used for each cultivar.

Effect of root extracts on sclerotial germination. Roots of six garlic cultivars which showed different resistances in above experiment were cut into 0.25, 0.5, 1.0 and 2.0 g. The roots were homogenized (Ace homogenizer, 10,000 rpm, 5 min) with 50 ml of distilled water, passed through filterpaper (Whatman No. 2), and diluted with distilled water to 100 ml. Five 1 ml samples were stored at -20°C in small tube. Fifty sclerotia were placed on the bottom of petridish and then were covered with gauze. Quantities of 25 g of air-dried unsterile soil (Table 1) were placed evenly on the gauze. A quantity of 1 ml extract was added every week to each petridish to stimulate sclerotial germination, and the petridishes were sealed with parafilm. Control received only 1 ml of distilled water. After 5 weeks of incubation in darkness at 15°C, the percentages of germinated sclerotia were calculated using dissecting microscope (10×).

Table 1. Chemical properties of soil used in this experiment

pH	Organic materials (%)	P_2O_5	Exchangeable cation (me/100 g)			
			Ca	Mg	Na	K
5.8	1.6	684	3.18	0.72	0.22	1.08

RESULTS

Effect of inoculum density on disease incidence. Disease incidence has been increased when the inoculum density was added gradually from 10 to 200 sclerotia per bulb. In pot inoculated with 10-sclerotium, the mild foliar symptom was observed on the cultivar and roots were being infected when they were examined by uprooting them. The foliar symptom was much severe, and easy to observe when 100 or more sclerotia were inoculated. Among the inoculum densities, the density using fifty sclerotia seemed to be appropriate to differentiate resistance or susceptibility of garlic cultivars (Table 2).

Disease incidence and growth of *S. cepivorum* in detached roots of garlic cultivars. There were some differences in disease incidence among garlic cultivars (Table 3). The cultivars collected from England, Japan, Nepal and Turkey, and cultivars such as common red, PI1356104, PI135693 showed less than the other cultivars in their disease incidence. The growth of *S. cepivorum* in detached roots varied from 23 to 33 mm according to garlic cultivar. The cultivars such as Jabong, Koheung and Nepal which have shown the growth of 23 mm, showed the disease incidence of 100.0, 70.0, 66.7%, respectively. This result means that there was

Table 2. Disease incidence of garlic cultivars inoculated with different numbers of sclerotia of *Sclerotium cepivorum*

Cultivar	Disease incidence (%)[x]					
	No. of inoculated sclerotia					
	10	25	50	100	150	200
Namdo	76.7[y]ab	90.0 a[z]	90.0 a	96.7 a	100.0 a	100.0 a
Jabong	70.0 b	76.7 b	80.0 b	100.0 a	96.7 a	100.0 a
PI135689	80.0 a	80.0 b	86.7 a	96.7 a	100.0 a	100.0 a
IT136644	73.3 ab	76.7 b	80.0 b	93.3 a	93.3 a	96.7 a
Daeseo	73.3 ab	76.7 b	80.0 b	93.3 a	100.0 a	100.0 a
Mean	74.7	80.0	83.3	96.0	98.0	99.3

[x] Disease incidence was measured 6 weeks after inoculation.
[y] The values are means of three replications of 10 cloves.
[z] The same letters in a column is not significantly different (P= 0.05) according to Duncan's multiple range test.

Table 3. Disease incidence and growth of *S. cepivorum* in detached roots of garlic cultivars

Cultivar or origin[a]	Disease incidence[b] (%)	Root infection (mm)
Namdo, Korea	96.7[c] ab[d]	27 defghi
Jabong, Korea	100.0 a	23 i
Koheung, Korea	70.0 def	23 i
Daeseo, Korea	93.3 abc	30 bcdef
Kodang, Korea	73.3 def	25 fghi
Changsan, Korea	70.0 def	30 bcdefg
Gajeong, Korea	80.0 cde	33 ab
Fengyuan, China	80.0 cde	24 hi
Gajeong, China	70.0 def	29 bcdehgh
Jeolgangpyoungho, China	73.3 def	30 bcdef
Sangdong, China	90.0 abc	30 bcdef
Hoeoon, China	93.3 abc	30 bcdef
Gangsosayang, China	100.0 a	35 a
Gangdo, China	96.7 ab	33 ab
Gangsosingeol, China	100.0 a	27 cdefghi
Jeolgangkaheung, China	100.0 a	33 ab
Bukyoung, China	93.3 abc	34 ab
Sangdong, China	100.0 a	25 ghi
Bukhaedo, Japan	63.3 f	34 ab
Common red	63.3 f	25 efghi
England	63.3 f	27 defghi
Paris, France	90.0 abc	33 ab
Italy	70.0 def	31 abcd
Spain	96.7 ab	31 abcd
Mexico	90.0 abc	29 bcdefgh
Nepal	66.7 ef	23 i
Turkey	63.3 f	26 defghi
PI1356104	66.7 ef	33 ab
PI135689	96.7 ab	31 abce
PI135693	66.7 ef	27 defghi

[a] Disease incidence was measured 6 weeks after inoculation.
[b] Mycelial growth of *S. cepivorum* in the root of each garlic cultivar. It was measured 7 days after inoculation.
[c] The values are means of three replications of 10 cloves.
[d] The same letters in a column is not significantly different (P= 0.05) according to Duncan's multiple range test.

Table 4. Germination of sclerotia of *S. cepivorum* treated weekly with 1 ml of root extracts of garlic with different concentrations

Cultivar	Germinated sclerotia (%)			
	0.25 g/ 100 ml	0.5 g/ 100 ml	1.0 g/ 100 ml	2.0 g/ 100 ml
Namdo	90[a] ab	92 a	93 a	93 a
Jabong	88 a	93 a	93 a	95 a
Gangsosinjeol	88 a	92 a	95 a	93 a
Pukhaedo	93 a	92 a	93 a	95 a
PI1356104	85 a	90 a	93 a	97 a
Common red	90 a	92 a	92 a	93 a
Control[c]			3	

[a] The values are means of three replications of 50 sclerotia.
[b] The same letter in a column is not significantly different (P= 0.05) according to Duncan's multiple range test.
[c] Control is treated with 1 ml of distilled water.

no relationship between the disease incidence and the growth of *S. cepivorum* in detached roots (correlation coefficient=0.384).

Effect of root extracts on sclerotia germination. The sclerotial germination was increased significantly when root extract was added (Table 4). The addition of only distilled water resulted in 13% of germination, but the addition of 0.25 g of root extract resulted in more than 85% of germination, and the addition of root extract showed little difference in stimulating sclerotial germination according to garlic cultivars which showed different resistance.

DISCUSSION

The first control measures of white rot consisted of cultural practices, including crop rotation, exclusion of contaminated material, application of lime, and planting in noninfected field soils (1). But, in spite of importance of this disease, there is no report on control methods in Korea.

Disease incidence was increased when the inoculum dose was raised, and the optimal inoculum density to differentiate resistance or susceptibility of garlic cultivars seemed to be 50 sclerotia. A high standardized method for testing garlic cultivars is necessary (3), as the differences are expected to be small. The test can be carried out in a growth chamber or greenhouse. In this way, it is possible to optimize and standardize environmental conditions for infection and disease development. Moreover, the time for each test is markedly shorter than for field test, which enables greater numbers of cultivars or

species to be screened (3). The density of using 50 sclerotia which was shown to be optimal to differentiate resistance in this test should be recalculated by the germinability of sclerotia when it was used in the other experiments. The cultivars which have shown resistance in this test may be susceptible in the field where the inoculum density and the germinability of sclerotia are high.

There were some differences in disease incidence among garlic cultivars (Table 3). It may be the indicative of difference in tissue reaction or stimulatory capacity of root exudates (2). However, there was no relationship between the disease incidence and the growth of *S. cepivorum* in detached roots.

The sclerotial germination was increased significantly when root extracts was added (Table 4). But, there was any difference among cultivars which showed different resistances. Differences in resistance to white rot between species of *Allium* are due mainly to different stimulation of sclerotial germination (4, 7). Brix (2) indicated that there was some difference in stimulating effect according to *Allium* species. The result in this experiment indicated that white rot in the commonly cultivated cultivars of garlic is unlikely to be affected by differences in stimulatory activity or in cultivar resistance. This is consistent with the result that there is no direct evidence that non-stimulatory species are resistant to *S. cepivorum* (11, 13).

Screening for disease resistance seemed to be limited in garlic which does not produce seed. But much concern may be needed to select cultivars resistant to *S. cepivorum*. The concern should include wild type of garlic and other *Allium* species, and field tests at different locations.

요 약

마늘 품종의 *S. cepivorum*에 대한 저항성을 검정하기 위하여 균핵수를 증가시키면서 접종한 결과 균핵농도를 10개에서 100개로 증가시킬수록 발병은 증가하였는데, 품종간의 저항성 검정을 위한 적정 접종 균핵 수는 50개였다. 균핵 50개를 인위적으로 접종하여 30개의 마늘 품종에 대한 저항성을 검정한 결과 품종에 따라 발병율과 뿌리 절편내에서의 균사생육이 차이가 있었다. 영국, 일본, 네팔, 터키에서 수집한 수집종과 common red, PI 1356104, PI135693이 다른 품종들에 비하여 발병율이 낮았다. 뿌리 절편내에서 균사는 23에서 33 mm까지 생육하여 품종에 따라 차이를 보였으나, 발병율과 뿌리 절편내에서의 균사생육은 관련이 없었다. 한편, 마늘 뿌리의 추출액을 첨가하였을 때 균핵발아율이 현저히 증가하였다. 증류수만을 첨가하였을 때 13%의 발아율을 보였으나, 뿌리 0.25 g 추출액 첨가시 85% 이상의 발아를 보여 균핵발아에 대한 효과는 인정되었으나, 저항성 품종과 감수성 품종간에 품종간 균핵발아에 미치는 영향에는 큰 차이가 없었다.

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