Density and Viability of Sclerotia of Rice Sheath Blight Pathogen Overwintering in Field

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金忠會·金章圭：벼잎집무늬마름病菌 越冬菌核의 密度와 活性

Korean J. Plant Prot. 26(2) : 99~106(1987)

ABSTRACT Three post-harvest fields each in four rice growing areas, Iri, Naju, Jinju and Taegu were randomly selected and surveyed during December 1986 to examine sclerotial density of *Rhizoctonia solani* overwintering in the field. Surface soil of 0.09m² area was sampled in each field with three replications and sieved to collect sclerotia. Germiability and pathogenicity of collected sclerotia were examined in the laboratory. Number of sclerotia ($\times 10^6$)/ha in Iri, Naju, Jinju, and Taegu was estimated from the sample as 2.7, 1.2, 0.7 and 0.6, respectively. Based on sample variance with simple random sampling in each area, number of sampling required for estimating average sclerotial density with the precision of 10% apart from a chance of 1 in 20 was calculated to 41, 132, 232, and 395 for Iri, Naju, Jinju and Taegu, respectively. Percentage of germination of sampled sclerotia on potato sucrose agar (PSA) ranged from 42 to 78% depending on the area, and averaged 60%. About 49% of the germinated sclerotia were pathogenic to a rice cultivar Jinheung that was used to test pathogenicity of the sclerotia. Proportion of viable sclerotia that have both germiability and pathogenicity was thus estimated to 0.29 of total sclerotia collected. *R. solani* cultures obtained from the sclerotia could be distinguished into three groups based on colony morphology on PSA. Size and number of sclerotia formed on PSA differed between groups but were not associated with pathogenicity to Jinheung.

INTRODUCTION

Rice sheath blight caused by *Rhizoctonia solani* Kuhn has been a serious rice disease responsible for appreciable yield losses in Korea. Yield loss due to this disease was estimated to 7.3% in 1973 (2). The fungus produces lesions on the leaf sheaths throughout the season that later often advance to severe blight. The fungus forms sclerotia on diseased plant parts in later growing season. These sclerotia overwinter in the field and serve as primary inocula in the next season.

Control of this disease is heavily dependent on fungicide applied on a regular basis in Korea, since no reliable resistance sources are available to date. Efficiency of fungicide application could be improved greatly by the ability to anticipate epidemic outbreaks. Despite, forecasting of this disease has been hampered because field epidemiological parameters required for the prediction have not been well understood or recognized. Until now, researches on this disease conducted in Korea have mostly concentrated on screening of either fungicides or resistant varieties (1, 3). Epidemiological study on sclerotia is needed to provide informations on the parameter associated with quantity of initial inoculum. This has little been attracted by researchers due in part to relatively more field labor required to collect sclerotia with adequate sample size. Some researches on physiology and sclerotia of *R. solani* could be found in Korea (7) and Japan (5, 6).

This study was conducted to examine the density of sclerotia overwintering under commercial production conditions and to assess their viability as an initial inoculum of rice sheath blight. In addition, appropriate sample

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size required for the sampling with desired precision in sclerotial density survey was determined using a proper method(4).

MATERIALS AND METHODS

Survey of sclerotial density. Four major rice growing regions, Iri, Naju, Jinju and Taegu were surveyed to examine density of sclerotia of *R. solani* in fields during December, 1986. In each region, three fields were randomly chosen and $0.3\times0.3m^2$ of surface soil in each field was sampled at random with 3 replications using a small garden spade. Soil samples were air-dried and sieved through 20 mesh screen to collect sclerotia in the laboratory. Whole number of sclerotia in the soil sample was counted and later converted into the density per hectare basis by appropriate multiplication. Collected sclerotia were separated into groups on the basis of collected region and their relative size for further studies.

Germination test of sclerotia. A total of 302 sclerotia obtained from four regions was tested their ability to germinate. Each sclerotium was put on the center of 5cm diam. petri dishes containing potato sucrose agar (PSA, potato 250g, agar 20g, water 1*l*) after dipping in 3% NaClO solution for 1 min for surface disinfection. The petri dishes were incubated at 26°C for 9 days and then germination of each sclerotium was determined based on mycelial characteristics of *R. solani* appeared in the culture.

Pathogenicity test of sclerotia. Cultures of *R. solani* obtained from germinated sclerotia were grouped into three on the basis of their colony morphology. Pathogenicity of a total 102 cultures in three morphological groups was tested with the susceptible cultivar, Jinheung. Possible association of pathogenicity with either sclerotial size or amount of sclerotia production on PSA was also examined. Jinheung was seeded in 2cm diam. test tubes containing water agar (agar 10g, distilled water 1*l*) after dipping in 3% NaClO solution for 30 min for seed disinfection. The test tubes were placed in a growth cabinet maintained at 20~22°C with diurnal fluorescent light illumination. Thirteen-day-old seedlings in the test tubes were inoculated with each sclerotia-originated *R. solani* isolate with three replications. Inoculation was performed by placing a sclerotium at the basal stem of seedling using a transfer needle. Prior to inoculation, seedlings contaminated with other microorganisms were excluded in the test. Inoculated seedlings were put in the growth cabinet at 28°C. Presence or absence of pathogenicity of each isolate was determined 13 days after inoculation on the basis of appearance of typical lesions on leaf and stem.

Viable sclerotia in this study were defined as sclerotia having both germiability and pathogenicity. Proportion of the viable sclerotia among the sclerotia collected was thus calculated from [1×(proportion of germinated sclerotia)×(proportion of pathogenic sclerotia among the germinated ones)].

Sclerotial size and production. Ability of sclerotial production and sclerotial size were compared between the pathogenic and nonpathogenic isolates. Each isolate was grown in 5cm diam. petri dishes containing PSA for 43 days at room temperature and then number of sclerotia produced in each petri dish culture was counted. Maximum length (mm) of each sclerotium was measured with a plastic scale. One hundred and sixty sclerotia of each isolate were separated into three group; large : 2.9~5.5mm(average=3.7), medium : 1.6~2.8mm(average=2.2), small : 0.6~1.5 (average=1.0) to calculate the ratio of each size group.

Estimation of sample size. Based on the sample variances obtained from the sclerotial density survey data, sample size required for the estimation of average sclerotial density with the defined precision was calculated following the method of Cochran (4). Desired precision for average sclerotial density in each region was predetermined as within 10% deviation from the average with a chance of 1 in 20. If $\bar{y}$ is the average of the sclerotial density observations from a simple random sample, estimation of $\bar{Y}$ with desired precision, d and α would be;

$$Pr(|\bar{y}-\bar{Y}|\geq d)=\alpha$$

where d is the chosen margin of error, α is a probability level and $\bar{Y}$ is a population average. Since $\bar{y}$ is assumed to be distributed normally, standard error of the population is:

$$\sigma\bar{y}=[(N-n)/N^{1/2}S/n^{1/2}$$

where N is population size, n is sample size and S is standard error of sample. Here, $\sigma\bar{y}=d/t$ where t is percentile of t distribution, so the above equation would be:

$$d=t[(N-n)]^{1/2}S/n^{1/2}$$

This gives:

$$n=(tS/d)^2/[1+(tS/d)^2/N]$$

When population size N is large enough to ignore $(tS/d)^2/N$, the equation with an approximation would be:

$$n=(tS/d)^2$$

In this study, $t_{\alpha=0.05}=2$, S^2 is sample variance of sclerotial density observations in each region, and d is 10% portion of the average sclerotial density observations in each region. For instance, desired sample size in Iri region (n_I) was calculated as:

$$n_I=[2^2(62.774)]/(0.1\times24.6)^2\fallingdotseq41$$

Table 1. Density and viability of scleortia of *Rhizoctonia solani* sampled from three post-harvest rice fields in each of four growing areas in December, 1986

Sampling area	Replication[a]	No. of sclerotia ($\times10^6$/ha)[b]	No. of viable sclerotia[c]	No. of samples needed[d]
Iri	I	2.35±1.12	—[e]	—
	II	2.83±1.06	—	—
	III	2.94±0.63	—	—
	Avr.	2.71±0.87	1.03±0.33	41
Naju	I	1.21±0.68	—	—
	II	1.02±0.89	—	—
	III	1.21±0.71	—	—
	Avr.	1.15±0.67	0.30±0.17	132
Jinju	I	1.10±0.19	—	—
	II	0.77±0.68	—	—
	III	0.22±0.11	—	—
	Avr.	0.69±0.53	0.14±0.11	232
Taegu	I	0.19±0.17	—	—
	II	0.88±0.55	—	—
	III	0.66±0.76	—	—
	Avr.	0.57±0.57	0.14±0.14	395

[a] Three fields randomly selected in each area were considered as replications.

[b] Values are averages of 3 replications and their standard deviations. In each replication, soil surface of 0.09m² was sampled to examine its sclerotial density.

[c] Values are the products of (sclerotial density)×(proportion of sclerotia having germiability in Table 2)×(average proportion of sclerotia having pathogenicity in Table 3).

[d] This was determined on the basis of sample variance obtained in each area following the method described by Cochran (4). Values are number of samples required for estimating average sclerotial density with the precision of 10% apart from a chance of 1 in 20.

[e] Dash indicates no calculation.

RESULTS

Sclerotial density. Average number of sclerotia per hectare was estimated to 2.94×10^6—0.19×10^6, depending on sampled fields over four surveyed regions (Table 1). Sclerotial density differed among regions with highest in Iri followed by Naju, Jinju and Taegu. Differences in sclerotial density among the regions of Naju, Jinju and Taegu, however, were not so great as those between Iri and other three regions.

Density of sclerotia also varied appreciably among replications in the same field. The variations in sclerotial density between soil samples collected within one field tended to decrease as sclerotial density increases. For this reason, coefficients of sample variance were largest with Taegu area, followed by Jinju, Naju and Iri. Large coefficients of variance led to a bigger number of samplings required for sclerotial density estimation in the population with desired precision. Sample size required in Taegu area was thus

Table 2. Germiability of sclerotia of *Rhizoctonia solani* sampled from four rice cultivation areas during December in 1986

Origin	No. of sclerotia examined[a]	No. of sclerotia germinated (% germination)[b]
Iri	114	89(78)
Naju	83	45(54)
Jinju	57	24(42)
Taegu	48	23(48)
Total	302	181(60)

[a] Sample sclerotia were randomly chosen from the bulk of sclerotia collected in each area.
[b] Germination was determined on potato sucrose agar 9 days after incubation at 26°C.

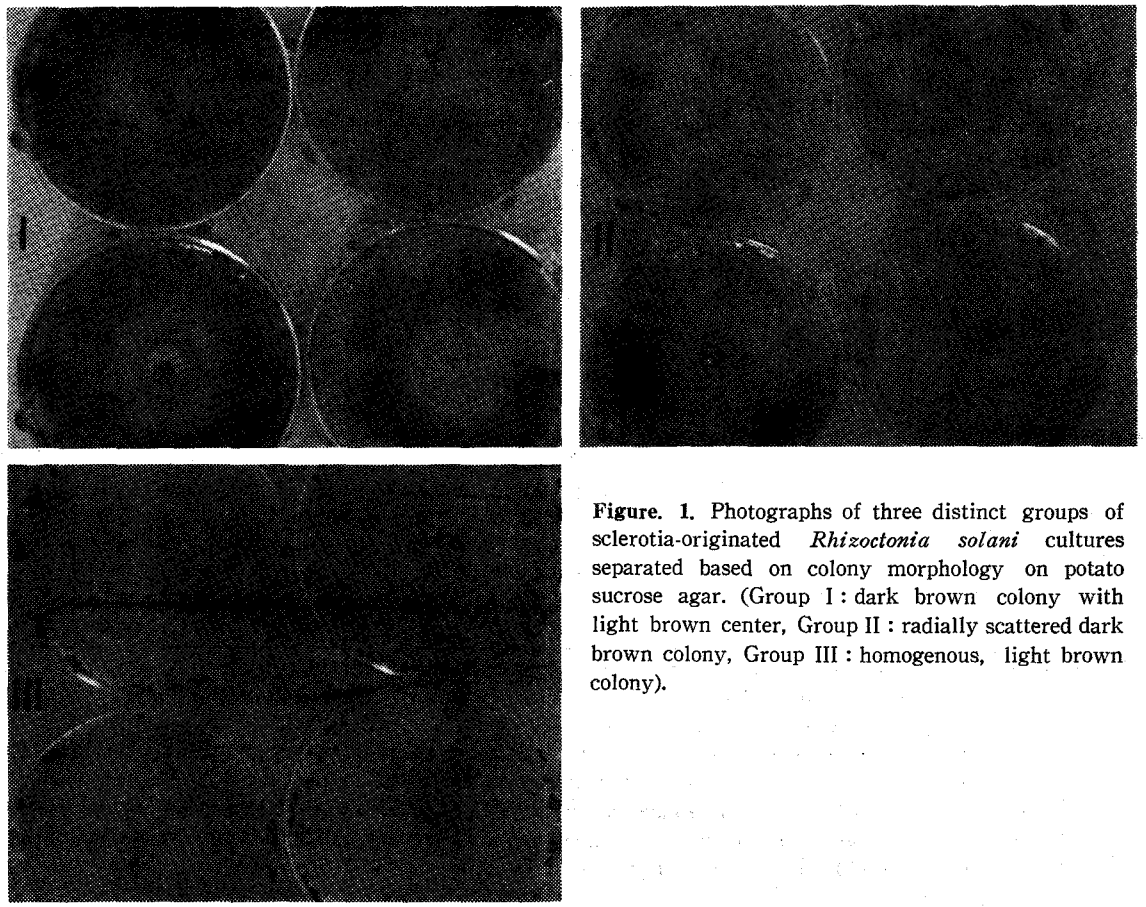

Figure. 1. Photographs of three distinct groups of sclerotia-originated *Rhizoctonia solani* cultures separated based on colony morphology on potato sucrose agar. (Group I : dark brown colony with light brown center, Group II : radially scattered dark brown colony, Group III : homogenous, light brown colony).

Table 3. Comparison of sclerotial production on potato sucrose agar (PSA) and pathogenicity on rice cultivar Jinheung among morphologically distinct groups of *Rhizoctonia solani* cultures obtained from the sclerotia sampled in the field

Morphological group[a]	No. of sclerotia examined[b]	No. of sclerotia pathogenic[c] (% pathogenic sclerotia)	No. of sclerotia produced/ 5cm diam. petri dish culture[d]			Sclerotial size ratio (large:medium:small)[e]
			Pathogenic isolate	Non-pathogenic isolate	Average	
I	10	4(40)	3.0±3.6	4.3±3.0	3.8±3.1	1 : 2 : 1
II	29	14(48)	6.8±4.8	7.5±4.4	6.9±4.5	1 : 1 : 1
III	63	32(51)	5.0±5.9	6.3±7.3	5.5±6.7	1 : 1 : 0.5

[a] Morphological characteristics of the culture group are shown in Fig. 1.
[b] Sampled sclerotia were the ones from the field.
[c] Pathogenicity was determined 13 days after inoculation with each sclerotium on 7-day-old seedling of rice cultivar Jinheung grown in 2cm diam test tubes containing water agar.
[d] Values are averages of the tested isolates and their standard deviations examined 43 days after incubation at room temperature.
[e] In maximum length (mm) of sclerotia developed on PSA, large: 2.9~5.5 (avr.=3.7), medium: 1.6~2.8 (avr.=2.2), small: 0.6~1.5 (avr.=1.0)

estimated nearly 10 times greater than that needed in Iri (Table 1).

Viability of sclerotia. Around 42 to 78% of field-collected sclerotia germinated on PSA (Table 2). Percentage of sclerotia germination was somewhat higher at Iri, compared with other three regions where similar germination percentage was observed. Percentage of sclerotia germination averaged 60% over the regions. Cultures from germinated sclerotia of *R. solani* were divided into three groups (Group I, II and III) on the basis of their colony morphology on PSA. Their colony characteristics are shown in Figure 1. Group I cultures have homogenous dark brown colony with small area of light brown center. Cultures belonging to group II are characterized by the presence of radially scattered dark brown pigment over the culture. In Group III cultures, dark brown pigment is not distinct and colony colour is homogenous light brown. Group III cultures were most common type among the cultures from field-collected sclerotia and followed by Group II and Group I.

Proportions of pathogenic isolates to Jinheung in each morphological group were similar between groups ranging from 40 to 51% with an average of 49% (Table 3).

Group II cultures generally produced more number of sclerotia than Group I and III, although the differences were not statistically significant(Table 3). Sclerotial production of Group III cultures was slightly better than that of Group I. Ability of sclerotial production did not differ between the pathogenic and nonpathogenic isolates, irrespective of cultural groups.

Size of sclerotia developed on PSA varied within an isolate, regardless of cultural groups (Table 3). When the size of sclerotia developed on PSA was divided, based on maximum length in mm, into large : 2.9~5.5 (avr. 3.7), medium : 1.6~2.8(avr. 2.2) and small : 0.6~1.5 (avr. 1.0), ratio of number of sclerotia belonging to each size group did not differ greatly between cultural groups (Table 3).

From the data obtained from both germination and pathogenicity tests, proportions of viable sclerotia among total field-collected scleroia were calculated and they were 38%, 26%, 21% and 24% for Iri, Naju, Jinju and Taegu, respectively, and averaged 29% over four regions. With these calculated proportion,

density of viable sclerotia was obtained by multiplication and listed in Table 1. Number of viable sclerotia per hectare ranged from 0.14×10^6 to 1.03×10^6, depending on the region, and averaged 0.40×10^6.

DISCUSSION

Assessment of amounts of initial inoculum is a very first step to undertake in the most prediction systems for plant disease epidemics. In many plant diseases, density of initial inoculum is difficult to estimate because inoculum sources are often disclosed or, if exposed, it is hard to collect for assessing their amounts. In case of rice sheath blight, these difficulties encountered commonly in the assessment of initial inoculum for other diseases are much lessened because survival form of the pathogen is mostly limited to sclerotia that can be collected from the fields without significant efforts. For this reason, severity of initial incidence of this disease could be predicted relatively easily by monitoring sclerotial density in fields on annual and regional basis.

In this study, sclerotial densities in the surveyed four major rice growing regions were 0.19×10^6 to 2.9×10^6 per hectare, depending on the sampling area. In Korean cultivation conditions, standard planting density of rice is around 0.24×10^6 plants (hills)/ha. These two figures lead to that sclerotial density available for a single plant (hill) level in fields as an inoculum is theoretically 0.79 to 12.25 sclerotia. These values were obtained considering the all sclerotia collected from the field. In actual situations, density of sclerotia may be less than this number because some of the sclerotia may lose germiability and/or pathogenicity during the course of overwintering. Data obtained in this study showed that about 40% of sclerotia in fields, in average, lost germiability and that about half of those germinated sclerotia might not retain pathogenicity on rice. This means that only one fifth of sclerotia overwintering in fields might maintain their viability as inoculum for the next season. When considering this facts, actual inoculum density for a single plant (hill) base would be reduced to 0.15 to 2.45 sclerotia. Although there is a possibility that actual inoculum density under the natural conditions is further reduced by various reasons such as tillage, these amounts of initial inoculum could be considered still enough theoretically to infect majority of plants in field at the same time when environmental conditions favor disease development. This might be the reason for uniform infections of rice plants in the paddy fields observed especially in early growing stages, since most of the secondary infections of the disease have been known to cause by mycelia of *R. solani* that might have limitations in spatial and temporal spread. These limitations, on the other hand, operate towards doubling the role of initial inoculum for the epidemic outbreaks and thereby enhance the importance of the parameter of initial inoculum for the predicting systems of rice sheath blight. In order to increase accuracy of prediction for severity of initial incidence, precise estimates of inoculum density would be required. Precision of the estimation largely depends on magnitude of sample variance which is affected by number of samplings and unit sample size. In various survey data of disease incidence, average values in each surveyed area are commonly used to compare several areas without considering precision of the averages. In a strict sense, those comparisons would not be of value unless degree of precision, i.e., sample variance is similar between surveyed areas.

This has not been considered usually in many survey experiments. One area may need more number of samplings than another area to achieve desired level of precision. Sample size required for accomplishing the fixed precision could be determined on the regional basis by pilot survey experiments that would give some informations on the variance of the population. The pilot survey conducted in this study to examine sclerotial density with random sampling of a sample unit of 0.09m² area indicates that number of samplings required for the precision of 10% deviation from the average at 95% confidence level ranged from 41 to 395, depending on the surveyed region. This means that with simple random sampling a great number of samplings may be required in some regions for reducing sample variance. In practical sense, sclerotial density estimations over large region, like in this study, may not be so meaningful because of presence of great variations within the region that may result in reduction of the accuracy of estimates to be used for prediction Size of sampling would be extensively reduced with increasing accuracy where the forecasting is intended to cover relatively small areas, i.e., a plain field, instead of a vast area. Increase of unit sample size could be also considered to reduce number of samplings into practical level. Actually in this study, sample variance was greatly reduced as number of sclerotia present in a sample unit increased. Bigger unit sample size would result in a bigger number of sclerotia to be collected, and hence would reduce sample variance. Lowering the level of precision in magnitude of deviation or in confidence level could also contribute to the reduction of sample size, but at the same time would sacrifice the accuracy of prediction due to inaccurate estimates.

The results obtained in this study indicate that simple random sampling may not be adequate for practical use to estimate sclerotial density in some areas because of large sample size. In those areas, applications of other sampling methods such as stratified sampling, cluster or double samplings are needed to be examined to maximize the efficieny of samplings under the considerations of cultural or varietal conditions of those areas.

摘 要

圃場에서 越冬하고 있는 벼 잎집무늬마름病菌 菌核의 密度와 그 活性을 調査하기 위하여 1986年 12月에 裡里, 羅州, 晋州, 大邱의 圃場에서 菌核을 採集하여 發芽力과 病原性을 調査하였다. 菌核의 密度는 裡里, 羅州, 晋州, 大邱 地域이 各各 ha당 2.7×10^6, 1.2×10^6, 0.7×10^6, 0.6×10^6개였으며 地域에 따라 그중 42~78% (平均 60%)가 發芽力이 있었고 發芽된 菌核中 平均 49%가 벼品種 振興에 病原性이 있었다. 따라서 發芽力과 病原性을 同時에 가진 菌核의 比率은 全體菌核의 29%였다. 菌核으로부터 分離한 잎집무늬마름病菌은 培地에서의 菌叢形態에 따라 3가지 類形으로 大別되었으나 類形이나 菌核의 크기, 菌核形成量은 振興에 對한 病原性과 아무런 相關이 없었다. 10%의 偏差안에서 平均菌核密度를 95% 信賴하기 위하여 必要한 單純任意 標本數는 裡里, 羅州, 晋州, 大邱에서 各各 41, 132, 232, 395個所로 算出되었다.

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