

ハウス栽培ホウレン草から分離されるRhizoctonia solani Kuhnの類別

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Grouping of *Rhizoctonia solani* Kühn causing Root Diseases of Spinach in Plastic House Cropping

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内記 隆・加納正和：ハウス栽培ホウレンソウから分離される *Rhizoctonia solani* Kühn の類別

Abstract

In plastic house cropping of spinach having its growing season from May to October, damping-off and foot rot diseases caused by *Rhizoctonia solani* Kühn were most serious from June to September. The occurrence of the fungus was more frequent at the earlier growth stage of the plant. The fungus was more dominant in the plastic house cropping in drained rice paddy fields than in upland fields or in open field cropping, and was less frequent in light clay soil than in clay loam or in loam soil. Isolates of *R. solani* from spinach were assigned to 5 anastomosis groups (AG). Isolates occurred most frequently and being most pathogenic to spinach seedlings were of AG-4, followed by ones of AG-2, type-2. The former group was most dominant at the earlier growth stage of the plant irrespective of the growing season, cropping histories of the plant and soil types of the fields. The latter group was frequently isolated from drained rice paddy fields and increased with the development of the plant. The groups, AG-1, AG-2, type-1 and AG-1 were additional ones which were found less frequently in plastic house cropping.

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Introduction

Successive commercial production of spinach (*Spinacia oleracea* L.) from May to October has commonly been practiced in mountainous and cool summer districts in and around Takayama City in Gifu Prefecture. In these areas, spinach is cultivated in a simple plastic house equipped with a watering system in order to escape from heavy and long-lasting rain. During last few years, some serious root diseases of spinach showing symptoms of damping-off, foot rot and wilting have been found in summer and early fall production, and the outbreak of these diseases is one of the most important factors which would limit the establishment of commercial spinach production. The occurrence of diseases causing loss of spinach yield and the causal fungi associated with the diseased spinach were reported in the previous paper⁴⁾. The pathogenic fungi isolated frequently from the diseased plants were *Rhizoctonia solani* and *Fusarium oxysporum*, followed by *Pythium* spp.. Damping-off of spinach seedlings caused by *R. solani* was most serious in house cropping⁴⁾. The isolates of *R. solani* in Japan were divided into 5 anastomosis groups⁶⁾: they were corresponded to the respective groups which were divided on the basis of their cultural appearance^{6, 10)}.

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We report here the occurrence and grouping of *R. solani* isolated from diseased spinach in plastic house cropping.

Materials and methods

Sampling fields. Spinach samples from 185 plastic houses and 15 open-field plots at the 8 sites in and around Takayama City from May to October, 1977, were used for this study. Sampling fields were selected according to their differences in cropping histories and soil types. Plastic houses selected for sampling plots differed in years of successive cropping and in cultivation in drained rice paddy fields and upland fields. They were divided into three soil types; light clay, clay loam and loam soil.

Isolation and grouping of *R. solani*. Diseased plants which varied in their growth stages (leaf number) and showed symptoms of damping-off, foot rot or wilting, were collected as samples. Hypocotyls and root segments (ca. 5 mm long) were dissected from each sample of 30 to 50 diseased plants per house, washed with running tap water for over 1 hr and then twice with sterilized water, blotted, and finally placed on water agar (pH 5.0) in plates without surface sterilization. Hyphal tips of the fungus growing from each segment were transferred directly to potato sucrose agar (PSA) slant after incubation for 1 to 3 days at 25 C. All isolates of the fungus were grouped on the basis of differences in cultural appearance on PSA. They showed the common characteristics of species criteria outlined by Parmeter *et al.*⁹⁾. Some of the representative isolates from each group were tested for anastomosis grouping by the method described by Ogoshi⁶⁾. In this study, isolates which were quite similar in cultural appearance to those grouped by anastomosis test were considered as the same group.

Pathogenicity test on spinach seedlings. Seven to twenty seven different isolates from each anastomosis group were selected. They were isolated from spinach differing in its growth stage, cropping season and sampling site, and tested for pathogenicity on seedlings of a spinach cultivar "Fukamidori" which was cultivated most commonly in summer and early fall production. The inoculum was prepared by homogenizing the mycelial mat from 5-day-old potato sucrose broth culture. Ten surface-disinfected and germinated seeds of the plant were seeded in the autoclaved spinach field soil in a 450 ml glazed pot infested with one mycelial mat homogenate of a given isolates per pot. Three replicates of each treatment were placed in a growth cabinet at 25 C. Spinach seedling emergence and damping-off were checked from 2 to 21 days after seeding and the percentage of damping-off was calculated.

Results

*The occurrence and anastomosis grouping of *R. solani* isolated from diseased spinach varied in growth stage during its growing season*

The occurrence of *R. solani* and its anastomosis groups were shown as the percentage of the total fungal isolates in each month during growing season (Fig. 1).

The isolates of *R. solani*, total number 3195, obtained from May to October were divided into 5 anastomosis groups (Fig. 2). The isolates which were most frequent throughout the growing season were of AG-4 and followed by AG-2, type-2. They were 88.3 % and 11.4 % of the total isolates, respectively. Isolates of another anastomosis groups (AG-1, AG-2, type-1 and AG-5) were less and obtained only 0.03-0.5 % of the total. No isolates were found that belonged to AG-3. The

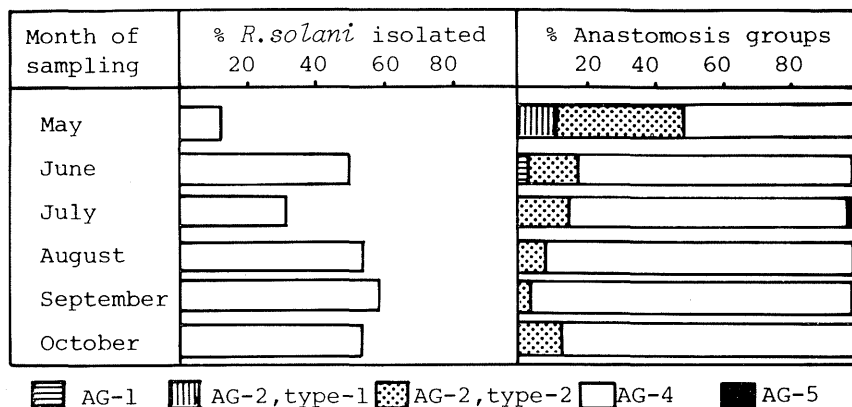


Fig. 1. Occurrence and anastomosis groups of *R. solani* isolated from spinach plants during the growing season.

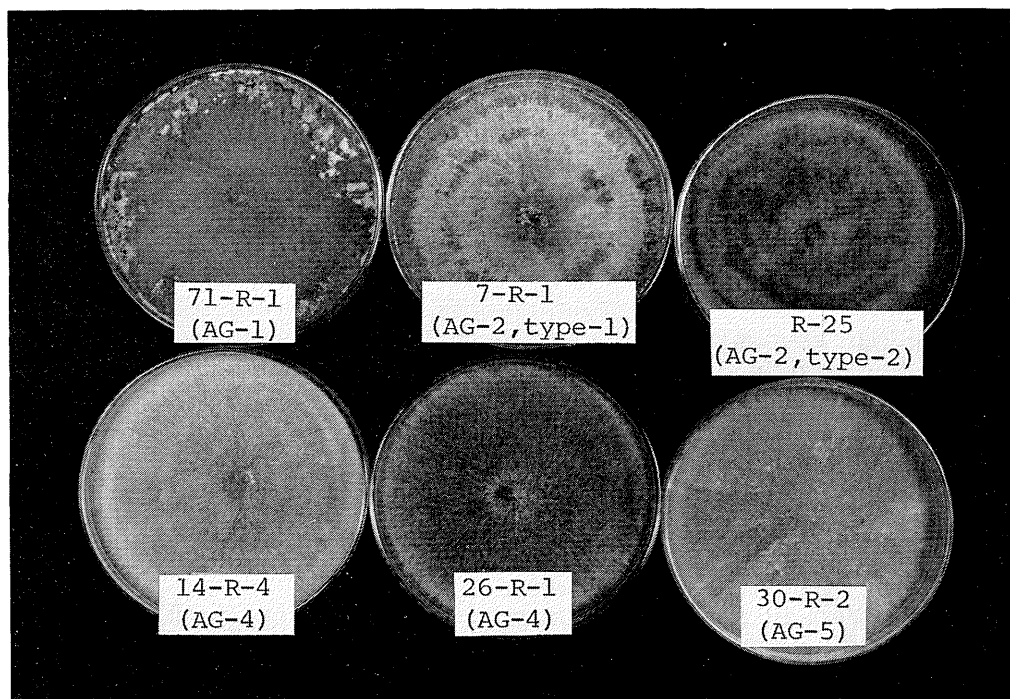


Fig. 2. Cultural appearance of each anastomosis group of *R. solani* isolated from spinach plants.

occurrence of the fungus was only 11.3 % in May in the first cropping season, but it was considerably increased up to 49.7 % in June. Although the frequency of isolation of the fungus was somewhat decreased in July, it was recovered in the following month and reached to 59.5 % in September. More than 50 % of the isolates were still obtained in October, the end of cropping season.

Fig. 3 shows the occurrence and anastomosis groups of *R. solani* isolated from spinach in different growth stages of each month. Samples of the plant were divided into 4 growth stages depending on leaf development, i. e. the cotyledon stage

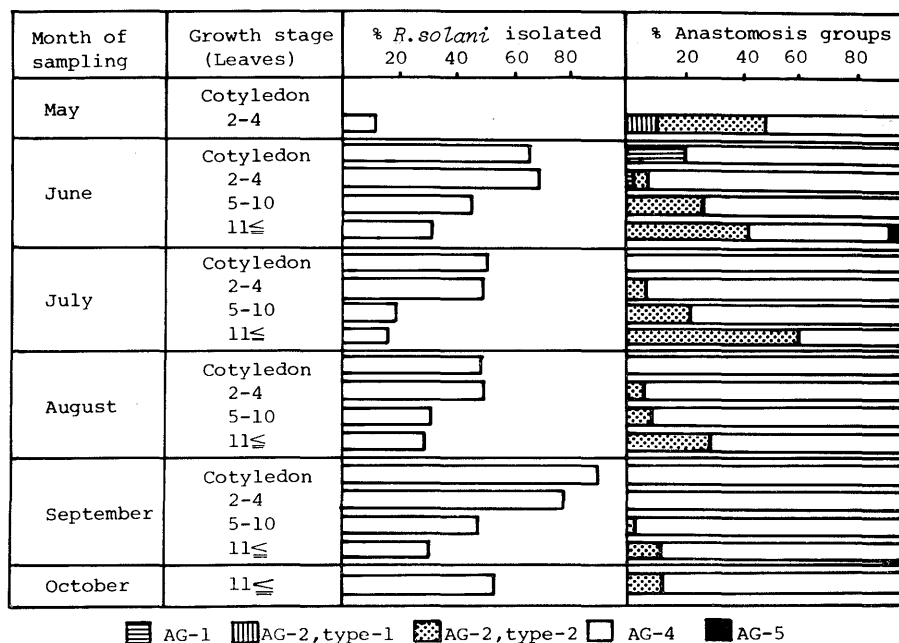


Fig. 3. Occurrence and anastomosis groups of *R. solani* isolated from spinach plants differing in their growth stages.

(the earlier growth stage), the 2-4 leaf-stage (increasing stage of leaf number), the 5-10 leaf-stage (the elongating and thickening of leaf blades), and the $11\leq$ leaf-stage (the growth down stage). Samples of the plant were obtained only from the cotyledon and the 2-4 leaf-stage in May and from the $11\leq$ leaf-stage in October, because these months corresponded to the beginning and the end of cropping season, respectively. The occurrence of *R. solani* varied with the growth stage of the plant in each month. From June to September, *R. solani* was isolated most frequently from both the cotyledon and the 2-4 leaf-stage of the plant. Conversely, the frequency of isolation of the fungus gradually decreased with the development of the plant. The most dominant isolates were of AG-4 in the earlier growth stage in each month, while the frequency of isolation of the isolates of AG-2, type-2 was increased with the development of the plant. It was most noticeable in the $11\leq$ leaf-stage of June and July. Isolates of AG-1 and AG-2, type-1 were obtained only in June and May, respectively. A few isolates of AG-5 were found in various growth stages from June to September.

The occurrence and grouping of R. solani from the fields differing in their soil types and cropping histories

The occurrence and anastomosis groups of *R. solani* from the respective types of the fields were shown in Table 1 as the percentage of the total fungal isolates obtained during spinach growing season. Although the AG-4 type was the most dominant group, the frequency of isolation of the fungus differed in the respective fields types. *R. solani* was obtained from 84.8 % of 185 plastic houses and 66.7 % of 15 open-field plots. Significantly more isolates and anastomosis groups were obtained in plastic house cropping compared with in open-field cropping. In open-fields, in addition to the AG-4 type, only one groups (AG-1) was dominant and 28.3 % of the isolates fell into this group. Otherwise, *R. solani* was less frequent in light clay soil than in clay loam or in loam soil. The occurrence of AG-2, type-2

Table 1. Occurrence and anastomosis groups of *R. solani* isolated from various fields differing in their cropping histories and soil types

Sampling fields	Number of examined fields	% fields in which <i>R. solani</i> was isolated	% <i>R. solani</i> isolated	% anastomosis groups				
				AG-1	AG-2		AG-4	AG-5
					type-1	type-2		
1. Cropping system								
Plastic house cropping	185	84.8	43.1	0.1	0.03	10.8	88.6	0.5
Open-field cropping	15	66.7	17.6	28.3	0	0	71.7	0
2. Plastic house cropping								
1) Soil texture								
Light clay soil	49	66.7	21.6	0.8	0	4.2	90.8	4.2
Clay loam soil	70	85.5	55.1	0	0.1	33.5	66.4	0.1
Loam soil	66	92.4	44.3	0.1	0	0	99.7	0.2
2) Cropping history								
Drained rice paddy field	58	82.7	52.2	0	0.1	42.3	57.6	0.1
Upland field	127	62.7	40.8	0.1	0	0.5	98.7	0.6
3) Successive cropping years								
2 years	71	94.2	48.1	0	0.1	38.6	61.3	0.1
3 years	66	92.4	44.3	0.1	0	0	99.7	0.2
6 years	48	76.9	31.9	0.5	0	2.8	93.8	2.8

was relatively high (33.5 %) in clay loam soil. Significantly more isolates of the fungus were obtained in plastic house cropping in drained rice paddy fields than in upland fields. It was noticeable that the frequency of isolation was increased up to 42.3 % of the isolates in drained rice paddy fields. On the other hand, the occurrence of *R. solani* slightly decreased contrary to increasing years of successive cropping. The fungus was the least frequent in 6-year successive cropping fields, and more isolates of AG-2, type-2 were obtained in 2-year ones. Most of the sampling fields of 6-year successive cropping were light clay soil and ones of 2-years were drained rice paddy fields, respectively.

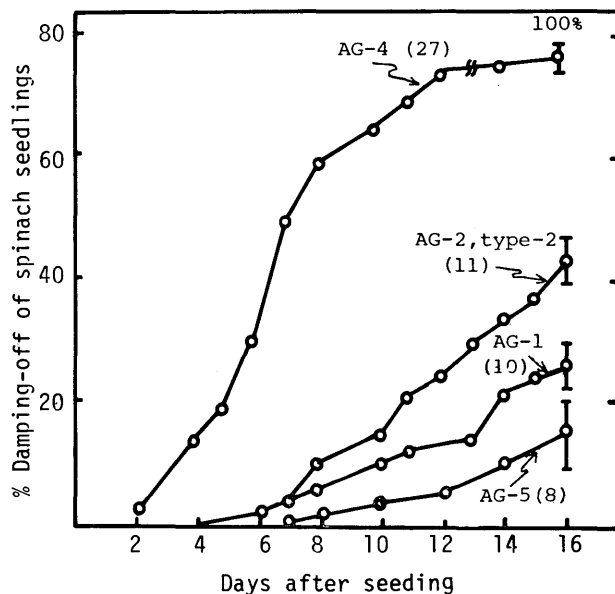


Fig. 4. Pathogenicity of isolates of *R. solani* in each anastomosis group on spinach seedlings. The number of isolates used for pathogenicity tests was given in parentheses.

Pathogenicity of the isolates from each of the anastomosis groups on spinach seedlings

The result of the pathogenicity test on spinach seedlings was shown as the average percentage of diseased plants caused by each isolate within the same group against healthy ones (Fig. 4). All isolates induced damping-off as compared with control seedlings. Significant differences in pathogenicity on the seedlings were found among anastomosis groups, and isolates most pathogenic, causing severe damping-off with 100 % of the seedlings within 16 days after seedling were of AG-4, followed by AG-2, type-2 (43.6 %), AG-1 (26.2 %) and AG-5 (15.6 %). The differences in pathogenicity among the isolates within AG-4 was less than those of the isolates in other groups. The high frequency of isolation of AG-4 from the seedling stage may be due to high pathogenicity of this group on the seedling.

Discussion

As the pathogenic fungi causing the root diseases of spinach, *Aphanomyces cochlioides*²⁾, *Phytophthora* sp.¹⁾, *Pythium* spp.^{1,2,4,7)}, *Rhizoctonia solani*^{1,4,7)} and *Fusarium oxysporum* f. sp. *spinaciae*³⁾ have been reported in Japan and the outbreak of damping-off of the seedlings caused by *R. solani* and *Pythium* spp. is most common⁸⁾. In plastic house cropping in summer and early fall production, damping-off and foot rot of the plant caused by *R. solani* were most serious and *Fusarium* wilting was next⁴⁾. In this study, it was shown that *R. solani* was isolated more frequently at the earlier growth stage of the plant from June to September. The occurrence of the fungus decreased with the development of the plant, and differed among the fields which varied in their cropping histories and soil types. Significantly more isolates were obtained from the plant grown in the plastic house than in the open field. Moreover, the fungus was more dominant in the house built in drained rice paddy fields than that built in upland fields, and less frequent in light clay soil than in clay loam or in loam soil. Otherwise, the frequency of isolation of *R. solani* did not differ considerably among the fields which varied in number of successive cropping years.

Spinach isolates of *R. solani* were assigned to 5 anastomosis groups. Isolates of AG-4 were obtained most frequently from the diseased plant, especially, at the earlier growth stage, irrespective of the growth season, cropping history of the plant and soil type of the field. AG-2, type-2 which is known as Rush type and Root rot type¹⁰⁾ was obtained frequently in drained rice paddy fields, and its occurrence gradually increased with the development of the plant. More anastomosis groups were found in plastic house cropping than in open field cropping. In addition to AG-4 and AG-2, type-2, a few isolates of AG-1, AG-2, type-1 and AG-5 were also found in house cropping. Conversely, AG-1 and AG-4 were the only ones found in open field cropping. Similar results were reported by Fukunishi¹⁾ in open field cropping in drained rice paddy fields in Tokushima Prefecture. Watanabe and Matsuda¹⁰⁾ assigned 3 spinach isolates of *R. solani* to 111 A (Praticola type) and 1V (Potato type and Root rot type) based on their cultural appearance. Thereafter, it has been shown that the 111 A type corresponded to AG-4 and the 1V to AG-2, type-2 or AG-3⁶⁾. In this study, no isolates was found that belonged to AG-3. On the other hand Ogoshi⁶⁾ assigned spinach isolates to AG-4 by anastomosis tests. In addition to these results, our survey revealed that isolates of AG-4 and AG-2, type-2 were dominant in various spinach cultivated areas in open-field cropping in Gifu and Aichi Prefectures from late fall to winter (unpublished

data).

The isolates of AG-4 were most pathogenic to the spinach seedlings and the next, AG-2, type-2. Although the pathogenicity of each isolate on mature spinach has not been tested, more isolates of AG-4 and AG-2, type-2 were obtained in the seedling stage and in the mature stage of the plant, respectively. It is interesting phenomenon that crown rot of sugar beet, which is the same genus as spinach was infected by isolates of AG-2, type-2⁵⁾.

From these results, it is apparent that isolates most closely associated with spinach were of AG-4, followed by AG-2, type-2 and AG-1.

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和 文 摘 要

ハウス栽培ホウレンソウから分離される *Rhizoctonia solani* Kühn の類別

内 記 隆・加 納 正 和

岐阜県高山市を中心とする冷涼地帯の簡易ハウス栽培ホウレンソウの立枯症状、株腐症状株から分離される *Rhizoctonia solani* Kühn の消長ならびに菌糸融合群 (AG) について検討した。栽培初期の5月には分離率が低く、6月より増加し、栽培末期の10月にも高い分離率を示した。ホウレンソウの生育時期毎に比較すると子葉期と本葉2～4葉期に多く分離され、生育時期が進むにつれて減少の傾向にあった。また調査期間中何れの生育時期においても AG-4 が最も多く、ついで AG-2, type-2 が多く分離された。AG-2, type-2 はホウレンソウの生育時期が進むにつれ多く分離される傾向にある。その他 AG-1, AG-2, type-1, AG-5 が僅かに分離された。またこれら分離率は作歴、土壌により異なる。軽塩土地帯よりも塩漬土および塩土地帯、畑作地帯よりも水田跡地のハウス栽培ホウレンソウから多く分離された。塩漬土地帯、水田跡地のハウス栽培では AG-2, type-2 の分離率が増加した。さらに露地栽培ホウレンソウではハウス栽培のそれと比較し分離率は低く、AG-4 の他 AG-1 が分離されたにすぎない。