Rhizoctonia solani Kuhn の菌核形成に及ぼす栄養源の影響(農学科)

The effect of nutrients on sclerotium formation of *Rhizoctonia solani* Kühn

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Introduction

Rhizoctonia solani Kühn is a causal agent of serious diseases such as a sheath blight of rice, damping off of eggplant, cucurbits and tomato, a root rot of sugar beet and banded sclerotial disease of sugar cane[7,9]. This fungus has a wide host range and widely spread in the world. Most strains of the fungus produce sclerotia as a mean of propagation and propagule for survival to adverse condition in soil. In this experiment the effects of nutrients on sclerotial formation of three isolates of this fungus were tested. The abstract has already appeared.[8].

Materials and Methods

Three isolates of the *Rhizoctonia solani* (C-14 isolated from *Cyperus rotundus* in Fukuoka, C-324 from sugar cane in Kagoshima and C-326 from rice plant in Fukuoka) were used. These isolates belong to anastomosis group AG-1 and cultural type IA. The isolates were precultured on potato dextrose agar medium (PDA) at 25 C for 2-3 days. Small discs of 5 mm in diameter were cut from the edge of mycelial mat with cork borrer and used as an inoculum. To test qualitative and quantitative effect of various nutrients on sclerotial formation, various substances were replaced or additionally amended to the basal medium. Hopkins medium (2 g KNO_3, 0.5 g $MgSO_4$, 0.1 g KH_2PO_4, 10 g glucose, 20 g agar, 1000 ml water) was used as a basal medium. Fifteen ml of each media (autoclaved at 110 C for 10 min.) was poured into petri dish and inoculated. Culturing was carried out at 25 C for 14 days in dark. After 14 days, sclerotia formed were taken out, dried at 60 C and weighed. Each values is the average of five dishes and every experiments were replicated for three times.

Results

1) Between isolates

To examine the ability of sclerotial formation between three isolates, the fungi were cultured on Hopkins medium for 14 days and sclerotia formed were weighed. As shown in Table 1, C-14 isolate produced 48.78 mg sclerotia, C-324 52.76 mg and C-326 36.67 mg per dish. The process of

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Table 1. Sclerotial formation of 3 isolates of *Rhizoctonia solani*

Exp.	Isolates		
	C-14	C-324	C-326
1	44.10	55.15	35.45
2	47.00	52.05	38.00
3	49.25	51.10	36.55

The mean weight (mg) of 5 petri dishes

sclerotium formation of each isolates was similar, however, the places on the medium where sclerotia formed were slightly different.

2) Different media

Production of sclerotia by three isolates on various media were studied. The composition of each media is as follows: (1) PDA medium: 200 g potato, 20 g glucose (2) Hopkins medium: 2 g KNO_3, 0.5 g $MgSO_4$, 0.1 g KH_2PO_4, 10 g glucose (3) Asparagine medium: 5 g KH_2PO_4, 2.5 g asparagine, 0.2 g $MgSO_4$, 10 g sucrose (4) Czapek medium: 0.5 g $MgSO_4$, 0.5 g $NaNO_3$, 0.01 g $FeSO_4$, 1 g K_2HPO_4, 0.5 g KCl, 50 g sucrose (5) Richard medium: 10 g KNO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4$, 50 g sucrose, 0.02 g $FeCl_2^-$ per 1 litre. Each media contained 2% agar and pH was adjusted at 6.0 with 1

Table 2. Sclerotial formation of *Rhizoctonia solani* on various kind of medium

Media	Isolates		
	C-14	C-324	C-326
PDA	48.85	88.50	64.45
Hopkins	44.10	51.15	35.45
Asparagin	51.55	68.15	39.00
Czapek	79.31	125.20	93.65
Richard	195.10	213.35	266.30

The mean weight (mg) of 5 petri dishes

N NaOH and 1 N HCl before autoclaving. As shown in Table 2, all three isolates well produced sclerotia, especially on Richard and Czapek medium. Hopkins medium was selected as a basal medium because of its simple composition and moderate sclerotial formation on it.

3) Inoganic ions

To examine the effect of inorganic elements on sclerotial formation, $CaCl_2$(0.1 g) and $CaHPO_4$ (0.1 g) as Ca ion sources, $FeCl_3$ (0.07 g) and $FeSO_4$(0.07 g) as Fe ion and $ZnSO_4$(0.07 g/l) as Zn ion were added to Hopkins medium. As shown in Table 3, Ca and Fe ions did not affect on the sclerotial formation. While Zn ion reduced the weight of sclerotia per dish in C-14 isolate.

Table 3. The effect of inorganic ions on sclerotial formation of *R. solani*

Inorganic ions	Isolates		
	C-14	C-324	C-326
$CaCl_2$	35.00	59.60	55.90
CaH_2PO_4	32.40	62.10	56.00
$FeCl_3$	28.30	60.08	51.95
$FeSO_4$	38.65	58.50	47.70
$ZnSO_4$	1.43	45.75	31.75
Cont.	44.15	51.15	35.45

4) Nitrogen sources

Seven nitrogenous compounds were tested. Ammonium citrate, ammonium tartrate and ammonium nitrate were better than ammonium sulfate and ammonium chloride (Table 4). The

Table 4. The effect of nitrogen sources on slectrotial formation of *R. solani*

Nitrogen sources	Isolates		
	C-14	C-324	C-326
Ammonium Citrate	57.50	59.75	22.70
Ammonium Tartrate	49.90	34.55	39.55
Ammonium Sulfate	18.65	17.40	25.15
Ammonium Nitrate	42.80	36.10	28.40
Ammonium Chloride	18.76	20.95	27.25
Sodium Nitrate	48.50	60.50	44.58
Potassium Nitrate (Check Medium)	44.15	51.15	35.45

sclerotial formation on potassium nitrate and sodium nitrate amended medium was well.

5) Carbon sources

Fourteen carbon sources were tested. As shown in Table 5, sclerotia were well formed on glucose, galactose amended medium and relatively well production were observed on mannose, xylose, levulose, maltose, lactose and sucrose amended media. While sclerotia were poorly formed on arabinose medium. The linear hyphal growth of three isolates on lactose amended medium was almost same, however, the poor sclerotium formation was observed in C-14 isolate. Although starch, inulin and dextrin were well utilized for mycelial growth, very little amount of sclerotia were produced on inulin medium. Higher alcohols such as glycerin and mannitol were used as carbon sources, however, hyphal growth and the sclerotium production were very poor, especially on glycerin amended medium.

Table 5. The effect of saccharides as carbon source on sclerotial formation of *R. solani*

Saccharide	Isolates		
	C-14	C-324	C-326
Monosaccharide			
glucose	62.85	41.30	47.45
mannose	25.50	41.40	41.15
galactose	57.35	57.45	36.30
arabinose	1.50	7.70	0.80
xylose	27.50	37.40	25.65
levulose	33.20	46.70	27.25
Disaccharide			
maltose	44.45	43.40	29.65
lactose	1.05	30.70	12.50
sucrose	53.95	44.55	30.45
Polysaccharide			
starch	43.85	44.95	29.55
inulin	1.90	4.35	1.60
dextrin	43.40	45.95	23.55
Higher alcohol			
mannitol	4.75	4.60	4.30
glycerin	0	0	0

6) Carbon concentration

To examine the effect of concentration of carbon source on sclerotial formation, glucose was

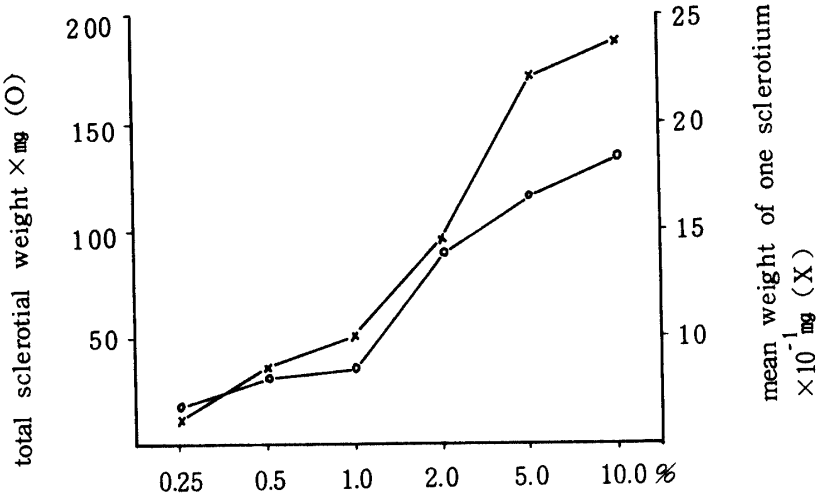

Fig. 1 The effect of glucose concentration on sclerotial formation of R. solani (C-326 isolate)

added at various concentration (0.25 - 10.0%). As shown in Fig. 1, total and one sclerotium weight were proportionally increased with glucose concentration. One sclerotium weight of C-326 isolate

increased 4-5 times and totally 3-4 times at ten times concentration of glucose.

7) Nitrogen concentration

Potassium nitrate was amended to examine the effect of nitrogen concentration on sclerotial

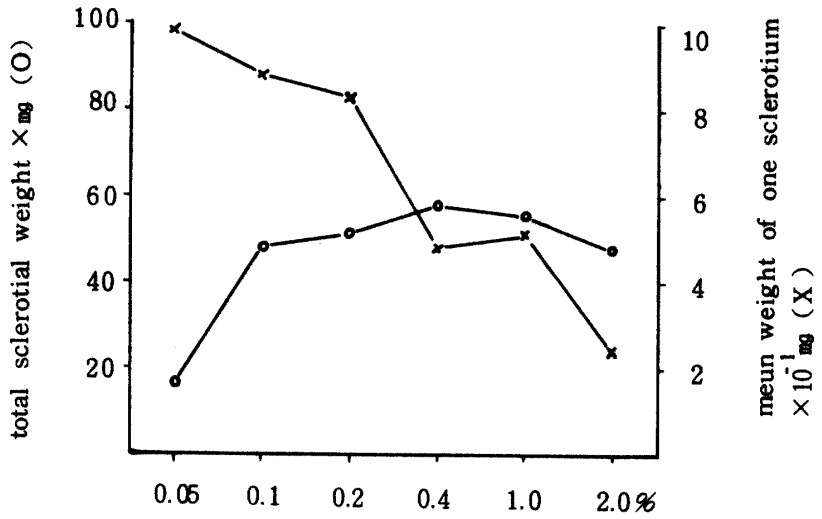

Fig. 2 The effect of KNO_3 concentration on sclerotial formation on R. solani (C−324 isolate)

formation. Quite few amount of sclerotia were formed at low concentration (0.05%). The weight of each sclerotium inversely decreased according with the increase of KNO_3 concentration.

Discussion

The effect of nutrients on fungal differentiation such as sporulation and sclerotial formation have been well documented[2,3]. Townsend reported that sclerotial initials formed even on a relatively poor medium but further development requires nutrients[12]. Furthermore, unfavorable conditions may enhance sclerotial formation. In this experiment, it was described that there are several aspects of sclerotial production by *R. solani* which are affected by change in nutritional composition.

Watanabe and Matsuda studied on the culture types of *R. solani* and reported that size and productive ability of sclerotia were different between culture types[17]. Although three isolates used in this experiment have an ability of sclerotium production, its degree was different. Well sclerotial production was obtained on Richard and Czapek medium. It can be considered that this phenomenon is due to the amount of carbon source, because both media contain 50 g of sucrose as carbon source. For basal medium, Hopkins medium was used because of its simple composition and moderate sclerotial formation. Several minerals were tested but no significant difference was

observed except for Zn ion. Vega et al reported that one mg/litre of Zn caused maximal sclerotium formation, whereas higher amounts increased their total dry weight but in a smaller number of sclerotia[14]. In this experiment, however, the addition of Zn ion inhibited the sclerotium formation, especially in C-14 isolate. Several workers showed that the source and concentration of nutrient, particulaly those supplying carbon and nitrogen affected sclerotial formation and that both NH_4^+ and NO_3^- can be utilized by sclerotia forming fungi for growth and sclerotial formation[1, 5,6,7, 10, 15, 16]. No different effect of inorganic nitrogen sources on sclerotial formation has been reported. However, in this experiment the formation on ammonium sulfate and ammonium chloride amended medium was worse than that of nitrate media. The effect of the carbon sources on sclerotial formation is well known. Heale and Isaac observed that the number of microsclerotium increased with the addition of carbohydrate, especially sucrose[5]. Working on *Sclerotinia sclerotiorum*, Bedi found that maltose was most suitable and that lactose and galactose were poor sources[1]. Furthermore he reported that mannitol, although it was well utilized for mycelial growth, totally inhibited the sclerotium formation[1]. On the other hand, Wang and LeTourneau repoted some different results, finding the highest sclerotial formation with raffinose, sucrose, maltose, lactose, mannose, glucose and fructose[15]. In my results, arabinose, lactose and inulin were most suitable for sclerotial formation but for hyphal growth. Mannitol and glycerin in higher alcohol were unuseful for mycelial growth and sclerotial production. The concentration of carbon and nitrogen source evidently affected on the formation. Increase of glucose concentration proportionally increased the total and one sclerotial weight. No obvious change of total sclerotial weight in addition of potassium nitrate over 0.1% was observed and the decrease of size of each sclerotium was occured. While the dependance of sclerotial formation on C/N ratio in the medium has been documented in several fungi[4,19]. Hashiba and Mogi reported that most suitable N/C ratio is 1.4 - 7.0% in *R. solani* (AG-1, IA)[4]. When nitrogen concentration was varied at constant carbon concentration, however, not so much alteration of sclerotium number was observed (Fig. 2). It was considered that carbon source concentration may be most effective factor on sclerotial formation of *R. solani*.

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Abstract

The effect of minerals, carbon and nitrogen sources and its concentration on sclerotial formation of *Rhizoctonia solani* was examined. The addition of Ca or Fe ion was not obviously effective while

Zn ion inhibited the formation of C-14 isolate. Most carbon sources tested were well utilized for the sclerotial formation, however, little formation on arabinose and inulin amended medium were observed, though hyphal growth was normal. The formation was scarcely observed on lactose medium in C-14 isolate. The hyphal growth was very poor on mannitol and glycerin amended medium. Therefore, very few or no sclerotial formation was observed. The formation on ammonium sulfate and ammonium chloride amended medium was worse than that of nitrate media. Sclerotial weight were proportionally increased with carbon concentration. However, no obvious change of total sclerotial weight in addition of potassium nitrate above 0.1% was observed and the decrease of size of each sclerotium was occured.

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Rhizoctonia solani Kühnの菌核形成に及ぼす栄養源の影響（摘要）

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Rhizoctonia solani Kühn の菌核形成におよぼす微量要素，炭素源，窒素源およびそれらの濃度の影響を検討した。Ca およびFe イオンの添加は菌核形成に明瞭な影響をおよぼさなかったが，Zn イオンの添加は，C－14菌株において著しく形成を阻害した。供試した大部分の炭素源は菌核形成によく利用されたが，アラビノースおよびイヌリン区では菌糸は良好に生育したにもかかわらず菌核は形成されなかった。また，2糖類のラクトース区では，C－14株において殆んど形成が認められなかった。マニトールおよびグリセリン区においては菌糸の生育自体悪く，したがって，菌核も全く又は殆んど形成されなかった。窒素源については，アンモニア態より硝酸態の方が，概して菌核形成に適するという結果が得られた。菌核形成量は炭素源濃度に比例して増加した。しかし，炭素源を一定にして窒素源濃度を高くしても，0.1％以上の添加は形成量に殆んど影響をおよぼさず，一個当たりの菌核量の減少をもたらした。