Helminthosporium oryzaeの完全世代 Cochliobolus miyabeanusの形成条件の検討

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者	津田, 盛也 上山, 昭則
巻/号	41巻5号
掲載ページ	p. 447-452
発行年月	1975年12月

日植病報 41: 447–452 (1975)
Ann. Phytopath. Soc. Japan 41: 447–452 (1975)

Culture Conditions and the Formation of Perfect State of *Helminthosporium oryzae*, *Cochliobolus miyabeanus**

Mitsuya TSUDA** and Akinori UEYAMA**

津田盛也**・上山昭則**: *Helminthosporium oryze* の完全世代 *Cochliobolus miyabeanus* の形成条件の検討

Abstract

The optimum culture conditions for pseudothecia formation by *Cochliobolus miyabeanus* have been determined: Sachs medium with rice straw, 20–24 C (development was slower at 20 C), pH 4.65–8.10 (in the case of Czapek's agar-no carbon source medium with rice straw: pH 3.7–5.9), Sachs medium with gramineous crop or weed plants added showed fairy good pseudothecia fromation as well as with rice straw. As a natural medium for perfect state formation, rice straw decoction agar (50 g, 10 g, or 1 g/l) or potato decoction agar (150 g, 30 g, or 3 g/l) was adequate when a rice straw was placed on the agar surface. Use of rice seed coats on Sachs medium gave scanty pseudothecia formation. (Received November 29, 1974)

1. Introduction

In a previous paper[7], the authors re-confirmed the formation of the perfect state of *Helminthosporium oryzae* Breda de Haan in culture. However, the original culture conditions used by Ito and Kuribayashi, rice straw decoction agar medium (100 g/l)[4,5], were not adequate. As we have not yet found the perfect state in nature, it is necessary to re-investigate the life cycle of the fungus and to clarify the physiological and genetical problems including pathogenicity, tolerance to fungicides etc.

In the present paper, results of our studies of different culture conditions for formation of the perfect state of *H. oryzae* are presented. Our objective was to learn the most suitable conditions for achieving maturity in culture; also to predict the most favourable environment for a field survey.

2. Perfect state formation on Sachs agar medium

Two isolates, A and C, were employed for the strain pairings as was done in previous experiments[7]. Unless otherwise mentioned, about 15 ml of Sachs agar medium was poured into each Petri dish. After solidifying, a 3–4 cm rice straw was placed in the centre of the medium. Tiny agar blocks containing the isolates were used to inoculate the agar. The plates were kept in an incubator at a constant temperature of 24 C for 25–30 days.

---

* Chemical biology of the sexual factors in the fungi (4). This paper was presented at the Annual Meeting of the Kansai Branch, Phytopathological Society of Japan held at Okayama-city, Nov. 1–2, 1973. This study has been supported in part by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education, Japan.

** Pesticide Research Institute, Kyoto University, Kyot 606, Japan. 京都市左京区北白川 京都大学農薬研究施設

2-1) Effect of temperature: as shown in Table 1, mature ascocarps were observed abundantly in the range of 20–24 C. At 10 C, mycelial growth was very slow, and pseudothecia or their primordia were not observed with the naked eye. At 15 C, small black spots, presumably undeveloped pseudothecia, were formed. The ostiolar beaks were not developed, and asci or ascospores had not yet differentiated. At 28 C, unripened pseudothecia were produced abundantly, covered with a large amount of aerial mycelium, and there was no indication of differentiation at that temperature.

Table 1. Relationship of temperature to formation of the perfect state of *H. oryzae*

Temp. (C)	Pseudothecia formed
10	0
15	0 a)
20	35
24	43
28	0 b)

a) Recognized only small spots.
b) The contents are not ripened.

2-2) Effect of volume of the medium in the Petri dish: ascocarp formation was reduced in the medium when more than 25 ml per Petri dish was used (Table 2).

Table 2. Relationship of volume of medium to formation of the perfect state of *H. oryzae*

Medium ml/Petri dish	Pseudothecia formed
10	52
15	70
20	51
25	17
30	19

Table 3. Relationship of agar content of medium to formation of the perfect state of *H. oryzae*

Agar content (%)	Pseudothecia formed
1.0	43
1.5	35
2.0	38
2.5	52
5.0	40

2-3) Effect of agar content (%) in the medium: as shown in Table, 3, no significant differences were observed in the range of these experiments.

2-4) Effect of pH of the medium: the pH of the medium was adjusted to various levels with 0.1 N NaOH and 0.1 N HCl. After autoclaving, the pH values were determined by the glass electrode method. Normal Sachs medium had a pH about 5.5.

As shown in Table 4, good ascocarp formation was obtained throughout the range of pH studied.

Table. 4. Relationship of pH of Sachs medium to formation of the perfect state of *H. oryzae*

pH[a)]	Pseudothecia formed
4.38	74
4.40	88
4.65	160
5.50	103
6.20	125
6.75	180
8.10	165

a) Measured after autoclaving.

3. Kinds of plant speceis to be placed on Sachs agar medium and their effect on formation of the perfect state

A 3–4 cm portion of a dried rice plant

Table 5. Relationship of numbers of rice culms placed on Sachs medium to formation of the perfect state of *H. oryzae*

Number of rice culms placed	Pseudothecia formed
1	100
2	105
3	80
4	40
5	60

Table 6. Relationship of rice plant part to formation of the perfect state of *H. oryzae*

Part	Pseudothecia formed
Seed	3.5
Chaff (seed coat)	1.0
Grain	24
Branches and pedicels	50
Uppermost internode	85
Leaves	90
Straw: upper parts	110
Straw: lower parts	75

or other plants was placed on Sachs medium. The plant parts were sterilized by autoclaving as previously described.

3-1) **Number of rice straws**: as shown in Table 5, pseudothecia formation rather decreased by an increase in numbers of rice straws placed on the agar.

3-2) **Kinds of parts of the rice plant**: it is very difficult to clearly compare each value obtained, because each volume or weight is different for the various plant parts used.

Good formation was obtained from all the parts except seed. These results are shown in Table 6. In the seed-medium, scanty pseudothecia were produced. This value was evidently due to the presence of the seed coat. Formation of pseudothecia on seeds withont coats was more abundant than on unscraped grains or on seed coats. Increasing the numbers of seed coats was also not effective.

3-3) **Use of gramineous plants**: the results are summarized in Table. 7.

All the parts used showed no inhibitory effect. Extremely scanty pseudothecia

Table 7. Relationship of plant species placed on Sachs medium to formation of the perfect state of *H. oryzae*

Plant species	Plant part		
	Culms	Leaves	Seeds
Barley	65	34	46
Wheat	57	26	—
Corn	90	11	3
Goosegrass	45	8	13
Fingergrass	78	51	51
Barnyard-grass	65	14	30
Foxtail-grass	73	33	60
Job's tears	55	77	54
Eulalia	270	96	—
Wheat-grass[a)]	125	5	26
Rice	92	—	—

a) *Agropyron tsukushiense* var. *transiens*.

formation was not obtained.

3-4) Use of non-gramineous plants: this experiment was conducted in order to confirm the formation of pseudothecia on non-gramineous plants growing in or near rice paddies.

As shown in Table 8, some plants showed no or scanty pseudothecia formation.

4. Kinds of media and pseudothecia formation

Several kinds of ordinary media[1] with or without a 3-4 cm piece of rice straw were

Table 8. Plant species placed on the medium and the perfect state formation of *H. oryzae*

Plant species	Plant part	
	Culms	Leaves
Morning-glory	0.3	0
Green-pigweed	34	0
Common ragweed	8	0
"Kudzu"-vine	3	0
"Yabukarashi"[a]	0	0
"Hinatainokozuchi"[b]	0.7	3
"Ooabunome"[c]	12	4
Smartweed	25	2
Daisy-fleabane	7	0
Smallflower umbrella plant	2	10
Soybean	46	2
Rice	37	—

a) *Cayratia japonica*
b) *Achyranthes fauriei*
c) *Gratiola japonica*

Table 9. Formation of the perfect stats of *H. oryzae* on several media

Medium	Rice straw	
	Placed	Not placed
Asparagine	—[a]	—[a]
Asparagine without sucrose	—	—
Sabouraud	—	—
Sabouraud without glucose	—	—
Richard	—	—
Richard without sucrose	—	—
Malt decoction	—	—
Apricot decoction (25 g/l)	—	—
Apricot decoction (2.5 g/l)	+[b]	—
Knop	+	—

a) Pseudothecia were not formed.
b) Pseudothecia were formed.

Table 10. Formation of the perfect state of *H. oryzae* on potato decoction agar

Potato (g/l)	Rice straw	
	Placed	Not placed
0.3	16	0
3.0	23	0
30	10	0
150	17	0

Table 11. Formation of the perfect state of *H. oryzae* on rice culm decoction agar

Rice culms (g/l)	Rice straw	
	Placed	Not placed
0.1	25	0
1.0	106	0
10	81	17
50	10	59
100	0[a]	0[a]
10+glucose 1.0 g	24	2

a) The contents were not mature.

Table 12. Formation of the perfect state of *H. oryzae* on Czapek agar medium[a]

pH[b]	Rice straw	
	Placed	Not placed
3.7	32	0
5.4	74	0
5.9	86	0
6.8	0[c]	0
7.5	0[c]	0

a) Without sucrose.
b) pH was measured after autoclaving.
c) The contents were not mature.

employed to compare pseudothecia formation.

As shown in Table 9, sugar-containing media gave good mycelial growth, but were not adequate for pseudothecia formation. Pseudothecia formation was obtained only in the dishes of Knop agar medium and apricot decoction agar medium (2.5 g/l) with a rice straw.

Tables 10 and 11 give the results of decreasing the concentration of ordinary rice straw decoction or potato decoction used in the agar medium employed for pseudothecia formation. In the case of the rice straw decoction agar medium, good formation was observed in the case of 10 g straw/l, when a rice straw was placed on the medium. In the case of 10–50 g/l, pseudothecia formation occurred both with or without a rice straw on the rice straw decoction medium. However, when one gram per liter of glucose was added on the medium composed of 10 g straw/l decoction agar, their formation was extremely depressed.

In the case of potato decoction agar, placing a rice straw on the medium is absolutely essential for pseudothecia formation. In the case of Czapek's medium wihout sucrose, pseudothecia formation was observed when a rice straw was placed on the medium (Table 12).

However, pseudothecia matured only under acidic conditions, when a rice straw was placed on the medium. In media with over pH 6.8, the authors observed only immature pseudothecia with short ostiolar beaks or protothecia (Table 12).

5. Discussion

From these experiments, the most favourable temperature range for formation of mature ascospores of *C. miyabeanus* was found to be 20–24 C, which was somewhat lower than 25–28 C, which is optimum for mycelial and conidial formation[2,3].

In the case of Sachs agar medium in a 9 cm Petri dish, 15 ml of 1.5% agar with a rice straw or a part from some other gramineous plants, was suitable for routine experiments.

Gramineous plant species were best for use on Sachs medium, wheares non-gramineous plants were often inadequate for pseudothecia formation. Further studies are necessary to clarify the reasons in relation to a field survey.

Use of rice seed coats on Sachs medium resulted in scanty pseudothecia formation. As a probable cause, we suggest that the seed coat may contain a substance (s) that inhibits pseudothecia formation, or a nutritional substance (s) diffuse into the agar from the seed coat in a very small amount. Ascocarp formation was good, when pairing was done on a medium containing a rice straw, but none or very little carbohydrates. In the case of potato decoction medium, mature pseudothecia developed, only when a rice straw was placed on the medium. These results suggest the ascocarp formation does not occur under simple conditions such as a nutritionally poor medium. Further research is needed to clarify the conditions necessary for pseudothecia formation.

In some cases, it was suggested that two phases are necessary for perithecia formation as already reported for *Pleospora*[6] and *Venturia*[8] for formation of ascocarp primordia and the ascocarp maturation. However, the present authors were not able to clearly detect this phenomenon in *C. miyabeanus*.

The possibility of formation of the perfect state of *H. oryzae* in nature has already

been pointed out by Ito and Kuribayashi. We also believe that pseudothecia may exist in nature, based on the laboratory studies described in the present paper.

Acknowledgement

We are indebted to Dr. G. Gooday, University of Aberdeen, Scotland, and to Dr. M. Kulik, U.S.D.A.-Beltsville, for reading the draft.

Literature cited

1) Asuyama, H. *et al.* (1962). Laboratory guide for plant pathologists (Asuyama, H. *et al. eds.*) Nippon Shokubutsu Boeki Kyokai, p. 764–779 (in Japanese).
2) Hemmi, T. (1947). Nogaku (Agricultural Science) 1: 325–331 (in Japanese).
3) Honda, Y. (1969). Bull. Inst. Agric. Res., Tohoku Univ. 21: 63–132 (in Japanese with English summary).
4) Ito, S. and Kuribayashi, K. (1927). Ann. Phytoapthol. Soc. Japan 2: 1–8.
5) Kuribayashi, K. (1929). Nogyo oyobi Engei (Agriculture and Horticulture, Tokyo) 4: 141–156.
6) Leach, C. M. (1971). Trans. Brit. mycol. Soc. 57: 295–315.
7) Ueyama, A. and Tsuda M. (1975). Ann. Phytopathol. Soc. Japan 41: 432–439.
8) Wilson, E. E. (1928). Phytopathology 18: 375–416.

和文摘要

Helminthosporium oryzae の完全世代, *Cochliobolus miyabeanus* の形成条件の検討

津田盛也・上山昭則

Cochliobolus miyabeanus の培地上における子のう殻形成の条件を検討した。Sachs 培地を用いた場合，9 cm ペトリ皿に 1.5% 寒天として 15–20 ml 分注し，3–4 cm に切ったイネわらを1本置床すれば，通常の実験には十分であった。子のう殻の形成は 20–24 C の温度範囲で認めたが，この範囲を越えると認められなかった。Sachs 培地に置床する植物種と形成との関係を調べた。イネ科植物ではおおむね良好であったが，イネ科以外の植物種では形成の不良な場合がしばしば認められた。

そのほか，Knop，アンズ煎汁 (2.5 g/l) などの寒天培地，ジャガイモ煎汁 (150 g, 30 g, 3 g/l)，イネわら煎汁 (50 g, 10 g, 1 g/l) などの寒天培地にイネわらを置床した場合に形成が認められた。なお，イネわら煎汁培地 (50 g, 10 g/l) ではイネわらを置床しなくても形成された。

Czapek-糖無添加培地上でも，pH 3.7–5.9 の場合には，イネわらを置床すれば形成が認められた。Sachs 培地にイネもみを置床した場合には，子のう殻の形成がきわめて悪い。これはもみがらによるものであるが，形成不良因子については現在検討中である。