한국식물병리학회지 12(2) : 142-146(1996)
Korean J. Plant Pathol. 12(2) : 142-146(1996)

Effects of Nutrient Source, Temperature, pH, and Light on Sporidial Production of *Ustilago maydis* and Its Viability for Long-term Storage

Kyung Seok Park and Choong Hoe Kim*
Plant Pathology Division, National Agricultural Science & Technology Institute, Suwon 441-707, Korea

*Ustilago maydis*의 소생자 형성에 미치는 영양원, 온도, 광의 효과 및 장기보존 후의 생존력

박경석 · 김충회*
농업과학기술원 작물보호부 병리과

ABSTRACT : Air-dried teliospores of corn smut, *Ustilago maydis*, maintained about 40 to 50% of their viability after 24 months when preserved at 4°C or lower temperatures with sand or silica gel as a carrier. Teliospores germinated easily and produced abundant sporidia on potato-dextrose agar at 28°C. The optimum media for sporidial production from teliospores of *U. maydis* were corn meal broth followed by carrot broth and potato-dextrose broth. Optimum conditions for sporidial production were 28~30°C and pH 5~7. Sporidial development appeared better under dark conditions.

***Key words* : corn smut, *Ustilago maydis*, teliospore, sporidium, germination.**

Smut often causes considerable damages when improperly managed in cereals such as barley, wheat and maize (2, 3 ,4, 7). Smut has been mainly controlled by seed dressing with highly specific systemic fungicides, such as carboxin. However, reduced effectiveness of such a fungicide has been often reported due to development of resistant pathogen strains (5). For this reason, there has been a demand for developing new fungicides effective against smut disease, as seen in many other diseases.

Screening of chemical compounds against smut disease has depended largely on field tests, and thus required much time and efforts for field experiments. In fact, lack of a simple screening technique hampered development of new fungicides effective against smut disease.

Development of screening technique requires understanding on the physiology of smut fungus, i.e. spore germination and host infection. So far, little study has been done on viability of smut fungus, particularly, on germination of teliospore and production of sporidia responsible for infection.

This study was performed to examine physiology of teliospore germination and sporidial production of *Ustilago maydis* necessary for inoculum production, as a part of the experiments for developing a fungicide screening technique against smut fungus. A preliminary report has been published elsewhere (6).

MATERIALS AND METHODS

Mycological observation on smut fungus. Corn smut samples were collected from Kangwon and Kyonggi provinces in September, 1993. Black masses of teliospores were collected from diseased plant samples after air-drying, and kept at 4°C for future use. For mycological observation, teliospores were placed on water agar and incubated at 28°C for 2 days. Morphology of teliospores and sporidia that were developed from the germinated teliospores was examined under a microscope. Size of teliospores and sporidia was measured based on 25 spores each using a hemacytometer.

*Corresponding author.

Preservation of teliospores and their viability measurement. Silica gel, sand and vermiculite were selected as carriers of teliospores for long-term storage. Each carrier was put into a 20 ml vial and autoclaved. Teliospores were mixed with each carrier at 10% rate (v/v). Teliospores without any carrier were used as a check. The vials were stored at room temperature, 10, 4, −20, and −60°C and checked for their viability after 6 and 24 months, respectively. To examine viability of teliospores, samples were taken from each vial, placed on water agar containing 100 μg/ml of streptomycin sulfate, and incubated at 28°C for 48 hr. Percentage of germinated teliospores that produced sporidia was examined under a microscope.

Selection of media for sporidial production. Five media, Czapek-dox broth (Difco), carrot broth (carrot extract 20 g, distilled water 1 l), corn meal broth (corn meal 17 g, distilled water 1 l), V-8 juice broth (V-8 juice 200 ml, distilled water 1 l, pH 6.5), and potato-dextrose broth (potato 200 g, dextrose 20 g, distilled water 1 l) and distilled water as a check were compared for sporidial development of *U. maydis*. An 1-day-old PDA culture disk of *U. maydis* was put into a 250 ml Erlenmeyer flask containing 100 ml of each medium after sterilization. The flask was incubated in a shaker at 150 rpm at 30°C, and 0.1 ml of the sample was taken from the flask every day, starting 1 day after incubation until 5 days. The sample culture was plated on PDA after dilution to count the number of colonies appeared. The treatment was replicated 5 times.

Examination of effects of temperature, pH, and light on sporidial production. To examine temperature effect, a culture disk of *U. maydis* was point-inoculated onto PDA plate and incubated at 4, 7, 14, 20, 24, 26, 28, 30, and 37°C. Sporidia were harvested 48 hr later and number of sporidia was determined.

To examine pH effect, potato-dextrose broth was adjusted to pH 4 to 10 at 1 intervals using 1N HCl and 1N NaOH, put in an 100 ml Erlenmeyer flask, inoculated with a culture disk of *U. maydis* and incubated in a shaker at 150 rpm at 30°C. Samples were taken at one-day intervals for 5 days to check sporidial production.

Light treatment was achieved by placing a PDA petri dish inoculated with *U. maydis* under a cool white fluorescent lamp at 15 cm high in an incubator at 30°C. For the dark treatment, the inoculated PDA plates wrapped with aluminum foil were used.

RESULTS

Mycological characteristics of *U. maydis*. Teliospores were olive brown to black, spherical to globose, bluntly echinulate, and 8.4×8.2 μm on average (Table

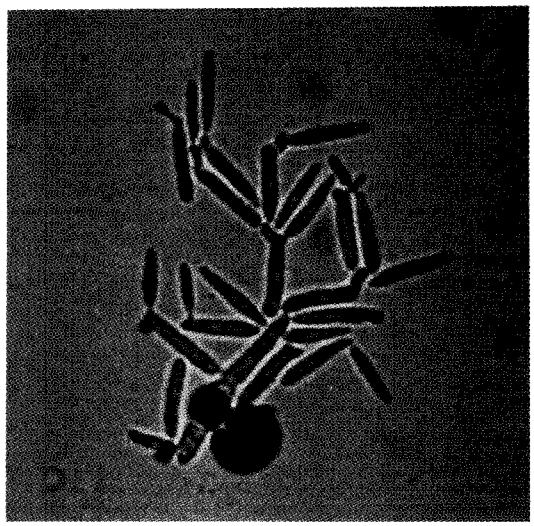

Fig. 1. Germination of a teliospore of *Ustilago maydis* which results in production of cylindrical sporidia.

Table 1. Comparison of mycological characteristics of *Ustilago maydis* isolates used in this study and those reported previously

Characteristics		This study	Ainsworth (1)
Teliospore (Chlamydospore)	shape	spherical to globose, bluntly echinulate	globose to subglobose, prominently and bluntly echinulate
	size (μm)	7.5~11.2×6.5~10.0 (8.4×8.2)	8.0×12.0
	color	olive brown to black	olive brown to black
Sporidium	shape	fusiform	fusiform
	size (μm)	10~20×2.0~3.75	–
	color	hyaline	hyaline

Table 2. Viability of teliospores of *Ustilago maydis* preserved with various carrying agents for up to 24 months under 5 different conditions

Carrying agent	Months after storage	% teliospore germinated[a]				
		−60°C	−20°C	4°C	10°C	Room temp.
None	6	47.8±8.9	56.0±11.6	50.5±13.9	49.4±11.8	41.6± 3.0
	24	40.2±6.0	47.9± 4.8	42.8± 6.6	−[b]	0
Silica gel	6	58.2±8.1	49.0±11.2	48.3± 8.5	51.9± 6.6	51.6±14.4
	24	38.4±5.5	48.4± 6.2	42.2± 2.7	−	14.6± 1.6
Sand	6	39.2±3.7	59.9±13.3	55.4± 4.3	47.3±10.0	54.1±11.9
	24	27.4±5.7	42.6± 4.5	41.8± 5.2	−	0.5± 0.4
Vermiculite	6	51.1±5.7	37.7± 4.8	21.5± 5.0	58.4±14.4	54.4±12.9
	24	34.6±3.2	17.4± 3.4	0.7± 0.5	−	0

[a] Values are means of 5 replications with their standard deviations.
[b] Not examined.

Table 3. Sporidial production from teliospores of *Ustilago maydis* on various culture media at 28°C

Medium (broth)	No. of sporidia produced (10^9/ml)[a] after				
	1 day	2 days	3 days	4 days	5 days
Water (control)	<0.001	<0.003	<0.001	<0.001	<0.01
Czapek-dox	0.02±0.01	0.01±0.002	0.032±0.02	0.039±0.005	0.004±0.0036
Carrot	0.28±0.10	0.30±0.07	15.0±5.0	40.0±22.3	52.5± 4.5
Corn meal	0.12±0.05	11.0±0.30	75.0±3.3	80.0±78.4	12.8± 7.9
V-8 juice	0.14±0.02	1.56±0.66	16.3±7.3	22.5±10.9	−[b]
Potato-dextrose	0.14±0.04	2.90±1.06	16.3±6.8	30.3±27.1	37.5±13.0

[a] Values are means of 5 replications with their standard deviations.
[b] Not examined.

Table 4. Effect of temperature on sporidial production from teliospores of *Ustilago maydis* 48 hr after incubation on potato-dextrose agar

Temperature (°C)	No. of sporidia[a] produced (10^3/ml)
4~24[b]	0
26	29.6±12.4
28	58.6±12.9
30	64.6±16.3

[a] Values are means of 5 replications with their standard deviations.
[b] Temperatures treated were 4, 7, 14, 20, and 24°C.

1). Sporidia borne from promycelia of germinated teliospores were hyaline, fusiform and 10~20×2.0~3.75 μm in size (Table 1 and Fig. 1). Mycological characteristics of the teliospores and sporidia observed in this study did not differ from those described by Ainsworth (1).

Viability of teliospores in long-term storage. Viable teliospores after 6 or 24 months of storage ranged 0~58%, depending on the combination of carrier, storage temperature, and storage duration (Table 2). Viability of teliospores was about 40~50% after 6 months preservation regardless of storage temperatures and carrying agents with an exception of 4°C preservation with vermiculite. After 24 months of the room temperature preservation, the viability of teliospore was decreased sharply to 0~15%. However, storage at 4°C or lower temperature conditions with sand or silica gel as a carrying agent resulted in a slight decrease in viability after 24 months of storage. Vermiculite showed the lowest survival compared to other carrying agents at temperatures of −20°C and 4°C.

Optimum media for sporidial production. Sporidial production was greatest on corn meal broth, followed by carrot broth and potato-dextrose broth, intermediate on V-8 juice broth, and poor on Czapek-dox broth (Table 3). Only few sporidia were developed in distilled water. Sporidial production was increased gradually as incubation time prolonged, and reached the maximum at 4~5 days, depending on the media used.

Temperature effect on sporidial production.

Table 5. Effect of pH on sporidial production from teliospores of *Ustilago maydis* on potato-dextrose broth at 28°C

pH	No. of sporidia produced[a] (10^9/ml) after				
	24 hr	48 hr	72 hr	96 hr	120 hr
4.0	0.023±0.008	0.067±0.021	3.00±1.50	20.50±9.00	20.00±15.0
5.0	0.077±0.023	0.155±0.040	3.37±2.15	225.00±21.7	29.50±4.03
6.0	0.113±0.018	0.236±0.162	51.8 ±21.5	145.00±30.4	15.00±5.00
7.0	0.135±0.036	0.403±0.127	5.03±2.09	10.50±1.50	72.00±16.0
8.0	0.079±0.019	0.208±0.053	0.43±0.15	72.50±22.8	17.50±0.83
9.0	0.011±0.038	0.075±0.014	0.35±0.26	382.50±156.3	35.00±22.9
10.0	0.012±0.008	0.044±0.012	2.82±0.99	33.25±8.92	29.00±1.87

[a] Values are means of 5 replications with their standard deviations.

Table 6. Effect of light illumination on sporidial production of *Ustilago maydis* on PDA at 30°C

Treatment	No. of sporidia produced (10^7/ml)[a]	
	24 hr	48 hr
Light	3.9±1.0	71.6± 5.2
Dark	1.8±1.1	140.3±80.8

[a] Values are means of 9 replications with their standard deviations.

Temperature greatly influenced sporidial development (Table 4). Teliospores germinated and began to produce sporidia from 26°C. The sporidial production was peaked at 28~30°C. Sporidia were not formed at 24°C and below, or at a high temperature such as 37°C.

pH effect on sporidial production. Sporidial production varied greatly among pH of media (Table 5). The maximum number of sporidia were developed 96 hr after inoculation in most of pH levels tested. The optimum pH for sporidial production was generally pH 5~6.

Light effect on sporidial production. Sporidial production was observed both in light and in dark conditions (Table 6), but appeared to be higher in the dark condition.

DISCUSSION

Cultivation of smut fungus in artificial media has been reported little (4). The lack of information on inoculum production of smut fungus, in fact, had hampered development of screening technique against this pathogen.

In this study, teliospores of *U. maydis* germinated well on common media, like PDA, and produced abundant sporidia that are directly responsible for host infection. Environmental factors such as nutrient sources, temperature, pH, and light were found to affect greatly teliospore germination and subsequent sporidial production. However, those factors were manageable for the optimum production. This study indicates that inoculum production of *U. maydis* in artificial media seemed possible, like other saprophytic fungi.

In this study, *Ustilago maydis* maintained as sporidial culture in PDA slant tubes, which were kept either at 4°C or at room temperature. The fungus survived well in this condition for a prolonged time. However, for the purpose of a long-term storage, teliospore masses itself from smut could be preserved. When they were placed in the laboratory after air-dried without specific carriers, they could maintain their viability for at least six months. However, both carrying agents such as silica gel or sand, and low temperatures as 4°C or lower appeared to be required for a long-term preservation. Under this condition, *U. maydis* could be preserved for at least two years with about 40 to 50% viability level.

요 약

옥수수 깜부기병균 *Ustilago maydis* 동포자를 풍건한 후 모래나 silica gel을 보존매체로 해서 4°C나 그 이하 온도에서 24개월 두었을 때 동포자 생존율은 40~50%였다. 동포자는 28°C PDA상에서 쉽게 발아하여 많은 소생자를 형성하였다. *U. maydis* 동포자로부터 소생자 형성의 최적배지는 corn meal broth였고, carrot broth와 potato-dextrose broth의 순으로 좋았다. 소생자 형성에 가장 적합한 조건은 온도 28~30°C, pH 5~7이었다. 소생자 형성은 암조건에서 보다 좋았다.

REFERENCES

1. Ainsworth, G. C. 1965. *Ustilago maydis. Description*

of Pathogenic Fungi and Bacteria, No. 79. Commonwealth Mycological Institute, Kew Surrey, England.

2. Christensen, J. J. 1963. Corn smut caused by *Ustilago maydis. Am. Phytopathol. Soc. Monograph* 2 : 1-41.
3. Fischer, G. W. and Holton, C. S. 1957. *Biology and Control of the Smut Fungi.* Ronald Press, New York.
4. Jones, D. G. and Clifford, B. C. 1983. *Cereal Diseases, Their Pathology and Control.* John Wiley and Sons, Chichester. 309pp.
5. Nyvall, R. F. 1989. *Field Crop Diseases Handbook*, pp. 105-106. Avi Book, New York.
6. Park, K. S. and Kim, C. H. 1994. Effects of some environmental factors on germination of teliospores of *Ustilago maydis*. (Abstr.) *Korean J. Plant Pathol.* 10(4) : 354.
7. Shurtleff, M. C. 1980. *Compendium of Corn Diseases*, 2nd ed. APS Press, Am. Phytopathol. Soc., St. Paul, Minnesota. 105pp.