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Smut Induction on Corn Seedlings by Inoculation of Sporidia and Teliospores of *Ustilago maydis* in Greenhouse Conditions

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온실조건에서 *Ustilago maydis*의 소생자 및 동포자 접종에 의한 옥수수 유묘의 깜부기병 발병유도

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ABSTRACT : Most successful smut development on corn seedlings was obtained when seedlings at the 4th leaf stage were inoculated with sporidial suspension of *Ustilago maydis* at the concentrations of 10^3~10^5 sporidia/ml, and subsequently incubated at 28~30°C in a dew chamber for 24 hr before placing in the greenhouse. Younger seedlings, higher inoculum concentrations as 10^9 sporidia/ml, or longer incubation period in a dew chamber resulted in early seedling death. Smut development was reduced as incubation temperature decreased. Both foliar spray and stem injection, but not soil drench, of the sporidial inoculum successfully induced the smut disease. Sporidial suspension alone developed smut sufficiently without addition of extra nutritional sources to the inoculum such as glucose, ammonium nitrate, yeast extract or their combinations. Teliospore inoculation also developed smut symptom by either foliar spray or stem injection, but far less severely compared to the sporidial inoculum.

***Key words* : corn, smut, *Ustilago maydis*, sporidia, teliospore, screening.**

Smut is a common disease found in most cereal-growing areas (1, 9). Corn smut fungus, *Ustilago maydis* usually attacks flowers and damages grains, but the smut occurs in all parts of plants including leaves and stems causing distortion and formation of various sizes of galls (2, 10). The smut fungus produces black teliospore masses within galls, which germinate to develop sporidia that are responsible for host infections. Host infections have been known to be successful only when two compatible haploid sporidia fuse and give rise to binucleate infection hyphae (4).

Smut has been mainly controlled by seed treatment with fungicides (3). Screening of chemical compounds against smut disease has been carried out so far based on field tests which require much time and effort. In fact, lack of a simple screening technique has hampered development of new fungicides against smut disease.

This study was performed to develop a simple technique applicable for screening fungicides against smut disease in the greenhouse. Parts of the researches have been published elsewhere (5~8).

MATERIALS AND METHODS

Raising corn plants. Corn cultivar Jaerae was seeded one in each in pots (10.5 cm diam., 10 cm high) containing standard soil (Flora gard, TKS 2) and grown in a greenhouse at 21~35°C until use.

Inoculum preparation. Smut fungus, *Ustilago maydis* isolated from a smut gall on diseased corn plants was grown in potato dextrose agar (PDA) at 28°C for 4 days. Sporidia were harvested by gently brushing the culture surface after adding sterile water. Concentration of sporidial suspension was adjusted to 10^5/ml and used as an inoculum in most experiments.

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In inoculum density test, three additional inoculum concentrations, 10^3, 10^7 and 10^9 sporidia/ml, were used. In an experiment, 10 mg of glucose, 1 mg of ammonium nitrate, 10 mg of yeast extract or a combination of glucose and ammonium nitrate were added in 1 ml of the sporidial inoculum to examine the effect of nutritional addition on smut development. Teliospore suspension at the concentrations of 10^3 and 10^7/ml was also used in some experiments.

Inoculation. Corn seedlings at the 4th leaf stage were inoculated by spraying the sporidial inoculum using a hand gun sprayer in most experiments. Inoculated corn seedlings were kept in a dew chamber at 28°C for 24 hr and then put in a greenhouse for smut development. Temperature of dew chambers was set at 20°C, 24°C, 28°C and 30°C to examine the effect of varying incubation temperature. To examine effect of growth stage of the plants, seedlings at the 2nd, 3rd, 4th, and 5th leaf stages were inoculated. In the experiment to examine effect of dew period duration, inoculated corn seedlings were put in a dew chamber for 12 hr, 24 hr, 48 hr and 72 hr. In one experiment, seedling stems were injected by a microsyringe at the rate of 0.1 ml of sporidial or teliospore inoculum per plant. Seedlings growing in pots were also drenched with the sporidial inoculum at the rate of 50 ml per plant. All experiments were replicated 5 times.

Data acquisition. Smut development on inoculated seedlings was examined 10 days after inoculation. Symptom severity appeared on each plant was recorded based on the following index of 0 to 5: 0: no symptom developed, 1: parts of seedling leaves turning red, 2: partial red-discoloration occurring along with minor leaf distortions, 3: severe leaf distortions accompanying with small gall formation, 4: plants becoming dwarf due to malformation of leaves with numerous galls. 5: plants becoming blighted to death.

RESULTS

Smut symptom developed on corn seedlings. Various types of symptom were observed usually 10 days after inoculation (Fig. 1a~d). Parts of some leaves first turned brown to red with minor distortion (Fig. 1a), and often splitted longitudinally, or sometimes rolled like a whip (Fig. 1b). Small galls were formed independently on leaf blades (Fig. 1c) or often formed along with leaf axis. Severely infected seedlings became gradually distorted with numerous galls (Fig. 1d) and did not grow further. Large smut galls containing a sooty teliospore mass occasionally developed on a basal stem and a main root were observed 30 days after inoculation (Fig. 1e), and the infected corn seedlings became severely dwarfed and malformed (Fig. 1f).

Effect of inoculum density and incubation temperature on smut development. Smut was developed severely with severity index of 3 to 5 at the concentrations of 10^3 to 10^9 sporidia/ml (Table 1). High inoculum level such as 10^9 sporidia/ml resulted in early death of corn seedlings and thus caused difficulty for examining symptom appearance. Smut severity did not vary greatly by inoculation of the four different inoculum levels, although some inoculated plants remained healthy depending on replications. Most appropriate inoculum density for smut development was 10^3 or 10^5 sporidia per ml.

Smut development was reduced at lower temperature such as 20°C and 24°C (Table 1). At the lower temperature corn seedlings developed the symptom severity index averaged 1.2 to 2.4, compared to 3.0 to 3.2 at 28°C and 30°C, respectively. However, variations were observed among replications because of no infection of the plants.

Effect of dew period and seedling growth stage. Most appropriate dew period after inoculation for smut development was around 24 hr, although great variations occurred among replications (Table 2). However, when the dew period became shorter than 24 hr, the possibilities for corn seedlings without symptom development became larger. When the dew period became longer than 24 hr, the percentage of seedling death was increased.

The 4th leaf stage of corn seedlings appeared to be most adequate for smut development (Table 2). Seedlings at younger leaf stages were apt to be killed early by the inoculation and were impossible to examine their symptom development. Older seedlings than the 4th leaf stage resulted in less degree of symptom severity on the average.

Effect of nutritional addition in sporidial inoculum. Addition of nutrient as a source of carbon or nitrogen in sporidial inoculum failed to bring greater smut development (Table 3). Smut development index was averaged 4.0 to 4.1, regardless of nutritional treatments.

Smut development by different inoculation methods with either sporidia or teliospore inoculum. Both foliar spray and stem injection with sporidial ino-

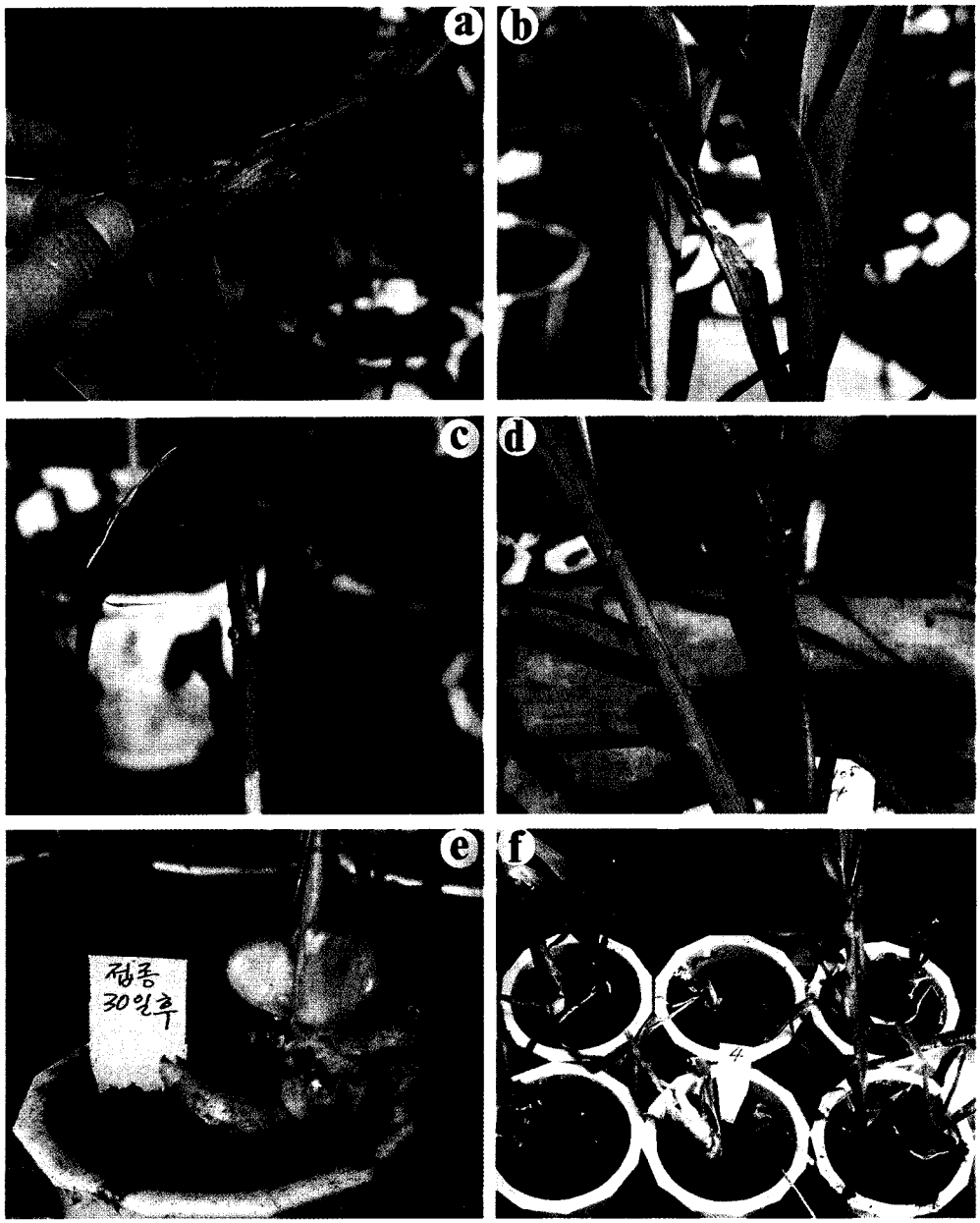

Fig. 1. Various types of smut symptoms developed on corn seedlings 10 days (a~d) and 30 days (e, f) after foliar spray inoculation. a: a partly red-discolored leaf blade with minor distortion, b: a rolled leaf blade like a whip, c: small galls formed around leaf axis, d: a severely distorted seedlings with numerous galls, e: large smut galls containing a sooty teliospore mass developed on a basal stem and a main root, f: severely malformed or dwarfed seedlings.

culum caused severe smut as rated averages of 4.0, while soil drenching failed to cause smut development (Table 4).

Inoculation with teliospore suspension by either foliar spray or stem injection developed the symptom severity index averaged 1.4 to 2.9 (Table 4). This was far less degree in severity compared with the sporidial inoculum. In case of foliar spray, two levels of ino-

Table 1. Effects of inoculum density of sporidial suspension of *Ustilago maydis* and incubation temperature on smut development of corn seedlings in the greenhouse

Experiment	Treatment	Smut development index (0~5)[a]					Average
		I[b]	II	III	IV	V	
Inoculum density	0	0	0	0	0	0	0± 0[d]
(No. sporidia/ml)	10^3	3	0	4	4	4	3.0±1.7
	10^5	0	4	4	4	4	3.2±1.7
	10^7	4	0	4	4	0	2.4±2.2
	10^9	5	4	5	5	5	4.8±0.4
Incubation temperature (℃)[c]	20	3	0	0	3	0	1.2±1.6
	24	4	4	4	0	0	2.4±2.2
	28	0	3	4	4	4	3.0±1.7
	30	4	0	4	4	4	3.2±1.8

[a] 0: healthy, 1: leaf discoloration, 2: leaf discoloration and mild distortion, 3: severe distortion and minor gall formation, 4: severe distortion and numerous gall formation, 5: death of whole plant.
[b] Replication.
[c] Temperature of a dew chamber where the corn seedlings were kept for 24 hr after spray-inoculation with sporidial inoculum.
[d] Standard deviation.

Table 2. Effects of duration of dew period and age of corn seedlings on smut development after spray-inoculation with sporidial suspension of *Ustilago maydis* in the greenhouse

Experiment	Treatment	Smut development index (0~5)[a]					Average
		I[b]	II	III	IV	V	
Duration of dew period (hr)[c]	0	2	0	0	0	2	0.8±1.1[d]
	12	0	2	0	0	0	0.4±0.9
	24	0	3	0	4	4	2.2±2.0
	48	5	4	2	1	3	3.2±1.6
	72	5	1	5	0	5	3.2±2.5
Leaf growth stage	2nd	1	5	0	5	5	3.2±2.5
	3rd	4	5	0	0	0	1.8±2.5
	4th	0	4	3	4	4	3.0±1.7
	5th	0	0	3	3	5	2.2±2.2

[a] 0: healthy, 1: leaf discoloration, 2: leaf discoloration and mild distortion, 3: severe distortion and minor gall formation, 4: severe distortion and numerous gall formation, 5: death of whole plant.
[b] Replication.
[c] Period of time for which the corn seedlings were kept in a dew chamber after inoculation.
[d] Standard deviation.

culum density of teliospore suspension resulted in a similar degree of symptom severity, whereas in stem injection higher inoculum density of 10^7 teliospores/ml brought a greater degree of smut development.

DISCUSSION

In this study smut was induced easily on corn seedlings by inoculation with low concentration of sporidial inoculum as 10^3 sporidia/ml. Between two methods of inoculations, such as foliar spray and stem injection, foliar spray appears more suitable since it is easy to handle both inoculum and plants, and it does not cause any damage to plants.

Direct use of teliospores as an inoculum, instead of sporidia, from sooty spore mass in smut samples seems to be unsuitable for screening tests, since it failed to develop sufficient amount of smut disease by either method of foliar spray and stem injection. Soil drenching with both sporidial and teliospore inocula suspension did not induce smut either.

Smut was developed most successfully at higher tem-

Table 3. Effect of nutritional sources in sporidial suspension *Ustilago maydis* on smut development of corn seedlings in the greenhouse[a]

Nutritional source	Added dose (mg/ml)	Smut development index (0~5)[b]					Average
		I[c]	II	III	IV	V	
Glucose (G)	10	4	4.5	4	4	4	4.1±0.2[d]
NH_4NO_3 (N)	1	4	4.5	4	4	4	4.1±0.2
Yeast extract	10	4	4.5	4	4	4.5	4.2±0.3
G+N	10+1	4	4	4	4	4	4.0±0
None	–	4	4	4	4	4	4.0±0

[a] Corn seedlings at the 4th leaf stage were spray-inoculated with sporidial suspension of 10^5/ml.
[b] 0: healthy, 1: leaf discoloration, 2: leaf discoloration and mild distortion, 3: severe distortion and minor gall formation, 4: severe distortion and numerous gall formation, 5: death of whole plant.
[c] Replication.
[d] Standard deviation.

Table 4. Effect of inoculation methods on smut development of corn seedlings with either sporidia or teliospores inoculum suspension of *Ustilago maydis* in the greenhouse[a]

Inoculation method	Inoculum applied	Inoculum concentration (/ml)	Smut development index (0~5)[b]					Average
			I[c]	II	III	IV	V	
Foliar spray	sporidia	10^5	4	4	4	4	4	4.0±0[d]
	teliospores	10^3	4.5	2	2	2	2.5	2.6±1.1
	teliospores	10^7	4	2.5	1	1	2	2.1±1.2
	water	–	0	0	0	0	0	0 ±0
Stem injection	sporidia	10^5	4	4	4	3.5	4.5	4.0±0.4
	teliospores	10^3	0	1.5	1	1	3.5	1.4±1.3
	teliospores	10^7	3	3	2.5	3.5	2.5	2.9±0.4
	water	–	0	0	0	0	0	0 ±0
Soil drenching	sporidia	10^5	0	0	0	0	0	0 ±0
Uninoculated	–	–	0	0	0	0	0	0 ±0

[a] Two separate inoculations, one with sporidia, the other with teliospores were done in the independent experiments, but the data were shown together in this table.
[b] 0: healthy, 1: leaf discoloration, 2: leaf discoloration and mild distortion, 3: severe distortion and minor gall formation, 4: severe distortion and numerous gall formation, 5: death of whole plant.
[c] Replication.
[d] Standard deviation.

perature such as 28°C and 30°C. This is thought to be due in part to rapid sporidial germination occurring under higher temperature conditions as observed by Kim and Park (6).

Smut attacks most favorably young tissues of plants (4). Based on this report, younger seedlings might be better for smut development. However, young seedlings at the 2nd or 3rd leaf stage were inadequate for screening purpose, since they were often killed early before the symptom severity was rated. Seedlings at the 4th leaf stage appears to be most suitable for inoculation to evaluate the efficacy of new chemical compounds.

In this study some of plants were not infected, while other plants were severely infected under the same inoculation. One of the reason for unsuccessful infections is thought to be due to the failure of diploid infection hyphae formation which resulted from the fusion of compatible haploid sporidia after foliar spray of sporidial suspension as reported by some workers (4). In fact, in this study most variations in smut development between replications originated from this phenomenon. One way to reduce this variation is to increase number of replications. This study suggests that the number of replications has to be at least five or more.

Based on the results of this study, most successful corn smut development could be obtained by foliar spray with sporidial suspension at the concentrations of

10^3~10^5 sporidia/ml on the 4th leaf stage of seedlings, and subsequently incubated in a dew chamber at 28~30°C for 24 hr before placing them in the greenhouse.

요 약

옥수수 유묘접종시 깜부기병이 가장 잘 발병하는 조건은 4엽기의 옥수수묘를 10^3~10^5/ml 농도의 소생자 현탁액으로 경엽살포한 후 28~30°C의 습실상에 24시간 둔 후 꺼내어 온실에 두었을 때였다. 유묘의 엽기가 이보다 어리거나 접종원 농도가 이보다 높을 때, 혹은 습실상내 처리기간이 길어지면 접종후 유묘의 이른 고사를 초래하였다. 또한 습실상의 온도가 낮아질수록 깜부기병의 발병정도도 감소하였다. 깜부기병의 발생은 소생자 현탁액의 경엽살포 및 줄기주사접종에 의해서는 가능하였지만 토양관주에 의해서는 발병하지 않았다. 소생자 현탁액은 별도의 영양원의 첨가 없이도 발병을 잘 유도하였다. 동포자 현탁액을 직접 경엽살포하거나 줄기주사접종하여도 발병하였으나 소생자 현탁액접종에 비하여 발병정도가 현저히 낮았다.

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