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Significance of Semame Seedborne Fungi, with special Reference to *Corynespora cassiicola*

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참깨의 種子傳染性 眞菌과 그 病原性 : *Corynespora cassiicola*를 中心으로

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Abstract

Alternaria sesami, *A. sesamicola*, *A. tenuis*, *A. longissima*, *Cercospora sesami*, *Cephalosporium* sp., *Corynespora cassiicola*, *Fusarium equiseti*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina* and *Myrothecium roridum* were detected from 40 seed samples of sesame. *A. sesami*, *A. sesamicola*, *A. tenuis* and *C. cassiicola* were the predominant fungi. Except *C. cassiicola*, all fungi were almost completly reduced and wiped out the infection by pretreatment with chlorine. Plating components also indicate that *C. cassiicola* was well-established infections. Seedborne infection of *C. cassiicola* caused heavy seed rot and seedling mortality.

Detailed description has been given on the habit character of *C. cassiicola* under stereoscopic microscope and the variation in colony character and spore morphology have been taken into account.

In inoculation experiments, *C. cassiicola* produced severe leaf and stem spots and blights on sesame plants resulted in ultimate death of the plants. *A. sesami*, *A. sesamicola*, *A. longissima* and *C. sesami* also produced mild to severe leaf spotting and leaf blight when suspension of their conidia were sprayed on to plants. In soil inoculation experiments, *F. oxysporum* and *M. phaseolina* were the most pathogenic causing seed rot and seedling blight.

Introduction

Sesame (*Sesamum indicum* L.) is an important oil crop in Korea. An increasing demand for sesame products is a stimulating factor for both the breeder and grower to seek higher yields. Diseases, however, constitute a limiting factor. So far, about nine sesame diseases have been reported in Korea,[7,17] but little is known regarding fungi associated with its seed and their role in causing diseases.

Corynespora blight, caused by *Corynespora cassiicola* (Berk. & Curt.) Wei. has been a prevalent disease of sesame in south-eastern United States[2] since it was identified by Stone and Jones.[14] They

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found that the pathogen was very virulent at seedling stage and indicated that the pathogen was carried both on and within sesame seed. Mathur and Kabeere[9] detected the pathogen in one seed sample of sesame from Uganda. So far, the seedborne nature of *C. cassiicola* has not been studied for any crops.

In this investigation forty seed samples of sesame collected from Crop Experiment Station, Chungnam Provincial Office of Rural Development, farmers and grain merchants in Korea were examined for seedborne fungi. Observations made on the habit characters of *Corynespora cassiicola* growing on seeds incubated on wet blotters. Variations in colony character and conidial morphology of the fungus are described in this paper. Inoculation experiments of fungi detected were conducted to study their significance in causing diseases.

Materials and Methods

Forty seed samples were tested for fungi by the standard blotter method. Two hundred untreated seeds from each sample were sown on moist blotters in plastic petri dishes at the rate of 25 seeds per dish, and incubated for seven days under 12 hours alternating cycles of near ultraviolet light (NUV) and darkness at 22±2°C. Blotter test with chlorine pre-treatment was made by soaking seed for five minutes in 2% sodium hypochloride solution.

Location of pathogens in seed.

Seeds of three samples were washed individually in sterilized water and soaked in distilled water for 4 hours. The soaked seeds were dissected aseptically with a pair of sterilized needles under stereoscopic microscope to seperate seed coat, endosperm and embryo. Each component was again rinsed in 1% chlorine water and plated directly on water soaked blotters in dishes. Different parts of a seed plated in one dish, quite apart from each other. After seven days of incubation under NUV/darkness cycles at 22±2°C, each component was examined under stereomicroscope for the presence of fungi.

Growing in soil.

Seeds of three samples having heavy, moderate and low infection of *Corynespora cassiicola* were sown in pots, and emergence and survival of seedlings were recorded.

Cultural characters of isolates of Corynespora cassiicola.

Seven single spore isolates were maintained on PDA. Observations on different colony characters were taken after 8 days of incubation at 27±1°C in 12 hours alternating cycles of NUV light and darkness.

Spore measurements of isolates of Corynespora cassiicola.

Fifty conidiophores and conidia from infected seeds and eight days old growth on PDA were measured for each isolate in lactophenol. The width of conidia was measured at the widest point and length from tips to hilum.

Pathogenicity tests.

Inoculation experiments with different fungi were carried out in two ways, i.e. by leaf inoculation and by soil inoculation.

For testing pathogenicity of *C. cassiicola*, five isolates which showed differences in cultural characters were tested on sesame plants. Spore suspension for spraying plants was made following the technique of Stone and Jones.[14] Other fungi included in the leaf inoculations were *Alternaria sesami*, *A. sesamicola*, *A. tenuis*, *A. longissima* and *Cercospora sesami*. Plants raised from surface disinfected seed of cv. Suweon #9 and Suweon # 26 were sprayed with spore suspension of different isolates, first when the plants were one month old and again when they were two months old. After inoculation the plants were kept for two days in a humid plastic bag. Check plants were sprayed with distilled water only.

For soil inoculation of *Fusarium* spp., the cultures were produced on autoclaved rice for a week, before they were introduced into the soil in pots. Surface disinfected seeds were sown in soil already inoculated with the fungus tested Pathogenicity of *Macrophomina phaseolina* was determined by the techniques of Thirumalachar et al.[15] All inoculation experiments were conducted in greenhouse, temperature ranging between 25~30°C.

Results

1. Occurrence of fungi

Thirteen species of fungi were observed from 40 seed samples of sesame tested. The number of samples infected with fungi and the ranges of infection are given in Table 1. *Alternaria sesami*, *A. sesamicola*, *A. tenuis* and *Corynespora cassiicola* were found in 26, 35, 38 and 31 samples out of 40, respectively. While *Fusarium* spp. were present only in a few samples with low infection.

Three different samples were used for comparative observations of untreated and pretreated seeds in the blotter test (Table 2). All fungi appeared in high counts on untreated seeds. The chlorine pretreatment almost completly reduced and wiped out the infections of *Alternaria* spp., *Fusarium* spp. and *Macrophomina phaseolina*. The chlorine pretreatment also reduced the infection of *C. cassiicola*, but percentage reductions were not strikingly evident.

Table 1. Percentage infection of fungi recorded in 40 samples of sesame seed tested by the blotter method.

Fungi	Number of samples infected	% incidence	
		range	average
Alternaria sesami	26	1.0～35.5	6.4
A. sesamicola	35	4.0～79.5	37.8
A. tenuis	38	2.0～40.0	18.0
A. longissima	17	1.0～20.5	4.7
Cercospora sesami	6	2.5～28.0	6.6
Cephalosporium sp.	3	0.5～ 2.0	0.8
Corynespora cassiicola	31	1.0～62.0	19.3
Fusarium equiseti	5	0.5～ 5.0	0.7
F. moniliforme	9	1.0～19.0	1.9
F. oxysporum	3	0.5～ 2.5	0.6
F. semitectum	4	0.5～ 5.5	1.5
Macrophomina phaseolina	15	0.5～ 8.5	2.9
Myrothecium roridum	3	0.5～ 1.0	0.6

Table 2. Percentage infection of fungi in three samples of sesame in blotter tests with or without chlorine pretreatment.

Fungi	Untreated seed			Pretreated seed		
	Sample number					
	1	2	3	1	2	3
Alternaria sesami	1.0	3.0	0	0	0	0
A. sesamicola	65.0	12.0	29.5	5.5	0	1.0
A. tenuis	8.0	7.0	12.0	0	0	1.5
A. longissima	1.0	0	0	0	0	0
Corynespora cassiicola	24.0	48.0	3.0	10.0	17.5	0
Fusarium equiseti	0	0	1.0	0	0	0
F. moniliforme	0.5	0	0	0	0	0
F. semitectum	1.5	0	0	0	0	0
Macrophomina phaseolina	0.5	0	1.0	0	0	0

2. Location of fungi

Fungi recovered from different parts of the seed are given in Table 3. Most fungi were recovered more frequently from the testa, and decreasing amounts of infection were observed in the endosperm. In the embryo only *C. cassiicola* was recovered in two of the three samples.

Since the seeds infected with *C. cassiicola* showed extremely poor germination on blotters and the pathogen was thought to be strongly associated with seed, the fungus looked more important than others. Pre- and post-emergence losses of three samples and infection percentages of *C. cassiicola* are give in Table 4. Seedborne infection of the fungus caused heavy seed rot and seedling mortality.

3. Growth habit and cultural characters of *Corynespora cassiicola*

(1) Growth habit: On seed, colonies apeared hairy, having greyish, pale brown, to olive brown colour. In heavily infected seeds, growth of the pathogen on seed was even visible to the naked eye and such seeds usually did not show any germination (Fig. 1, 2). In case of light infection the seed germinated and the developing roots and shoots turned brown and dead and profuse sporulation of the pathogen was observed in these roots and shoots as the incubation period was prolonged (Fig. 3).

The conidiophores were brown to dark brown, with successive cylindrical proliferations bearing conidia solitarily or in short chains, apically and

Table 3. Percentage recovery of fungi from different seed parts of three samples of sesame. 100 seeds were tested per sample.

Sample number	Fungi	Testa	Endosperm	Embryo
1	*Alternaria sesami*	1.0	0.5	0
	A. sesamicola	68.0	60.0	0
	A. tenuis	8.0	6.0	0
	Corynespora cassiicola	26.0	24.0	6.0
2	*A. sesami*	2.0	1.0	0
	A. sesamicola	12.0	15.0	0
	A. tenuis	8.0	8.0	0
	C. cassiicola	52.0	48.0	18.0
3	*A. sesamicola*	30.0	22.0	0
	A. tenuis	12.0	14.0	0
	C. cassiicola	2.0	2.0	0
	Macrophomina phaseolina	1.0	1.0	0

Table 4. Infection of *Corynespora cassiicola* and pre- and post-emergence losses observed in three samples of sesame seed, when lots were directly sown in soil. 200 seeds were sown in each sample.

Sample number	Percentage incidence of *C. cassiicola*	Percentage loss		Total loss (%)
		Pre-emergence	Post-emergence	
1	24.0	15	21	36
2	48.0	27	20	47
3	3.0	4	5	9

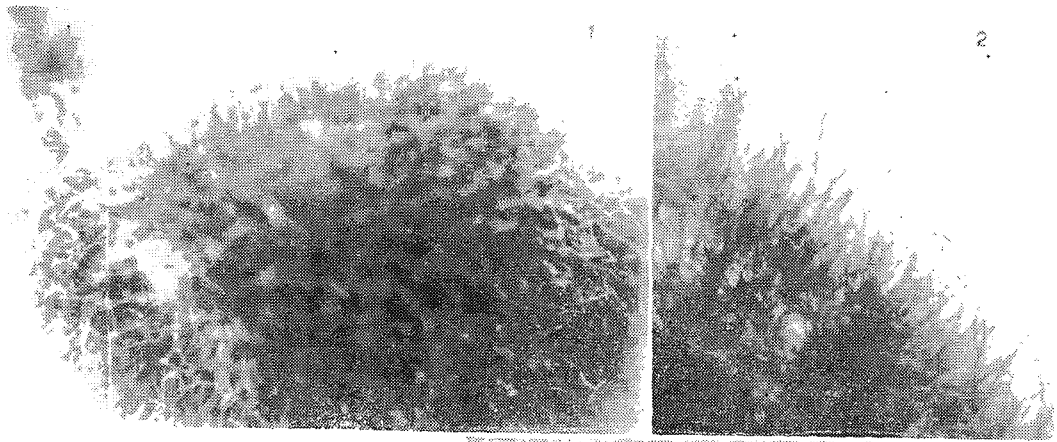

Fig. 1. Heavy growth of *C. cassiicola* on sesame seed. (25X)

Fig. 2. A portion of seed magnified to show long conidiophores and conidia. (40X)

F.g. 3. Germinating seed showing growth of *C. cassiicola* on seedcoat and radicle. (15X)

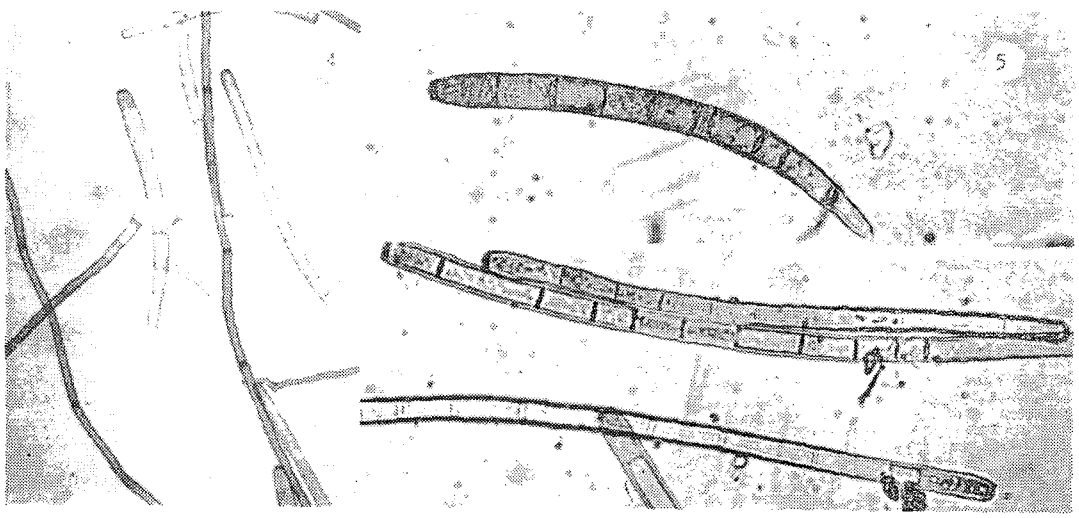

Fig. 4. Conidiophores and conidia of *C. cassiicola*. (200X)

Fig. 5. Conidia of *C. cassiicola*. (400X)

laterally (Fig. 4). Conidia were obclavate to cylindrical, straight or curved with septa or pseudosepta (Fig. 4,5). The conidia, which are subhyaline to rather pale brown or olivaceous brown, had a dark basal hilum or truncate base.

(2) Cultural characters: On PDA, two types of colonies were observed.

Type A (Isolate C-A-1, C-A-2) : Colonies were velvety or felted, grey or dark blackish brown in colour, abundant sporulation.

Type B (Isolate C-B-1, C-B-2, C-B-3, C-B-4, C-B-5) : Colonies were wooly, grey or grayish brown to olive brown in colour, abundant aerial mycelium and poor sporulation.

(3) Spore measurements: Much variation in size of conidiophores and conidia within the same isolate was noticed. From the seed, the length and width of conidiophores varied from 66 to 1354μ and 4 to 11μ, respectively, and the same measurements for conidia varied from 42 to 450μ, and 4 to 19μ, respectively. In culture, the range in the length and width of conidia was 22~380μ and 4~19μ, respectively.

4. **Pathogenicity tests.** The results of the infection experiments are summarized in Table 5.

(1) Leaf inoculations

Corynespora cassiicola: All the five isolates tested produced brown, irregular leaf lesions or brick-red leaf spots, usually within 3 to 4 days after inoculation. Leaf lesions developed purple or reddish-brown margins and centers blanched with age. Such lesions appearing scorched or form bigger irregular-shaped, concentrically zoned, light brown lesions which later coalesce and caused defoliation. Stem lesions were reddish-brown streaks, sometimes spread over the entire stems causing death of the plants. There were no marked differences in pathogenicities among the isolates to sesame plants.

Alternaria sesami: The first symtoms were observed 3 to 4 days after inoculation. Lesions on the leaves were produced in the form of dark brown to black, round or irregular, often zonate spots. Such spots gradually were increased in size, resulting in complete leaf blight. Stem lesions were produced on plants in the form of dark brown spots and streaks.

A. sesamicola: The first symptoms were observed 4 to 5 days after inoculation. The spots on the leaves were light brown to mid brown, circular to irregular, often zonate. Such spots gradually were increased in size up to 1.5cm in diameter.

A. longissima: The lesions on the leaves were similar to those of *A. sesamicola*, but they developed faster, resulting in blight and drying up of plants.

Table 5. Fungi isolated from sesame seed and their pathogenicity to sesame plants and seedlings.

Fungi	Pathogenicity[a]
Alternaria sesami	+(3)[b]
A. sesamicola	+(2), ±(1)
A. tenuis	−(3)
A. longissima	+(3)
Cephalosporium sp.	n.t.[c]
Cercospora sesami	+(1)
Corynespora cassiicola	+(5)
Fusarium equiseti	−(2)
F. moniliforme	±(2)
F. oxysporum	+(2)
F. semitectum	−(2)
Macrophomina phaseolina	+(3)
Myrothecium roridum	n.t.

[a] +, ±, − : pathogenic, weakly pathogenic, not pathogenic, respectively.
[b] Figures in parenthesis indicate number of isolates used for pathogenicity test.
[c] n.t.: not tested.

Cercospora sesami: On leaves, it caused small, whitish or pale brown spots with dark borders.

(2) Soil inoculations.

Fusarium moniliforme: In soil inoculation tests, this species reduced germination to about 10~15 per cent, but seedlings remained healthy.

F. oxysporum: There was about 45 per cent reduction in the germination of the seeds in the infested soil and the seedlings remained stunting.

F. equiseti, F. semitectum: Almost all the seeds sown in the inoculated soil produced normal, healthy seedlings.

Macrophomina phaseolina: The young cultures of isolates were highly pathogenic causing damping-off of germinating sesame seeds but old cultures bearing microsclerotia did not readily bring about infection.

Discussion

Using incubation techniques, forty seed samples of sesame tested were found infected by about 13

fungi and a number of which are known to be seed-transmitted in the crop[8,9,11,14,16,17]. *Alternaria sesami*, *A. sesamicola*, *A. tenuis* and *Corynespora cassiicola* were the predominant fungi but the frequence and infection per cent of the others were low.

Association of *C. cassiicola* with seeds of sesame was reported earlier by Stone & Jones[14] and Mathur & Kabere[9]. In the present investigation, the fungus was found in 31 samples out of 40. The results of chlorine pretreatment and component plating indicate that the pathogen may invade all parts of seed. The pathogen is capable of affecting the seed health adversely. Seeds showing heavy infection show initial germination but soon radicle and hypocotyl are invaded by the fungus. Variation was observed in colony characters and spore morphology, but the culture type was not related to pathogenicity.

C. cassiicola has been reported to cause leaf spots and blights, premature defoliation and death in sesame[2,14] and also cause target spots in soybean, cowpea[12] and cotton[5]. In the present inoculation experiment, all isolates used caused severe leaf and stem spots and blights on sesame plants. In USA, Corynespora blight has been a prevelent disease of sesame and the disease usually appears and rapidly reaches epiphytotic conditions as plants approach physiological maturity under field conditions[2].

Survey of the sesame crop in the cultivators fields was conducted in August and September in 1980 and revealed that Corynespora blight was widely spread and caused severe damage to sesame plants in most of the fields. So far, the significance of this pathogen has not been known for other crops in Korea.

Considering the frequency and high incidence of this pathogen in sesame seed lots and the severe losses due to the fungus in the field, the damage resulting from seedborne infection of *C. cassiicola* should be reduced by seed treatment and use of disease-free seed.

Alternaria sesami was known to be seed-transmitted in the crop and produced severe losses in various countries.[1,4,6,8,9,10,16] But *A. sesamicola* which was found in high counts in the Korean samples has never been reported from sesame seeds in other countries. In the inoculation experiment, *A. sesami* gave more severe symptoms on plants than those caused by *A. sesamicola*, but both of them showed seed and seedling rot in the blotter test. *A. longissima* which was found in low counts also gave severe symptoms on plants. Taxonomy and pathogenicity of these species of *Alternaria* from sesame seed has been described earlier[16].

Of the species of *Fusarium* recorded, only *F. oxysporum* was known to cause wilt and damping off in the field[13]. *F. moniliforme*, *F. equiseti* and *F. semitectum* are known as seedborne in a number of crops[11]. In soil inoculation tests, *F. moniliforme* reduced seed germination by about 10~15 per cent. *F. oxysporum* was found to be the most important since this species reduced seed germination by about 45 per cent and some seedlings remained stunted. Occurrence of *Macrophomina phaseolina* in Korean seed samples of sesame and its significance was reported earlier by author[17].

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적 요

작물시험장, 충남농촌진흥원 및 일반농가에서 수집한 40개 참깨종자표본의 종자전염진균을 Blotter 법으로 조사하였던 바 *Alternaria sesami*, *A. sesamicola*, *A. tenuis*, *A. longissima*, *Cercospora sesami*, *Cephalosporium* sp., *Corynespora cassiicola*, *Fusarium equiseti*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina* 그리고 *Myrothecium roridum*이 검출되었다. 그 중 *A. sesami*, *A. sesamicola*, *A. tenuis*, *C. cassiicola*가 많은 비율로 검출되었다. 참깨 종자내의 감염부위를 알아본 결과, *C. cassiicola*가 가장 깊숙히 배에까지 감염되어 있었으며 Chlorine으로 종자표면소독을 하였을 경우에도 완전히 제거되지 않았다. 이균에 이병된 종자를 살균토양에 파종하였을 경우 심한 발아지해와 유묘고사를 초래하였다.

*C. cassiicola*의 종자상에서의 생육상과 배양기상에서의 배양적성질 및 포자의 형태를 기술하였다.

병원성을 조사하기 위하여 배양균의 포자현탁액을 참깨에 분무접종한 결과 *C. cassiicola*는 참깨의 잎과 줄기에 암갈색 또는 자색의 병반을 나타내며 이것이 급격히 확대되어 고사하였다. *A. sesami*, *A. sesamicola*, *A. longissima*, *C. sesami*도 참깨 잎에 반점 또는 blight의 병징을 나타내었으나 *A. tenuis*은 병원성이 없었다. 토양접종실험의 결과 *F. oxysporum*과 *M. phaseolina*는 강한 병원성을 나타내어 심한 종자부패와 유묘고사를 초래하였다.