市販のインゲン種子から分離された糸状菌とその病原性

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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巻/号	38巻2号
掲載ページ	p. 111-116
発行年月	1972年3月

日植病報 38: 111-116 (1972)
Ann. Phytopath. Soc. Japan 38: 111-116 (1972)

Fungi Associated with Commercial Kidney Bean Seed and their Pathogenicity to Young Seedlings of Kidney Bean

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渡辺恒雄*：市販のインゲン種子から分離された糸状菌とその病原性

Abstract

Eighteen to 23% of commercial kidney bean seeds tested failed to emerge, and 7.5–14.5% of them produced diseased seedlings when seeded in pasteurized soil. *Rhizoctonia solani* or *Colletotrichum lindemuthianum* were isolated from these diseased seedlings. Fungi associated with 3 different lots of surface-sterilized commercial kidney bean seeds (variety Topcrop) were examined to study fungal flora of these seeds.

A total of 1,036 fungal isolates obtained from 6,390 seeds of these 3 seed lots were classified into 24 genera. The major 5 genera were *Alternaria, Fusarium, Colletotrichum, Chaetomium,* and *Rhizoctonia,* making up 86% of the total isolates.

Kidney bean plants became diseased in the pasteurized soil artificially infested with *Colletotrichum lindemuthianum, Rhizoctonia solani* or *Macrophomina phaseoli* isolated from these seeds. *Fusarium oxysporum* showed very weak pathogenicity.

One per 22–41 commercial kidney bean seeds was infected with at least one of the aforementioned pathogenic fungi. (Received September 17, 1971)

Significance of seed-borne pathogens, toxicogenic fungi from seed and deleterious effect of seed-contaminated fungi on seed quality and seedling vigor have been discussed[1,3,8]. Microflora of seed of some crops have been clarified in relation to those works[2,4,5,6,7].

In this investigation, fungi isolated from commercial kidney bean seed of the local product and microflora were studied in order to examine plant pathogens among them.

Materials and methods

Kidney bean seed (variety Topcrop) of 3 different seed lots produced in 3 different areas of Nagano prefecture were clean and visually healthy. The first lot of seed was harvested in December, 1968, and the second and the third in September to December, 1970.

Rates of germination and fungal and bacterial contamination of these seed samples were analyzed by placing some seeds on potato dextrose agar (PDA) (5 per plate) and incubating at 26°C for 3 days. Seeds were previously washed by running tap water over 1 hr, treated with 1% sodium hypochlorite solution for 3 min, and rinsed more than 3 times with sterilized water. A total of 1,910 seeds of the 2nd lot, and 3,570 of the 3rd lot were examined in 4 and 9 separate experiments, 2–9 months after harvest and within 3 months after purchase, respectively. In a sample of the, first seed lot, 400 or 510 seeds were tested in 4 separate experiments within 8 months and 29 months after harvest, respectively.

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Fungi were generally isolated by cutting single hyphal tips elongated onto PDA plate from the seeds tested, and identified directly or in growing them in some conventional laboratory media.

For the pathogenicity tests, inoculum was prepared by growing a fungus isolate in 20 m*l* of potato dextrose broth in stationary culture for 10 days at 26°C and harvesting the mycelium-spore mat by draining-off the cultural filtrate. The inoculum was placed on pasteurized soil in a 15 cm clay pot, and 4 certified seeds were placed around the inoculum without direct contact each other. These seeds and the inoculum were covered in 2 cm-depth with pasteurized soil. The certified seeds indicate visually microbes-free germinated seeds in 3 days after incubation on PDA. As a control, certified seeds were planted in noninfested pasteurized soil. In one experiment, 12 seeds per isolate were tested under the greenhouse condition, and experiments were repeated 2–8 times.

A total of 89 isolates listed in Table 1 were tested for the pathogenicity to kidney bean plants in the young seedling stage. Twenty days after seeding, inoculated plants were rogued, and fungi from the diseased plants were isolated on PDA after surface-sterilization. The temperature in the greenhouse was 25±7°C. Plants were watered once a day.

Results

Emergence rate and suspected seed-borne pathogens A total of 120 or 130 of the respective nonsterilized or surface-sterilized seeds of the 3rd lot were seeded in pasteurized soil in 2 separate experiments. Ten days after seeding, about 77–82% of nonsterilized seeds emerged, whereas 86–88% of surface-sterilized seeds emerged. The rate of disease incidence in 15 days old seedlings of nonsterilized and sterilized seeds were 13.3 and 7.5–14.0%, respectively (Table 1). Their symptoms were damping-off, blight, wilt or abnormal growth. From the diseased plants, *Rhizoctonia solani*, *Colletotrichum lindemuthianum*, and *Botrytis* sp. were isolated, and these fungi were suspected to be the causes of the diseases.

Table 1. Rate of emergence and disease incidence of commercial kidney bean seed in pasteurized soil

Treatment	Seed no.	Emergence %	Disease incidence %
Unsterilized	60	81.7	13.3
	60	76.7	13.3
Sterilized	80	87.5	7.5
	50	86.0	14.5

Table 2. Rate of germination, and bacterial and fungal contamination of commercial kidney bean seed on PDA after 3 days incubation at 26°C

Seed lot	Time after harvest month	Seed no.	Rate: Germination %	Rate: Bacteria %	Rate: Fungi %
1	8	400	71.6	—	76.5
	29	510	17.6	53.3	1.0
2	6–9	1,910	78.6	31.0	20.3
3	2–6	3,570	73.8	9.4	7.0

Rates of germination, and fungal and bacterial contamination Rate of germination of kidney bean seeds of the 2nd and 3rd lot was 74.5–86.5% (average 78.6%) and 65.2–85.4% (73.8%), respectively. The seed in the 1st seed lot germinated 71.6% within 6 months after harvest, whereas 17.6% of the seed germinated 29 months after harvest (Table 2).

Rates of fungal contamination of 2nd, and 3rd lot were 20.3 and 7.0%, and bacterial contamination 31.0 and 9.4%, respectively. In the 1st seed lot, fungi were isolated from 76.5% of 400 seeds tested within 6 months after harvest, whereas only 1.0% of seed were contaminated with fungi, and

53.3% of seed with bacteria after 29 months after harvest.

Fungi isolated from the surface-sterilized seed A total of 1,036 isolates of fungi, 311, 391, or 334 isolates from the respective 1st, 2nd, or 3rd seed lot were isolated, and among them were 122 isolates of Ascomycetes, 1 of Phycomycetes, and the rest of Fungi Imperfecti. They were classified into 24 genera. The most frequently isolated 5 genera were *Alternaria, Fusarium, Colletotrichum, Chaetomium,* and *Rhizoctonia*. These 5 genera made up 25.7, 25.4, 18.4, 11.8 and 6.2% of the total isolates, respectively (Table 3).

Table 3. Fungi isolated from surface-sterilized kidney bean seed and their pathogenicity to kidney bean seedlings

Fungus	Number of isolates				Pathogenicity[a]
	Seed lot			Total	
	1	2	3		
Alternaria tenuis Nees. (Gray type)	52	76	5	133	−(9)[b]
A. tenuis Nees. (Black type)		133		133	−(2)
Arthrinium sp.			1	1	n.t.[c]
Aspergillus niger van Tieghem			10	10	−(1)
Aspergillus spp.			17	17	−(4)
Botrytis sp.		2	2	4	−(1)
Cephalosporium sp.		1	1	2	−(1)
Chaetomium aterrimum Ellis & Everhart			1	1	−(1)
C. aureum Chivers		2		2	n.t.
C. brasiliense Batista & Pontual			8	8	−(1)
C. cochliodes Palliser	1		6	7	−(2)
C. funicolum Cooke		26	11	37	−(5)
C. dolichotrichum Ames		1	2	3	−(1)
C. globosum Kunze	8	10	37	55	−(4)
C. indicum Corda		6	1	7	−(1)
C. spirale Zopf			2	2	−(2)
Cladosporium sp.		2	2	4	−(1)
Colletotrichum cocodes (Wallr.) Hughes			1	1	−(1)
C. dematium (Pers, ex Fr.) Grove	19	1		20	−(4)
C. lindemuthianum Briosi & Cavara		8	161	169	+(6)
Colletotrichum sp.		1		1	n.t.
Epicocum nigrum Link.		1		1	−(1)
Fusarium oxysporum (Sheld.) emend. Snyd. & Hans.	118	8		126	±(4)
F. roseum (Lk.) emend. Snyd. & Hans.	23	48	12	83	−(6)
F. solani (Mart.) Appel & Wr. emend. Snyd. & Hans.	53	1		54	−(1)
Glomerella glycines (Hori) Lehman & Wolf		1		1	−(1)
Lacellina macrospora Ellis		12		12	−(5)
Macrophomina phaseoli (Maubl.) Ashby	13	1		14	+(5)
Monocillium indicum Saksena		1	1	2	−(2)
Myrothecium verrucaria Ditmar			3	3	−(1)

Paecilomyces sp.	3			3	n.t.
Penicillium spp.	2	7	34	43	−(4)
Phoma sp.		2	5	7	−(5)
Phomopsis sp.		1		1	n.t.
Rhizoctonia solani Kühn (White type)	18	22	8	48	+(4)
R. solani Kühn (Brown type)		16		16	+(1)
Rhizopus oryzae Went & Geerlings			1	1	−(1)
Trichoderma viride Pers.	1		1	2	−(1)
Trichothecium roseum Link		1		1	n.t.
Ulocladium botrytis Preuss			1	1	n.t.
Total	311	391	334	1,036	

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

Some 200 nonsterilized seeds of each seed lot were also examined for the contaminated fungi. The fungi found but not listed in Table 3 were *Torula* sp. and *Mucor* sp., while others were identical with the isolates from the surface-sterilized seeds.

Pathogenicity of the isolates from seed Most of isolates tested have not caused any disease symptom onto kidney bean seedlings within 20 days after seeding under the greenhouse conditions at least in 2 separate experiments. Some of certified seeds grown in noninfested soil became diseased, and the causes of the diseases were ascribed to *Colletotrichum lindemuthianum* or *Rhizoctonia solani*, which was probably originally contaminated in the certified seeds which could not have been detected during 3 days certification period.

In the inoculation experiment, isolates of *R. solani*, and *C. lindemuthianum* tested were proved to be pathogenic to kidney bean seedlings (Table 4). One isolate of *Fusarium oxysporum* was weakly pathogenic. Pathogenicity of *Macrophomina phaseoli* has been already demonstrated elsewhere[9].

Table 4. Pathogenicity of *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, and *Rhizoctonia solani* to kidney bean seedlings

Fungus	Isolate no.	No. diseased plants / No. plants tested				Avg. %	Reisolation %
Colletotrichum lindemuthianum	1	8/8	7/8	3/20		50.0	55.5
	25	8/8	8/8	8/20		66.7	55.5
	48	8/8	4/8	9/20		58.3	38.1
Fusarium oxysporum	54	3/8	2/8	12/30	3/8		
		0/8	6/20			36.1	86.7
Rhizoctonia solani	17	4/10	11/25	8/8	15/20		
(White type)		2/10	4/15	8/8	16/20	58.6	100.0
	62	8/8	15/20			82.1	86.4
Rhizoctonia solani (Brown type)	53	8/8	16/20			85.7	95.8
Control		0/10	0/15	3/8	2/8		
		3/20	0/20	1/20	0/20	7.4	44.4[a]

a) *Rhizoctonia solani* or *Colletotrichum lindemuthianum* was reisolated.

Discussion

Fungi isolated from the surface-sterilized seed were thought to be strongly associated with seed, but just not the temporary air-contaminants. Most of these fungi are common soil-inhabitants, and so-called field fungi. These fungi are also very common as members of microfloras of other seed[4,5,6,7].

Microfloras of kidney bean seed in 3 different seed lots tested were a little different, showing a certain characteristic; *Fusarium, Alternaria* or *Colletotrichum* was isolated most frequently from the 1st, 2nd, or 3rd seed lot respectively. As for the plant pathogens, the seed in the 1st seed lot were rather frequently associated with *R. solani* (white type), and *Macrophomina phaseoli,* the 2nd with *R. solani* (both white and brown type), and the 3rd with *C. lindemuthianum.*

Twenty-nine months after harvest, *Macrophomina phaseoli, Fusarium oxysporum, Chaetomium cochliodes,* and *Penicillium* spp. were isolated from the 1st seed lot, and over 50% of seed were contaminated with bacteria. This result is consistent with Kurata's observation that aged seed were more frequently associated with bacteria[7], and less with fungi.

Most of seed-contaminated fungi were demonstrated not to be pathogenic even under rather heavy inoculum infestation although they may cause more or less slight damage *in vitro.* If tested in different methods or conditions, or onto different hosts, some of them may be pathogenic.

One isolate of *Fusarium oxysporum* caused weak discoloration and streak lesions on the lower hypocotyles of kidney bean seedlings, and from the diseased tissues, the fungus was rather consistently reisolated, but further work is needed to confirm its pathogenicity (Table 4).

If all isolates of *C. lindemuthianum, R. solani,* and *M. phaseoli* were assumed to be pathogenic, these pathogenic fungi may make up 10.0,12.0, and 50.6% of the respective of the total isolates of 1st, 2nd, or 3rd seed lot. As all fungi were isolated more or less at the rate of 1 isolate per seed, 1 seed per 30, 41 and 22 seeds of the respective 1st, 2nd and 3rd seed lot may be assumed to harbor at least one of the aforementioned plant pathogens.

The results in this investigation may present additional data on microflora of kidney bean seed, and significance of plant pathogens among them.

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和文摘要

市販のインゲン種子から分離された糸状菌とその病原性

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市販のインゲン種子（品種，トップクロップ）を低温殺菌した土壌（70℃で1時間蒸気殺菌）に播種したところ18-23%が発芽せず，また発芽した子苗の約 7.5-14.5% が何らかの病徴を示した。その発病株からは *Colletotrichum lindemuthianum*, や *Rhizoctonia solani* などの病原菌が分離された。そこで長野県産で採種圃の異なる3群の市販の種子を表面殺菌後PDA培地上に置いて糸状菌を分離し，その菌相を調べた。これらの3群の種子からは合計1,036菌株を分離したが，それらは24属に分類された。おもな属は *Alternaria*, *Fusarium*, *Colletotrichum*, *Chaetomium*, *Rhizoctonia* で，これらは全分離菌株数の約86%を占めた。

また種子から分離した合計89菌株を土壌に接種して，インゲンに対して病原性試験を行なったところ，強い病原性を示したのは *Colletotrichum lindemuthianum*, *Macrophomina phaseoli*, *Rhizoctonia solani* の3種であった。供試した市販の種子は22-41粒につき1粒の割合で上記の病原菌のいずれかによって汚染されていた。