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Mating Types of *Phytophthora capsici* Leonian from Red-pepper (*Capsicum annuum* L.) in Korea

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고추疫病菌(*Phytophthora capsici* Leonian)의 配偶子型 分布

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ABSTRACT: Each of 103 isolates of *Phytophthora capsici* was obtained from diseased red pepper plants randomly belonged to either the mating type A_1 or the mating type A_2. Fifty four isolates were classified as mating type A_1, and 49 isolates were classified as mating type A_2. Oospores were formed in each combination of isolates between A_1 or A_2 on 5% V-8 juice agar except one combination.

KEYWORDS: *Phytophthora capsici*, Mating type, Oospores.

The Pytophthora blight of red pepper has been one of the main destructor for red pepper production in Korea. The Phytophthora blight occurred 50% in average in 12 fields among 50 fields investigated at the main red pepper production area: Euiseong, Jungweon and Imshil in Korea(Lee *et al*; 1987).

Phytophthora capsici was known as one of heterothalic *Phytophthora* species which has two mating types A_1 and A_2(Ribeiro *et al.*; 1975). Savage(1968) and Kamjaipai *et al.*(1978) reported oospore formation by the pair-culturing of the mating type A_1 and A_2. Oospore formation was also reported between different species such as *P. capsici* and *P. arecae, P. cinamomi*(Savage *et al.*; 1968).

Oospores play an important role as overwintering structure and primary inoculum source. Since Korean farmers cultivate the red pepper continuously in their field, increased primary inocula were suggested to attribute to epidemics of the *Phytophthora* blight. Vulnerability of some red pepper resistant to *P. capsici* also suggested that pathogenic variation might be due to frequent oospore formation possibly pathogenically different mating types in farmers fields. This study aimed to determine the mating types and their frequency of *P. capsici*.

Materials and Methods

Isolation of *P. capsici:* Diseased plants were collected from Euiseong in Kyeongbuk province, Geumsan in Kyeongnam province, Imshil in Jeonbuk province, Kwangsan in Jeonnam province, Jungweon and Eumseong in Chungbuk province, Cheonan in Chungnam province; Suwon and Anseong in Kyeonggi province. Diseased samples were collected from the mid-May through the early October. Diseased samples included plants showing wilting symptom only, discolaration on the base of stem with root rot symptom, and discoloration on the base of stem without root rot symptom. Two or three diseased red pepper plants were collected from each field. The total 103 isolates of *P. capsici* were obtained from the diseased samples: fifty eight isolates from Euiseong, 21 isolates from Imshil, 10 isolates from Jungweon and 14 isolates from other six areas. A piece of infected tissue was dipped in 5% sodium hypochloride for 2-3 min and blotted dry after rinsing with sterilized water. The infected tissue was placed on water agar and incubated at 27℃. After two days the mycelium tip grown on water agar was transferred to the fresh water agar for 2-3 times for pure isolation. For the isolation from old infected tissues, red pepper seedlings were used as baiting for *P. capsici* and isolation was made with newly infected tissues. Identification of *P. capsici* was made under microscope by investigation of swollen and club-like mycelium with no septa, and globose with taper sporangium. The culture on PDA showed no aerial mycelium growth. The culture was maintained on PDA. For determination of oospore formation, the 5% V-8 juice agar containing 0.2% $CaCO_3$ was prepared by filtering with three folds of filter paper. The clarified media was added with 2% Bacto agar powder.

Mating of the isolates: The mating type A_1 and A_2 were provided from Dr. Y.H.Yu, the Korean Jinseng and Tabacco Research Institute, originally obtained from Dr. W.H.Ko, University of Hawaii, USA. Each of *P. capsici* isolates was transferred to the same Petri-dish approximately 2 cm apart and then incubated for three days at 24C in dark. Presence of oospores was investigated in the zone of both isolates mycelium present under microscope. Each of ten isolates of mating type A_1 and mating type A_2 was chosen randomly for oospore formation test.

Results and Discussion

Both mating type A_1 and A_2 were identified based on oospore formation when 103 isolates of *P. capsici* were tested by pair-culturing on V-8 juice agar. Frequency of each mating type appears to be similar; 52.4% of the mating type A_1 and 47.6% of the mating type A_2(Table 1). Similar distribution frequency of both mating types of *P. capsici* supports that oospore is a means of continuous presence of soilborne inoculum in the Phytophthora blight of red pepper. Increase of oospore density following cultivation of red pepper without rotation might be one of the major reasons of the epidemics of Phytophthora blight especially in Euiseong area.

Number of oospores was variable depending

Table 1. Number of isolates determined as mating type A_1 and A_2 of *P. capsici* obtained from diseased samples of different areas in Korea.

Diseased samples from	No .of isolates	No. of mating type A_1	A_2
Euiseong	58	31	27
Imshil	21	10	11
Anseong	5	2	3
Jungweon	10	4	6
Kwangsan	3	2	1
Eumseong	2	1	1
Cheonan	2	2	0
Kumsan	1	1	0
Suwon	1	1	0
Total	103	54 (52.4%)	49 (47.6%)

upon each combination of the isolates between mating type A_1 and A_2(Table 2). No oospore formation was also observed in a combination between the isolate I-2 and J-2 even when repeated twice. Although oospores were reported to be produced either by matings on a semisynthetic medium(Ribeiro *et al*; 1975) or by maintaining a single zoospore culture in different condition (Savage *et al.*: 1968), our results indicated that V-8 juice agar medium was useful for oospore formation and that both mating types were present among isolates of *P. capsici* obtained from the diseased plants in the field.

Oospores of *P. capsici* were produced at the end zone where each mycelium met in pair culturing(Fig.A). The oospore was pale yellow and globular in shape, which included the humplike antheridium. The size of fully mature oospores was 32.5-35.0 μm in diameter, however, oospores at the early stage for growth of 2-3 days after mating were 26.0-28.0 μm in diameter. The average diameter of oospores was 33.75 μm for mature oospores and 27.5 μm for developing oospores. Oospores of Phytophthora species were generally 7-10 μm at the oogonial expansion stage and 20-30 μm at dense mature stage(Erwin *et al.*: 1971). The size of *P. capsici* ranged from 20.4 to 30.0 μm when the isolates from pumpkin were measured(Kamjaipai *et al.*: 1978). The size of oospores was also variable depending upon Phytophthora species and ranged

Table 2. Differences in oospore formation frequency of each combination between the mating type A_1 and A_2 of *P. capsici*.

Mating type	Mating type A_2 and oospore formation									
A_1	E-1-4	E-3-2	E-9	I-1	I-5	J-2	M-1-1	K-2	A-1-3	C-1
E-1-1	++ⓐ	+	++	++	++	+	++	+	+	+
E-2-1	++	+	++	++	++	+	++	++	++	+
E-3-2	++	++	++	++	++	+	++	++	++	+
I-2	++	+	+	+	++	−	+	+	+	+
I-7	++	+	++	++	++	+	++	+	+	+
J-7	++	+	++	++	++	+	++	++	++	+
J-4	++	+	++	++	++	+	++	++	++	+
M-2	++	+	++	++	++	+	++	++	++	+
K-1	++	+	++	++	++	+	++	++	+	+
A-1-4	++	+	++	++	++	++	++	+	++	+
S-2	++	+	++	++	++	+	++	++	++	+

ⓐ-: No oospore formation was observed; +: one to ten oospores were observed, and ++: more than 10 oospores were observed culture on V-8 juice agar per a petri-dish.

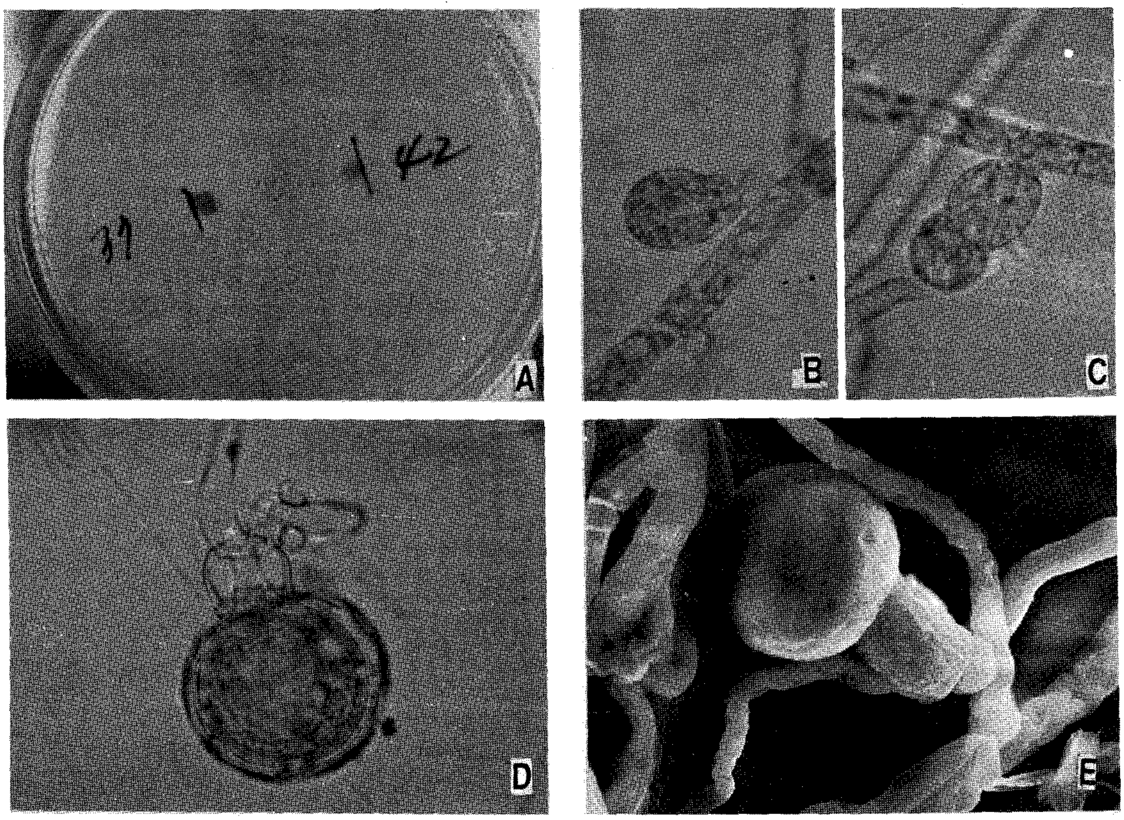

Fig A-E. Development of oospores in pair-culturing of *P. capsici* on 5% V-8 juice agar containing 0.2% $CaCO_3$(A: Pair-culturing, B and C: Oogonium formation, D: Oospore formation by fusion antheridium with oogonium, E: Oospore viewed with the Scanning electron microscope, Hitachi S-570, 25 KV, 2,000X) from 18-20 μm for *P. nicotianiae* var. *pariasitica* to 37-44 μm for *P. megasperma*(Ribeiro *et al.*: 1975).

Oospores of *P. capsici* were produced by the antheridium penetration to oogonium(Fig.B), growing through antheridium and developing into globose oogonium(Fig.C). The oospore was observed with the typical outer and inner oogonial membrane, lipid and ooplast(Fig.D). The antheridium remained like a funnel shape structure around the base of mature oogonium(Fig.E).

Yu *et al.*(1981) investigated on cultural conditions such as cultural temperatures, media for growth and sporulation for Korean isolates of *P. capsici*. The temperature 25C and pH 6.0 appeared to be proper for mycelial growth. Sporulation required for culturing under continuous fluorecent light(Yu *et al.*: 1981). For chemical control of the Phytophthora blight, Kim *et al.*(1982) tested several fungicide application showing some efficacy of the application. Since then ten fungicides were registered for control of the Phytophthora blight in pepper.

In terms of the primary inoculum sources, factors affecting survival and germination of oospores might be important in disease cycle. Erwin and McCormick(1971) reported that germination of oospores of *P. megasperma* var. *sojae* was favored around 24-27C than 15, 18 or 30C. In nature, it appears undoubtedly to be an important factor to determine what soil temperature is more conducive for germination of oospores and causing diseases early in the corp season. Kim *et al.*(1975) reported that incidence of the Phytophthora blight occurred from Mid-July in Suweon, however, Cho *et al.* (1987) observed the incidence of the disease was as early as Mid-May right after transplanting of the pepper. Therefore, further understanding on overwintering nature like oospores and possibly reduction in the primary inoculum by cultural or chemical means might contribute to establish a satisfactory control measure in the fight against the disease.

摘　要

고추疫病菌 103個菌株를 分離하여 配偶子型을 分類하였다. 分離된 菌株中 54個菌株가 mating type A_1으로, 49個菌株가 mating type A_2로 分類되었다. 서로 다른 配偶子型인 mating type A_1과 A_2菌株를 各各 5% V-8 쥬스培地에 對置培養한 結果 有性世代인 卵胞子를 形成하였다.

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