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Changes in Frequencies and Distribution of A2 Mating Type and Metalaxyl-Resistant Isolates of *Phytophthora infestans* in Korea

Young Jin Koh*, Hee Jeong Chung and William E. Fry[1]
Department of Agricultural Biology, College of Agriculture, Sunchon National University, Sunchon 540-742, Korea
[1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA

우리나라 감자 역병균 A2 교배형 및 Metalaxyl 저항성균의 빈도 및 분포의 변화

고영진* · 정희정 · W. E. Fry[1]
순천대학교 농과대학 농생물학과
[1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA

ABSTRACT : *Phytophthora infestans* populations collected from various geographical locations of Korea in 1991 and 1993 were analyzed for mating types and responses to metalaxyl. Both A1 and A2 mating type isolates were detected in 1991. The majority of the isolates were A2 mating type, but no A1 mating type was detected in 1993. About 40% of the isolates collected in 1991 were resistant to metalaxyl, and the distribution of metalaxyl-resistant isolates of *P. infestans* was strongly associated with their geographic origins in Korea. Metalaxyl-resistant isolates with EC_{50} values >50 μg/ml were collected from the northern provinces of Kangwon, Kyungbuk, and Chonbuk, but not from the southern provinces of Kyungnam, Chonnam, and Jeju in 1991. The drastic increase in the degree of quantitative resistance to metalaxyl was detected among the isolates from the southern provinces during 1991~1993. More than 50% of the isolates collected from the southern provinces of Kyungnam and Chonnam in 1993 had EC_{50} values >50 μg/ml. The province of Kangwon had isolates with the greatest resistance to metalaxyl. This alpine areas might be the origin of metalaxyl-resistant isolates of *P. infestans* in Korea. The A2 genotype with metalaxyl resistance appears to be displacing the A1 genotype which is presently the predominant genotype in Korea.

***Key words* : Phytophthora infestans, late blight, mating type, metalaxyl resistance.**

Potato late blight is remarkably explosive disease which can destroy potato fields in a few days. *Phytophthora infestans* (Mont.) de Bary, which causes late blight of potatoes and tomatoes, primarily reproduces asexually and the infection of foliage or tubers is initiated by asexual sporangia and/or zoospores. However, the fungus is heterothallic with two compatible mating types, A1 and A2. When two compatible mating types exist in the same place, they mate sexually, producing hard-hulled oospores that can survive in soil in the absence of a host (8).

Migrations and displacements of *P. infestans* populations have played an essential role in the entire history of potato late blight (10, 11, 21). In order to analyze the changes in the population structure of the fungus, characterization of populations has relied on a series of markers. Mating type is one of the useful markers investigating major changes of *P. infestans* populations. In addition to mating type, metalaxyl resistance is an agriculturally important trait that can be used as a helpful marker to characterize *P. infestans* populations.

Occurrence of A2 mating type and metalaxyl-resistant isolates of *P. infestans* was recently reported in Korea (3, 14, 15, 20), but their geographical distri-

*Corresponding author.

bution and frequencies were not investigated. The goal of this study was to investigate the frequencies and geographical distribution of A2 mating type and metalaxyl-resistant isolates of *P. infestans* and analyze the changes in the population structure of the fungus in Korea.

MATERIALS AND METHODS

Sources of isolates. Fifty-seven isolates were collected from most of the major potato growing areas in Korea. Nine isolates from Chonbuk province were obtained from Chonbuk National University, and some isolates from Kangwon province in 1991 were provided by Korea Research Institute of Chemical Technology. Eleven isolates were collected from Chonnam and Kyungnam provinces in 1993. The geographic origins of the isolates used in this study are described in Table 1.

P. infestans was isolated from diseased samples by placing blighted leaf fragments on 20% non-clarified V-8 agar (200 ml V-8 juice, 20 g agar, 800 ml H_2O, 4.5 g $CaCO_3$) containing 500 ppm ampicillin, 200 ppm vancomycin, 100 ppm pimaricin, 50 ppm rifampicin, and 10 ppm benomyl. Purified isolates were maintained on rye A agar at 18℃ in the dark.

Mating type determination. To determine the mating type of an isolate, mycelial agar disk (8 mm in diameter) was cut from the advancing edge of a 7～10 day-old colony growing on rye A agar and placed on 10% clarified V-8 agar (100 ml V-8 juice, 15 g agar, 900 ml H_2O, 1 g $CaCO_3$, 0.05 g β-sitosterol) 4 cm distant from a standard A1 isolate and A2 isolate on a different plate. Mexican isolates, A1 mating type isolate 1100 and A2 mating type isolate 575 maintained at Fry's Lab., Department of Plant Pathology, Cornell University were used as A1 and A2 tester isolates, respectively. After 5～10 days the presence or absence of oospores at the hyphal interface between the isolates was examined microscopically. Those isolates forming oospores with the A1 tester but not with the A2 tester were designated A2, and those forming oospores with the A2 tester, but not the A1 were designated A1. All mating type assays were performed twice.

Metalaxyl resistance. The response to metalaxyl was determined by an *in vitro* growth test. Radial growth of an isolate on 10% clarified V-8 agar with metalaxyl at final concentrations of 5, 100, and 500 μg a.i./ml was compared to the growth in the abse-

Table 1. Geographic origins of *Phytophthora infestans* isolates collected in Korea in 1991 and 1993

Year	Province	Location	Number of collection site	Collection date	Sample size
1991	Kangwon	Jinbu	2	4 Aug.	5
		Kangneung	2	4 Aug.	2
		Hoenggye	5	4 Aug.	17
	Kyungbuk	Cheongsong	1	17 Aug.	2
		Youngyang	3	17 Aug.	8
	Chonbuk	Kimje	–	13 Jun.	2
		Muju	–	30 Jul.	7
	Kyungnam	Milyang	2	15 Nov.	2
	Chonnam	Wando	1	3 Nov.	1
		Haenam	1	3 Nov.	3
		Kangjin	2	3 Nov.	5
		Muahn	1	8 Jul.	11
	Jeju	Hallim	2	18 May	2
	Subtotal				57
1993	Kyungnam	Milyang	1	24 Oct.	4
	Chonnam	Sunchon	1	14 Jul.	1
		Muahn	2	16 Oct.	6
	Subtotal				11
	Total				68

nce of metalaxyl. One liter of medium was added with one ml of dimethlysulfoxide (DMSO) containing the appropriate concentration of technical grade metalaxyl to achieve the desired concentration. The control (no metalaxy) received 1 ml of pure DMSO. Agar disks (8 mm) containing mycelium from the edge of an actively growing colony were transferred to the center of petri dishes (9 cm) containing the test medium. Cultures were incubated at 18℃ in the dark for 10~20 days until the colony in the absence of metalaxyl was at least 3 cm in diam. The mean radial growth of two replicates of each isolate was measured. All assays were performed twice.

Two types of statistics relating to metalaxyl sensitivity were claculated. The effective concentration of metalaxyl for inhibiting 50% of mycelial growth of an isolate (EC_{50}) was calculated on the basis of linear regression of the response (mean radial growth) plotted against dose (log concentration of fungicide). Classes of responses similar to those described by Dagget *et al.* (5) and Therrien *et al.* (23) were also calculated: Sensitive (S=relative growth at 5 and 100 μg/ml less than 40% that of control); Intermediate (I=relative growth at 5 μg/ml greater than 40% that of control, but at 100 μg/ml relative growth was less than 40% of control); and Resistant (R=relative growth at 5 and 100 μg/ml greater than 40% that of control).

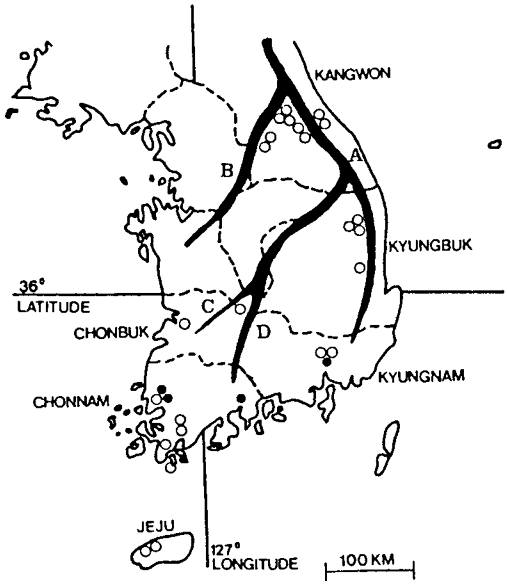

Fig. 1. Locations in Korea of sites from which isolates of *Phytophthora infestans* were collected in 1991 (○) and 1993 (●), respectively. The heavy lines describe the locations of mountain ranges (A; Taebaek Mountains, B; Charyung Mountains, C; Noryung Mountains, D; Sobaek Mountains).

RESULTS

The broad geographical distribution of the locations in Korea of sites from which isolates of *P. infestans* were collected in 1991 and 1993 is illustrated in Fig. 1. Both A1 and A2 mating type isolates were detected in 1991 (Table 2). The majority of the isolates were A2 mating type and only one isolate from Chonnam province was A1. However, no A1 mating type was detected in the samples collected in 1993. The mating types of the isolates of *P. infestans* collected from different geographical locations in 1991 and 1993 are shown in Table 2.

Approximately 40% of the isolates collected from the different geographical locations in 1991 were resistant and intermediate to metalaxyl, respectively, and less than 20% were sensitive to metalaxyl (Table 3). The isolation frequencies of the metalaxyl-resistant isolates from the northern provinces of Kangwon, Kyungbuk, and Chonbuk were 75%, 30%, and 22.2%, respectively. Metalaxyl-resistant isolates were not detected in the southern provinces of Chonnam,

Table 2. Frequency of mating types of *Phytophthora infestans* isolates collected from different geographical locations in Korea in 1991 and 1993

Year	Province	Number of isolates	Mating type	
			A1	A2
1991	Kangwon	24	0	24
	Kyungbuk	10	0	10
	Chonbuk	9	0	9
	Kyungnam	2	0	2
	Chonnam	10	1	9
	Jeju	2	0	2
	Subtotal	57	1 (1.8%)	56 (98.2%)
1993	Kyungnam	4	0	4
	Chonnam	7	0	7
	Subtotal	11	0 (0 %)	11 (100%)

Table 3. Frequency of metalaxyl-resistant isolates of *Phytophthora infestans* collected from different geographical locations in Korea in 1991 and 1993

Year	Province	Number of isolates	Metalaxyl response[a]		
			Sensitive	Intermediate	Resistant
1991	Northern provinces				
	Kangwon	24	1 (4.2%)	5 (20.8%)	18 (75.0%)
	Kyungbuk	10	3 (30.0%)	4 (40.0%)	3 (30.0%)
	Chonbuk	9	1 (11.1%)	6 (66.7%)	2 (22.2%)
	Subtotal	43	5 (11.6%)	15 (34.9%)	23 (53.5%)
	Southern provinces				
	Kyungnam	2	1 (50.0%)	1 (50.0%)	0 (0 %)
	Chonnam	10	4 (40.0%)	6 (60.0%)	0 (0 %)
	Jeju	2	1 (50.0%)	1 (50.0%)	0 (0 %)
	Subtotal	14	6 (42.9%)	8 (57.1%)	0 (0 %)
	Total	57	11 (19.3%)	23 (40.4%)	23 (40.4%)
1993	Southern provinces				
	Kyungnam	4	0 (0 %)	4 (100%)	0 (0 %)
	Chonnam	7	0 (0 %)	6 (100%)	0 (0 %)
	Total	11	1 (0 %)	10 (100%)	0 (0 %)

[a] Based on the percentage of hyphal growth relative to the control, individual isolates were classified as three classes of responses similar to those described by Dagget *et al.* (5) and Therrien *et al.* (22): Sensitive=relative growth at 5 and 100 μg/ml less than 40% that of control; Intermediate=relative growth at 5 μg/ml greater than 40% that of control, but at 100 μg/ml relative growth was less than 40% of control; and Resistant=relative growth at 5 and 100 μg/ml greater than 40% that of control.

Table 4. Degree of resistance to metalaxyl of *Phytophthora infestans* isolates collected from different geographical locations in Korea in 1991 and 1993

Year	Province	No. of isolates with EC_{50} (μg/ml)[a] range of:				Total
		0~5	5~49	50~499	500~	
1991	Northern provinces					
	Kangwon	3	2	10	9	24
	Kyungbuk	4	3	1	2	10
	Chonbuk	4	3	1	1	9
	Subtotal	11 (25.6%)	8 (18.6%)	12 (27.9%)	12 (27.9%)	43
	Southern provinces					
	Kyungnam	1	1	0	0	2
	Chonnam	5	5	0	0	10
	Jeju	1	1	0	0	2
	Subtotal	7 (50.0%)	7 (50.0%)	0 (0 %)	0 (0 %)	14
1993	Southern provinces					
	Kyungnam	0	1	3	0	4
	Chonnam	0	4	3	0	7
	Subtotal	0 (0 %)	5 (45.5%)	6 (54.5%)	0 (0 %)	11

[a] The effective concentration of metalaxyl for inhibiting 50% of mycelial growth of an isolate (EC_{50}) was calculated on the basis of linear regression of the response (mean radial growth) plotted against dose (log concentration of metalaxyl).

Kyungnam, and Jeju in 1991 and 1993. More than 40% of the isolates collected from the southern provinces were sensitive to metalaxyl in 1991, but no metalaxyl-sensitive isolate was collected in 1993.

The degree of metalaxyl resistance varied greatly with the isolates collected in 1991 (Table 4). Eighteen out of 57 isolates were sensitive to metalaxyl with EC_{50} values ≤ 5 μg/ml, whereas 12 isolates were resistant with EC_{50} values >500 μg/ml. Most of the highly metalaxyl-resistant isolates were collected from the northern provinces, especially Kangwon. Metalaxyl-resistant isolates with EC_{50} values >50 μg/ml were not detected in the southern provinces in 1991, but more than 50% of the isolates collected in 1993 had EC_{50} values >50 μg/ml.

DISCUSSION

P. infestans populations in Eurasia, Africa, and South America were known to consist of a single clonal lineage until the A2 mating type was first found in western Europe in 1981 (10, 12). Since then, the occurrence of the A2 mating type outside Mexico has been reported from many countries (7, 9, 16, 18, 21). The A2 mating type has now been reported from countries representing all continents except Antractica and Australia (10, 11). In this study, 98% of *P. infestans* populations in 1991 were A2, but no A1 mating type was detected in the samples collected in 1993 (Table 2). Therefore, the A2 genotype appears to be displacing the A1 genotype which is now the predominant genotype, since there was no evidence for sexual reproduction between both mating types in Korea (16). Occurrence of the A2 mating type in Korea was also reported by So and Lee in 1993 (20). The isolation frequency of the A2 genotype in Japan was also reported to be increasing in recent years (17).

Metalaxyl is an oomycete-specific fungicide used for effectively controlling potato late blight and is highly inhibitory to mycelial growth and sporangium formation of *P. infestans* even at low concentrations (1). Despite the excellent fungicidal effects of metalaxyl, the intensive use of the fungicide resulted in the development of resistance to the fungicide of *P. infestans*. Failures of the metalaxyl to control naturally occurring metalaxyl-resistant isolates of *P. infestans* have been reported in many countries (4, 6, 18). The occurrence and spread of metalaxyl-resistant isolates of the fungus may cause serious difficulties in controlling late blight of potatoes and tomatoes.

About 40% of the isolates of *P. infestans* collected from the different geographical locations in 1991 were resistant to metalaxyl (Table 3). There was a strong association between geographic origin and occurrence of metalaxyl resistance. Highly metalaxyl-resistant isolates were collected from the northern provinces of Kangwon, Kyungbuk, and Chonbuk, but not from the southern provinces of Chonnam, Kyungnam, and Jeju. Most of the sampling sites in the northern provinces of Kangwon, Kyungbuk, and Chonbuk such as Hoenggye, Youngyang, and Muju are major seed potato production areas in Korea (Fig. 1), where severe epidemics of late blight occur annually due to cool and humid weather and metalaxyl has been used intensively. Since the province of Kangwon had isolates with the greatest resistance to metalaxyl, this alpine areas might be the origin of metalaxyl-resistant isolates of *P. infestans* in Korea (16). Therefore, nearly all of the resident old populations appear to be displaced by new A2 populations with metalaxyl resistance probably originated from the alpine areas of Kangwon province.

The drastic increase in the degree of metalaxyl resistance was detected among the isolates obtained from the southern provinces during 1991～1993 (Table 4). Metalaxyl-resistant isolates with EC_{50} values >50 μg/ml were not detected in the southern provinces in 1991, but more than 50% of the isolates collected in 1993 had EC_{50} values >50 μg/ml. Kim *et al.* (13) reported that composite fitness index of metalaxyl-resistant isolates were higher than that of the metalaxyl sensitive isolates. Therefore, the recent shift in the population structure of *P. infestans* in Korea seems to be due to the increased fitness, and predominance of the newly migrating population of the fungus with metalaxyl resistance requires new strategies of late blight management.

The mechanism of migration was probably via infected potato tubers, since most isolates commonly survive as mycelium in infected tubers. It seems likely that global migrations were via infected seed tubers associated with the seed trade among countries on other continents (10). New A2 populations of *P. infestans* in Korea were probably originated from infected seed tubers imported from other countries such as Japan, since Korea had imported Japanese potato cultivar 'Dejima' as seed tubers from the middle 1980s (16). However, Chang and Ko (2) postulated the conversion of A1 to A2 by metalaxyl as a possible origin of A2 isolates of *P. infestans* in Europe. New A2 populations are also likely to be migrated from neighbour countries such as Japan, China and the Commonwealth of Independent States to Korea, vice versa. Therefore, further studies are needed to elucidate the origins and the mechanism of the migration or displacement of new A2 populations of *P. infestans* in Korea.

요 약

1991년과 1993년 우리나라 여러 지역으로부터 수집된 감자 역병균 집단의 교배형과 metalaxyl에 대한 반응을 분석하였다. 1991년에는 A1과 A2 교배형 균주들이 모두 검출되었지만 대다수 균주들이 A2 교배형이었고, 1993년에는 A1 교배형이 검출되지 않았다. 1991년에 수집한 균주들의 약 40%가 metalaxyl에 저항성이었는데, 우리나라에서 감자 역병균 metalaxyl 저항성균의 분포는 지리적 기원과 깊은 관련이 있었다. 1991년에 metalaxyl에 대해 저항성균(EC_{50}>50 μg/ml)들은 강원도, 경상북도 및 전라북도에서만 수집된 반면에 경상남도, 전라남도 및 제주도에서는 수집되지 않았다. 1991~1993년 동안 경상남도와 전라남도에서 수집한 균주들에서 metalaxyl에 대한 양적 저항성 정도는 급격하게 증대되어 1993년에 수집된 균주의 50% 이상이 metalaxyl에 대해 저항성(EC_{50}>50 μg/ml)을 나타내었다. 강원도에서 metalaxyl에 대해 가장 높은 저항성을 지닌 균주들이 수집되었는데, 강원도의 고령지가 우리나라에서 감자 역병균의 metalaxyl 저항성균의 진원지로 추정된다. 우리나라에서는 metalaxyl 저항성을 가진 A2 유전자형이 A1 유전자형을 대체하여 지금은 A2 유전자형이 지배적인 것으로 보인다.

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