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FUNGAL DISEASES

New multilocus genotypes of *Phytophthora infestans* in Japan

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Abstract Twelve isolates of Japanese *Phytophthora infestans*, which differed from the major genotypes US-1, JP-1, JP-2, and JP-3, were analyzed for RG57 fingerprints, mtDNA haplotypes, two allozyme genotypes, and mating types. Genotypes JP-1.1, JP-2.1, JP-2.2, JP-3.1, and JP-4 were newly defined. JP-1.1 and JP-2.1 were isolated discontinuously from potato fields in several years, and JP-1.1 was found in Hokkaido and Kagoshima. These results show that some minor genotypes can overwinter and disperse from their original site.

Keywords Multilocus genotyping · Late blight · *Phytophthora infestans* · RG57 fingerprint

In Japan, the population diversity of *Phytophthora infestans* is low compared to that in other countries such as Poland (Sujkowski et al. 1994) or the Netherlands (Drenth et al. 1994). Only four major multilocus genotypes of Japanese *P. infestans* had been identified: US-1, JP-1, JP-2 (Japanese A1-A), and JP-3 (Japanese A1-B) (Kato 2001; Gotoh et al. 2005). In addition, there were some isolates for which genotypes could not be defined because of small sample numbers. We describe the characteristics of these "minor" isolates here and propose new genotypes for *P. infestans* in Japan.

Twelve isolates of Japanese *P. infestans* were selected from 409 of the stock cultures (1956–2002) in the Laboratory of Plant Pathology, Hokkaido University. These isolates had different fingerprints from the known genotypes US-1, JP-1, JP-2, and JP-3 (results of preliminary investigations). Each isolate was obtained from a single lesion as described in Gotoh et al. (2005), maintained on rye A agar (Caten and Jinks 1968) at 15°C in the dark, and transferred onto new agar at 6 month intervals. The multilocus genotype of each isolate was determined using RG57 fingerprints (Goodwin et al. 1992), mtDNA haplotypes, and two allozymes [*glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*)]. The mating type was also determined. These tests were performed according to the protocol of Gotoh et al. (2005).

In brief, total DNA was extracted and used for RG57 fingerprint analysis as described by Goodwin et al. (1992). The total DNA was digested with *Eco*RI, subjected to agarose gel electrophoresis, and transferred onto nylon membranes. The RG57 probe was labeled with digoxigenin-11-UTP using a PCR DIG Probe Synthesis kit (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) and detected using the manufacturer's instructions. The mtDNA haplotypes were identified using the method of Griffith and Shaw (1998). The P2 and P4 polymerase chain reaction (PCR) products were digested with *Hpa*II, instead of the previously reported *Msp*I (Griffith and Shaw 1998), which digests the same site, and *Eco*RI, respectively, and analyzed using 2% agarose gel electrophoresis. The allozyme genotypes for *Gpi* and *Pep* were determined by cellulose–acetate electrophoresis (CAE) (Goodwin et al. 1995a). The identities of the allozyme alleles were determined using the zymograms scores of Mosa et al. (1993) as references. The mating

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types were determined by pairing each isolate with A1 and A2 tester isolates on 10% clarified V8-juice agar medium.

Eight multilocus genotypes were found (Tables 1, 2), six of which had not been previously defined (Table 1). These new multilocus genotypes were discriminated using RG57 fingerprints, but not using the mtDNA haplotypes or allozyme genotypes (Table 2). The *Pep* pattern for Japanese A1-B (= JP-3) was corrected to 96/100 from 98/98 reported in a previous study (Akino et al. 2005). In addition, the patterns of Japanese A1-C and D (Kato 2001) were redetermined in this study.

Genotypes US-1.4 (Goodwin et al. 1994a) and KR-1 (Gotoh et al. 2005), which had not previously been discovered in Japan, were confirmed in this study (Table 1).

The multilocus genotype of three isolates, OB996, KG1-2-2, and DN0217-2 were named JP-1.1, according to the nomenclature of Forbes et al. (1998). The RG57 fingerprint of this multilocus genotype was identical to that of JP-1, except for a distinct signal between bands 8 and 9/bands 21 and 24 (Fig. 1, Table 2). Previously, the genotype of two isolates OB996 and KG1-2-2 was identified as JP-1 (Gotoh et al. 2005). In this study, we confirmed the reproducibility and stability of these bands and designated them 8a and 23a, similar to descriptions of other extra bands (Forbes et al. 1998). JP-1.1 and JP-1 coincided in mating type, allozyme genotypes, and mtDNA haplotype.

Isolates SS0115 and 98B3 had the same RG57 fingerprints as JP-2, except for the absence of band 14, and we designated this multilocus genotype JP-2.1; the genotype of 98B3 had been identified as JP-2 in a previous study (Gotoh et al. 2005). The RG57 fingerprint of KM0103 was identical to that of JP-2 except for bands 5 and 11, and this multilocus genotype was designated JP-2.2. The RG57 fingerprint of KS0108 differed from JP-3 in band 6; we designated this multilocus genotype JP-3.1. These differences could have resulted from minor genetic changes, e.g., mutation, parasexual recombination, or both. Isolate KM0102, previously reported to be Japanese A1-C (Kato 2001), had unique

Table 1 Sources of Japanese *Phytophthora infestans* used in this study

Isolate	Location	Year	Mating type	Provisional name[a]	Genotype[b]
IFO9174	Hokkaido	1958	A1	–	US-1.4
TK301	Shintoku, Hokkaido	1993	A2	–	KR-1
OB996	Hokkaido	1996	A2	–	JP-1.1
98B3	Memuro, Hokkaido	1998	A1	–	JP-2.1
KG1-2-2	Nagashima, Kagoshima	1999	A2	–	JP-1.1
KM0102	Tanno, Hokkaido	2001	A1	A1-C	JP-4
KM0125	Bihoro, Hokkaido	2001	A1	A1-C	JP-2
TK0120	Makubetu, Hokkaido	2001	A1	A1-D	JP-3
KM0103	Tokoro, Hokkaido	2001	A1	–	JP-2.2
KS0108	Teshikaga, Hokkaido	2001	A1	–	JP-3.1
SS0115	Kyogoku, Hokkaido	2001	A1	–	JP-2.1
DN0217-2	Kumaishi, Hokkaido	2002	A2	–	JP-1.1

[a] These provisional names were determined by Kato (2001)

[b] The genotype was determined using RG57 fingerprinting, *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) allozyme genotypes, mtDNA haplotype and mating type. US-1.4 was named by Goodwin et al. (1994a). JP-2, JP-3 and KR-1 were named by Gotoh et al. (2005). JP-1 was named by Koh et al. (1994). JP-1.1 / JP-2.1 / JP-2.2 / JP-3.1 and JP-4 were determined and named in this study

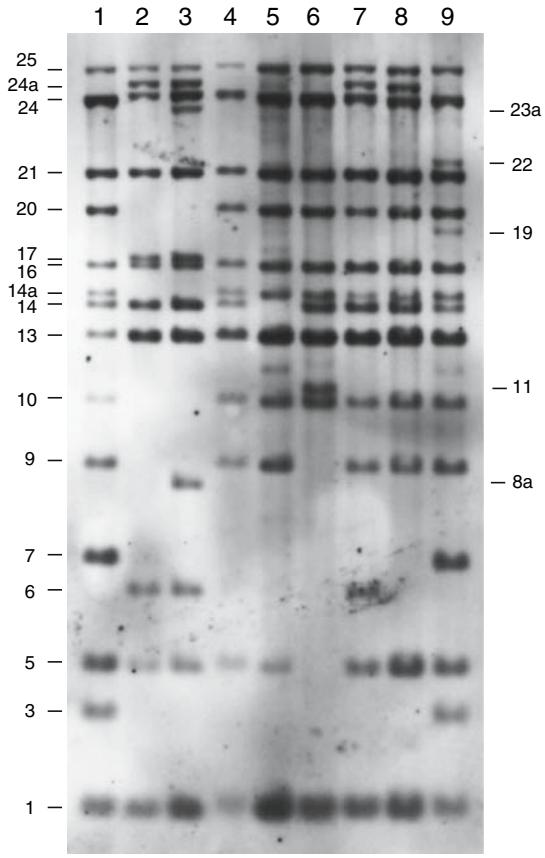

Fig. 1 Genomic restriction fragment length polymorphisms (RFLPs) using the RG57 probe of each isolate. Lanes: *1* ATCC940501 (US-1 multilocus genotype, control); *2* HK0112 (JP-1); *3* DN0217-2 (JP-1.1); *4* KM0125 (JP-2); *5* SS0115 (JP-2.1); *6* KM0103 (JP-2.2); *7* TK0120 (JP-3); *8* KS0108 (JP-3.1); *9* KM0102 (JP-4). The numbers on the left and right are band numbers, as indicated in Forbes et al. (1998)

Table 2 Multilocus genotypes of *Phytophthora infestans* based on mating type, two allozymes, RG57 fingerprint and mtDNA haplotype

Genotype	Mating type	*Gpi*[d]	*Pep*[d]	RG57 fingerprint (RG57)[e]	mtDNA haplotype
US-1[a]	A1	86/100	92/100	101 010 101 100 110 100 011 001 101 00	Ib
US-1.4[b]	A1	86/100	100/100	101 010 101 000 110 100 011 001 101 00	Ib
KR-1[c]	A2	100/100	96/96	100 010 000 000 110 110 001 001 100 01	IIa
JP-1[c]	A2	100/100	96/96	100 011 000 000 110 110 001 001 100 01	IIa
JP-1.1	A2	100/100	96/96	100 011 000 000 110 110 001 001 110 11	IIa
JP-2[c]	A1	100/100	100/100	100 010 001 100 110 100 011 001 101 00	IIa
JP-2.1	A1	100/100	100/100	100 010 001 100 100 100 011 001 101 00	IIa
JP-2.2	A1	100/100	100/100	100 000 001 110 110 100 011 001 101 00	IIa
JP-3[c]	A1	100/100	96/100	100 011 001 100 110 100 011 001 101 00	IIa
JP-3.1	A1	100/100	96/100	100 010 001 100 110 100 011 001 101 00	IIa
JP-4	A1	100/100	96/100	101 010 101 100 110 100 111 101 101 00	IIa

[a] Goodwin et al. (1994b)

[b] Goodwin et al. (1994a)

[c] Gotoh et al. (2005)

[d] *Gpi* glucose-6-phosphate isomerase, *Pep* peptidase

[e] Fingerprints were generated by the RG57 probe (Goodwin et al. 1992). The presence or absence of bands is indicated by 1 or 0, respectively. Bands 1–25, 8a, 14a, 23a and 24a are listed from left to right. Bands 8a and 23a were generated in this study

polymorphisms: A1 mating type, 100/100 (*Gpi*), 96/100 (*Pep*), mtDNA haplotype IIa, and the presence of the rare bands 19 and 22 on RG57 fingerprints. We designated this multilocus genotype JP-4. The mechanism(s) generating these polymorphisms of this isolate was not clear.

Japanese A1-C genotype was defined by the characters of KM0125 and KM0102 in a previous report (Kato 2001). In this study, the genotype was separated into two distinct multilocus genotypes, JP-2 and JP-4, using two allozyme genotypes and RG57 fingerprints (Fig. 1, Table 2). Our results suggest that cultural characteristics do not always coincide with genetic polymorphisms. Japanese A1-D genotype was defined by the characters of TK0120 in previous report (Kato 2001). In this study, it was confirmed that the *Pep* pattern of TK0120 was not 100/100, but 96/100. Accordingly, we identified this isolate as the JP-3 genotype. The misreading may have resulted from the low resolution of starch gel electrophoresis for *Pep*.

Genotypes JP-1.1, JP-2.1, JP-2.2, and JP-3.1 were newly defined. In particular, JP-1.1 and JP-2.1 were detected in fields in 1996, 1999, 2002, and in 1998 and 2001, respectively. In addition, JP-1.1 was found in Hokkaido and Kagoshima, which are separated by about 1500 km. These results show that some minor genotypes can overwinter and disperse from their original sites. Accordingly, these minor genotypes should not be neglected in any survey of *P. infestans* populations in Japan.

Some differences were noted among characteristics of the Japanese genotypes of *P. infestans* (Gotoh et al. 2005). The detailed characteristics of these genotypes in terms of aggressiveness and fitness (Tooley and Fry 1985) are still under investigation.

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