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FULL PAPER

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Phylogenetic analyses of *Uromyces viciae-fabae* and its varieties on *Vicia*, *Lathyrus*, and *Pisum* in Japan

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Abstract A pea rust fungus, *Uromyces viciae-fabae*, has been classified into two varieties, var. *viciae-fabae* and var. *orobi*, based on differences in urediniospore wall thickness and putative host specificity in Japan. In principal component analyses, morphological features of urediniospores and teliospores of 94 rust specimens from *Vicia*, *Lathyrus*, and *Pisum* did not show definite host-specific morphological groups. In molecular analyses, 23 *Uromyces* specimens from *Vicia*, *Lathyrus*, and *Pisum* formed a single genetic clade based on D1/D2 and ITS regions. Four isolates of *U. viciae-fabae* from *V. cracca* and *V. unijuga* could infect and sporulate on *P. sativum*. These results suggest that *U. viciae-fabae* populations on different host plants are not biologically differentiated into groups that can be recognized as varieties.

Key words *Lathyrus* · Phylogeny · *Pisum* · Rust fungus · Taxonomy · Uredinales · *Uromyces viciae-fabae* · *Vicia*

Introduction

Uromyces viciae-fabae (Pers.) J. Schroet., causing pea rust disease, is an autoecious and macrocyclic rust fungus occurring on wild and cultivated *Vicia*, *Lathyrus*, *Pisum*, and *Lens* throughout the world (Wilson and Henderson 1966; Cummins 1978; Duke 1981; Hiratsuka et al. 1992). Currently, *U. viciae-fabae* is separated into two varieties, var. *viciae-fabae* (= *U. fabae* de Bary) and var. *orobi* (Schumach.) Jørst. (= *U. orobi* Lév.), based on wall thickness difference of urediniospores and putative host specificity (Wilson and Henderson 1966; Hiratsuka et al. 1992).

The species name, *U. orobi* Lév. (= *U. viciae-fabae* var. *orobi*), was originally applied to a rust occurring on *Lathyrus montanus* Bernh. by Léveillé (1847, cited from Wilson and Henderson 1966), and it was noted that the urediniospore wall was thicker than that of *U. fabae* (= *U. viciae-fabae* var. *viciae-fabae*). Later, Jørstad (1936, cited from Wilson and Henderson 1966) merged *U. orobi* with *U. viciae-fabae*, designating the former fungus as a variety of the latter. On the other hand, *U. fabae* was originally applied to a rust on *Vicia faba* L. (de Bary 1863, cited from Wilson and Henderson 1966), having been reported on *Vicia*, *Lathyrus*, *Pisum*, and *Lens* (Wilson and Henderson 1966; Duke 1981). However, Gäumann (1934) stated that urediniospores of *U. fabae* on *Vicia sepium* showed different wall thickness and that those on stems were thicker than those on leaves. He listed six formae speciales for *U. fabae* and three formae speciales for *U. orobi*.

In Japan, Ito (1922) classified a rust fungus on *V. unijuga* Al. Br. as *U. orobi* because it had a thicker urediniospore wall than *U. fabae* on *Vicia*, *Lathyrus*, and *Pisum*. Later, Hiratsuka (1933) classified rust fungi on *V. unijuga*, *V. nipponica* var. *capitata* Nakai, and *L. davidii* Hance as *U. orobi*. Furthermore, *U. orobi* was stated not to infect the host plants of *U. fabae* (Hiratsuka 1933). Recently, the fungi on these three host species were transferred into *U. viciae-fabae* var. *orobi* (Hiratsuka 1973; Hiratsuka et al. 1992). Although the difference in urediniospore wall thickness of *U. viciae-fabae* was considered an important taxonomic characteristic, it is not necessarily distinct among the species compared. Therefore, there has been confusion about the identity of, and relationship between, the two varieties causing the rust disease in different geographic areas and occur-

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ring on closely related host plants (Gäumann 1934; Wilson and Henderson 1966; Hiratsuka et al. 1992).

Recently, molecular methods have been applied to filamentous fungi for the study of genetic variation and the phylogeny of species that are morphologically indistinct (Foster et al. 1993). Molecular phylogenetic analyses of rust fungi were not widely carried out because they are obligate parasites that are impossible or difficult to obtain or maintain in pure culture. However, it is now possible to extract DNA from a single spore of dry herbarium specimens and to amplify target DNA by polymerase chain reaction (PCR) (Bruns et al. 1990; Lee and Taylor 1990). Virtudazo et al. (2001) modified DNA extraction methods from Suyama et al. (1996) and extracted genomic DNA from spores from a single uredinium, then amplified the template DNA by PCR. Ribosomal repeat units are generally informative for species and generic differentiation (Bruns et al. 1991; O'Donnell 1993; Piepenbring et al. 1999; Tehler et al. 2000; de Jong et al. 2001). Accordingly, ribosomal DNA sequences of rust fungi have been analyzed and registered in genetic databases (Zambino and Szabo 1993; Vogler and Bruns 1998; Ayliffe et al. 2001; Pfunder et al. 2001; Virtudazo et al. 2001).

The current study reevaluates morphological variations of urediniospores and teliospores of *Uromyces viciae-fabae* populations on *Vicia*, *Lathyrus*, and *Pisum* in Japan and analyzes their molecular phylogeny in relation to their taxonomy. The scientific names of cultivated and wild legumes follow Ohwi and Kitagawa (1992).

Materials and methods

Morphological observations and statistical analyses

Fresh material or dry herbarium specimens were used for light microscopic (LM) and scanning electron microscopic (SEM) observation. Most specimens examined were loaned from the Hiratsuka Herbarium, Tokyo, Japan (HH); the National Fungus Collections, the United States Department of Agriculture, Beltsville, MD, USA (BPI); the Mycological Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan (TSH); the Herbarium of Systematic Mycology, the College of Education, Ibaraki University, Mito, Japan (IBA); and the Herbarium of the National Institute of Agro-Environmental Science, Tsukuba, Japan (NIAES).

Ninety-four specimens were gathered from seven species of *Vicia*, three species of *Lathyrus*, and *P. sativum* in Japan (Table 1). These host plants are reported to be susceptible to *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* (Hiratsuka et al. 1992). Specimens were examined for morphological characteristics of urediniospores and teliospores. Statistical analyses, including multivariate analyses of measured continuous numerical variables, were performed using the software package SPSS (SPSS Japan, Tokyo, Japan). Discrete numerical or qualitative attributes, or host species, were superimposed on two- or three-dimensional scatter diagrams generated from the analyses to detect possible groups.

For SEM, spores obtained from dry specimens were dusted onto double-sided adhesive tape on specimen holders, and then coated with platinum-palladium with a Hitachi E-1030 Ion Sputter Coater. The spores were examined by SEM with a Hitachi S-4200 instrument operating at 15 kV.

Fungal taxa for rDNA sequencing

Twenty-three specimens were selected for large subunit (LSU) rDNA (D1/D2) and internal transcribed spacer (ITS) sequence analyses (Table 2). For comparison, and as outgroup taxa, the fabaceous rusts *U. appendiculatus* (Pers.) Unger, *U. vignae* Barclay, *U. fabae* de Bary, *U. pisi* (DC.) Otth, and *U. minor* Schroet., a rust of *Gagea lutea* (L.) Ker Gawl., *U. gageae* Beck, and a rust of *Miscanthus sinensis*, *Puccinia miscanthi*, were also included in the analyses. These GenBank sequences of fungi used for phylogenetic comparison are listed in Table 3.

DNA extraction, PCR amplification, and purification and sequencing of LSU rDNA (D1/D2) and ITS regions

Genomic DNA was extracted from about 150–200 urediniospores or teliospores from a single uredinium or

Table 1. Specimens of *Uromyces viciae-fabae* used for morphological observations

Host plants	Locality (no. of specimens)	Stage[a]
Vicia amoena Fisch.	Honshu (3)	II, III
V. cracca L.	Honshu (22), Hokkaido (3), Kyushu (1)	0, I, II, III
V. faba L.	Honshu (7), Hokkaido (4), Kyushu (4), Okinawa (1)	II, III
V. japonica A. Gray	Honshu (7), Hokkaido (6)	II, III
V. nipponica var. *capitata* Nakai	Honshu (1)	II, III
V. pseudo-orobus Fish. & C.A. Mey.	Honshu (2)	II, III
V. unijuga Al. Br.	Honshu (5), Kyushu (1)	II, III
Lathyrus davidii Hance	Honshu (2)	II, III
L. maritimus Bigel.	Honshu (5), Hokkaido (4), Kyushu (3)	II, III
L. palustris L.	Honshu (6), Hokkaido (1)	II, III
Pisum sativum L.	Honshu (3), Hokkaido (1), Kyushu (2)	I, II, III
Total	94	

[a] 0, spermogonia; I, aecia; II, uredinia; III, telia

Table 2. Specimens of *Uromyces viciae-fabae* sequences used for phylogenetic analysis

Host plants	Locality in Japan	Voucher specimens[a]	GenBank accession no.	
			D1/D2	ITS
Vicia amoena	Yamanashi	TSH-R13227	AB115592	AB115650
V. cracca	Nagano	TSH-R16998	AB115597	AB115654
	Ibaraki	TSH-R16999	AB115598	AB115655
	Hokkaido	TSH-R16269	AB115595	AB115652
	Nagano	TSH-R18187	NA[b]	AB115659
	Nagano	TSH-R2986	NA	AB115660
V. faba	Chiba	BPI-0005425	AB115607	AB115663
	Fukuoka	BPI-0005454	AB115608	AB225664
V. pseudo-orobus	Yamanashi	TSH-R1743 (IBA-2652)	AB115601	AB115656
V. japonica	Hokkaido	TSH-R1738 (IBA-5836)	AB115600	AB085194
	Yamanashi	TSH-R13306	AB115592	NA
V. unijuga	Yamanashi	TSH-R1747 (IBA-3068)	AB115603	AB115657
	Kanagawa	H40928	AB115605	AB115661
	Yamaguchi	H67023	AB115611	AB115666
	Nagano	TSH-R18185	AB115604	AB115658
V. nipponica var. *capitata*	Tottori	BPI-0005541	AB115609	NA
Lathyrus maritimus	Ibaraki	TSH-R6320	AB115610	AB115665
	Hokkaido	TSH-R1736 (IBA-5842)	AB115599	AB085193
	Hokkaido	TSH-R1739 (IBA-3004)	NA	AB085195
	Ibaraki	TSH-R1744 (IBA-2894)	AB115602	AB085192
L. palustris	Iwate	BPI-0005266	AB115606	AB115662
	Hokkaido	TSH-R16270	AB115596	AB115653
Pisum sativum	Hokkaido	TSH-R16268	AB115594	AB115651

ITS, internal transcribed spacer region
[a] TSH, Mycological Herbarium, University of Tsukuba, Japan; BPI, USDA National Fungus Collections, USA; HH, Hiratsuka Herbarium, Tokyo, Japan; IBA, Herbarium of Systematic Mycology, Ibaraki University, Japan
[b] No analyses

Table 3. Additional taxa selected for D1/D2 and ITS analysis

Species	Host plants	GenBank accession no.	
		D1/D2	ITS
Uromyces minor Schroet.	*Trifolium lupinaster* L.	NA[a]	AB115737
U. vignae Barclay	*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*	AB115629	AB115720
U. appendiculatus (Pers.) Unger var. *appendiculatus*	*Phaseolus vulgaris* L.	AB115644	AB115741
U. appendiculatus var. *azukicola* (Hirata) Hiratsuka, f.	*Vigna angularis* (Willd.) Ohwi & Ohashi var. *angularis*	AB115619	AB115710
U. fabae de Bary	*Vicia pannonica* Crantz	AF426199	NA
U. pisi (DC.) Otth	*Euphorbia cyparissias* L.	AF426201	NA
U. pisi	*Euphorbia cyparissias*	NA	AF180165
U. gageae Beck	*Gagea lutea* (L.) Ker Gawl.	AF426208	NA
Puccinia miscanthi Miura	*Miscanthus sinensis* Anderss.	AJ296546	NA

[a] No analyses

telium. DNA extraction methods followed Virtudazo et al. (2001). From this crude extract, 2–3 μl was used directly for each PCR amplification. Amplifications were done using 40-μl PCR reactions, each containing 0.2 μM of each primer, 1 unit TaKaRa Ex Taq DNA polymerase (Takara, Tokyo, Japan), and a commercial dNTP mixture (containing 2.5 mM of each dNTP) and Ex Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the conditions used by Virtudazo et al. (2001). The D1/D2 region was amplified using primers NL1 and NL4 (O'Donnell 1993). For ITS and 5.8 S regions, the primers used at the beginning of this study were ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). However, because amplification using these primers was successful for only a few specimens, we chose primers that worked better for *Uromyces* spp., ITS5-u (5′-AAGGTTTCTGTAGGTG-3′) and ITS4-u (5′-GGCTTTTCCCTCTTCAT-3′) (Pfunder et al. 2001). PCR products were run on 1% agarose gels containing 0.5 μg/ml ethidium bromide in TAE [Tris-acetate, ethylene diaminetetraacetic acid (EDTA)] or TBE (Tris-borate, EDTA) buffers. PCR products were first purified by spin columns (MicroSpin S-400 HR Columns). Purified PCR products were reacted with BigDie Terminator v3.0 Cycle Sequencing (Applied Biosystems) under the following conditions: 25 cycles of 96°C for 10 s, 50°C for 5 s,

and 60°C for 4 min. Cycle sequencing reaction products were purified by ethanol precipitation, and then analyzed by ABI PRISM 3100 automated sequencers (Applied Biosystems).

Sequence alignment and analyses

DNA sequences were aligned using Clustal X v1.8 (Thompson et al. 1997). Further visual alignments were done in Sequence Alignment (Se-Al) Editor v.2.0 (Rambaut 2000). Specimen sequences were analyzed together with sequences from GenBank.

The aligned sequences data file can be obtained from the authors. Phylogenetic analyses of the data were done by distance and parsimony methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter method (Kimura 1980) and was analyzed with the neighbor-joining (NJ) method (Saitou and Nei 1987) using the program PAUP v 4.0 b (Swofford 1999), excluding positions with gaps and correcting for multiple substitutions. Reliability of the inferred trees was estimated by 1000 bootstrap resamplings using the same program. Parsimony analysis was also done by PAUP v 4.0 b using the heuristic search option with 100 random stepwise-addition sequences to search for the most parsimonious tree. Bootstrap (Felsenstein 1985) values were generated with 1000 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program.

Inoculation experiments

Urediniospores from *Vicia cracca* L. and *V. unijuga* were dusted with a scalpel onto pieces of wet filter paper (about 5 × 5 mm), which were then placed on the lower surface of healthy leaves of *V. amoena* Fisch., *V. cracca*, *V. faba*, *V. unijuga*, *Lathyrus maritimus* Bigel., *L. palustris* L., and *Pisum sativum* L. The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Results

Morphological features and principal component analyses

Morphology of urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum* was not significantly different. The urediniospores were globose, subglobose, or ellipsoid; the spore wall was pale yellow or yellow, and echinulate (Fig. 1A,B). Mean urediniospore length in individual specimens ranged from 20.7 to 30.5 μm and mean width from 16.5 to 26.7 μm. However, no discrete groups were detected for urediniospore length and width (Fig. 2A). Urediniospores on *V. unijuga*, *V. nipponica* var. *capitata*, and *L. davidii* did not show significant difference in wall thickness, ranging from 1.1 to 2.8 μm.

The teliospores were subglobose, ovate, or ellipsoid (Fig. 1C,D). Mean length of teliospores in individual specimens ranged from 29.1 to 37.1 μm and mean width from 18.4 to 28.3 μm. Mean teliospore apical thickness in individual specimens ranged from 4.1 to 8.4 μm and mean wall thickness from 1.7 to 3.2 μm. However, no discrete groups were detected for teliospore size and apical thickness (Fig. 2B). The wall surface was smooth in all specimens observed, and color was pale brown, brown, or dark brown. The principal component analyses were undertaken with various combinations of urediniospore size, wall thickness and teliospore size, apical thickness, and side-wall thickness. After the Varimax rotation, the calculated factors 1 and 2 explained 30.4% and 26.5% of the total variance, respectively. The scatter diagram with factors 1 and 2 did not form discrete groups (Fig. 2C).

Molecular phylogenetic analysis inferred from rDNA D1/D2 and ITS

The DNA sequences of the entire LSU rDNA (D1/D2) region of pea rust fungi, ranging from 604 to 607 bases, were used for phylogenetic analysis. Of the 617 aligned bases, 19 sites were variable and 24 sites were parsimony-informative characters. The NJ tree constructed from the LSU (D1/D2) rDNA regions showed that *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* formed a single genetic clade with high bootstrap support (Fig. 3). The LSU (D1/D2) bootstrap phylogram has a consistency index (CI) of 0.900, a retention index (RI) of 0.906, retention consistency (RC) of 0.815, and a tree length of 50.

The DNA sequences of the entire ITS regions of cultivated and wild legume rust, ranging from 615 to 623 bases, were used for phylogenetic analyses. Of the 660 aligned bases, 107 sites were variable and 70 sites were parsimony-informative characters. In the NJ tree constructed from the ITS and 5.8S rDNA regions, the rust fungi on *Vicia*, *Lathyrus*, and *Pisum* also formed a single genetic clade, with high bootstrap support (Fig. 4). The ITS bootstrap phylogram has a CI of 0.809, an RI of 0.667, an RC of 0.539, and a tree length of 251. Therefore, the sequence analyses of *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* revealed virtually no genetic variation based on the D1/D2 and ITS regions.

Host specificity of *Uromyces viciae-fabae* on *Vicia*, *Lathyrus*, and *Pisum*

Three fungal isolates, TSH-R16998, TSH-R16999, and TSH-R18187, from *V. cracca* could infect and sporulate on *V. cracca* and *P. sativum*; TSH-R16998 and TSH-R18187 could also infect and sporulate on *V. faba*; and TSH-R18187 could also infect and sporulate on *V. amoena*. One fungal isolate, TSH-R18185, from *V. unijuga* could infect and sporulate on *V. unijuga* and *P. sativum*. However, urediniospores of four fungal isolates from wild *Vicia* were also pathogenic to cultivated *P. sativum* (Table 4).

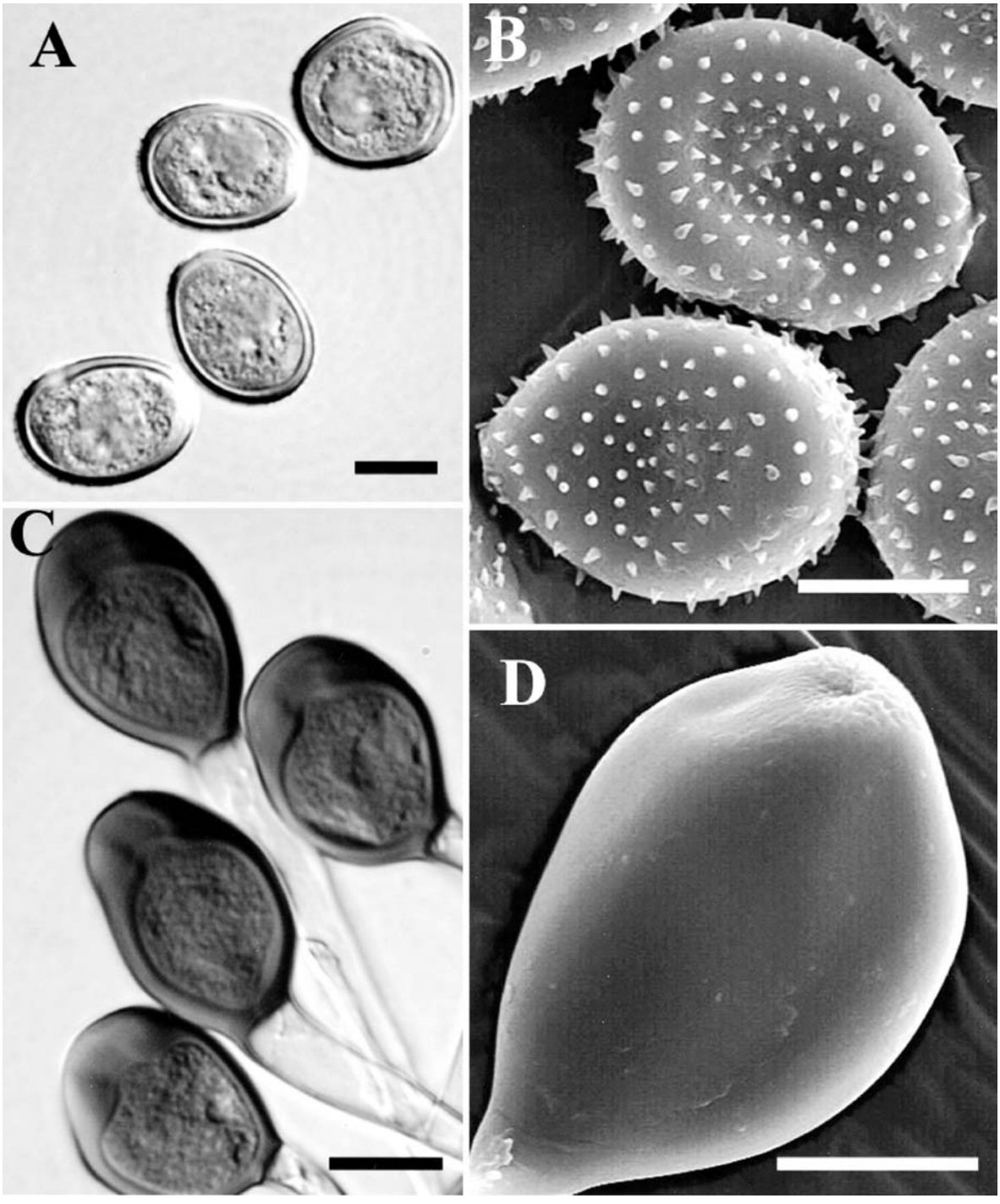

Fig. 1. *Uromyces viciae-fabae* (TSH-R18185) on *Vicia unijuga*. **A,B** Urediniospores. **C,D** Teliospores. *Bars* **A–D** 10 μm

Discussion

Uromyces viciae-fabae populations in Japan on *Vicia*, *Lathyrus*, and *Pisum* had morphologically similar urediniospores and teliospores. Likewise, molecular phylogenetic analyses revealed that specimens used for LSU rDNA D1/D2 and ITS regions analyses formed a single clade. Also, *Uromyces* isolates on wild *Vicia* showed the same pathogenicity on cultivated *P. sativum*, and the variations in virulence on *Vicia*, *Lathyrus*, and *Pisum* are recognized in our inoculation results.

In Japan, a rust on *V. unijuga* was first stated to have urediniospores with the wall 2–3 μm thick, thicker than those rusts on most other *Vicia*, *Lathyrus*, *and Pisum* (1.5–2.5 μm thick), and the fungus was classified into *U. orobi* (= *U. viciae-fabae* var. *orobi*) (Ito 1922). Hiratsuka (1933) stated that the rust on *V. nipponica* var. *capitata* and *L.*

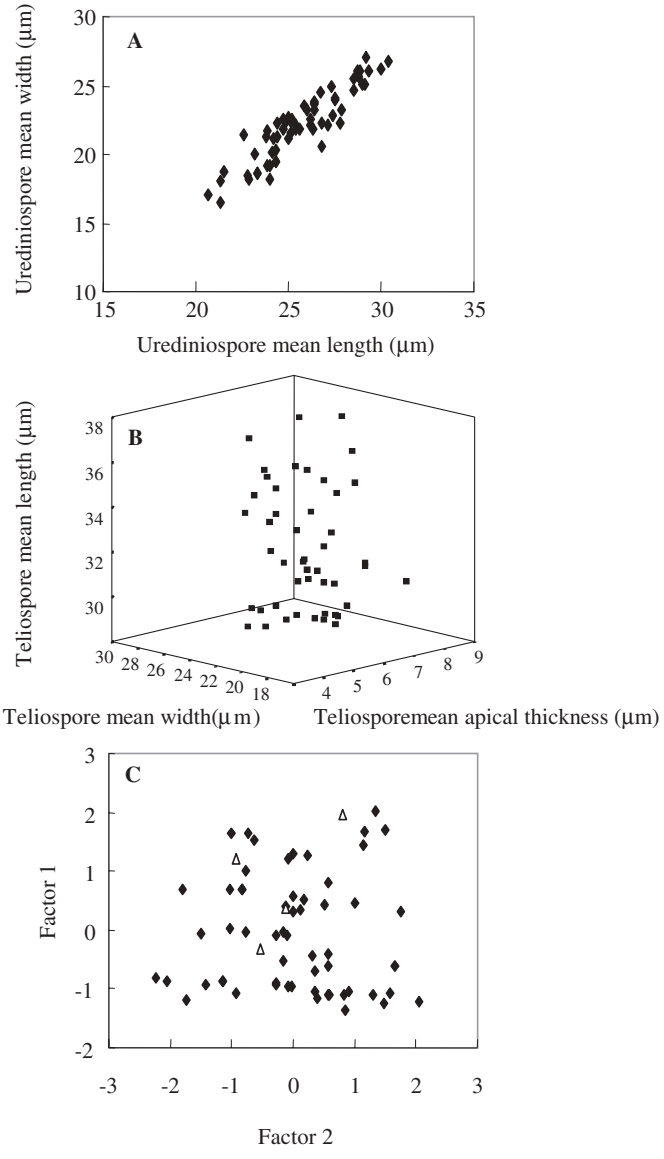

Fig. 2. Variations in characteristics of urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum*. **A** Urediniospore mean lengths against urediniospore mean widths. **B** Correlation of teliospore mean lengths, widths, and apical thickness. **C** Results of a principal component analysis of urediniospores and teliospores (△, specimens on *Vicia unijuga*, *V. nipponica* var. *capitata*, and *Lathyrus davidii*)

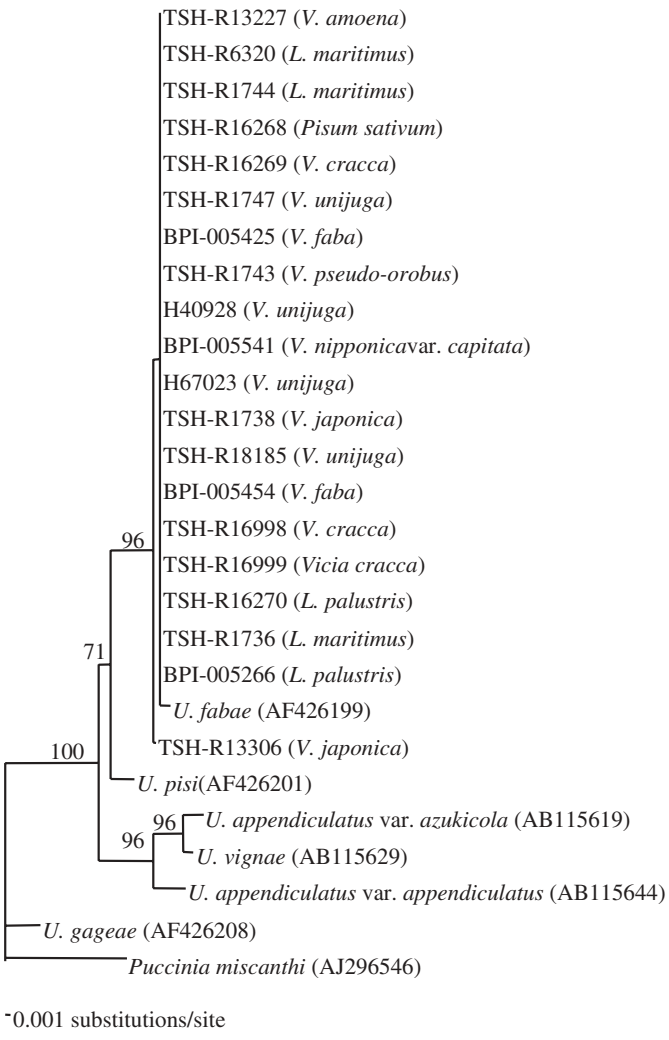

Fig. 3. A neighbor-joining tree inferred from sequences of large subunit (LSU) rDNA (D1/D2) regions using Clustal X. *Values above the branches* indicate percentage bootstrap support for 1000 resamplings. *Length of branches* is proportional to number of base changes, indicated by the *scale at bottom*

davidii also had thicker urediniospore walls and could be distinguished from *U. fabae*. Hiratsuka (1973) and Hiratsuka et al. (1992) agreed with Jørstad's taxonomic system and transferred *U. fabae* and *U. orobi* into *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi*, respectively, because these fungi resemble each other except for the urediniospore wall thickness and putative host specificity (Hiratsuka 1973; Hiratsuka et al. 1992). Our morphological observations revealed that urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum* are similar. Similarly, principal component analyses did not show significant variation among these specimens. Although only six specimens of rust on *V. unijuga*, one on *V. nipponica* var. *capitata*, and two on *L. davidii* were measured in this study, statistical analyses showed that these specimens could not be grouped separately from other rust specimens on *Vicia*, *Lathyrus*, and *Pisum*. We do not consider variation in urediniospore wall thickness to be a useful and stable character to separate var. *viciae-fabae* and var. *orobi* in Japan.

Twenty-three rust specimens on *Vicia*, *Lathyrus*, and *Pisum* were used for molecular phylogenetic analyses. The analyses revealed that the 23 specimens have high homology values based on the D1/D2 and ITS regions. For D1/D2 analysis, homology of these specimens ranged from 95 to 100, and they formed a single genetic clade. Likewise, homology of ITS regions ranged from 97 to 100, and also formed a single genetic clade. Parsimony analysis of the two regions showed the same genetic tree as the NJ analysis (tree not showed). Moreover, *Uromyces* on *V. unijuga* and *V. nipponica* var. *capitata* did not have a significant genetic difference from other analyzed specimens based on D1/D2 and ITS regions. Furthermore, we also analyzed *U. fabae*

Table 4. Results of inoculation experiments on *Vicia*, *Lathyrus*, and *Pisum*

Plants inoculated	Voucher specimen number			
	TSH-R16999[b]	TSH-R16998[b]	TSH-R18187[b]	TSH-R18185[c]
Vicia faba	−[a]	+	+	−
V. cracca	+[a]	+	+	−
V. amoena	−	−	+	−
V. unijuga	−	−	−	+
Lathyrus maritimus	−	−	−	−
L. palustris	−	−	−	−
Pisum sativum	+	+	+	+

[a] −, no infection; +, uredinium production
[b] *Uromyces* isolates from *V. cracca*
[c] *Uromyces* isolate from *V. unijuga*

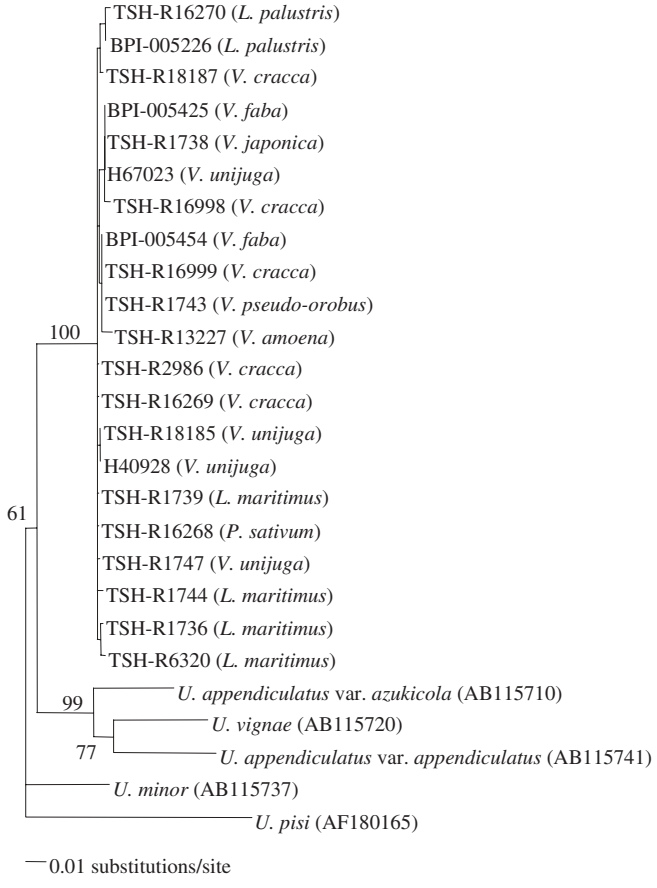

Fig. 4. A neighbor-joining tree inferred from sequences of internal transcribed spacer (ITS) and 5.8S rDNA regions using Clustal X. *Values above the branches* indicate percentage bootstrap support for 1000 resamplings. *Length of branches* is proportional to number of base changes, indicated by the *scale at bottom*

(GenBank: AF426199), *U. appendiculatus* var. *appendiculatus* (GenBank: AB115644, AB115741), *U. appendiculatus* var. *azukicola* (GenBank: AB115619, AB115710), *U. vignae* (GenBank: AB115629, AB115720), *U. pisi* (GenBank: AF426201, AF180165), *U. gageae* (GenBank: AF426208), and *P. miscanthi* (GenBank: AJ296546). Results revealed that our specimens formed a single genetic clade with *U. fabae* (GenBank). Our specimens are closely related to other *Uromyces* and distant from *Puccinia*, inferred from D1/D2 regions. Moreover, *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* are closely related genetically with *U. appendiculatus* and *U. vignae*, based on molecular phylogenetic analysis (see Figs. 3, 4).

For host specificity, Hiratsuka (1973) and Hiratsuka et al. (1992) reported var. *orobi* only on *V. unijuga*, *V. nipponica* var. *capitata*, and *L. davidii*. Historically, Gäumann (1934) stated that *Uromyces* on the three host plants were formae speciales of *U. fabae*. However, the host ranges of *U. fabae* and *U. orobi* are obscure (El-Gazzar 1981). Our inoculation results revealed that three *Uromyces* isolates from *V. cracca* showed variation in virulence on different *Vicia* species and *P. sativum*. We repeated the inoculation of urediniospores from *V. unijuga* onto *P. sativum*, then inoculated urediniospores from *P. sativum* onto *V. unijuga*. The cross-inoculations were successful (data not showed). However, the inoculation results suggest that host specificity is also not an appropriate characteristic for classification of our specimens.

Morphological observations, inoculation experiments, and molecular phylogenetic analyses revealed that *U. viciae-fabae* did not show significant variation among our specimens on *Vicia*, *Lathyrus*, and *Pisum* in Japan. According to these results, we suggest that *U. viciae-fabae* var. *viciae-fabae* and var. *orobi* should be included in *U. viciae-fabae* (= *U. fabae*).

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