I. Introduction

Legumes are important crops and are cultivated throughout the world. The edible parts include seeds, pods, leaves, flowers and tuberous roots that afford high energy to human and animals (Yuasa and Maekawa 1987). Rust diseases are a serious problem in the cultivation of legumes, which cause severe damage to the edible parts and reduce either quantity or quality of legumes products. *Uromyces* is recognized as one of the most important rust genera known to occur on legumes, and when occurring in epidemic proportions it may cause heavy economic losses around growing area, especially, on pea, bean, cowpea and related legumes (Duke 1981; Thurston 1998).

The pea rust fungus, *Uromyces viciae-fabae* (Pers.) Schroet., is an autoecious and macrocyclic rust fungus occurring on wild and cultivated *Vicia, Lathyrus, Pisum* and *Lens* plants throughout the world (Wilson and Henderson 1966; Cummins 1978; Duke 1981; Azbukina 1984; Guo and Wang 1986; 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 in Japan (Ito 1922, 1950; Hiratsuka 1930, 1933, 1973; Hiratsuka et al. 1992) and Europe (Gäumann 1934; Wilson and Henderson 1966).

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 diseases in different geographic areas and occurring on closely related host plants (Gäumann 1934; Wilson and Henderson 1966; Azbukina 1984; Hiratsuka et al. 1992) (Table 1.1).

On the other hand, *Uromyces appendiculatus* (Pers.) Unger and *U. vignae* Barclay

are also autoecious and macrocyclic rust fungi that occur on beans, cowpea and related legumes across the world (Cummins 1978; Duke 1981; Guo and Wang 1986; Hiratsuka et al. 1992; Thurston 1998).

Uromyces appendiculatus (Pers.) Unger was first found in Europe in 1795 on *Phaseolus vulgaris* L. (cited from Arthur 1934) and *U. vignae* Barclay in India in 1891 on *Vigna vexillata* (L.) A. Rich. (Barclay 1891). Fromme (1924) considered that *U. vignae* was distinct from *U. appendiculatus* because the former species possessed superequatorial germ pores in urediniospores and occurred on a host genus different from one for the latter. Contrarily, Arthur (1934) believed that the morphological distinction of the two rust fungi on *Phaseolus* and *Vigna* were not sufficient enough to warrant specific separation and, thus, classified them under a single species, *U. phaseoli* (Pers.) Winter, with two varieties, var. *typica* Arth. and var. *vignae* (Barclay) Arth.

In Japan, Ito (1922) classified a rust fungus found on species of *Phaseolus* and *Vigna* into *U. appendiculatus* because of the morphological similarity in urediniospores. Later, Hiratsuka (1937) separated a fungus on *Phaseolus* from a fungus on *Vigna* as a distinct species without stating a reason. Later, Ito and Murayama (1943) described a rust fungus on *Apios fortunei* Maxim. as a new species, *U. dispersus* Hiratsuka f. ex S. Ito & Murayama. Hirata (1952) separated a rust fungus on *V. angularis* (Wild.) Ohwi & Ohashi var. *angularis*, *V. angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi and *V. umbellata* (Thunb.) Ohwi & Ohashi from *U. appendiculatus* and described it as a new species, *U. azukicola* S. Hirata.

Hiratsuka (1973) changed his taxonomic opinion and followed Arthur's (1934) taxonomic treatment of *U. phaseoli*; and, thus, *U. appendiculatus*, *U. azukicola*, *U. dispersus* and *U. vignae* became a variety of *U. phaseoli* (Pers.) Winter. Hiratsuka and his colleagues (Hiratsuka et al. 1992), however, followed Cummins's (1978) circumscription of *U. appendiculatus* and *U. vignae* and recognized three varieties under the former species. The major morphological characteristics of *U. appendiculatus* including varieties and *U. vignae* in Japan were listed in Table 1.2. Since Cummins's (1978) taxonomic treatment, *U. appendiculatus* has been said to differ from *U. vignae* in the position of urediniospore germ pores, the teliospore-wall ornamentation and putative host specificity (Cummins 1978; Fernandez and Heath 1985; Guo and Wang 1986; Hiratsuka et al. 1992).

This taxonomic conclusion, however, has not aided at the proper identification of the rust species that occur on *Phaseolus* and *Vigna* across the world, particularly the host specificity having been a source of confusion (El-Gazzar 1981; Duke 1981). Therefore, identification of *U. appendiculatus* with three varieties and *U. vignae* has often been difficult and even erroneous. The current difficulty in the taxonomy and identification of the *U. appendiculatus* and *U. vignae* species complex necessitates re-evaluation of morphological features and physiological specialization of the concerned fungi for their proper circumscription and taxonomic distinction.

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 since 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 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, and 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).

DNA regions coding ITS regions including 5.8 S rRNA, 18 S rRNA, 28 S rRNA, β-tubulin genes and mitochondrial DNA are often used to analyze filamentous fungi (Guadet et al. 1989; Bruns et al. 1991; Zambino and Szabo 1993; Vogler and Bruns 1998; Piepenbring et al. 1999; Begerow et al. 2000; Dahlman et al. 2000; Kahlman et al. 2000; O'Donnell et al. 2000; Taylor et al. 2000; Tehler et al. 2000; de Jong et al. 2001; Ko and Jung 2001; Pfunder et al. 2001; Virtudazo et al. 2001; Almaraz et al. 2002; Maier et al. 2003; Weber et al. 2003; Chung et al. 2004). Using these DNA regions, taxonomic revisions of filamentous fungi with indistinct morphologically characters were carried out recently (O'Donnell et al. 2000; de Jong et al. 2001; Zambino and Szabo 1993; Taylor et al. 2000; Pfunder et al. 2001).

For taxonomic reexamination, specimens of *U. viciae-fabae*, *U. appendiculatus*,

and *U. vignae* were collected from different places of Japan, and the morphological features of urediniospores and teliospores were clarified using light and electron scanning microscopes. Subsequently, molecular phylogenetic analyses were done based on large subunit rDNA (D1/D2) and internal transcribed spacer (ITS) regions including 5.8 S rRNA gene. Using the morphological characteristics and molecule data, the taxonomy of *U. viciae-fabae*, *U. appendiculatus* and *U. vignae* are discussed in the present study.

Table 1.1. Morphological characteristics of *Uromyces viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* on *Vicia, Lathyrus* and *Pisum* in Japan, Europe and Russia.

Varieties of *U. viciae-fabae*	Japan (Hiratsuka et al. 1992)		Europe (Gaumann 1934; Wilson and Henderson 1966)		Russia (Azbukina 1984)	
	Urediniospore wall thickness	Host plants	Urediniospore wall thickness	Host plants	Urediniospore wall thickness	Host plants
var. *viciae-fabae*	1.5-2.5 µm	*Lathyrus maritimus* *L. palustris* *Pisum sativum* *Vicia amoena* *V. cracca* *V. deflexa* *V. faba* *V. fauriae* *V. japonica* *V. tanakae*	1.2-2.5 µm	*Lathyrus pratensis* *L. palustris* *Pisum sativum* *Vicia angustifolia* *V. cracca* *V. bithynica* *V. faba* *V. hirsuta* *V. lutea* *V. sativa* *V. sepium* *V. unijuga* *V. nipponica* var. *capitata*	1.5-2.5 µm	*Lathyrus maritimus* *L. komarovii* *L. palustris* *L. pilosus* *Pisum sativum* *Vicia amoena* *V. amurensis* *V. multicaulis* *V. pseudo-orobus* *V. venosa* *V. cracca* *V. faba* *V. japonica*
var. *orobi*	2-3 µm	*Lathyrus davidii* *Vicia unijuga* *V. nipponica* var. *capitata*	3-4 µm	*Lathyrus montanus* *L. vernus* *L. maritimus*	2-3.5(4) µm	*Lathyrus davidii* *Vicia unijuga*

Table 1.2. Morphological characteristics of two *Uromyces* species on bean and cowpea and related legumes (Hiratsuka et al. 1992).

Uromyces species and varieties	Urediniospores		Teliospores	Host plants
	Size(µm)	Germ pores	Size(µm)	
U. appendiculatus				
var. *appendiculatus*	18-28x18-24	2, equatorial or subequatorial	24-40x20-30	*Phaseolus vulgaris*
var. *azukicola*	18-34x14-26	2, equatorial or subequatorial	20-38x10-28	*Vigna angularis* var. *angularis, V. angularis* var. *nipponensis, V. umbellata*
var. *dispersus*	25-32x20-27	2(3), equatorial or subequatorial	25-40x17-26	*Apios fortunei*
U. vignae	20-36x18-35	2(3), near apex	27-36x20-28	*Vigna unguiculata, Lablab purpureus*