# Studies on the ecology and pathogenic specialization of soilborne pathogens affecting adzuki bean (*Vigna angularis*)

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## Introduction

The commercial production of adzuki bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] began in the early 1900s on the northern island of Hokkaido, Japan. The cultivation area, comprising more than 60,000 ha in 1960, has been maintained at about 20,000 ha even in recent years, indicating that adzuki bean has been one of the most important upland crops in Hokkaido. Diseases caused by soilborne pathogens, as well as other microbial pathogens and insect pests, have been factors that limit the production of adzuki bean. The most important diseases are brown stem rot, Phytophthora stem rot, and Fusarium wilt caused by *Cadophora gregata* (formerly *Cephalosporium gregatum* or *Phialophora gregata*), *Phytophthora vignae*, and *Fusarium oxysporum*, respectively.

Crop rotation as a cultural control method can effectively prevent these diseases, but may not dramatic reduce these diseases on adzuki bean because of its short intervals of cultivation. Therefore, growers in Hokkaido have been hoping for the development of varieties that are resistant to these diseases. Extensive research to develop such resistant varieties has been carried out for more than 40 years. Successful breeding of disease-resistant varieties generally requires knowledge not only of the sources of heritable resistance, but also of the biology and genetics of pathogens. In this review, I present the current understanding of the ecology and pathogenic

specialization of the three pathogens and advances in breeding for resistance in adzuki bean.

**Brown stem rot**

Brown stem rot (BSR) caused by *C. gregata* Harrington and McNew f. sp. *adzukicola* Kobayashi, Yamamoto, Negishi, and Ogoshi (Harrington and McNew 2003; Kobayashi et al. 1991) is characterized by wilting and a reddish-brown discoloration of vascular and pith tissues of the stem and the petiole, often in conjunction with leaf chlorosis, necrosis, or defoliation. The current epidemic of BSR was first observed in the late 1960s in Tokachi District in eastern Hokkaido, then the infested area extended to Kamikawa and Shiribeshi districts in central and western Hokkaido during the 1970s. Even in recent years, the proportion of severely diseased fields has remained at about 8% in Hokkaido.

The pathogenicity of isolates from adzuki beans in Japan and from soybeans in the United States has been tested in naturally infested fields in Hokkaido, Japan and in Ames, Iowa, respectively (Kobayashi et al. 1983). Pathogenic strains affecting adzuki bean and soybean differed in their ability to infect and incite brown stem rot in each host, whereas there were no differences in the morphological and cultural characteristics between both strains. These results suggested the existence of formae speciales of the fungus, then Yamamoto et al. (1990) and Kobayashi et al. (1991) named *C. gregata*

f. sp. *adzukicola* and *C. gregata* f. sp. *sojae* for adzuki bean and soybean, respectively, in subsequent phylogenetic and pathogenicity experiments.

Although survival organs such as chlamydospores are not formed by the fungus, conidia can survive for more than 68 and 28 weeks in field soils at continuous temperatures 15°C and 4°C, respectively (Kondo and Kobayashi 1983). Thus, in field soils, the fungus apparently survives in the crop residues in the form of mycelia, which produces conidia on residue surfaces at low temperatures that will then serve as inoculum.

Three races of *C. gregata* were identified in Hokkaido by their responses to the differential adzuki bean varieties Kita-no-otome and Acc259: race 1, avirulent to both varieties; race 2, virulent to Kita-no-otome but not to Acc259; and race 3, virulent to Acc259 but not to Kita-no-otome. Races 1 and 2 were the first to be identified (Kondo et al. 1998); race 3 was later confirmed among isolates previously identified as race 1 (Kondo et al. 2005a). The distribution of races 1 and 2 was examined using a total of 483 isolates obtained from 39 fields in 19 locations in Hokkaido. Race 1 was predominant (416 isolates, 86.1%) in the commercial fields tested. Race 2 isolates were widely distributed in most of the production areas (25 fields, 64.1%) and were sympatric with race 1 isolates in most fields surveyed (Kondo et al. 2002), although the frequency of

isolation was lower than for race 1.

Variety Acc259 in the adzuki bean collection in Tokachi Agricultural Experiment Station, Hokkaido has been used as a source of resistance to both races 1 and 2. However, an outbreak of BSR on Acc259 was found in a plot in the experimental field in which Acc259 had been cultivated successively to explore the effect of resistant adzuki beans on the race frequency of the population in the plot soil. Although during the first year no disease was found on Acc259, a slight incidence (4%) was observed in the second year and severe BSR (about 100% of diseased plants) was found in the third year and thereafter. Consequently, the existence of a new race of the pathogen, designated race 3, was determined; its frequency in the plot soil was shown to increase from 16.7% before planting Acc259 to 100% after the third year of cultivation. Of 140 isolates from the commercial production area that were formerly identified as race 1, 13 isolates were actually race 3 and were restricted to certain limited fields in Tokachi and Shiribeshi districts (Kondo et al. 2005a).

From the results of amplified fragment length polymorphism (AFLP) analysis with representative isolates selected using population-based race data from Shiribeshi, Ishikari, Kamikawa and Tokachi districts, cluster analysis revealed no close correlation between races and AFLP groups. Gene diversity analysis detected high levels of gene differentiation among four regional populations,

115 which is likely due to genetic drift after the introduction of a limited
116 number of genotypes into each population, indicating low levels of
117 interpopulation gene flow (Kondo et al. 2002).

118 Isolates of *C. gregata* avirulent to both adzuki bean and
119 soybean were obtained from soils in Tokachi District. However,
120 polymerase chain reaction with primers specific for *C. gregata* f. sp.
121 *adzukicola* detected a specific DNA fragment in these isolates, and
122 cluster analysis with intersimple sequence repeat markers revealed
123 that the isolates were phylogenetically closer to strains that are
124 virulent to adzuki bean (Tanaka et al. 2010). A few selected isolates of
125 the nonpathogenic *C. gregata* were highly effective in reducing BSR
126 incidence and have potential for development as biological control
127 agents.

128 A molecular marker that was developed to separate and
129 identify specific populations of the soybean pathogen collected in the
130 United States (Chen et al. 2000) was used to analyze populations of
131 the adzuki bean pathogen and the nonpathogenic fungus. Soybean
132 isolates were separated into A or B genotypes, and adzuki bean
133 isolates were placed in the C genotype (Chen et al. 2000). However,
134 98 adzuki bean isolates, including nonpathogenic ones, were
135 separated not only into the A, B, or C genotypes, but also into an
136 additional D genotype. These genotypes were also not associated with
137 races of the adzuki bean pathogen. All four genotypes were found in

Tokachi District, while genotypes A and B were detected in Kamikawa and Ishikari districts (Ito and Kondo 2007).

**Phytophthora stem rot**

Phytophthora stem rot (PSR) caused by *P. vignae* Purss f. sp. *adzukicola* Tsuchiya, Yanagawa et Ogoshi (Tsuchiya et al. 1986), which is phylogenetically close to the pathogen (*P. vignae* f. sp. *vignae*) of cowpea (Kondo et al. 2005b), is also an economic constraint on adzuki bean production (Kondo et al. 2004). This disease was first found in Kamikawa District in 1967 and became the main limitation to the production of adzuki beans throughout Hokkaido in the 1980s. Overproduction of rice in the 1960s became a problem, leading to the establishment of an act to equilibrate supply with demand for rice. Hence, during the 1970s, producers were obliged to convert rice paddies into upland fields to cultivate upland crops. Adzuki bean was grown widely in these converted fields in central and western Hokkaido. Poorly drained fields usually provide the moist environmental conditions that favor infection by the PSR pathogen and the development of this disease. In 1977, this disease was epidemic in the converted fields in Kamikawa District.

The PSR pathogen infects the roots, epicotyls, and stems of young seedlings and mature adzuki plants, causing water-soaked lesions that eventually enlarge and turn reddish-brown. Symptoms

include leaf yellowing and blight with stem-girdling lesions followed by wilting and death. The pathogen persists in soils as oospores either in crop residues or free in soil after residues decompose, and can survive for many years without a host. Although infection through leaves was not observed in field conditions, detached leaves can be infected in the laboratory after inoculation with zoospores (Harada and Kondo 2009); reactions of leaf tissues are characterized by speckled lesions in resistant adzuki bean varieties and water-soaked, spreading lesions in susceptible.

There are three races of the pathogen based on their reactions to differential varieties of adzuki bean: race 1 virulent to var. Erimo-shozu, but avirulent to var. Kotobuki-shozu and var. Noto-shozu; race 2 virulent to vars. Erimo-shozu and Kotobuki-shozu, but not var. Noto-shozu; and race 3 virulent to vars. Erimo-shozu, Kotobuki-shozu, and Noto-shozu. Extensive surveys to determine race distribution from 1974 to 1977 (Tsuchiya et al. 1986) and 1994–1995 (Makino et al. 1997) revealed that races 1 and 3 were predominant in Hokkaido, while race 2 was present at a low frequency or not found. Additionally, no isolates were pathogenic to var. Urasa-Shimane, which was shown to be resistant to all three races in a subsequent survey. However, in 1999 when the resistant var. Syumari (previously known as Toiku no. 140), derived from var. Urasa-Shimane, was first grown in several experimental fields, PSR

was unexpectedly observed on that variety. The presence of a new race, designated race 4, was confirmed, and Erimo-shozu, Kotobuki-shozu, Noto-shozu, and Syumari (or Urasa-Shimane) were proposed as differential varieties for the races (Notsu et al. 2003).. Using these differential varieties, race 4 isolates were proved to be widely distributed in the adzuki bean-producing regions in Hokkaido, especially in central and western Hokkaido (Kondo et al. 2004).

**Fusarium wilt**

Adzuki bean Fusarium wilt (AFW) is caused by *F. oxysporum* Schl.: Fr. emend. Snyder & Hansen f. sp. *adzukicola* Kitazawa & Yanagita (Kitazawa and Yanagita 1989). A destructive AFW occurred first in upland fields converted from paddy fields in Ishikari District, and the epidemic extended to Sorachi and Kamikawa districts in central and western Hokkaido (Kitazawa and Yanagita 1984). Chlorosis, vein necrosis, and wilting of primary and true leaves usually become evident 1 to 2 months after seeding in fields. The pathogen invades the root tissues of young plants and then spreads upward in the vascular tissues to cause wilting and death or rugose leaves of mature plants. The disease is also characterized by a reddish-brown discoloration of vascular and pith tissues of the stem and the petiole.

Although as mentioned above, Kitazawa and Yanagita (1989) identified a novel *forma specialis* of *F. oxysporum* for the pathogen

collected from wilted plants, this conformed to “adzuki tachigare-byo” (the Japanese name for the disease related to the damping-off symptom of adzuki bean caused by *F. oxysporum* f. sp. *phaseoli*; Matsuo 1980). In contrast, Kondo and Kodama (1989) suggested the more accurate term “adzuki icho-byo” (wilt in English) for the disease after confirming the existence of a new *forma specialis* based upon host range tests and comparing the pathogenicity of authentic *F. oxysporum* f. sp. *phaseoli* with that of *F. oxysporum* f. sp. *adzukicola.* Additionally, Kondo and Kodama (1989) identified three races and showed the usefulness of race 3, the most virulent, for the screening of resistant varieties.

Isolates pathogenic and nonpathogenic to adzuki bean were grouped using vegetative compatibility tests with nitrate non-utilizing mutants to analyze the genetic relationships among them (Kondo et al. 1997a). Of 112 pathogenic isolates, 105 collected from 20 fields in Hokkaido were either classified into one large vegetative compatibility group (VCG) consisting of two subgroups (a total of 102 isolates) or one small VCG (three isolates), and the other were self-compatible. All three races were included within the largest VCG. A total of 199 nonpathogenic *F. oxysporum* isolates were incompatible with the two VCGs of *F. oxysporum* f. sp. *adzukicola* and 184 isolates examined were classified into 25 VCGs; the other 15 single were self-compatible isolates. Of these 25 VCGs, 16 were

common among the nonpathogenic *F. oxysporum* populations from soil samples of both non-infested (Tokachi District) and infested fields with *F. oxysporum* f. sp. *adzukicola*. Moreover, 162 isolates (81.3% of all nonpathogenic isolates tested) belonged to one of these 16 VCGs. No marked difference in the population structure of nonpathogenic *F. oxysporum* was observed between the infested and non-infested locations.

Chlamydospores of the pathogen survived on infected plant residues placed on and under the soil (5–30 cm depth) for more than 251 weeks in upland fields (Kondo et al. 1997b). In contrast, in paddy fields converted from infested upland fields, after 5 years of continuous rice cultivation, the pathogen population decreased to undetectable levels. No incidence of AFW in adzuki beans that were planted in soil collected before flooding in the fifth year of rice cultivation was observed, indicating the efficacy of this method for suppressing the disease (Kondo and Kodama 1993).

**Breeding for multiple resistances**

At the Tokachi Agricultural Experiment Station in Hokkaido, varieties resistant to BSR, PSR, and AFW have been bred since 1976, 1981, and 1988, respectively. After identifying races of these pathogens, new potential sources of resistance have been sought from a diverse germplasm collection conserved at the Tokachi Agricultural

Experiment Station (Fujita et al. 2007; Kondo et al. 2009). Furthermore, adzuki bean collections and related species of *Vigna* from various Asian countries from the Genetic Resources Center of the National Institute of Agrobiological Sciences, Tsukuba have been evaluated for resistance (Kondo and Tomooka 2012). The development of DNA markers closely linked to BSR and AFW resistance has enabled breeders to effectively select and identify resistant progenies of the recombinant plants (Suzuki et al. 2013). As a result, multiple-resistance varieties of adzuki bean have been successfully developed.

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## Conflicts of interest

The author has no conflicts of interest to declare.

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