Brown Stem rot and Phytophthora Stem Rot: Limiting Factors in Azuki Bean Production

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Summary

Information on the distribution of races of *Cadophora gregata* f. sp. *adzukicola* and *Phytophthora vignae* f. sp. *adzukicola* is important for the management of brown stem rot and Phytophthora stem rot of azuki beans, respectively. Potential lines or cultivars with multiple resistances are developed using information on virulence of races and infection methods.

Introduction

Brown stem rot (BSR) of azuki bean [*Vigna angularis* (Willd.) Ohwi et Ohashi] caused by *Cadophora gregata* (syn. *Phialophora gregata*) Harrington & McNew f. sp. *adzukicola* Kobayashi, Yamamoto, Negishi, and Ogoshi (Harrington and McNew, 2003; Kobayashi *et al*. 1991), a soil-borne fungus, is a serious problem in Hokkaido, Japan (Fujita *et al*., 2007). BSR is characterized by wilting, and a reddish brown discoloration of vascular and pith tissues of the stem and the petiole that often occur in conjunction with leaf chlorosis, necrosis or defoliation. BSR was first observed in late 1960's in Tokachi district, the eastern part of Hokkaido, and then the infested area extended to Kamikawa and Shiribeshi, the central and western parts during 1970's. In 1977 and 1981 about 75% of the commercial fields in Tokachi district, accounting for about 40% of the total production area of Hokkaido, were infected with the BSR pathogen. Even in recent years the proportion of severely diseased fields has remained about 10 to 15% (Fujita, 2004). BSR is estimated to reduce the actual seed weight per plant by up to 77%.

Phytophthora stem rot (PSR) caused by *Phytophthora vignae* Purss f. sp. *adzukicola* Tsuchiya, Yanagawa et Ogoshi (Tsuchiya *et al*. 1986) is also an economically important constraint to azuki bean production (Kondo *et al*. 2004). The PSR pathogen, an oomycete that is classified in the kingdom Stramenopiles, infects the roots, epicotyls, and stems of young seedlings and mature azuki plants, eventually causing wilt or leaf blight. Leaves can be infected by deliberate inoculation in the laboratory (Harada and Kondo, 2008). Beginning in the 1960's overproduction of rice became a problem in Japan leading to the national government establishing an act to equilibrate supply with demand for rice. Hence, during the 1970's farmers were obliged to convert rice paddies into upland, non-flooded, fields. Azuki bean was grown widely in the converted fields in the central and western parts of Hokkaido. Those poorly drained fields provide the moist environmental conditions that favor infection by *P. vignae* and disease development. This disease was first reported in Hokkaido in 1967 and became the main constraint in the production of azuki beans in Hokkaido including Tokachi district.

Generally, practical methods of controlling these diseases are crop rotations and the use of resistant cultivars. With these disease pressures, producers hoped for the development of cultivars with resistance to both pathogens. Hence, the development of multiple resistant cultivars with good agronomic qualities has been continued since 1981 in collaboration with the Tokachi and Central Agricultural Experiment Stations in Hokkaido, and recently resulted in the release of some promising cultivars. In this paper it is shown how to identify physiological races of the BSR and PSR pathogens. It is important to know the frequency and distribution of races of these pathogens for managing the damage to azuki bean production by new races in the future.

Methods for inoculation of *C. gregata*

Inoculum is grown in V-8 juice broth (200 ml V-8 juice and 2 g $CaCO_3$ centrifuged at 5,000 rpm for 15 min, and the supernatant is diluted to

1 L with distilled water) at 25°C on a reciprocal shaker at 120 oscillations/ min. After 2 to 3 weeks of incubation, mycelia and spores are collected by filtration through Whatman No.1 filter paper, and washed with distilled water. The fungal pellets are homogenized with distilled water (10,000 rpm for 3 min) and diluted to 10^7 spores and mycelial fragments per milliliter. Seedlings grown in sterilized vermiculite (about 10 to 14 days) are removed, washed gently, dipped into each inoculum suspension for 12 hr, then transplanted into a disinfested soil / vermiculite mixture (1:1) in 12-cm diameter plastic pots. The response to the pathogen was evaluated after 8 weeks growth in the greenhouse. The severity of symptoms is assessed in terms of the mean disease severity index (DSI) of plants with a scale where 0 = no symptoms, 1 = a slight vascular discoloration without foliar symptoms, 2 = foliar symptoms or severe vascular discolorations (above the first node or in petioles) , and 3 = death. Plants with a DSI < 0.5 are rated resistant (R), plants with 0.5 ≤ a DSI < 1.0 are regarded as intermediate (I) and plants with a DSI ≥ 1.0 were considered susceptible (S).

Races of *C. gregata* and their distribution

There are three races of *C. gregata* in Hokkaido so far; race 1, virulent on Erimo-shozu and avirulent on Kita-no-otome and Acc259; race 2, virulent on Erimo-shozu and Kita-no-otome but avirulent on Acc259; and race 3, virulent on Erimo-shozu and Acc259 but avirulent on Kita-no-otome (Table 1). Race 1 is predominant in the commercial fields in Hokkaido. Although the frequency of isolation is lower than that of race 1, race 2 was widely distributed in most of the production areas (Kondo *et al*., 2002). Meanwhile, race 3 was actually restricted to certain fields only (Kondo *et al*., 2005).

Methods for Inoculation of *P. vignae*

Root dipping

One gram (fresh weight) of mycelia from 2-week-old cultures of each isolate, grown in pea broth at 25°C, is homogenized (Nissei AM-5, 5,000 rpm, 3 min.) with 100 ml of sterilized distilled water. Seven- to ten-day-old seedlings, grown in sterilized vermiculite, are washed gently with running tap water before inoculation. Seedlings of each cultivar grown in sterilized vermiculite are removed and soaked for 12 hr in inoculum suspensions Then, the seedlings are transplanted into a mixed soil (vermiculite/ Pot Ace, Katakura Chikkarin K. K., Tokyo, Japan (1/1) v/v mixture) in plastic pots (18 cm in diameter). Three weeks after transplanting under greenhouse conditions (12 to 25°C), the incidence of disease (dead or alive) is evaluated.

Epicotyl inoculation

Fourteen-day-old seedlings (planted in vermiculite and grown in growth chamber at 25℃ under fluorescent light for 15 h and 18℃ in the dark for 9 h) are removed from the vermiculite and placed horizontally in plastic containers (15 × 20 × 5 cm) with their roots covered with moistened paper towels. An approximately 1.5 cm long slit wound is made on the upper part of the epicotyl (about 1 cm below the primary node) with a sterilized razor blade. Then, 200 µl drops of minced mycelial (10 mg/ml distilled water) suspension from 3-day-old cultures grown in V8 juice medium (200 ml V8 juice and 2.6g $CaCO_3$ centrifuged at 5000 rpm for 15 min, and the supernatant diluted to 1 L with distilled water) are inoculated into the wounded site. After inoculation, the containers are kept closed with plastic (poly vinylidene chloride) cling film (Asahi kasei chemicals, Tokyo) to maintain high humidity and incubated at 25°C in the dark. The severity is evaluated as described above. The disease severity is assessed two days after inoculation using the mean disease severity index (DSI) on a scale of 0 to 3, where 0 = no symptoms, 1 = a slight or restricted lesion on the epicotyl, 2 = an expanding lesion on the epicotyl, and 3 = death.

Table 1. Races of *Cadophora gregata* f. sp. *adzukicola* and differential cultivars/line

	Erimo-shozu	Kita-no-otome	Acc259
Race 1	S	R	R
Race 2	S	S	R
Race 3	S	R	S

S: susceptible, R: resistant

Races of *P. vignae* and their distribution

The extensive surveys of the races distribution of *P. vignae* f. sp. *adzukicola* during 1977 to 1979 and 1994 to 1995, showed that race 1 (virulent on cv. Erimo-shozu, but avirulent on cv. Kotobuki-shozu and cv. Noto-shozu) and race 3 (virulent to cvs. Erimo-shozu, Kotobuki-shozu and Noto-shozu) are predominant in Hokkaido, while race 2 (virulent on cvs. Erimo-shozu and Kotobuki-shozu, but not cv. Noto-shozu) is present at a low frequency or not observed. Race designation tests in the latter survey used cv. Urasa-Shimane, which is one of the parents of cv. Syumari, as a resistant host to all races. No isolates were virulent on cv. Urasa-Shimane. However, in 1999 when cv. Syumari was first grown in several experimental fields, PSR was observed on it (Notsu *et al*., 2003). The presence of a new race, designated race 4, was confirmed. The differential cultivars for the races were proposed, i.e. Erimo-shozu, Kotobuki-shozu, Noto-shozu, Urasa-shimane or Syumari (Table 2). Race 4 isolates had the broadest host range and were widely distributed in the azuki bean-producing regions, especially in the central and western parts of Hokkaido (Kondo *et al*., 2004). The appearance of race 4 immediately after the release of cv. Syumari has raised concerns about promoting this new cultivar, and the effectiveness and durability of its resistance.

Conclusion

At Tokachi agricultural experiment station in Hokkaido, breeding for BSR and PSR resistance has been carried out in the infested fields since the late 1970's. Field screening for BSR and PSR resistance can be difficult as infection and subsequent disease development are highly influenced by the environmental conditions. Factors such as inoculum levels and isolate aggressiveness, along with air temperature, soil fertility, and soil moisture all impact disease severity. At least race frequency in field soils should be clarified before screening in the field. Screening in the greenhouse allows for control of these factors, thereby improving the chance of discovering truly resistant lines. After identifying races of the BSR and PSR pathogens, new sources of BSR and PSR resistance have been sought(Fujita *et al*., 2007; Kondo *et al*. 2009). Azuki bean or wild azuki bean germplasm from some Asian countries including Japan have been evaluated so far for resistance genes and some potential candidates have been identified. Thus, multiple resistant lines are developed from the cross with new resistant gene sources. In addition, now we focus on using partial resistance (also termed field resistance) against PSR as well as Phytophthora stem and root rot of soybean caused by *P. sojae*.

Table 2. Races of *Phytophthora vignae* f. sp. *adzukicola* and differential cultivars

	Erimo-shozu	Kotobuki-shozu	Noto-shozu	Syumari
Race 1	S	R	R	R
Race 2	S	S	R	R
Race 3	S	S	S	R
Race 4	S	S	S	S

S: susceptible, R: resistant

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