New Sources of Resistance to *Cadophora gregata* f. sp. *adzukicola* and *Fusarium oxysporum* f. sp. *adzukicola* in *Vigna* spp.

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Abstract

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Adzuki bean brown stem rot (BSR), caused by *Cadophora gregata* f. sp. *adzukicola*, and adzuki bean Fusarium wilt (AFW), caused by *Fusarium oxysporum* f. sp. *adzukicola*, are serious problems in Hokkaido, Japan, and have been managed using cultivars with multiple resistance. However, a limited number of adzuki bean varieties are resistant to these pathogens because of the frequent appearance of new races; thus, new sources of resistance have been sought in related *Vigna* spp., particularly in the section *Angulares* to which adzuki bean belongs. An analysis of selected *Vigna* accessions (JP81231 to JP235420) conserved in the Genebank of the National Institute of

Agrobiological Sciences, Tsukuba, Japan, revealed wide variation in resistance spectra and resistance combinations, and eight disease response groups (A to H) were identified. Four of eight were newly detected response groups, suggesting the existence of novel resistance genes. Of 252 accessions from 26 species, 28 accessions in *Vigna angularis* var. *nipponensis*, *V. hirtella*, *V. minima*, and *V. tenuicaulis* (section *Angulares*) from group D, which were cross-compatible with adzuki bean, are expected to be potential sources of multiple resistance genes. They were resistant to all races of BSR and AFW pathogens examined.

Brown stem rot (BSR) of domesticated adzuki bean (*Vigna angularis* var. *angularis*), caused by the soilborne fungus *Cadophora gregata* (syn. *Phialophora gregata*) f. sp. *adzukicola* (8,11), is a serious problem in Hokkaido, Japan (2). Adzuki bean Fusarium wilt (AFW), caused by *Fusarium oxysporum* f. sp. *adzukicola* (10), another soilborne fungus, is often observed in the western part of Hokkaido (2,14). Both diseases are characterized by wilting and a reddish-brown discoloration of vascular and pith tissues in the stem and petiole that often occurs in conjunction with leaf chlorosis or necrosis (14,18). AFW is an early-season disease usually appearing in early July, whereas BSR occurs from late August to early September. In both cases, the pathogen invades the root tissues of young plants, then spreads upward to the vascular and pith tissues via mycelia and conidia (14,18), leading to wilting in susceptible plants.

The multiple-resistant adzuki bean 'Kita-no-otome' (registered in 1996) and 'Syumari' (registered in 2003) have contributed greatly to the control of both diseases, especially AFW (4,5,14). However, race 2 of *C. gregata* f. sp. *adzukicola*, which is virulent on both cultivars, was discovered in 1995 (12,13). To find a new source of resistance to race 2, Fujita et al. (3) screened 236 cultivated and 33 wild adzuki bean plants and selected several cultivated (e.g., Acc259) and wild (*V. angularis* var. *nipponensis*; e.g., Acc2515) adzuki bean accessions resistant to both races 1 and 2 (3). However, Acc259 (a local cultivar from Ehime prefecture, Shikoku, Japan), one of the accessions used as a resistance gene donor for races 1 and 2, became completely susceptible to a new race (designated race 3) after continuous field cultivation at the Tokachi Agricultural Experiment Station (TAES) in Memuro, Hokkaido, Japan (15). As the number of designated races has increased in Hokkaido, the need to find additional sources of resistance and to incorporate multiple sources of resistance into commercial culti-

vars has become essential. Kondo et al. (16) screened a total of 67 accessions using race 3 of BSR and found 39 resistant accessions. However, only two accessions showed resistance to all three races of BSR and AFW (16): Akamame (cultivated accession) and Acc2515 (a wild accession collected in Ishikawa prefecture, Japan).

The work of Fujita et al. (3) and Kondo et al. (16) suggested that wild relatives of adzuki bean have strong potential as novel sources of resistance; therefore, further screening of wild species belonging to the genus *Vigna* was conducted in the present study. From hybridization studies in domesticated adzuki bean, several wild *Vigna* spp. in the section *Angulares* were revealed to be cross-compatible and were classified as the primary gene pool of cultivated adzuki bean (23) based on the concept of Harlan and De Wet (7). This *Vigna* gene pool is expected to contribute to the development of resistant cultivars of adzuki bean. The purpose of this study was to identify new sources of resistance to *C. gregata* f. sp. *adzukicola* and *F. oxysporum* f. sp. *adzukicola* in the wild *Vigna* gene pool.

Materials and Methods

Plant materials. The *Vigna* spp. germplasm examined in this study consisted of 252 accessions from 26 species (JP accession numbers JP81231 to JP235420) conserved in the Genebank of the National Institute of Agrobiological Sciences (NIAS Genebank) in Tsukuba, Japan (Table 1). This *Vigna* spp. collection includes three subgenera and six sections (i.e., subgenus *Ceratotropis* [sections *Angulares*, *Ceratotropis*, and *Aconitifoliae*], subgenus *Vigna* [sections *Catiang* and *Vigna*], and subgenus *Plectotropis* [section *Plectotropis*]; 23,24). In the collection, seven species (Table 1, bold) in the section *Angulares* are cross-compatible with adzuki bean; therefore, a large number of accessions were tested for resistance. Four cultivars and lines of adzuki bean or wild adzuki bean conserved in the TAES ('Erimo-shozu', 'Kita-no-otome', Acc259, and Acc2515) were used as standards to represent disease reaction groups (16). Seedlings were grown in the greenhouse in plastic containers (15 by 20 by 5 cm) containing sterilized vermiculite for 10 to 14 days to fully expand their primary leaves. The roots were then washed gently with running tap water before inoculation with the pathogens. Greenhouse temperatures were a minimum of 15°C at night and a maximum of 32°C during daytime.

Fungal isolates and preparation of the inocula. The inoculations were carried out according to Kondo et al. (16). The follow-

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ing isolates of *C. gregata* f. sp. *adzukiicola* were used: T96-1 (race 1, deposited as MAFF 241056 in the NIAS Genebank), T96-5 (race 2, MAFF 241057), and T650 (race 3, MAFF 241055; 16). Inocula for *C. gregata* were produced in liquid V8 juice medium (200 ml of V8 juice containing 2 g of CaCO₃ was centrifuged at 1,400 × g for 15 min and the supernatant was diluted to 1 liter with distilled water) at 25°C on a reciprocal shaker (TB-128; Takasaki Scientific Instrument, Kawaguchi, Japan) at 120 oscillations/min. After 3 weeks, mycelia and bud cells were collected by filtering through filter paper (Whatman number 1), washed in distilled wa-

ter, and centrifuged. The fungal pellets were homogenized in distilled water with a homogenizer (10,000 rpm for 3 min; AN-5, Shin-nihonseiki, Tokyo). The concentration of mycelial fragments and bud cells was determined using a hemocytometer; mycelial fragments of all sizes were included in the counts. Homogenized cultures were then diluted with distilled water to a concentration of 10⁷ propagules/ml. Isolate 96-3K2 (MAFF 241054) of *F. oxysporum* f. sp. *adzukiicola*, a race 3 virulent on many varieties (14), was also used. The inoculum was produced in potato sucrose broth medium (Difco, Lawrence, KS) with 2% yeast extract (Difco) at

Table 1. *Vigna* plant accessions in disease response groups representing resistance or susceptibility to *Cadophora gregata* f. sp. *adzukiicola* and *Fusarium oxysporum* f. sp. *adzukiicola*

Accessions ^y	Total	Disease response group (reaction to each pathogen) ^x							
		A (S,S,S,S)	B (R,S,R,R)	C (R,R,S,R)	D (R,R,R,R)	E (S,S,S,R)	F (R,S,S,R)	G (R,S,R,S)	H (R,R,R,S)
<i>Ceratotropis</i>									
<i>Angulares</i>									
<i>Vigna angularis</i> ^z	80	4	38	14	12	9	...	1	2
<i>V. hirtella</i>	35	...	2	3	10	19	1	...	...
<i>V. minima</i>	21	2	1	3	2	13	...	...	...
<i>V. nakashimae</i>	25	...	...	5	...	16	4	...	...
<i>V. nepalensis</i>	6	...	...	...	...	6	...	...	...
<i>V. riukiensis</i>	24	...	...	...	...	24	...	...	...
<i>V. tenuicaulis</i>	30	2	3	...	4	20	1	...	...
<i>V. dalzelliana</i>	1	...	...	...	...	1	...	...	...
<i>V. exilis</i>	2	...	...	...	...	2	...	...	...
<i>V. glabrescens</i>	1	...	...	...	1	...	...	...	...
<i>V. reflexo-pilosa</i>	1	...	...	...	...	...	1	...	...
<i>V. trinervia</i>	1	...	...	...	...	...	1	...	...
<i>V. umbellata</i>	5	...	...	...	...	4	1	...	...
<i>Vigna</i> sp. (JP210811)	1	...	...	...	...	1	...	...	...
<i>Ceratotropis</i>									
<i>V. grandiflora</i>	1	...	...	...	...	1	...	...	...
<i>V. mungo</i>	2	...	...	...	...	2	...	...	...
<i>V. radiata</i>	2	...	...	...	...	2	...	...	...
<i>V. subramaniana</i>	3	...	...	...	...	3	...	...	...
<i>Vigna</i> sp. (JP235420)	1	...	...	...	...	1	...	...	...
<i>Aconitifoliae</i>									
<i>V. aconitifolia</i>	1	...	...	...	...	1	...	...	...
<i>V. aridicola</i>	3	...	...	...	...	2	1	...	...
<i>V. stipulacea</i>	1	...	...	...	...	1	...	...	...
<i>V. trilobata</i>	1	...	...	...	...	1	...	...	...
<i>Plectotropis</i>									
<i>V. vexillata</i>	1	...	...	...	1	...	...	...	...
<i>Vigna</i>									
<i>V. luteola</i>	1	...	...	...	...	1	...	...	...
<i>Catiang</i>									
<i>V. unguiculata</i>	2	...	...	1	...	1	...	...	...
Total	252	8	44	26	30	131	10	1	2

^x Disease response groups E–H are newly identified groups. Groups A–D were designated in Kondo et al. (16). Reaction (R = resistant and S = susceptible) are shown to *C. gregata* f. sp. *adzukiicola* race 1, race 2, race 3, and *F. oxysporum* f. sp. *adzukiicola* race 3, respectively.

^y Subgenus, section, species. Species shown in bold are cross compatible with domesticated adzuki bean (*V. angularis* var. *angularis*).

^z *V. angularis* var. *nipponensis*.

Table 2. Group designation of *Vigna* spp. based on differential reactions to races of *Cadophora gregata* f. sp. *adzukiicola*, cause of adzuki bean brown stem rot, and *Fusarium oxysporum* f. sp. *adzukiicola*, cause of adzuki bean Fusarium wilt

Group	Differential cultivar or line	Reaction ^y				Number of <i>Vigna</i> spp.
		<i>C. gregata</i> f. sp. <i>adzukiicola</i>			<i>F. oxysporum</i> f. sp. <i>adzukiicola</i>	
		Race 1	Race 2	Race 3	Race 3	
A	Erimo-shozu	S	S	S	S	3
B	Kita-no-otome	R	S	R	R	4
C	Acc259	R	R	S	R	5
D	Acc2515	R	R	R	R	6
E	...	S	S	S	R	22 ^z
F	...	R	S	S	R	7
G	...	R	S	R	S	1
H	...	R	R	R	S	1

^y R = resistant and S = susceptible.

^z Two unidentified species are included.

25°C on a reciprocal shaker at 120 oscillations/min. After 5 days of incubation, bud cells were collected by centrifugation at 1,400 × g for 10 min, washed in distilled water, and centrifuged. The concentration was adjusted to 10⁶ bud cells/ml in distilled water using a hemocytometer.

Screening for resistance. The roots of five to eight seedlings of each accession and adzuki bean cultivar, depending upon the seed numbers provided, were soaked in a suspension (50 ml) of each inoculum for 12 h. Two to four seedlings were then placed in each

2- to 3-cm-deep depression in sterilized potting soil (1:1 [vol/vol] vermiculite/Pot-ace; Katakura Chikkarin K. K., Tokyo) in plastic pots (12 cm in diameter) with two replicates. The remaining inoculum was then poured over the roots of the seedlings to maximize coverage, and the roots were covered with sterilized potting soil. All tests were conducted at least twice in all combinations and the data were combined. The nighttime low and daytime high temperatures in the greenhouse during testing were 15 and 32°C, respectively. The plants received supplemental lighting from

Table 3. *Vigna* spp., geographic sources, and accession numbers in disease response groups representing resistance or susceptibility to *Cadophora gregata* f. sp. *adzukicola* and *Fusarium oxysporum* f. sp. *adzukicola*

Group ^y	Subgenus	Section, species	Source	Accession (JP number) ^z				
A	<i>Ceratotropis</i>	<i>Angulares</i>						
		<i>Vigna angularis</i> var. <i>nipponensis</i>	Honsyu Island, Japan	87907	201464	211827		
			Kyusyu Island, Japan	225143				
		<i>V. minima</i>	Laos	222402				
			Papua New Guinea	226877				
B	<i>Ceratotropis</i>	<i>Angulares</i>						
		<i>V. angularis</i> var. <i>nipponensis</i>	Honsyu Island, Japan	87896	87898	87908	87909	87919
				90832	90837	90842	90843	90844
				90845	90846	90857	90860	90861
				90863	90866	90869	110658	110659
				201458	201461	201462	201466	211839
				211840	211844	225126	225132	225137
			Shikoku Island, Japan	81646	110690	110691	110693	110694
			Kyusyu Island, Japan	110676	110680			
			Myanmar	226056				
		<i>V. hirtella</i>	Myanmar	210805				
			Laos	220137				
		<i>V. minima</i>	Laos	224430				
		<i>V. tenuicaulis</i>	Myanmar	217444	217476	217519		
C	<i>Ceratotropis</i>	<i>Angulares</i>						
		<i>V. angularis</i> var. <i>nipponensis</i>	Honsyu Island, Japan	87905	87906	87913	87914	87917
				87918	90833	90834	90835	91231
				201457	225140			
			Kyusyu Island, Japan	110670	110679			
		<i>V. hirtella</i>	Laos	224435	224436	226680		
		<i>V. minima</i>	Thailand	205888				
			Myanmar	210806				
			Laos	220141				
		<i>V. nakashimae</i>	South Korea	212329	212333	212339	212340	212349
	<i>Vigna</i>	<i>Catiang</i>						
		<i>V. unguiculata</i>	Sri Lanka	81610				
D	<i>Ceratotropis</i>	<i>Angulares</i>						
		<i>V. angularis</i> var. <i>nipponensis</i>	Honsyu Island, Japan	87910	90836	90849	90850	90856
				107861	110662	201465		
			Kyusyu Island, Japan	110674				
			Myanmar	217478	217479	217480		
		<i>V. hirtella</i>	Thailand	202274				
			Myanmar	217447				
			Sri Lanka	218935				
			Laos	220133	220134	220135	224426	224432
				224443	226687			
		<i>V. glabrescens</i>	Philippine	109684				
		<i>V. minima</i>	Laos	220136	220139			
		<i>V. tenuicaulis</i>	Thailand	109682				
			Myanmar	217502				
			Laos	226649	226682			
E	<i>Plectotropis</i>	<i>Plectotropis</i>						
		<i>V. vexillata</i>	Papua New Guinea	230747				
	<i>Ceratotropis</i>	<i>Angulares</i>						
		<i>V. angularis</i> var. <i>nipponensis</i>	Honsyu Island, Japan	90855	90862	201456		
			Kyusyu Island, Japan	110675	110682			
			Shikoku Island, Japan	110686				
			India	110827				

(continued on next page)

^y A, susceptible to all pathogens tested; B, susceptible to *C. gregata* f. sp. *adzukicola* race 2 but resistant to the other pathogens; C, susceptible to *C. gregata* f. sp. *adzukicola* race 3 but resistant to the other pathogens; D, resistant to all pathogens tested; E, resistant to *F. oxysporum* f. sp. *adzukicola* race 3 but susceptible to the other pathogens; F, susceptible to *C. gregata* f. sp. *adzukicola* races 2 and 3 but resistant to *C. gregata* f. sp. *adzukicola* race 1 and *F. oxysporum* f. sp. *adzukicola* race 3; G, susceptible to *C. gregata* f. sp. *adzukicola* race 2 and *F. oxysporum* f. sp. *adzukicola* race 3 but resistant to *C. gregata* f. sp. *adzukicola* races 1 and 3; and H, susceptible to *F. oxysporum* f. sp. *adzukicola* race 3 but resistant to the other pathogens.

^z JP = Japan number identifying accessions used in the Genebank of the National Institute of Agrobiological Sciences. Numbers in bold letters indicate domesticated accession.

metal halide sodium lamps (400 W) to maintain a 16-h photo-period.

The disease severity of BSR (3) was assessed 8 weeks after inoculation as the mean disease severity index (DSI), where 0 = no symptoms, 1 = slight vascular discoloration without foliar symptoms, 2 = foliar symptoms or severe vascular discoloration (above the first node or in the petioles), and 3 = death. Plants with DSI < 0.5 were rated resistant (R), plants with DSI $\geq$ 0.5 to < 1.0 were regarded as intermediate (I), and plants with DSI $\geq$ 1.0 were

considered susceptible (S). To evaluate AFW (14), we scored the reactions of individual plants 8 weeks after inoculation according to a disease severity rating (DSR) of 1 to 10³, where 1 = healthy, no external symptoms, and no vascular discoloration; 10 = plants showing vascular discoloration but no external symptoms; 10² = plants showing leaf necrosis or stunting or wilting; and 10³ = dead plants. The DSI was calculated using the following formula: DSI = log₁₀ [mean (DSR)]. Plants with DSI $\leq$ 1.0 were rated resistant (R), whereas plants with DSI > 1.0 were considered susceptible (S).

Table 3. (continued from preceding page)

Group ^y	Subgenus	Section, species	Source	Accession (JP number) ^z				
F	<i>Ceratotropis</i>		Myanmar	217477	217481			
		<i>V. dalzelliana</i>	India	235419				
		<i>V. exilis</i>	Thailand	205884	210684			
		<i>V. hirtella</i>	Thailand	108551	108558	108562	109681	110839
				205885				
			Myanmar	210823	211876	217435	217451	217491
			Laos	220130	220131	224442	226635	226637
				226663	226669			
		<i>V. minima</i>	Thailand	107869	108129	205890	205891	210655
				210669	210683			
			Indonesia	202289	218938			
			Myanmar	210821	210824			
			Laos	220140	220142			
		<i>V. nakashimae</i>	South Korea	81231	212325	212326	212327	212328
				212331	212334	212335	212336	212338
				212345	212346	212348	212352	212353
			Kyusyu Island, Japan	107879				
		<i>V. nepalensis</i>	Nepal	107881	109687	109689		
			India	110826	110827			
			Bhutan	109688				
		<i>V. riukiensis</i>	Taiwan, China	107884				
			Ryukyu Islands, Japan	108810	201442	201446	201476	201477
				201479	201482	201484	201490	201495
				201498	201499	201500	201502	201503
				201508	201514	201521	201522	201526
				201532	201533	201542		
		<i>V. tenuicaulis</i>	Thailand	108552	109683	205883	210671	
			Myanmar	210795	210807	210808	210818	217448
				217453	217465	217471	217475	217476
				217486	217496	217497	217519	227438
			Laos	224441				
		<i>V. umbellata</i>	Thailand	109675	210639	210644		
			India	99485				
		<i>Vigna</i> sp.	Myanmar	210811				
		<i>Ceratotropis</i>						
		<i>V. grandiflora</i>	Thailand	107862				
		<i>V. mungo</i> var. <i>silvestris</i>	India	107874				
		<i>V. mungo</i> var. <i>mungo</i>	Thailand	109668				
		<i>V. radiata</i> var. <i>sublobata</i>	Madagaskal	107877				
		<i>V. radiata</i> var. <i>radiata</i>	Thailand	110830				
		<i>V. subramaniana</i>	India	110836	229278	229284		
		<i>Vigna</i> sp.	India	235420				
		<i>Acontifoliae</i>						
		<i>V. aconitifolia</i>	Pakistan	104332				
		<i>V. aridicola</i>	Sri Lanka	205894	207977			
		<i>V. stipulacea</i>	Sri Lanka	205892				
		<i>V. trilobata</i>	Sri Lanka	205895				
		<i>Catiang</i>						
		<i>V. unguiculata</i>	Mali	89083				
		<i>Vigna</i>						
		<i>V. luteola</i>	Papua New Guinea	230741				
		<i>Angulares</i>						
		<i>V. hirtella</i>	Laos	220137				
		<i>V. nakashimae</i>	South Korea	212343	212344	212350	212351	
		<i>V. reflexo-pilosa</i>	Malaysia	108867				
		<i>V. tenuicaulis</i>	Myanmar	217448				
		<i>V. trinervia</i>	Malaysia	108840				
		<i>V. umbellata</i>	Myanmar	217439				
		<i>Acontifoliae</i>						
		<i>V. aridicola</i>	Sri Lanka	205896				
G	<i>Ceratotropis</i>	<i>Angulares</i>						
H	<i>Ceratotropis</i>	<i>V. angularis</i> var. <i>nipponensis</i>	Laos	226665				
		<i>V. angularis</i> var. <i>nipponensis</i>	Laos	226667	226676			

Results

In all trials, the varieties of adzuki bean that were used as controls showed resistant or susceptible reactions to each pathogen (*data not shown*), as was demonstrated previously (16). Most of accessions showed clear and identical responses over the two trials. However, some accessions had reactions that differed between trials. For 15 accessions of *V. angularis* var. *nipponensis*, 20 of *V. tenuicaulis*, 1 of *V. minima*, and 3 of *V. nepalensis*, an additional trial was conducted to confirm the disease response. However, no intermediate evaluation was shown in the study. Consequently, eight disease response groups were created for 252 accessions examined from 26 species of *Vigna*, including 2 unidentified species (Tables 1 and 2): A, susceptible to all pathogens tested; B, susceptible to BSR race 2 but resistant to the other pathogens; C, susceptible to BSR race 3 but resistant to the other pathogens; D, resistant to all pathogens tested; E, resistant to AFW race 3 but susceptible to the other pathogens; F, susceptible to BSR races 2 and 3 but resistant to BSR race 1 and AFW race 3; G, susceptible to BSR race 2 and AFW race 3 but resistant to BSR races 1 and 3; and H, susceptible to AFW race 3 but resistant to the other pathogens. Of the eight groups, E, F, G, and H were newly recognized in the present study; groups A, B, C, and D were described previously (16).

Of the 26 *Vigna* spp. examined, 22 species, including 2 unidentified species, were placed in group E (Table 2), which contained the largest number of accessions (131 accessions), followed by groups

B (44 accessions), D (30 accessions), C (26 accessions), F (10 accessions), A (8 accessions), H (2 accessions), and G (1 accession) (Table 1). Accession numbers in each disease response group and the geographic source of the accession are shown in Table 3. Within the subgenus *Ceratotropis* and *Aconitifoliae* were mostly restricted to group E, except for an accession of *V. aridicola* in the section *Aconitifoliae* belonging to group F. Contrary to previous reports (11,12,15), two accessions of *V. unguiculata* were susceptible to *C. gregata* f. sp. *adzikicola* and were classified into group C or E.

V. angularis var. *nipponensis*, *V. hirtella*, *V. minima*, *V. nakashimae*, and *V. tenuicaulis* in the section *Angulares*, closely related and cross-compatible with adzuki bean, were largely differentiated into a variety of response groups ($n = 7, 5, 5, 3$, and 5 groups, respectively; Table 1). In particular, accessions of wild adzuki bean (*V. angularis* var. *nipponensis*) were broadly distributed throughout all of the groups except group F. The geographic distribution of five response groups for *V. angularis* var. *nipponensis* collected from Japan is shown in Figure 1. There was no clear geographic limitation to the range of wild adzuki bean collections, except accessions in group E, which were found only in southwestern Japan.

In contrast, *V. nepalensis* (6 accessions) and *V. riukiensis* (24 accessions) in the section *Angulares* did not show a diverse reaction and were restricted to group E. The accessions of *V. angularis* var. *nipponensis* in group D, which is a potential source of multiple resistance genes for the development of new adzuki bean cultivars,

Fig. 1. Geographic distribution of wild adzuki bean (*Vigna angularis* var. *nipponensis*) accessions from Japan in disease response groups A–E. Black circles indicate the sampling places where each accession was collected. Groups correspond to the descriptions in Table 1.

were collected from Japan and Myanmar, whereas those of the other species in group D were collected from several south and southeast Asian countries (Sri Lanka, Myanmar, Thailand, Laos, and the Philippines) (Table 3).

Discussion

To identify new sources of multiple resistance to *C. gregata* f. sp. *adzukicola* and *F. oxysporum* f. sp. *adzukicola*, we inoculated 252 accessions of *Vigna* spp. using isolates with specific pathotypes. Fujita et al. (3) screened a total of 36 accessions of *V. angularis* var. *nipponensis*, *V. nakashimae*, *V. riukiensis*, and *V. umbellata* (from the TAES collection) by artificial inoculation or a field test using two races (1 and 2) of *C. gregata* f. sp. *adzukicola*. They found 16 resistant accessions in *V. angularis* var. *nipponensis* but not in the other *Vigna* spp. However, the reactions of these accessions to race 3 were not examined.

Eight disease response groups were detected in this study, of which four were novel. Altogether, 29 accessions from five *Vigna* spp. (*V. angularis* var. *nipponensis*, *V. hirtella*, *V. glabrescens*, *V. minima*, and *V. tenuicaulis*) in the section *Angulares* belonged to group D, with resistance to all of the pathogens. These species are potential sources of multiple resistance genes (1). Crosses between adzuki bean and these species, with the exception of *V. glabrescens* (an allotetraploid derived from *V. trineria* × *V. minima*), are reported to produce fertile F₁ hybrids (23).

V. angularis var. *nipponensis* showed greater genetic diversity in resistance patterns compared with cultivated adzuki bean (16) and, within this species, three newly designated disease reaction groups (E, G, and H) were identified. In these three new groups, the reaction to race 1 of *C. gregata* f. sp. *adzukicola* was opposite to that of race 3 of *F. oxysporum* f. sp. *adzukicola*. This pattern of reaction was not observed previously in cultivated adzuki bean cultivars or lines (16). On the other hand, the reaction to the former pathogen was the same as that to the latter one in groups A, B, C, and D, and common resistance was explained by pleiotropy of the same gene or a strong linkage between the resistance loci involved in the reaction against the specific pathogens (22). The resistance sources to *F. oxysporum* found in group E and those to *C. gregata* found in groups G and H are considered to be novel. In particular, groups G (one accession) and H (two accessions) were found only in *V. angularis* var. *nipponensis* accessions collected from Laos. Their resistance could be polygenic to the pathogens in Hokkaido (9).

Adzuki bean cultivars in group B and Acc259 in group C were presumed to possess the putative resistance genes *Pga1* and *Pga2*, respectively (21; unpublished data). Recently, a specific locus for *Pga1* resistance to race 1 of *C. gregata* f. sp. *adzukicola* was mapped to adzuki bean linkage group 3 (6,20). The availability of molecular markers tightly linked to resistance loci based on genome maps (19) will enable the use of marker-assisted selection in adzuki bean breeding programs. In the near future, inheritance and mapping studies should be carried out on the accessions in the newly detected groups D, E, F, G, and H. In addition, studies to elucidate the mechanism of resistance would be helpful.

Contrary to a prior study (11), in which *C. gregata* f. sp. *adzukicola* was avirulent to domesticated *V. unguiculata*, each accession of *V. unguiculata* examined in the present study was designated as group C (domesticated) or E (wild), and was susceptible to at least one of two races of *C. gregata* f. sp. *adzukicola*. Although the complete host range of *C. gregata* f. sp. *adzukicola* must be verified, it is likely that the strains of *C. gregata* and *F. oxysporum* used in the present study evolved to be mainly pathogenic to *V. angularis* var. *nipponensis*. Sources of resistance to host-specific plant pathogens are often found in regions of the greatest differentiation of the host species (17,25). In theory, the host and pathogen coevolved in these regions over a long period of time, and this protracted association led to their diversity. The pathogens used in the present study were isolated from domesticated adzuki bean grown in Hokkaido. The natural distribution of *V. angularis* var. *nipponensis* includes Japan, the Korean peninsula, China, the northern

mountainous region of southeast Asia (Myanmar and Laos), Bhutan, Nepal, and northern India. Further study may find new races of these pathogens from wild adzuki bean growing in regions outside Hokkaido. Examining wild adzuki bean for resistance and the genetic diversity of the pathogens in their natural habitat will ultimately expand researchers' understanding of where these species coevolved.

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