Pathogenicity, anastomosis groups, host range, and genetic diversity of Rhizoctonia species isolated from soybean, pea, and other crops in Alberta and Manitoba, Canada

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ARTICLE

Pathogenicity, anastomosis groups, host range, and genetic diversity of *Rhizoctonia* species isolated from soybean, pea, and other crops in Alberta and Manitoba, Canada

Haitian Yu, Qixing Zhou, Sheau-Fang Hwang, Andrew J. Ho, Kan-Fa Chang, Stephen E. Strelkov, Yuhua He, Robert L. Conner, and Michael W. Harding

Abstract: Root rot is a common disease in soybean (*Glycine max*) and field pea (*Pisum sativum*), which restrain increased production in Canada. Sixty-seven isolates of *Rhizoctonia* were recovered from various diseased plants in Alberta, Canada along with three isolates from diseased soybean plants in Manitoba, Canada. According to their anastomosis behavior, 23 (32.9%) of the isolates were identified as anastomosis group (AG) 4 (AG4), 7 (10.0%) were AG2-1, 10 (14.3%) were AG2-2, 7 (10.0%) were AG5, 3 (4.3%) were AG-E and the AGs of the remaining 20 (28.6%) isolates could not be determined. Isolates belonging to AG4 produced typical symptoms of stem rot and root rot on seedlings of soybean and pea and were more aggressive than the AG2-1, AG2-2, AG5 and AG-E isolates. Selected isolates of AG4, AG2-1, AG2-2, AG5 and AG-E were to some degree able to infect common crops in Alberta, which included barley, canola, corn, faba bean, flax, lupin, lentil, pea, potato, soybean, and wheat. The genetic variability among these isolates was evaluated using phylogenetic analysis based on the rDNA ITS sequences and inter-simple sequence repeat (ISSR) markers. For the ITS sequence analysis, a neighbour-joining tree was constructed using the PAUP program, which clustered the *Rhizoctonia* isolates into five groups (Groups I to V). However, no correlation was observed between AGs, locations, aggressiveness or host origins. For the ISSR analysis, 54 polymorphic ISSR patterns were identified, indicating a high level of diversity among the isolates.

Key words: Rhizoctonia, soybean, pea, pathogenicity, anastomosis group, ITS, ISSR.

Résumé : Le pourridié est une maladie courante du soja (*Glycine max*) et du pois (*Pisum sativum*), ce qui en restreint la production au Canada. Les auteurs ont récupéré 67 isolats de *Rhizoctonia* sur divers plants de soja attaqués par la maladie en Alberta (Canada) et trois isolats sur des plants eux aussi affectés, au Manitoba (Canada). Si l'on se fie aux anastomoses, 23 (32,9 %) de ces isolats se classaient dans le quatrième groupe anastomosique (AG4), sept (10,0 %) dans le groupe AG2-1, dix (14,3 %) dans le groupe AG2-2, sept (10,0 %) dans le groupe AG5, trois (4,3 %) dans le groupe AG-E, le groupe des vingt isolats restants (28,6 %) n'ayant pu être déterminé. Les isolats du groupe AG4 produisent les symptômes typiques du pourridié chez les plantules de soja et de pois. Ils sont aussi plus agressifs que ceux des groupes AG2-1, AG2-2, AG5 et AG-E. Quelques isolats des groupes AG4, AG2-1, AG2-2, AG5 et AG-E parviennent à infecter à un certain degré diverses cultures courantes en Alberta, en l'occurrence l'orge, le canola, le maïs, la fèverole, le lin, le lupin, la lentille, le pois, la pomme de terre, le soja et le blé. Les auteurs ont évalué la variabilité génétique des isolats par analyse phylogénétique des séquences de l'espaceur transcrit interne (ITS) de leur ADNr ainsi que des marqueurs ISSR (*inter-simple sequence repeat*). Pour analyser les séquences de l'ITS, les

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auteurs ont bâti un arbre des séquences voisines et liées avec le logiciel PAUP, ce qui a permis de rassembler les isolats de *Rhizoctonia* en cinq groupes (numérotés de I à V). Néanmoins, on n'a pu établir de corrélation entre les groupes anastomosiques, l'emplacement, l'agressivité du cryptogame ou l'hôte. En ce qui concerne les marqueurs ISSR, les auteurs ont identifié 54 polymorphismes, ce qui indique un taux de diversité élevé chez les isolats. [Traduit par la Rédaction]

Mots-clés : Rhizoctonia, soja, pois, pathogénicité, groupe anastomosique, ITS, ISSR.

Introduction

Soybean [*Glycine max* (L.) Merr.] and field pea (*Pisum sativum* L.) are important leguminous crops that are widely grown in Canada (Pesticide Risk Reduction Program 2008; Masuda and Goldsmith 2009; Gabruch and Gietz 2014). Root rot diseases are important constraints to the expansion of soybean and sustainable production of field pea in Alberta, Canada (Hwang and Chang 1989; Gossen et al. 2016; Chang et al. 2018). The root rot pathogens cause damping-off, seedling blight, and root rot of soybean and field pea, which has resulted in large yield losses in Canada (Hwang et al. 2007; Xue et al. 2007; Chang et al. 2018). The root rot pathogen complex includes *Rhizoctonia solani* Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk], *Fusarium* spp., *Pythium* spp., *Aphanomyces euteiches* Drechsler, and *Sclerotinia sclerotiorum* (Lib.) De Bary (Xue 2003; Chatterton et al. 2015; Gossen et al. 2016; Zhou et al. 2018).

Among all the pathogens, *R. solani* is the most frequently isolated fungi from soybean and pea roots with root rot symptoms in western Canada (Hwang et al. 2007; McLaren et al. 2015; Nyandoro et al. 2014; Chang et al. 2018). *Rhizoctonia solani* has a broad host range that includes pulse crops, canola, cereals, and ornamental crops (Sneh et al. 1996). In the family of Leguminosae, *R. solani* caused seedling blight and root rot of beans (*Phaseolus vulgaris* L.) (Engelkes and Windels 1996), chickpea (*Cicer arietinum* L.) (Hwang et al. 2003), field pea (Xi et al. 1995; Hwang et al. 2007), lupin (*Lupinus angustifolius* L.) (Zhou et al. 2009), and soybean (Zhao et al. 2005). In southern Alberta, the occurrence of root rot of soybean was documented in all of the 29 fields surveyed in 2014 and 2015, and *R. solani* was shown to be one of the components of the root rot disease complex (Nyandoro et al. 2015, 2017).

Rhizoctonia solani is a species complex with various morphological and physical characters. Based on the characterization of hyphal fusion of the isolates via somatic incompatibility (i.e., anastomosis grouping, AG), *Rhizoctonia* spp. can be classified into 14 AGs (AG1 to AG13 and AG-BI) (Sneh et al. 1991; Carling 1996; Cubeta and Vilgalys 1997; Kuninaga et al. 1997; Carling et al. 2002*a*; Stodart et al. 2007). *Rhizoctonia* isolates belonging to different AG often have different host ranges and differences in aggressiveness (Carling et al. 2002*a*). Previously, *R. solani* AG2-1, AG2-2, AG4, AG5, and *Rhizoctonia* AG-E have been isolated from soybean and pea, causing seedling blight and root rot (Zhao et al. 2005; Hwang et al. 2007).

To evaluate genetic variability and to characterize *R. solani*, numerous genetic markers were applied, which included restriction fragment length polymorphisms (RFLP) (Rosewich et al. 1999; Ceresini et al. 2002), random amplified polymorphic DNA (RAPD) (Cubeta et al. 1991; Zhou et al. 2009; Dubey et al. 2012; Mirmajlessi et al. 2012), amplified fragment length polymorphism (AFLP) (Taheri et al. 2007), inter-simple sequence repeats (ISSR) (Sharma et al. 2005; Stodart et al. 2007; Khodayari et al. 2009; Dubey et al. 2012; Zhou et al. 2014), and sequence analysis of internal transcribed spacers (ITS) (Carling et al. 2002*b*; Justesen et al. 2003; Zhou et al. 2009, 2014; Çebi Kiliçoğlu and Özkoç 2010). ISSR employs a dominant inheritance marker and can generate a great number of highly informative and reproducible alleles, which can be used to rapidly differentiate closely related individuals. ISSR analysis can be highly variable within a species and has high repeatability and stability (Stodart et al. 2007). The ITS region of rDNA, which contains the ITS1 spacer, 5.8S rRNA gene, and the ITS2 spacer, has been used to identify various AGs of *R. solani* (Boysen et al. 1996; Kuninaga et al. 1997) and evaluate genetic variation within this species (Zhou et al. 2009, 2014).

In the present study, a total of 70 *Rhizoctonia* isolates were recovered from various pulse and special crops in Alberta and Manitoba. The objectives of the present study were to: (1) characterize the isolates recovered from the infected roots of soybean or pea, (2) determine their pathogenicity to pea and soybean, (3) to evaluate the host range of the major AG types found in Alberta and Manitoba, and (4) to examine the genetic diversity of those isolates and evaluate molecular methods for grouping *Rhizoctonia* isolates using their ITS sequences and ISSR markers.

Materials and Methods

Isolation of the pathogens

During the summers of 2015 and 2016, disease surveys were conducted across the southern (for soybean fields) and central (for pea isolates fields) regions of Alberta to assess the occurrence and impact of root rot on soybean and pea crops (Nyandoro et al. 2015, 2017). For pathogen isolation, roots of the plants with root rot symptoms were washed under running tap water and cut into small pieces (~1 cm in length) with a sharp knife. The root pieces were placed in a 0.5% sodium hypochlorite solution for 30 s to 1 min to surface-sterilize the tissue, rinsed three times with sterile distilled water, and then placed

Table 1. Location of sample collection, host, AGs, and isolate ID of *Rhizoctonia* isolates.

Location	Host[a]	AG group	Isolate ID
Bow Island	Dry bean	AG2-2	R104
	Dill	AG2-1	R98
		AG2-2	R100
	Soybean	AG4	R57
		Not determined	R12, R55
Brooks	Pea	AG4	R71, R73
		Not determined	R77
	Mint	AG2-2	R113
	Soybean	AG2-1	R01, R07, R38a
		AG2-2	R08, R22, R50
		AG4	R04, R13, R15, R24, R34, R48, R56, R64
		AG5	R06, R14b, R19
		Not determined	R10, R14a, R21a, R27a, R75
Edmonton	Faba bean	AG-E	R97a, R102
	Lupin	AG4	R21b, R31, R35b, R38b
Fort Saskatchewan	Soybean	AG2-1	R89
		AG4	R93
Leduc	Cauliflower	AG2-2	R117
		Not determined	R124, R127
Lethbridge	Pea	Not determined	R147, R148, R151, R168
Morden, Manitoba	Soybean	AG4	R35a, R52, R53
Namao	Pea	AG4	R92b
		Not determined	R95, R97b
Parkland	Soybean	AG4	R16
Redwater	Pea	AG2-1	R131
Seven Persons	Soybean	AG5	R70
Spruce Grove	Alstroemeria	AG2-2	R90
Stettler	Echinacea	AG4	R86
Strathcona	Soybean	AG2-1	R32
		AG5	R09, R20, R36
Sturgeon	Soybean	Not determined	R88
Tofield	Lupin	AG2-2	R42
		AG4	R44
Vauxhall	Soybean	AG2-2	R02
		AG4	R11
Westlock	Soybean	AG2-1	R126
		AG4	R91
		AG-E	R92a

[a]Scientific names are given below: Alstroemeria (*Alstroemeria* sp.), bean (*Phaseolus vulgaris* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), dill [*Peucedanum graveolens* (L.) C.B. Clarke], echinacea [*Echinacea purpurea* (L.) Moench], faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), lupin (*Lupinus angustifolius* L.), mint (*Mentha spicata* L.), soybean [*Glycine max* (L.) Merr.].

on sterile paper towels to remove excess water. The surface-sterilized root pieces were transferred into Petri dishes onto water agar medium (WA) amended with 50 $\mu g \cdot mL^{-1}$ streptomycin sulfate in a laminar flow hood. The Petri dishes were incubated under laboratory conditions at a temperature of 20 °C ± 2 °C and an 8 h/16 h light and dark period, and monitored daily for the growth of fungal colonies. A total of 70 *Rhizoctonia* isolates were collected from diseased soybean, pea plants and other plants in those fields, which are listed in Table 1. A hyphal tip of each isolate was transferred to fresh potato–dextrose agar media (PDA) and incubated for 1 wk at room temperature. All the isolates were stored on PDA at 4 °C.

Identification and anastomosis grouping

The nuclei number of the young hyphal cells, the septa and the branching of the hyphae of *Rhizoctonia* isolates were examined to identify *R. solani* (Sneh et al. 1991). Each *Rhizoctonia* isolate was paired with tester isolates representing AG1, AG2-1, AG2-2, AG3, AG4, AG5, AG6, AG7, AG8 and binucleate *Rhizoctonia* AG-E following the protocol of Zhou et al. (2009, 2014). Testers were obtained from Department of Agriculture, Food and Nutritional Science, at the University of Alberta. When hyphae of the two isolates touched, slides were removed from the Petri dishes and examined under a microscope. The AG type of *Rhizoctonia* isolate was determined based on the hyphae fusion between the testing isolate and tester

isolate. Two slides were prepared for each isolate under investigation. Anastomosis behaviour was determined at a 400× magnification.

Pathogenicity

All of the 70 *Rhizoctonia* isolates were tested for pathogenicity on the pea cv. Midas and soybean cv.900Y61 in two separate experiments following the protocols of Zhou et al. (2009, 2014). Plants were grown in 500-mL cups filled with Pro-Mix potting soil (Westgro Horticultural Supplies Inc., Calgary, AB, Canada). To inoculate the soil, *Rhizoctonia* isolates were grown on PDA plates (90 mm) for 10 d. The cultures with agar were cut into small pieces (~3 mm × 3 mm) with a sharp knife before inoculation. One-half of a plate of a culture with agar was added in replicate cup. Five replicate cups were used for each crop. Five seeds were planted 2 cm deep along with inoculum. Similar number of cups was included as control where the same amount of PDA pieces was added with the seeds. The experiments were laid out in a randomized complete block design in a greenhouse set for a 20/15 °C day/night regime with a 16-h photoperiod. Pots were watered daily. Seedling emergence was recorded 10 d after seeding. At 21 d after seeding, plant height was recorded. The plants were uprooted and root rot severity (0–4) and total dry biomass were recorded (Table 2). Root rot severity was assessed on a scale of 0–4, where; 0 = no lesion, 1 = small brown lesions on <25% of exterior circumference of tap root, 2 = lesions on 25%–49% of tap root, 3 = lesions on 50%–74% and tap root constricted, and 4 = tap root girdled (75%–100%) and plant wilted or dead (Zhou et al. 2009, 2014). The experiment was repeated once under the same conditions in a greenhouse. Data were subjected to analysis of variance using the SAS program and, where appropriate, Duncan's new multiple range test at $P \leq 0.001$ of significance was performed for means comparison (SAS Institute, Inc., NC, USA).

Host range of *Rhizoctonia*

Eleven isolates which belonged to *R. solani* AG2-1, AG2-2, AG4, AG5 and a binucleate *Rhizoctonia* AG-E were selected for the pathogenicity test following the inoculation protocol described above (Table 3). Prior to inoculation, each *Rhizoctonia* isolate was grown on sterilized wheat grains for 12 d, air dried and ground, and incorporated at the time of seeding at the rate of 10 mL per pot. Ten plant species, which were in crop rotations systems in western Canada, were tested as potential hosts of barley (*Hordeum vulgare* L.), canola (*Brassica napus* L.), corn (*Zea mays* L.), faba bean (*Vicia faba* L.), flax (*Linum usitatissimum* L.), lentil (*Lens culinaris* L.), lupin (*Lupinus angustifolius* L.), pea, soybean, and wheat (*Triticum aestivum* L.) (Table 4). Ten seeds of each plant species (except for 5 seeds of faba bean per cup) were inoculated with *Rhizoctonia* inoculum grown in a 500-mL cup (except 5-inch square pot for growing potato) filled with Promix potting soil (Westgro Horticultural Supplies Inc., Calgary, Canada). The cups (pots) were arranged following a split-plot design with the host crops in the main plots and the isolates in the sub-plots on the bench in a greenhouse set for a 20/15 °C day/night regime with a 16-h photoperiod. Plants were watered daily and fertilized once a week. Four replicate cups were used for each crop. Plant grown with non-inoculated PDA pieces was served as non-inoculated control. To evaluate the aggressiveness of *Rhizoctonia* isolate on different crops, seedling emergence, height, and disease severity of individual plant were recorded 10, 15 and 21 d after seeding, respectively. At 21 d after seeding, the plants were uprooted and root rot severity (0–4) and total dry biomass were recorded. Root rot severity was assessed on a scale of 0–4 as described above.

ITS sequencing and analysis

The genomic DNA of all the *Rhizoctonia* isolates was extracted from the mycelial mats which grown in potato-dextrose broth for 7 d following the method of CTAB (Doyle and Doyle 1987). The DNA concentration of each *Rhizoctonia* isolate was adjusted to 10–20 ng·μL^{-1} after estimation with a Thermo Scientific NANODROP™ 1000 Spectrophotometer (Fisher Scientific, Nepean, ON, Canada).

The ITS region was amplified with primers of ITS4 and ITS5 (White et al. 1990) in a 25 μL reaction volume containing PCR Master Mix (Promega, Madison, WI, USA), 0.5 μM of each primer and 10–20 ng of genomic DNA. The PCR amplification in the thermocycler included initial denaturation at 94 °C for 5 min followed by 35 denaturation cycles of 25 s at 94 °C, annealing for 30 s at 58 °C, and extension for 60 s at 72 °C, with a final extension of 10 min at 72 °C. The amplicons were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega) and transformed into the competent *Escherichia coli* strain JM109 using the *Eco*RV-digested and T-tailed pGEM®-T EasyVector System I (Promega). Plasmid DNA containing the insert was extracted with a PureYield Plasmid Miniprep System (Promega) and sequenced in the Department of Biological Sciences, University of Alberta.

The ITS sequences were analysed and edited with BioEdit, version 7.2.6.1 (Hall 1999), and then submitted to GenBank (Accession numbers MK084631–MK084700). The 70 ITS sequences and one reference sequence from GenBank (*Rhizoctonia zeae* KC620581) were aligned using BioEdit. To evaluate the genetic variation among the *Rhizoctonia* isolates, the best-fit nucleotide substitution model was selected using the program jModelTest 2.1.10 v 20160303 (Darriba et al. 2012). Distance tree was produced with PAUP 4.0b10 software (Swofford 2002) using the neighbour-joining (NJ) approach (Saitou and Nei 1987). The NJ tree was rooted by user-specified outgroup and displayed using the TreeViewX version 0.5.0 (Page 1996).

Table 2. Pathogenicity of *Rhizoctonia* isolates and their effects on seedling emergence, plant height, plant biomass and root rot severity on soybean and pea.

		Soybean					Pea				
AG	Isolate	Emergence	Height (mm)	Disease (0–4)	Root (g)	Shoot (g)	Emergence	Height (mm)	Disease (0–4)	Root (g)	Shoot (g)
—	CK	10.0	58.5	0.0	0.38	1.52	10.0	63.4	0.0	0.82	0.73
AG2-1	R01	7.0	40.9	1.0	0.29	1.21	8.0	36.9	1.5	1.12	0.27
	R07	8.0	44.2	2.2	0.33	1.24	10.0	55.3	1.2	0.77	0.59
	R32	3.0	28.3	2.3	0.18	0.81	0.0	0.0	4.0	0.00	0.00
	R38a	7.0	39.4	2.1	0.34	1.20	8.0	49.9	1.7	0.75	0.47
	R89	8.0	44.9	1.4	0.34	1.38	7.0	42.1	1.6	0.62	0.3
	R126	8.0	42.9	1.0	0.36	1.41	10.0	59.9	0.1	0.75	0.57
	R98	7.0	37.7	0.1	0.29	1.12	9.0	46.3	0.3	0.77	0.51
AG2-2	R02	9.0	42.7	0.6	0.32	1.30	7.0	49.7	1.1	0.89	0.39
	R08	6.0	36.1	1.9	0.24	0.80	1.0	17.1	3.3	0.03	0.01
	R22	8.0	48.8	2.8	0.39	1.46	8.0	48.2	2.2	0.73	0.42
	R42	9.0	46.6	0.0	0.35	1.23	9.0	52.3	0.0	0.64	0.56
	R50	8.0	48.9	2.0	0.34	1.29	9.0	52.4	1.4	0.76	0.57
	R90	5.0	46.6	1.3	0.29	0.99	7.0	35.1	2.2	0.96	0.31
	R100	8.0	42.0	0.1	0.28	1.21	10.0	53.6	0.1	0.75	0.63
	R104	9.0	45.4	0.1	0.33	1.32	9.0	56.3	0.0	0.81	0.61
	R113	8.0	46.7	0.1	0.31	1.29	8.0	44.4	0.2	0.71	0.49
	R117	8.0	42.5	0.4	0.28	1.12	9.0	53.1	0.8	0.75	0.54
AG4	R04	4.0	34.7	1.7	0.19	0.94	1.0	16.4	3.7	0.17	0.01
	R13	6.0	37.2	2.3	0.25	1.06	1.0	21.8	2.9	0.06	0.04
	R16	4.0	32.8	2.6	0.19	0.68	0.0	0.0	4.0	0.00	0.00
	R24	7.0	36.4	3.8	0.38	1.06	2.0	25.0	3.2	0.26	0.06
	R34	4.0	30.9	2.0	0.25	0.88	1.0	6.4	3.7	0.13	0.01
	R35	2.0	29.3	2.5	0.18	0.65	1.0	11.3	3.6	0.06	0.01
	R48	4.0	32.3	2.3	0.18	0.75	1.0	3.2	3.8	0.07	0.01
	R52	4.0	28.0	2.9	0.22	0.62	0.0	0.0	4.0	0.00	0.00
	R53	7.0	35.6	1.5	0.27	1.08	2.0	15.4	3.8	0.11	0.06
	R56	4.0	29.7	3.2	0.21	0.93	0.0	0.0	4.0	0.00	0.00
	R57	8.0	37.6	0.3	0.33	1.23	10.0	52.2	0.2	0.90	0.54
	R64	4.0	26.1	2.0	0.17	0.68	1.0	20.6	3.9	0.15	0.02
	R91	6.0	34.5	2.0	0.25	0.95	1.0	11.2	3.7	0.04	0.02
	R93	4.0	27.1	2.5	0.20	0.81	0.0	0.0	4.0	0.00	0.00
	R21b	5.0	30.0	1.7	0.22	0.83	1.0	18.3	3.5	0.13	0.02
	R31	8.0	35.2	1.5	0.32	1.07	1.0	19.9	3.4	0.15	0.02
	R35	7.0	36.6	1.6	0.27	0.96	1.0	10.5	3.6	0.04	0.01
	R38b	2.0	24.8	2.6	0.17	0.61	0.0	0.0	4.0	0.00	0.00
	R44	5.0	39.0	1.4	0.23	0.82	1.0	21.1	3.6	0.15	0.03
	R71	6.0	42.4	1.2	0.26	0.92	6.0	37.7	2.2	0.59	0.22
	R73	4.0	35.4	1.6	0.17	0.63	1.0	16.5	3.8	0.10	0.03
	R86	9.0	55.0	0.1	0.31	1.17	10.0	54.6	0.0	0.89	0.62
	R92b	4.0	32.4	1.7	0.23	0.76	0.0	0.0	4.0	0.00	0.00
AG5	R06	3.0	26.2	2.3	0.24	0.62	0.0	0.0	4.0	0.00	0.00
	R09	8.0	46.0	0.5	0.33	1.30	7.0	44.4	1.5	1.06	0.37
	R14b	8.0	46.5	1.5	0.28	1.12	7.0	36.2	1.8	1.07	0.31
	R19	7.0	42.3	1.5	0.24	0.91	8.0	40.6	1.5	1.00	0.3
	R20	7.0	44.6	1.2	0.26	0.96	7.0	32.2	1.5	1.21	0.22
	R36	8.0	41.3	1.0	0.37	1.24	8.0	32.5	1.2	0.97	0.28
	R70	5.0	30.5	2.5	0.24	0.91	8.0	39.2	1.1	0.99	0.32

Table 2. (*concluded*).

		Soybean					Pea				
AG	Isolate	Emergence	Height (mm)	Disease (0–4)	Root (g)	Shoot (g)	Emergence	Height (mm)	Disease (0–4)	Root (g)	Shoot (g)
AG-E	R92a	9.0	41.4	3.4	0.33	1.26	10.0	52.3	3.5	0.81	0.55
	R97b	3.0	28.8	3.9	0.16	0.71	0.0	0.0	4.0	0.00	0.00
	R102	8.0	57.0	4.0	0.29	1.30	9.0	49.4	3.5	0.67	0.57
Undetermined	R10	5.0	35.9	2.1	0.19	0.81	1.0	6.5	3.9	0.02	0.00
	R11	7.0	44.2	0.5	0.31	1.24	9.0	50.8	0.6	0.75	0.55
	R12	4.0	40.0	0.7	0.42	1.23	10.0	59.8	0.1	0.80	0.65
	R14a	9.0	46.9	0.7	0.3	1.26	10.0	59.4	0.0	0.85	0.66
	R15	5.0	33.6	2.5	0.2	0.63	0.0	0.0	4.0	0.00	0.00
	R21a	7.0	42.3	1.1	0.29	1.07	8.0	45.5	1.3	0.89	0.33
	R27a	8.0	44.0	0.9	0.32	1.22	10.0	58.1	0.1	0.77	0.57
	R55	0.0	0.0	4.0	0.00	0.00	0.0	3.9	4.0	0.00	0.00
	R75	7.0	34.5	1.5	0.29	1.03	9.0	47.9	1.1	0.91	0.42
	R88	8.0	38.8	1.2	0.37	1.16	10.0	53.7	0.0	0.80	0.53
	R97a	7.0	37.8	0.9	0.32	1.16	10.0	51.7	0.2	0.76	0.51
	R131	8.0	37.4	0.8	0.29	1.15	10.0	50.2	0.5	0.86	0.55
	R77	9.0	55.1	0.1	0.29	1.13	10.0	53.9	0.0	0.88	0.61
	R95	7.0	45.5	1.1	0.26	1.09	5.0	36.7	2.4	0.73	0.19
	R124	6.0	41.6	0.8	0.27	1.11	9.0	54.2	0.5	0.72	0.59
	R127	8.0	45.5	0.7	0.32	1.14	9.0	55.3	0.5	0.78	0.62
	R147	5.0	37.2	1.9	0.27	1.01	7.0	38.6	2.7	0.46	0.26
	R148	6.0	39.8	1.9	0.25	0.95	3.0	13.8	3.6	0.53	0.09
	R151	4.0	31.9	2.1	0.24	0.96	2.0	29.2	3.4	0.46	0.08
	R168	8.0	44.4	0.3	0.31	1.12	10.0	58.6	0.2	0.66	0.60
	SE	0.8	2.1	0.1	0.03	0.70	0.4	3.1	0.2	0.04	0.03

Note: Data are the means of 2 trials × 4 replications. SE, standard error of the means.

Support for groups in the NJ tree was assessed using a bootstrap analysis with 1000 replicates.

ISSR analysis

A total of 100 ISSR primer sequences were obtained from the Biotechnology Laboratory, University of British Columbia (UBC sets 9) and synthesized by Integrated DNA Technologies Inc. (IDT, Coralville, IA, USA) (Zhou et al. 2014). All 100 primers were screened against a mixture of DNA randomly chosen from the 70 *Rhizoctonia* isolates from the preliminary experiments. Ten primers that produced 2–10 polymorphic bands were selected for further ISSR PCR procedures with the genomic DNA of the 70 *Rhizoctonia* isolates (Table 4).

ISSR PCR was performed in a 25 μL reaction volume containing PCR Master Mix (Promega, Madison, WI, USA), 0.5 μM of each primer and 10–20 ng of genomic DNA. Amplification conditions in the thermocycler include a 5-min initial denaturation at 94 °C, followed by 35 cycles of 30 s at 94 °C to denature genomic DNA, 45 s for annealing of the primer at 42 °C, and 90 s at 72 °C for primer extension, with a final extension of 10 min at 72 °C. The PCR products were visualized by gel electrophoresis on 2.5% agarose gels in 1 × TAE buffer, at 110 V for 2 h. Gels were photographed with a Gel Doc™ XR System (BIO-RAD, Columbus OH, USA).

In the ISSR analysis, amplified fragments were scored for the presence (1) or absence (0) of homologous bands (Zhou et al. 2014). Only reproducible bands of strong intensity were included in the analysis. The gene frequency, genetic diversity, and genetic distance of 70 *Rhizoctonia* isolates were calculated. A dendrogram was also constructed for illustration of the phenetic relationships among these isolates (Zhou et al. 2014). All calculations and the dendrogram were generated using the POPGENE program (Yeh and Boyle 1997).

Results

Anastomosis grouping

According to the anastomosis behavior with *Rhizoctonia* tester isolates, seven isolates (10.0%) were *R. solani* AG2-1 (Table 1). One isolate (R98) was isolated from dill roots [*Peucedanum graveolens* (L.) C.B. Clark] in Bow Island, the other six isolates were isolated from soybean in Brooks (3), Fort Saskachewan (1), Strathcona (1) and Westlock (1). Ten isolates (14.3%) were AG2-2 of which two isolates from dill (1) and bean roots (1) in Bow Island, four isolates from mint (*Mentha spicata* L.)

Table 3. Host range of *Rhizoctonia* spp. and their effect on root rot severity.

		Barley (*Hordeum vulgare*)		Corn (*Zea mays*)		Wheat (*Triticum aestivum*)		Canola (*Brassica napus*)		Flax (*Linum usitatissimum*)	
AG	Isolate	CDC Copeland	AAC Synergy	Alto	Espresso	AAC Penhold	5700PR	1950	74-47RR	Prairie Thunder	CDC Neela
Control	CK	0.0f	0.00g	0.00d	0.00e	0.00c	0.00f	0.00d	0.00f	0.00f	0.00g
AG2-1	R07	1.3de	1.13cde	1.08c	0.48de	0.74b	1.33bcd	4.00a	4.00a	2.20e	1.59ef
AG2-1	R38a	1.7cde	0.93def	1.60bc	0.58de	0.95b	1.20bcd	4.00a	4.00a	3.31bc	3.30bc
AG2-2	R22	2.0bc	1.46cd	1.59bc	0.99bcd	1.11b	1.05cd	4.00a	4.00a	3.68ab	3.21bc
AG2-2	R50	1.8bcd	1.20cde	2.14ab	0.51de	0.86b	1.18bcd	3.95a	4.00a	3.09c	2.86c
AG4	R24	2.4b	1.66c	2.78a	1.93a	1.00b	1.68b	3.94a	3.75a	3.88a	3.78a
AG4	R56	2.4b	1.30cde	2.41ab	1.25bc	1.03b	1.44bc	3.84ab	3.98a	3.83a	3.66ab
AG5	R36	1.1e	0.58f	2.21ab	0.81cd	0.70b	0.41ef	1.83c	0.40e	2.26e	1.24f
AG5	R70	1.7cde	0.83ef	1.81bc	1.94a	1.05b	0.85de	1.46c	1.43d	2.50de	1.78e
AG-E	R92	3.3a	3.41a	1.71bc	1.58ab	3.14a	3.24a	3.65ab	3.20b	2.89cd	2.86c
AG-E	R97	3.3a	2.43b	1.79bc	1.90a	2.79a	3.33a	3.31b	2.80c	2.85cd	2.29d
AG-E	R102	3.2a	2.44b	1.93abc	1.53ab	2.60a	3.49a	3.40ab	2.58c	3.29bc	3.23bc

		Faba bean (*Vicia faba*)		Lentil (*Lens culinaris*)		Lupin (*Lupinus angustifolius*)		Pea (*Pisum sativum*)		Soybean (*Glycine max*)	
AG	Isolate	Snowbird	Malik	CDC Maxim	CDC Proclaim	Rose	Arabella	Midas	CDC Striker	DKB008-81	900Y61
Control	CK	0.00e	0.00e	0.00h	0.00f	0.00e	0.00f	0.00f	0.00f	0.00g	0.00f
AG2-1	R07	2.75c	2.80c	1.94f	1.29d	0.68d	0.46e	1.40e	1.23e	1.94e	2.26cd
AG2-1	R38a	3.88ab	3.98a	3.14cde	2.55c	2.46bc	1.39d	1.65de	1.48de	1.95e	2.08d
AG2-2	R22	3.48b	3.93a	2.86e	2.61c	2.61b	2.66b	1.80de	2.21c	2.73c	2.99b
AG2-2	R50	3.78ab	3.95a	3.04de	2.46c	2.05c	1.90c	1.84d	3.20b	1.71e	2.58c
AG4	R24	4.00a	4.00a	3.89a	3.64ab	4.00a	3.98a	3.44c	3.66a	3.26b	3.26b
AG4	R56	4.00a	4.00a	3.88a	3.63ab	4.00a	4.00a	3.71abc	3.81a	3.36b	3.24b
AG5	R36	1.63d	2.08d	1.43g	0.68e	0.61d	0.10f	0.18f	0.25f	0.58f	1.20e
AG5	R70	3.48b	3.25b	1.90f	1.51d	2.05c	2.89b	1.64de	1.78d	2.35d	2.35cd
AG-E	R92	3.99a	3.75a	3.69ab	3.74a	3.89a	3.94a	3.94a	4.00a	4.00a	4.00a
AG-E	R97	4.00a	3.79a	3.56abc	3.24b	3.88a	3.84a	3.51bc	3.94a	4.00a	4.00a
AG-E	R102	4.00a	3.85a	3.35bcd	3.85a	3.83a	3.83a	3.89ab	4.00a	4.00a	4.00a

Note: Data are the means of 2 trials × 2 host cultivars/lines × 4 replications. Means within a column followed by the same lowercase letter are not significantly different at $P \leq 0.05$ according to Duncan's new multiple range test.

Table 4. ISSR primers used to detect polymorphisms in *Rhizoctonia* isolates.

Primer	Sequence[a] (5′–3′)	No. of loci
UBC808	AGA GAG AGA GAG AGA GC	3
UBC809	AGA GAG AGA GAG AGA GG	6
UBC812	GAG AGA GAG AGA GAG AA	2
UBC826	ACA CAC ACA CAC ACA CC	6
UBC830	TGT GTG TGT GTG TGT GG	5
UBC861	ACC ACC ACC ACC ACC ACC	8
UBC862	AGC AGC AGC AGC AGC AGC	8
UBC865	CCG CCG CCG CCG CCG CCG	10
UBC881	GGG TGG GGT GGG GTG	10
UBC889	DBD ACA CAC ACA CAC AC	6

[a]B = (C, G, or T); D = (A, G, or T).

(1) and soybean (3) in Brooks, one from cauliflower in Leduc, one from *Alstroemeria* sp. in Spruce Grove, one from lupin (*Lupinus angustifolius* L.) in Tofield, and one from soybean in Vauxhall. Twenty-three (32.9%) isolates were identified as AG4 of *R. solani*, which was the dominate AG in this study. All the isolates belonging to AG4 were isolated from the family of Leguminosae (including field pea, lupin and soybean) in various locations except one isolate from *Echinacea* sp. in Stettler. Another seven (10.0%) isolates were collected from soybean roots and identified as AG5. These isolates were from Brooks (3), Seven Persons (1) and Strathcona (3). Three isolates were identified as binucleate *Rhizoctonia* AG-E were also identified from fababean in Edmonton and soybean in Westlock. However, the anastomosis groups of a total of 20 isolates remained un-determined based on anastomosis reactions with the tester strains. These isolates were recovered from field pea, soybean and cauliflower at various locations.

Pathogenicity

Pathogenicity tests revealed that out of 70 isolates of *Rhizoctonia* isolates, 60 isolates were able to infect soybean (DS ≥ 0.5), and 55 isolates were able to infect pea (DS ≥ 0.5) (Table 2). Among the 70 isolates tested on soybean, only six isolates (R24, R55, R56, R92a, R97b and R102) were highly aggressive (DS 3.2–4.0), 32 isolates were moderately aggressive (DS from 1.5 to 2.9), and 22 isolates were weakly aggressive on soybean (DS from 0.5 to 1.5) pathogenic. The other ten isolates were considered as non-aggressive (DS 0.0–0.4). On pea, 30 isolates were highly aggressive (DS from 3.2 to 4.0), 11 isolates were moderately aggressive (DS from 1.5 to 2.9), 13 isolates were weakly aggressive (DS from 0.5 to 1.5), and the other isolates were considered as non-aggressive (DS 0.0–0.5). Generally, the highly aggressive isolates significantly reduced seedling emergence, plant height, root and shoot biomass and increased the disease severity of both pea and soybean compared with the non-inoculated control.

Host range of *Rhizoctonia*

Ten plant species belonging to four families were infected (Table 3). Plant species belonging to families of Brassicaceae (canola), Graminaceae (barley, corn and wheat), Leguminosae (faba bean, lentil, lupin, pea and soybean) and Linaceae (flax) produced typical symptoms of root rot.

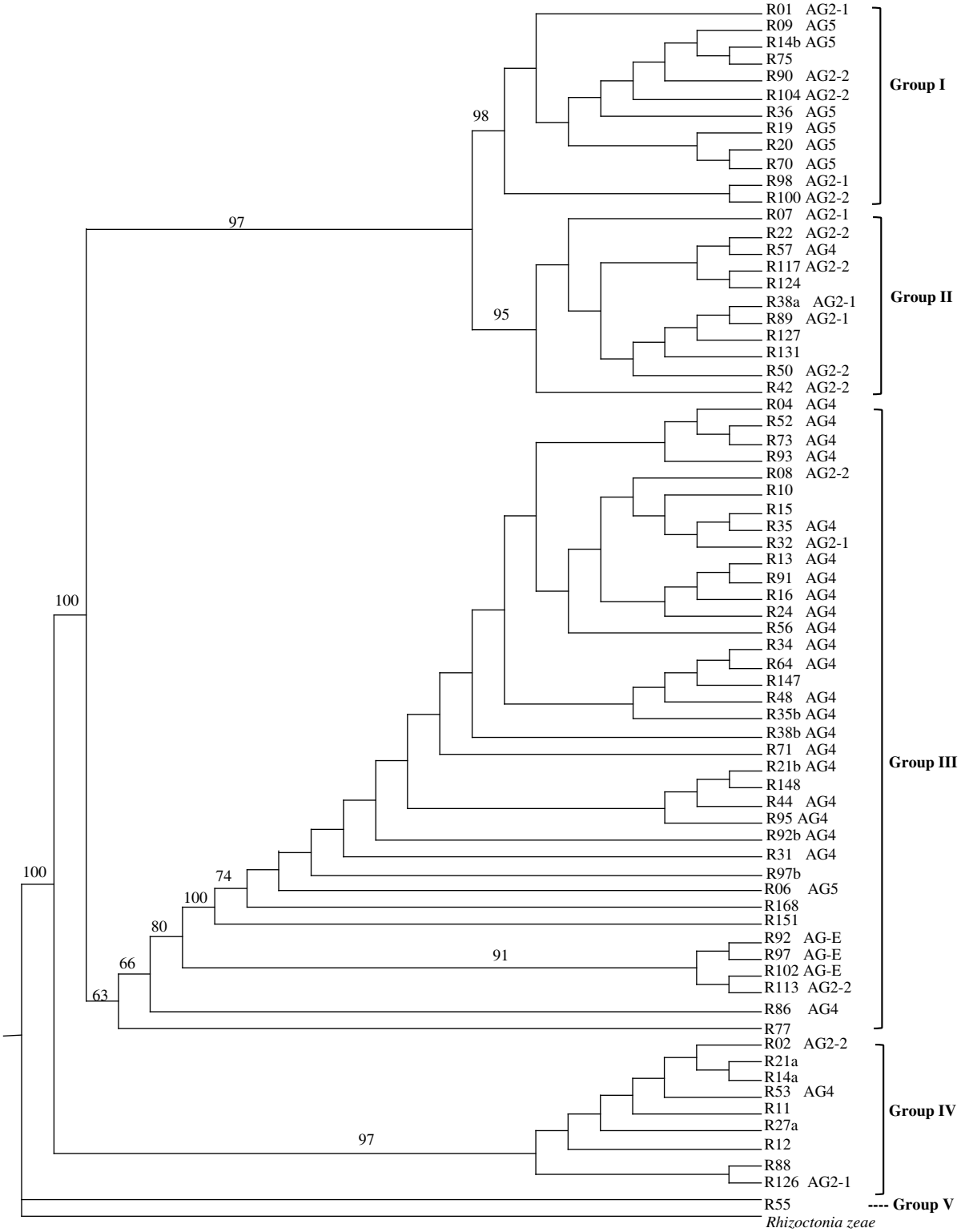
Host range studies indicated that 11 of the *Rhizoctonia* isolates which belonged to *R. solani* AG2-1, AG2-2, AG4, AG5 and binucleate *Rhizoctonia* AG-E aggressively infected almost all the plant species (Table 3). Pre-emergence damping-off, plant height and root and shoot biomass were recorded for the evaluation the aggressiveness (Data not shown). Root rot disease severity (DS) was also recorded at 21 d after inoculation.

Two isolates of AG4 (R24 and R56) were highly aggressive on canola and legume species (DS 3.2–4.0) and moderate aggressive on cereal species (1.0–2.8). The isolates of AG2-1 produced significantly different DS values on some species. The AG2-1 isolate R07 was highly aggressive on both canola cultivars (DS 4.0), moderately aggressive on flax, faba bean, lentil and soybean (DS 1.3–2.8), but less aggressive on the other plant species (0.5–1.4). The other isolate of AG2-1 (R38a) was more aggressive compared with R07 of AG2-1. R38a also was highly aggressive on both cultivars of canola (DS 4.0), faba bean (DS 3.9–4.0), flax (DS 3.3) and one cultivar of lentil (DS 3.1), moderately aggressive on another cultivar of lentil (DS 2.6), soybean (DS 2.0–2.1), lupin (DS 1.4–2.5), pea (1.5–1.7), and one cultivar of barley (DS 1.7), corn (DS 1.6) and weakly aggressive on the other plant species (0.6–1.2). Two isolates of AG2-2 were highly aggressive on canola, flax, faba bean and lentil (DS 2.5–4.0), moderate aggressive on lupin, pea, soybean and barley (DS 1.7–3.2) and weakly aggressive on corn and wheat (DS 0.4–2.1). The isolates that belonged to AG5 varied in aggressiveness on different plant species. R70 of AG5 was highly aggressive on both cultivars of faba bean (DS 3.3–3.5) and moderately aggressive on both cultivars of corn (DS 1.8–1.9), flax (DS 1.8–2.5), lentil (DS 1.5–1.9), lupin (DS 2.1–2.9), pea (DS 1.6–1.8) and soybean (DS 2.4), but weakly aggressive on barley and wheat (DS 0.8–1.7). The other isolate of AG5 (R36) was moderately aggressive on corn cv. ‘Alto’ (DS 2.2), canola cv. ‘1950’ (DS 1.8), flax cv. ‘Prairie Thunder’ (DS 2.3), two cultivars of faba bean (DS 1.6–2.1), and weakly aggressive on the other plant species (DS 0.1–1. 5). Three isolates of *Rhizoctonia* AG-E were highly aggressive on faba bean, lentil, lupin, pea and soybean (DS 3.2–4.0), moderately aggressive to canola, flax and the cereal crops (DS 1.6–3.7).

Sequence analysis of ITS regions

After alignment with the BioEdit program, the ITS fragments from 70 *Rhizoctonia* isolates were 690 bp in length. Nucleotide frequencies were A = 0.2736, C = 0.2053, G = 0.1910 and T = 0.3301. After alignment, the data optimal nucleotide substitution model was applied to the data

Fig. 1. Phylogenetic tree of *Rhizoctonia* isolates from soybean and other crops in Alberta were conducted using the neighbour-joining method based on ITS sequence data. Trees were rooted with *R. zeae* (KC620581). Bootstrap values, which are given on the branches, were based on 1000 replicates.



matrix. The best-fit model (TPM2uf+G) was selected using the program jModelTest 2.1.10 v 20160303 (Darriba et al. 2012). Then, a neighbour-joining (NJ) tree was produced from the ITS sequence data, and the isolates were clustered into five groups (Groups I–V, Fig. 1). Group I was composed of two AG2-1 isolates (R01 and R98), three AG2-2

isolates (R90, R104 and R100), six AG5 isolates (R09, R14b, R36, R19, R20 and R70) and one isolate that had undetermined AG grouping (R75), with a bootstrap value of 98. Group II consisted of three AG2-1 isolates (R07, R38a and R89), four AG2-2 isolates (R22, R117, R50 and R42), one AG4 isolate (R57) and three undetermined AG isolates (R124, R127 and R131), with a bootstrap value of 95. Group III was the largest group, which included one AG2-1 isolate (R32), two AG2-2 isolates (R08 and R113), 21 AG4 isolate, one isolate of AG5 (R06), three isolates of AG-E (R92, R97 and R102) and nine undetermined AG isolates with a bootstrap value of 63. Group IV included one AG2-1 isolate (R126), one AG2-2 isolate (R02), one AG4 isolate (R53) and six undetermined AG isolates, with a bootstrap value of 97. Only one undetermined AG isolate (R55) formed a separate clad as Group V.

ISSR analysis

Of the 100 primers tested, ten primers generated reproducible banding patterns and these were selected for ISSR-PCR (Table 4). A total of 64 bands were consistently produced in repeated amplifications using the DNA of the 70 *Rhizoctonia* isolates as templates. All of those bands were polymorphic. The gene frequency of those 54 loci ranged from 0.0143 to 0.9857, and the genetic diversity was 0.3013 ± 0.1697. The genetic distance of these isolates ranged from 0.0000 (between R02, R09, R11, R89, R91, R42, R71 and R104; and between R21a and R131) to 1.2809 (between R07 and R55). The phenetic relationships among the 70 *Rhizoctonia* isolates were illustrated in a dendrogram (Fig. 2). The isolates were clustered into four groups (Groups I–IV). Group I was composed of six isolates including two AG2-1 isolates (R01 and R98), two AG2-2 isolates (R100 and R117) and two undetermined AG isolates (R124 and R127). Group II included five AG2-1 isolates (R89, R32, R126, R08 and R07), six AG2-2 isolates, 16 AG4 isolate, six AG5 isolates, three AG-E isolates (R92, R97 and R102) and 12 undetermined AG isolates. In Group III, one isolate (R38a) belonged to AG-2-1, six isolates belonged to AG4 and the other eight were undetermined AG isolates. Group IV included only the undetermined isolate of R55.

Discussion

Rhizoctonia root rot has been reported as a common disease on soybean and pea in many countries and areas (Hwang and Chang 1989; Jones and Belmar 1989; Yang et al. 1990; Muyolo et al. 1993; Naito et al. 1995; Nelson et al. 1996; Fenille et al. 2002; Ajayi-Oyetunde and Bradley 2018). In the present study, a total of 70 *Rhizoctonia* isolates were recovered and some of the isolates were classified as *R. solani* AG2-1 (7 isolates), AG2-2 (10), AG4 (23), AG5 (7), binucleate *Rhizoctonia* AG-E (3), but the AG designation of 20 isolates was difficult to determine. Pathogenicity of all the isolates was tested on soybean and pea separately. The tests revealed that out of 70 *Rhizoctonia* isolates, 60 isolates were able to infect soybean (DS > 0.50), and 55 isolates were able to infect pea (DS > 0.50).

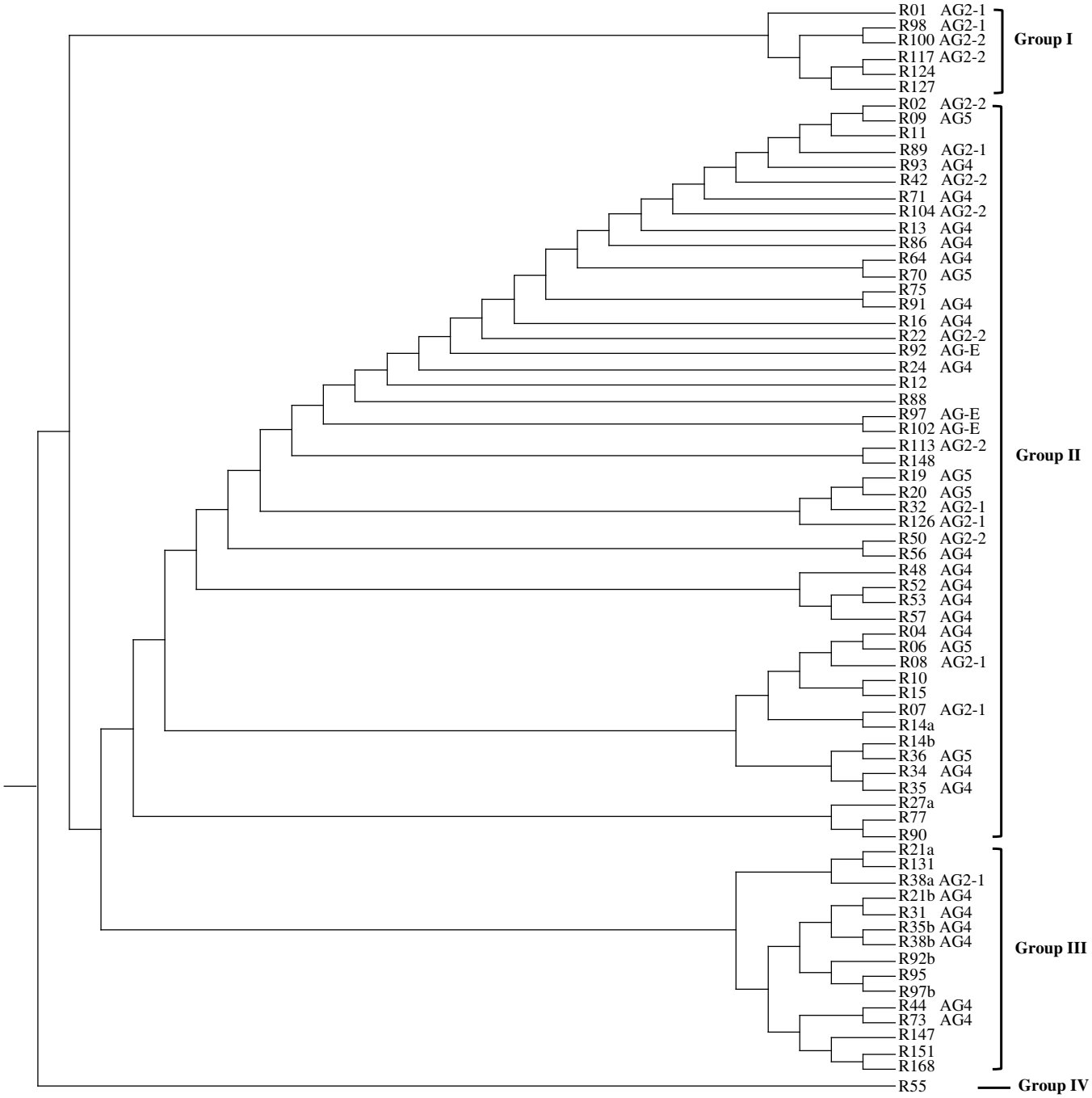
Rhizoctonia solani AG2-1 has a broad host range that includes lupin, pea, soybean, canola, wheat, turf grass, potato, tomato, and the ornamental plant *Matthiola incana* (Kataria and Verma 1992; Nicoletti et al. 1999; Hwang et al. 2007; Lehtonen et al. 2009; Zhou et al. 2009, 2014; Broders et al. 2014; Gómez et al. 2015; Sharma-Poudyal et al. 2015). Seven isolates of *R. solani* AG2-1 were originally collected from dill (1), pea (1) and soybean (5) in the current study, and they were less aggressive on both pea and soybean except one isolate originally from soybean (R32), which was highly aggressive to both soybean and pea.

Rhizoctonia solani AG2-2 also has been reported to have a broad host range that includes beans, lupin, pea, soybean, canola, turf grass, potato, and sugar beet (Kataria and Verma 1992; Engelkes and Windels 1996; Aoyagi et al. 1998; Lehtonen et al. 2009; Zhou et al. 2009, 2014). Ten isolates of *R. solani* AG2-2 in the current study were originally obtained from Alstroemeria (1), bean (1), cauliflower (1), dill (1), lupin (1), mint (1) and soybean (4). All the isolates were non-pathogenic or weakly pathogenic to soybean and pea except R08 (from soybean) and R90 (from *Alstroemeria*), which were highly and moderate aggressively on pea, respectively.

Rhizoctonia solani AG4 was reported to infect leguminous crops like pea, chickpea, lupin, and soybean (Hwang et al. 2003, 2007; Mathew et al. 2012; Sharma-Poudyal et al. 2015). It also has been recovered from canola, potato, and wheat (Mathew et al. 2012; Woodhall et al. 2012; Zhou et al. 2014; Muzhinji et al. 2015). Twenty-three isolates of *R. solani* were identified as AG4 making it the dominate AG in the current study. Most isolates were originally collected from leguminous crops including lupin (4), pea (3), and soybean (15). One isolate from Echinacea (R86) was recovered and identified as AG4. All the isolates except R57 (from soybean) and R86 (from Echinacea) were highly aggressive on pea and moderately aggressive on soybean.

Rhizoctonia solani AG5 were reported as pathogens of couch grass [*Elymus repens* (L.) Gould] (Woodhall and Lees 2004), stem canker of buckwheat (*Fagopyrum esculentum* Moench) in Maine, USA (Zhang et al. 2016) and potato (*Solanum tuberosum* L.) in UK (Yang and Wu 2012; Muzhinji et al. 2015), wheat (*Triticum aestivum* L.) in the UK, Turkey, Canada, and USA (Woodhall et al. 2012; Broders et al. 2014), soybean in Canada and USA (Nelson et al. 1996; Zhao et al. 2005). In fact, *R. solani* AG5 was most frequently detected in the soil samples from Europe (Goll et al. 2014). In Canada, *R. solani* AG5 isolates were isolated from soybean in Ontario (Zhao et al. 2005) and from wheat in Alberta and Manitoba (Broders et al. 2014). In the current study, seven isolates of AG5 were originally isolated from soybean in Alberta. These isolates could infect, but were weakly aggressive on soybean and pea except for one isolate (R06), which was

Fig. 2. Phenetic tree of *Rhizoctonia* isolates from soybean and other crops was constructed using the POPGENE program based on ISSR analysis.



highly aggressive on pea. This appears to be the first report of AG5 from soybean in Alberta.

Three isolates identified as the binucleate *Rhizoctonia* AG-E were recovered from soybean (R92a) and faba bean (R97b and R102) in the current study. All these isolates were highly aggressive on pea and soybean. AG-E had been reported to infect bean (Demirci and Döken 1995), soybean (Ploetz et al. 1985; Erper et al. 2011), but was considered to be moderately or weakly pathogenic.

In the current study, there were 20 *Rhizoctonia* isolates for which the AG designation could not be determined. These isolates were originally collected from cauliflower, pea, and soybean, whose aggressiveness on soybean and pea also varied. Four isolates (R10, R15, R55 and R151) were highly or moderately aggressive on soybean. The other isolates were non-pathogenic or weakly pathogenic on soybean. When tested on pea, five isolates (R10, R15, R55, R148 and R151) were highly aggressive, five

isolates (R12, R14a, R27a, R77 and R88) appeared to be non-pathogenic and the others were weakly or moderately aggressive.

Generally, in Alberta, *R. solani* most commonly occurs as AG4, AG2-1, AG2-2 in pulse crops (Hwang et al. 2003; Chang et al. 2005; Zhou et al. 2009), wheat, and canola (Broders et al. 2014; Zhou et al. 2014), AG5 in wheat (Broders et al. 2014) and AG9 in soil samples (Yang et al. 1996). Also, in pulse crops, AG4 isolates was the dominate AG and highly aggressive on host plants in Alberta (Hwang et al. 2003). The findings of the current study support that AG4 was the dominate AG infecting leguminous crops and causing severe seedling blight and root rot disease.

From the host range study, isolates belonging to AG4 and AG-E were more aggressive than the isolates of other AGs. Isolates belonging to AG2-1 and AG2-2 were moderately aggressive on most host plant species and highly aggressive on canola and faba bean. Isolates of AG5 displayed the lowest aggressiveness to all the plant species except R70, which was highly aggressive on faba bean and moderately aggressive on soybean. From the eleven plant species, canola, faba bean, and flax were more susceptible while potato and wheat were highly resistant to the *Rhizoctonia* isolates.

To evaluate the genetic variation among all the *Rhizoctonia* isolates, sequencing and phylogenetic analysis of the ITS regions and the ISSR were used in the current study. Sequencing of ITS regions has been shown to be a reliable method to divide isolates of *R. solani* into distinct groups, corresponding to the different anastomosis groups (Boysen et al. 1996; Kuninaga et al. 1997; Carling et al. 2002*b*). Lehtonen et al. (2008) reported that differences in the ITS regions were sufficiently large to reliably differentiate the AGs, but the region could not be used to detect differences between isolates within the same AG. Based on the ITS sequences, all the isolates were clustered to five groups. Unfortunately, there was no delineation between AGs and the clades based on the sequence analysis. Because ISSR markers produced greater polymorphisms with greater reproducibility than RAPD (Parsons et al. 1997; Chowdhury et al. 2002), ISSR was used to analyze the genetic diversity of *R. solani* and binucleate *Rhizoctonia* AG-E (Dubey et al. 2012; Zheng et al. 2013; Shu et al. 2014; Zhou et al. 2014). Based on the ISSR analysis in the current study, four groups could be recognized, but there was no correlation between the AGs and the clades.

In summary, *Rhizotonia* was important pathogen, which restrains the production of soybean and pea in Alberta and Manitoba. AG4 was the dominate anastomosis group, followed by AG2-2, AG2-1, AG5 and AG-E, but the AG of 20 isolates could not be determined. Isolates belonged to AG4 were more aggressive on soybean and pea. The selected isolates belonging to AG4, AG2-1, AG2-2 and AG5 were able to infect common crops in Alberta which including barley, canola, corn, faba bean, flax, lupin, lentil, pea, potato, soybean and wheat. AG4 isolates were more highly aggressive than the isolates of the other AGs. There was no correlation between AGs, location and host origin based on the ITS and ISSR analysis.

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References

Ajayi-Oyetunde, O.O., and Bradley, C.A. 2018. *Rhizoctonia solani*: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. Plant Pathol. **67**: 3–17. doi:10.1111/ppa.12733.

Aoyagi, T., Kageyama, K., and Hyakumachi, M. 1998. Characterization and survival of *Rhizoctonia solani* AG2-2 LP associated with large patch disease of Zoysia grass. Plant Dis. **82**: 857–863.

Boysen, M., Borja, M., del Moral, C., Salazar, O., and Rubio, V. 1996. Identification at strain level of *Rhizoctonia solani* AG-4 isolates by direct sequence of asymmetric PCR products of the ITS regions. Curr. Genet. **29**: 174–181. doi:10.1007/BF02221582. PMID:8821665.

Broders, K.D., Parker, M.L., Melzer, M.S., and Boland, G.J. 2014. Phylogenetic diversity of *Rhizoctonia solani* associated with canola and wheat in Alberta, Manitoba, and Saskatchewan. Plant Dis. **98**: 1695–1701. doi:10.1094/PDIS-02-14-0146-RE. PMID:30703878.

Carling, D.E. 1996. Grouping of *Rhizoctonia solani* by hyphal anastomosis reaction. Pages 37–47 in B. Sneh, S. Jabaji-Hare, S. Neate, and G. Dijst, eds. *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishers, Boston, MA, USA.

Carling, D.E., Baird, R.E., Gitaitis, R.D., Brainard, K.A., and Kuninaga, S. 2002*a*. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology, **92**: 893–899. doi:10.1094/PHYTO.2002.92.8.893.

Carling, D.E., Kuninaga, S., and Brainard, K.A. 2002*b*. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. Phytopathology, **92**: 43–50. doi:10.1094/PHYTO.2002.92.1.43.

Çebi Kiliçoğlu, M., and Özkoç, İ. 2010. Molecular characterization of *Rhizoctonia solani* AG4 using PCR-RFLP of the rDNA-ITS region. Turk. J. Biol. **34**: 261–269.

Ceresini, P.C., Shew, H.D., Vilgalys, R.J., and Cubeta, M.A. 2002. Genetic Diversity of *Rhizoctonia solani* AG-3 from potato n and tobacco in North Carolina. Mycologia, **94**: 437–449.

Chang, K.F., Hwang, S.F., Gossen, B.D., Howard, R.J., Lopetinsky, K., and Olson, M. 2005. First report of *Rhizoctonia solani* AG-4 and AG-2-2 on *Lupinus angustifolius* in Canada. Plant Dis. **89**: 685. doi:10.1094/PD-89-0685C. PMID:30795408.

Chang, K.F., Hwang, S.F., Ahmed, H.U., Strelkov, S.E., Harding, M.W., Conner, R.L., et al. 2018. Disease reaction to *Rhizoctonia solani* and yield losses in soybean. Can. J. Plant Sci. **98**: 115–124. doi:10.1139/cjps-2017-0053.

Chatterton, S., Bowness, R., and Harding, M.W. 2015. First report of root rot of field pea caused by *Aphanomyces euteiches* in Alberta, Canada. Plant Dis. **99**: 288. doi:10.1094/PDIS-09-14-0905-PDN. PMID:30699595.

Chowdhury, M.A., Vandenberg, B., and Warkentin, T. 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica, **127**: 317–325.

Cubeta, M.A., Echandi, E., Abernethy, T., and Vilgalys, R. 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. Phytopathology, **81**: 1395–1400. doi:10.1094/Phyto-81-1395.

Cubeta, M.A., and Vilgalys, R. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology, **87**: 480–484.

Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods, **9**(8): 772. doi:10.1038/nmeth.2109. PMID:22847109.

Demirci, E., and Döken, M.T. 1995. Anastomosis groups of *Rhizoctonia solani* Kühn and binucleate *Rhizoctonia* isolates from various crops in Türkiye. J. Turk. Phytopathol. **24**: 57–62.

Doyle, J.J., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11–15.

Dubey, S.C., Tripathi, A., and Upadhyay, B.K. 2012. Molecular diversity analysis of *Rhizoctonia solani* isolates infecting various pulse crops in different agro-ecological regions of India. Folia Microbiol. **57**(6): 513–524. doi:10.1007/s12223-012-0165-y. PMID:22653790.

Engelkes, C.A., and Windels, C.E. 1996. Susceptibility of sugar beet and beans to *Rhizoctonia solani* AG-2-2 IIIB and AG-2-2 IV. Plant Dis. **80**(12): 1413–1417. doi:10.1094/PD-80-1413.

Erper, I., Ozkoc, I., and Karaca, G.H. 2011. Identification and pathogenicity of *Rhizoctonia* species isolated from bean and soybean plants in Samsun, Turkey. Arch. Phytopathol. Plant Prot. **44**(1): 78–84. doi:10.1080/03235400903395427.

Fenille, R.C., de Souza, N.L., and Kuramae, E.E. 2002. Characterization of *Rhizoctonia solani* associated with soybean in Brazil. Eur. J. Plant Pathol. **108**: 783–792. doi:10.1023/A:1020811019189.

Gabruch, M., and Gietz, R. 2014. The potential for soybeans in Alberta. [Online]. Available from https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/bus15100/$file/soybeans-1.pdf?OpenElement.

Goll, M.B., Schade-Schutze, A., Swarta, G., Oostendorpa, M., Schotta, J.J., Jaserb, B., and Felsenstein, F.G. 2014. Survey on the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. Plant Pathol. **63**: 148–154. doi:10.1111/ppa.12063.

Gómez, L., Valdeolmillos, E.S., Perkins, K., Henderson, D., Barnes, A.V., and Woodhall, J.W. 2015. First report of *Rhizoctonia solani* AG2-1 on *Matthiola incana* in the United Kingdom. New Dis. Rep. **32**: 11. doi:10.5197/j.2044-0588.2015.032.011.

Gossen, B.D., Conner, R.L., Chang, K.F., Pasche, J.S., McLaren, D.L., Henriquez, M.A., et al. 2016. Identifying and managing root rot of pulses on the northern Great Plains. Plant Dis. **100**: 1965–1978. doi:10.1094/PDIS-02-16-0184-FE. PMID:30683014.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. **41**: 95–98.

Hwang, S.F., and Chang, K.F. 1989. Incidence and severity of root rot disease complex of field pea in northeastern Alberta in 1988. Can. Plant Dis. Surv. **69**: 139–141.

Hwang, S.F., Gossen, B.D., Chang, K.F., Turnbull, G.D., Howard, R.J., and Blade, S.F. 2003. Etiology, impact and control of Rhizoctonia seedling blight and root rot of chickpea on the Canadian prairies. Can. J. Plant Sci. **83**: 959–967. doi:10.4141/P02-165.

Hwang, S.F., Gossen, B.D., Conner, R.L., Chang, K.F., Turnbull, G.D., Lopetinsky, K., and Howard, R.J. 2007. Management strategies to reduce losses caused by Rhizoctonia seedling blight of field pea. Can. J. Plant Sci. **87**: 145–155. doi:10.4141/P04-172.

Jones, R.K., and Belmar, S.B. 1989. Characterization and pathogenicity of *Rhizoctonia* spp. isolated from rice, soybean and other crops grown in rotation with rice in Texas. Plant Dis. **73**: 1004–1010.

Justesen, A.F., Yohalem, D., Bay, A., and Nicolaisen, M. 2003. Genetic diversity in potato field populations of *Thanatephorus cucumeris* AG-3, revealed by ITS polymorphism and RAPD markers. Mycol. Res. **107**: 1323–1331. doi:10.1017/S0953756203008517. PMID:15000234.

Kataria, H.R., and Verma, P.R. 1992. *Rhizoctonia solani* damping off and root rot in oilseed rape and canola. Crop Prot. **11**: 8–13.

Khodayari, M., Safaie, N., and Shamsbakhsh, M. 2009. Genetic diversity of Iranian AG1-IA isolates of *Rhizoctonia solani*, the cause of rice sheath blight, using morphological and molecular markers. J. Phytophatol. **157**: 708–714. doi:10.1111/j.1439-0434.2009.01541.x.

Kuninaga, S., Natsuaki, T., Takeuchi, T., and Yokosawa, R. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. Curr. Genet. **32**: 237–243. doi:10.1007/s002940050272. PMID:9339350.

Lehtonen, M.J., Ahvenniemi, P., Wilson, P.S., German-Kinnari, M., and Valkonen, J.P.T. 2008. Biological diversity of *Rhizoctonia solani* (AG-3) in a northern potato-cultivation environment in Finland. Plant Pathol. **57**: 141–151. doi:10.1111/j.1365-3059.2007.01694.x.

Lehtonen, M.J., Wilson, P.S., Ahvenniemi, P., and Valkonen, J.P.T. 2009. Formation of canker lesions on stems and black scurf on tubers in experimentally inoculated potato plants by isolates of AG2-1, AG3 and AG5 of *Rhizoctonia solani*: a pilot study and literature review. Agric. Food Sci. **18**: 223–233. doi:10.2137/145960609790059415.

Masuda, T., and Goldsmith, P.D. 2009. World soybean production: area harvested, yield, and long-term projections. Int. Food and Agribus. Manage. Rev. **12**: 143–162.

McLaren, D.L., Henriquez, M.A., Conner, R.L., Chang, K.F., Henderson, T.L., Penner, W.C., and Kerley, T.J. 2015. Soybean root rot and Phytophthora root rot in Manitoba in 2014. Can. Plant Dis. Surv. **95**: 180–181.

Mathew, F.M., Lamppa, R.S., Chittem, K., Chang, Y.W., Botschner, M., Kinzer, K., et al. 2012. Characterization and pathogenicity of *Rhizoctonia solani* isolates affecting *Pisum sativum* in North Dakota. Plant Dis. **96**: 666–672. doi:10.1094/PDIS-02-11-0087. PMID:30727512.

Mirmajlessi, S.M., Safaie, N., Mostafavi, H.A., and Mahmoudy, S.B. 2012. Genetic diversity among crown and root rot isolates of *Rhizoctonia solani* isolated from cucurbits using PCR-based techniques. Afr. J. Agr. Res. **7**: 583–590. doi:10.5897/AJAR11.1453.

Muyolo, N.G., Lipps, P.E., and Schmitthenner, A.F. 1993. Anastomosis grouping and variation in virulence among isolates of *Rhizoctonia solani* associated with dry bean and soybean in Ohio and Zaire. Phytopathology, **83**: 438–444. doi:10.1094/Phyto-83-438.

Muzhinji, N., Truter, M., Woodhall, J.W., and van der Waals, J.E. 2015. Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* from potato in South Africa. Plant Dis. **99**: 1790–1802. doi:10.1094/PDIS-02-15-0236-RE. PMID:30699508.

Naito, S., Mochida, H., Nakajima, T., and Ohto, Y. 1995. Infection with basidiospores of *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*) and development of soybean foliar blight lesions. Ann. Phytopathol. Soc. Jpn. **61**: 362–368. doi:10.3186/jjphytopath.61.362.

Nelson, B., Helms, T., Christianson, T., and Kural, I. 1996. Characterization and pathogenicity of *Rhizoctonia* from soybean. Plant Dis. **80**: 74–80. doi:10.1094/PD-80-0074.

Nicoletti, R., Lahoz, E., Kanematsu, S., Naito, S., and Contillo, R. 1999. Characterization of *Rhizoctonia solani* isolates from tobacco fields related to anastomosis groups 2-1 and BI (AG 2-1 and AG BI). J. Phytopathol. **147**: 71–77. doi:10.1111/j.1439-0434.1999.tb03810.x.

Nyandoro, R., Chang, K.F., Hwang, S.F., Strelkov, S.E., Turnbull, G.D., Howard, R.J., et al. 2014. The occurrence of soybean root rot in southern Alberta, Canada in 2013. Can. Plant Dis. Surv. **94**: 198–200.

Nyandoro, R., Chang, K.F., Hwang, S.F., Ahmed, H., Strelkov, S.E., Turnbull, G.D., and Harding, M.W. 2015. The occurrence of soybean root rot in southern Alberta in 2014. Can. Plant Dis. Surv. **95**: 182–184.

Nyandoro, R., Li, N., Chang, K.F., Hwang, S.F., Akter, I., Ahmed, H.U., et al. 2017. The occurrence of soybean root rot in southern Alberta in 2016. Can. Plant Dis. Surv. **97**: 210–213.

Page, R.D.M. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. **12**: 357–358. doi:10.1093/bioinformatics/12.4.357. PMID:8902363.

Parsons, B.J., Newbury, H.J., Jackson, M.T., and Ford-Lloyd, B.V. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breeding, **3**: 115–125.

Pesticide Risk Reduction Program. 2008. Crop profile for field pea in Canada. Pest Management Centre, Agriculture and Agri-Food Canada. [Online]. Available from http://publications.gc.ca/collections/collection_2009/agr/A118-10-3-2009E.pdf.

Ploetz, R.C., Mitchell, D.J., and Gallaher, R.N. 1985. Characterization and pathogenicity of *Rhizoctonia* species from a reduced-tillage experiment multicropped to rye and soybean in Florida. Phytopathology, **75**: 833–839. doi:10.1094/Phyto-75-833.

Rosewich, U.L., Pettway, R.E., McDonald, B.A., and Kistler, H.C. 1999. High levels of gene flow and heterozygote excess characterize *Rhizoctonia solani* AG-1 IA (*Thanatephorus cucumeris*) from Texas. Fungal Genet. Biol. **28**: 148–159.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425. doi:10.1093/oxfordjournals.molbev.a040454. PMID:3447015.

Sharma, M., Gupta, S.K., and Sharma, T.R. 2005. Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. J. Phytopathol. **153**: 449–456. doi:10.1111/j.1439-0434.2005.01000.x.

Sharma-Poudyal, D., Paulitz, T.C., Porter, L.D., and du Toit, L.J. 2015. Characterization and pathogenicity of *Rhizoctonia* and *Rhizoctonia*-like spp. from pea crops in the Columbia basin of Oregon and Washington. Plant Dis. **99**: 604–613. doi:10.1094/PDIS-08-14-0803-RE. PMID:30699678.

Shu, C.W., Zou, C.J., Chen, J.L., Tang, F., Yi, R.H., and Zhou, E.X. 2014. Genetic diversity and population structure of *Rhizoctonia solani* AG-1 IA, the causal agent of rice sheath blight, in South China. Can. J. Plant Pathol. **36**(2): 179–186. doi:10.1080/07060661.2014.913685.

Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. American Phytopathological Press, St. Paul, MN, USA. 133 pp.

Sneh, B., Jabaji-Hare, S., Neate, S., and Dijst, G. 1996. *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology, and disease control. Kluwer Academic Publishers, Dordrecht, the Netherlands. 578 pp.

Stodart, B.J., Harvey, P.R., Neate, S.M., Melanson, D.L., and Scott, E.S. 2007. Genetic variation and pathogenicity of anastomosis group 2 isolates of *Rhizoctonia solani* in Australia. Mycol. Res. **111**: 891–900. doi:10.1016/j.mycres.2007.05.008. PMID:17707626.

Swofford, D.L. 2002. PAUP: phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sinauer, Sunderland, MA, USA.

Taheri, P., Gnanamanickam, S., and Höfte, M. 2007. Characterization, genetic structure, and pathogenicity of *Rhizoctonia* spp. associated with rice sheath diseases in India. Phytopathology, **97**: 373–383. doi:10.1094/PHYTO-97-3-0373.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds. PCR protocols: a guide to methods and amplifications. Academic Press, New York, NY, USA.

Woodhall, J.W., and Lees, A.K. 2004. First report of *Rhizoctonia solani* anastomosis group 5 (AG5) on couch grass in Britain. Plant Pathol. **53**: 538. doi:10.1111/j.1365-3059.2004.01041.x.

Woodhall, J.W., Laurenson, L., and Peters, J.C. 2012. First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. New Dis. Rep. **26**: 9. doi:10.5197/j.2044-0588.2012.026.009.

Xi, K., Stephens, J.H.G., and Hwang, S.F. 1995. Dynamics of pea seed infection by *Pythium ultimum* and *Rhizoctonia solani*: effects of inoculum density and temperature on seed rot and preemergence damping-off. Can. J. Plant Pathol. **17**: 19–24. doi:10.1080/07060669509500715.

Xue, A.G. 2003. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* Strain ACM941. Phytopathology, **93**(3): 329–335. doi:10.1094/PHYTO.2003.93.3.329. PMID:18944343.

Xue, A.G., Cober, E., Morrison, M.J., Voldeng, H.D., and Ma, B.L. 2007. Effect of seed treatments on emergence, yield, and root rot severity of soybean under *Rhizoctonia solani* inoculated field conditions in Ontario. Can. J. Plant Sci. **87**: 167–174. doi:10.4141/P05-192.

Yang, J., Kharbanda, P.D., Wang, H., and McAndrew, D.W. 1996. Characterization, virulence, and genetic variation of *Rhizoctonia solani* AG-9 in Alberta. Plant Dis. **80**: 513–518. doi:10.1094/PD-80-0513.

Yang, X.B., Berggren, G.T., and Snow, J.P. 1990. Types of Rhizoctonia foliar blight on soybean in Louisiana. Plant Dis. **74**: 501–504. doi:10.1094/PD-74-0501.

Yang, Y., and Wu, X. 2012. First report of potato stem canker caused by *Rhizoctonia solani* AG-5 in China. Plant Dis. **96**: 1579. doi:10.1094/PDIS-04-12-0388-PDN. PMID:30727329.

Yeh, F.C., and Boyle, T.J.B. 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. Belg. J. Bot. **129**: 157–163.

Zhang, X.Y., Zhang, X.M., Jiang, H.H., and Hao, J.J. 2016. First report of *Rhizoctonia solani* AG-5 causing stem canker on buckwheat in Maine. Plant Dis. **100**(6): 1241. doi:10.1094/PDIS-09-15-1060-PDN.

Zhao, G., Ablett, G.R., Anderson, T.R., Rajcan, I., and Schaafsma, A.W. 2005. Anastomosis groups of *Rhizoctonia solani* associated with soybean root and hypocotyl rot in Ontario and resistance of accession PI 442031 to different anastomosis groups. Can. J. Plant Pathol. **27**(1): 108–117. doi:10.1080/07060660509507201.

Zheng, L., Shi, F., and Hsiang, T. 2013. Genetic structure of a population of *Rhizoctonia solani* AG 2-2 IIIB from *Agrostis*

stolonifera revealed by inter-simple sequence repeat (ISSR) markers. Can. J. Plant Pathol. **35**(4): 476–481. doi:10.1080/07060661.2013.830148.

Zhou, Q., Chang, K.F., Hwang, S.F., Strelkov, S.E., Gossen, B.D., and Chen, Y.Y. 2009. Pathogenicity and genetic diversity of *Rhizoctonia solani* isolates from lupin and other crops in Alberta, Canada. Can. J. Plant Pathol. **31**: 340–347. doi:10.1080/07060660909507608.

Zhou, Q., Hwang, S.F., Fu, H., Strelkov, S.E., and Gossen, B.D. 2014. Genetic variation of *Rhizoctonia solani* isolates from canola in Alberta, Canada. Can. J. Plant Sci. **94**: 671–681. doi:10.4141/cjps2012-114.

Zhou, Q., Li, N., Chang, K.F., Hwang, S.F., Strelkov, S.E., Conner, R.L., et al. 2018. Genetic diversity and aggressiveness of *Fusarium* species isolated from soybean in Alberta, Canada. Crop Prot. **105**: 49–58. doi:10.1016/j.cropro.2017.11.005.