Improving Evaluation of Potato Resistance to *Rhizoctonia solani* infection by Optimizing Inoculum Method Combined with Toxin-based Assay

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Abstract

Background: *Rhizoctonia solani* causes stem canker and significantly impacts the production of potato. Conventional assay to evaluate potato resistance using *R. solani* inoculum is time consuming. To establish an effective and fast assay, 20 potato germplasms were examined using both *R. solani* inoculum and *R. solani*-derived toxin (RS toxin).

Results: In field trials of 2009 and 2010, wheat-bran-based inoculum of *R. solani* was incorporated at either 0, 2, 3, 4 or 5 g per seed piece in the soil followed by sowing potato seed pieces in the furrow. Stem canker was evaluated in the growing season. Inoculum of wheat-bran-based 2, 3, or 4 g could well distinguish resistance of potato germplasms. For a quick assay of resistance screening, a toxin-based method was established by treating potato seedlings with the toxin of *R. solani* (RS toxin). RS toxin was prepared by heating *R. solani* culture. Potato seedlings were obtained through tissue culture and grown in Murashige and Skoog medium. Seedlings at the stage of 12 cm in height were transferred into agar medium amended with RS toxin and incubated for eight days. The inhibition caused by RS toxin was positively correlated with toxin concentration. By evaluating various potato cultivars that have different sensitivities to toxin, the inhibition of potato stems sections and seedlings was from 33% to 100% and from 32% to 148%, respectively. Results of toxin-based evaluation were highly correlated with the field data using pathogen inoculum ($r = 0.731$, $P < 0.01$).

Conclusions: Inoculation with wheat bran-mediated *R. solani* of 2, 3 or 4 g per seed piece was an effective method for the evaluation of potato resistance in field trials. The toxin-based assay could improve efficiency and speed of disease resistance evaluation of potato germplasms. Both assays showed that none of the 20 potato materials was completely resistant to *R. solani*. However, cultivar ‘Desiree’ had the lowest level of disease, whereas ‘Atlantic’, ‘Favorita’, and ‘Shepody’ showed the high susceptibility.

Background

Rhizoctonia solani is an economically important pathogen of potato (Solanum tuberosum). It contains a complex of organisms with discriminately genetic variation, including 14 anastomosis groups (AGs) [1]. AG3 and AG2-1 are the most predominant groups associated with potato infection [2–5]. The pathogen can infect all belowground portions of potato plant and cause lesions and decay on potato tubers, sprouts, roots, stolons, and stems [2, 3, 6]. The stem canker is characterized by brown and black sunken lesions on the stem and severely girdled stem, which can result in reduced growth of plants, and wilting or early dying. Dark-colored sclerotia are formed on the surface of progeny tubers, which is referred to as black scurf [3, 7, 8]. China is the largest potato-producing country in the world [9]. In most of the production areas, potato stem canker has become an increasing problem. In 2009, 85% stem canker and 100% tubers black scurf were observed in Wulanchabu city of Inner Mongolia.

Controlling R. solani is difficult because of the extremely wide host range [3, 6, 10, 11] and the saprophytic life cycle of the pathogen [12]. Resistant cultivars is a long-term consideration in order to economically and effectively control stem canker and tuber black scurf [13–15]. However, availability of resistant genotypes is very limited [11, 13, 14, 15, 16, 17, 18, 19]. There are few potato cultivars that have high resistance or tolerance to R. solani [13, 16, 17, 18, 19]. On a list of potato cultivars in Canada, only 10% of the 111 cultivars are considered resistant [20] (Anonymous. 1986). Pietkiewicz & Chorozewski (1983) have found seven out of 44 potato cultivars are resistant [21]. Khandaker et al. (2011) reported 6 out of 25 potato germplasms show moderate resistance [14].

In screening potato germplasms for R. solani resistance, field evaluation of potato is a reliable method, but it takes a long growing season and is highly affected by environmental factors. Because of this reason, different methods of resistance evaluation, inoculation, and field operation vary depending on the researchers [13, 16, 17], which may lead to different results to same cultivar in different place. There is a need to establish a standard protocol that is fast and consistent for resistance evaluation. In this study, we proposed optimizing field evaluation using pathogen inoculum coupled with phototoxic-based assay. The latter can be used for primary screening on large collection of potato germplasms before field evaluation, which would greatly reduce the amount of work load and time consuming, therefore improve the efficiency of resistance screen.

Rhizoctonia solani produces toxin (RS toxin). The toxin is a host-selective pathogenicity determinant, meaning it only induces characteristic symptoms of corresponding hosts [22, 23]. We anticipated that RS toxin could correlate with inoculum for resistance evaluation, as shown in rice pathosystem, where toxin of R. solani is a significantly correlated with phototoxic sensitivity and disease susceptibility [24–25]. Frank and Francis (1976) also reported that the RS toxin can be used for potato resistance evaluation. Since then, no other reports have been documented in potato [26].

In this study, we used Rhizoctonia solani and its toxin as material equivalent to inoculum. The objectives of were to: (1) optimize inoculum levels for field inoculation; (2) develop a rapid method for disease resistance study using RS toxin of R. solani; (3) and validate the methods by evaluating the susceptibility of 20 potato germplasms against Rhizoctonia stem canker.

Methods

Potato materials

Commercial cultivars, including ‘Longshu 3’, ‘Longshu 5’, ‘Longshu 6’, ‘Longshu 7’, ‘Shuixihong’, ‘Sepihong’, ‘Zihuabai’, ‘LK99’, ‘Kexin 1’, ‘Heimeiren’, ‘Qingshu 168’ were bred in China. ‘Desiree’ and ‘Favorita’ bred by Hollander ,‘Atlantic’ and ‘Shepody’ breeded by USA and Canada, and potato lines J08-1, J08-2, J08-4, J07-2, and J07-5 which were provided by obtained from the Japanese Hokkaido University. These materials were all virus free assured by using tissue culture transfer, and maintained in the Research Center of Potato Breeding in Inner Mongolia Agricultural University, Hohhot, China.

Fungal Inoculum

Rhizoctonia solani AG2-1 strain WC-16 was isolated from an infected potato tuber from a field in Wuchuan county of Inner Mongolia where potato, which was confirmed to be highly pathogenic on stems and tubers of ‘Atlantic’ potato. The fungal culture was stored on potato sucrose agar (PSA) at 4°C for later use.

To prepare the inoculum, wheat bran (100 g) was soaked in distilled 200 ml water in a 500 ml conical flask, and autoclaved at 121℃ for 40 min. Each flask was inoculated with 10 discs (0.8 cm diameter) of 4-day-old R. solani culture, and incubated at 25℃ in the dark for 30 days. The wheat bran mixed with mycelia and a pseudosclerotia of R. solani was air dried and then manually rubbed into fine pieces.

Inoculum-based evaluation on potato resistance to R. solani under field conditions

Field trials were conducted at two locations. In 2009, the trial was conducted in the Horticulture Science and Technology Demonstration Area of Hohhot City. potato seed tubers of different potato germplasms were planted in the field covered with a nylon screen in May. Soil properties were analyzed by the Testing Center of Agricultural Products Quality and Safety, Hohhot, Inner Mongolia. The field is composed of light sandy soil with 18.5 g/kg organic matter, 74 mg/kg hydrolysis nitrogen, 23.0 mg/kg available phosphorus, and 116 mg/kg rapidly available potassium. Prior to planting, potato tubers were disinfested for 20 min with 0.5% $KMnO_4$, followed by rinsing with tap water and cutting into 30 to 50 g seed-tuber pieces. At the seeding site, 0 (control), 2, 3, 4, or 5 g of wheat bran inoculum was applied per seed piece, followed by planting. All 20 potato germplasms were planted in a randomized complete block design, with two rows per germplasm, and 20 plants per row. Plant space was 30 cm within rows and 60 cm between rows. Each treatment had three replications. During the growth period of 133 days, the potato entries were not fertilized but irrigated three times.

In May of 2010, the trial was repeated at the Inner Mongolia Agricultural University Farm, where there was higher soil fertility than that in 2009. Soil properties were as follows: 18.7 g/kg organic matter, 75 mg/kg hydrolysis nitrogen, 24.50 mg/ kg available phosphorus, and 118 mg/kg rapidly available potassium. The wheat bran inocula were applied at 0, 2 and 4 g per seed piece.

Sixty-five days after planting, potato underground stems were dug out and examined for canker. Total of 100 stems were processed. Disease severity was expressed as following rating scale based on the percentage of lesion on the stem [27]: 0 (no lesion), 1 (1–5%), 2 (6–25%), 3 (26–50%), 4 (51–75%), and 5 (76–100%). Disease index (DI) was calculated as: $DI = 100 \times \Sigma(r_i \times n_i)/(N \times 5)$, where N = total number of plants evaluated, r is the level of severity from 0 to 5, and i = specific level of severity (from 0 to 5), n = number of corresponding grade plants evaluated. Relative resistance index (RRI) was calculated as: $RRI = 1\text{-}DI_X/DImax$, where DIx = disease index of the observed stem and DImax = the maximum disease index of all cultivars or lines in the same repetition. Stem canker resistance was measured with the relative resistance index (RRI) as follows: 1: immune (I); 0.99 to 0.80: highly resistant (HR); 0.79 to 0.50: moderately resistant (MR); 0.49 to 0.20: moderately susceptible (MS); and 0.19 to 0.00: highly susceptible (HS).

Potato resistance evaluation using RS toxin

RS toxin derived by heating. Fifty milliliters of improved Richard medium [28–29] was added into a 250 ml conical flask, and autoclaved for 20 min. After cooling down, each flask was inoculated with 5 discs (0.5 cm diameter) of 4-day-old R. solani culture, and incubated for 20 days at 25℃ in the dark, with hand shaking daily. After incubation, the culture was filtered through a filter paper at 50 μm pores. The filtrate was examined for absorbance peak at wavelength 258.8 nm to confirm the presence of toxin, and collected for later use. The filtrate was added into agar at concentration 0.4% by volume, and poured into test tubes (18 cm × 1.8 cm) at 12 ml/tube, and autoclaved for 20 min at 115℃. This was used as toxin derived by heating [28]. The trial was repeated once.

RS toxin derived by active carbon adsorbtion. Equal amount of liquid active carbon was added into the filtrate as described above, and kept for 12 h at 4℃, followed by centrifugation for 15 min at 3500 r/min. The precipitated pellet of active carbon was mixed with equal amount of methanol, and then concentrated with a rotary evaporator at 45℃, which was yellow slurry. It was diluted with distilled water to the original volume, then filtrated through 0.22 μm membrane for sterilization. The derived product was used a toxin by adsorption [29]. The absorbance peak of toxin was examined at wavelength 258.8 nm for confirmation of toxin and poured into sterilized test tubes (18 cm × 1.8 cm) at 12 ml/tube contained agar at concentration of 0.4%. The trial was repeated once.

Disease evaluation. Virus-free potato 'Atlantic' seedlings were grown in Murashige and Skoog medium until their height reached around 12 cm. The seedlings were cut into stem sections with one leaf bud attached per stem section. This was a standard for stem section used for inoculation throughout the study, unless otherwise stated. The stem sections were placed into media amended with one of the toxins prepared either by heating or absorption. MS medium without toxin was used for control. Total of 60 stem sections were used for each treatment, which was replicated three times. After incubation at 25℃ with 16 h light (4000 lux)/day arrangement, the seedlings were observed for necrotic symptom from four to eight days of incubation, seedlings length were measured and growth inhibition rate was calculated after eight days. Growth inhibition = (seedling length of control - seedling length of treatment) / seedling length of control × 100%.

Comparison of inoculation with Rhizoctonia solani inoculum and its toxin. Water agar (0.4%) was autoclaved and cooled to 40℃, mycelia of R. solani in above culture were collected and ground into small pieces in a mortar with a pesto using aseptic operation, then added into the water agar with equal volumes culture, and shaken well. This suspension was used as a pathogen inoculum. The pathogen inoculum and toxin derived by heating containing 0.4% agar were separately put into test tubes (18 cm × 1.8 cm) at 12 ml/tube. The seedling of 12-cm long and stem sections of potato 'Atlantic' were transferred into tubes containing pathogen inoculum, toxin, and MS medium. There were 60 plants per treatment. The tubes were incubated at 25℃ with 16 h light (4000 lux)/day. The seedlings and stem sections were observed for necrotic symptom from four to eight days of incubation. Seedlings and stem section length were measured and growth inhibition was calculated. The trial was conducted three times.

Effects of toxin concentration on symptom expression. The above filtrate was diluted into 1/2 and 1/4 concentrations with either MS liquid medium or distilled water. To concentrate the toxin, the filtrate was treated with heat to obtain 2 or 4 times of concentration. The prepared different concentration toxin were added into 0.4% agar in tubes at 12 ml/tube, then autoclaved at 115℃ for 20 min. MS medium and distilled water agar were used as a control. Seedlings and stem sections of 'Atlantic' were transferred into the tubes containing different concentrations of toxin. In another trial, the filtrate was made into toxin and

toxin of 3/4 concentration with distilled water. Distilled water agar was control. Potato seedlings and stem sections of 12 cultivars were transferred into different concentration toxin.

There were 60 plants each treatment. Then incubated at 25℃ with 16 h light (4000 lux)/day, the symptoms were observed for necrotic from four to eight days of incubation, seedlings length were measured and growth inhibition rate was calculated after eight days. The trials were conducted three times.
Resistance Identification Of Potato Cultivars Using Toxin-based Assays

After preliminary trials, optimized assay was determined as follow. Virus-free potato seedlings were grown in Murashige and Skoog medium until their height reached around 12 cm. The seedlings were transferred into a medium amended with heat-derived toxin, with one seedling per tube. Distilled water agar was used as a control. Total of 60 seedlings were transferred for each variety or line, which was replicated three times. After incubation at 25℃ with 16 h light (4000 lux)/day, the seedlings were observed for necrotic symptom from four to eight days of incubation, and disease was measured after eight days. The disease was scored using the following scale based on the percentage of girdled stem and wilted or dead leaves: 0 (no girdled stem and wilted or dead leaves), 1 (1–5%), 2 (6–25%), 3 (26–50%), 4 (51–75%), and 5 (76–100%). Disease index (DI), relative resistance index (RRI), and seedling resistance measured using relative resistance index (RRI) were as described above in the field trials.

Statistical Analysis

Data were analyzed using the SPSS 17.0 statistical software (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp. Armonk, NY, USA). GLM procedure was used for analysis of variance. LSR multiple comparisons was used for mean separation, at significance level 0.05.

Results

Resistance evaluation in fields using R. solani inoculation

Stem canker resistance was evaluated on 20 potato materials with wheat bran inoculum at different levels in 2009 (Table 1). Plants without R. solani infection were not or slightly necrotic, with disease indices lower than 13.9. Potato materials inoculated with R. solani inoculums had significant high disease severity for most of the cultivars, and the disease indices of cultivars at 2, 3, 4, or 5 g inoculums all showed significant differences ($P < 0.01$). The resistance scores for 2-, 3-, and 4-g inoculum levels showed generally consistent proportions of resistant and susceptible, at 30–35% of resistance and 65–70% of susceptibility. When the inoculum was 5 g the proportions of resistant and susceptible cultivars reached 10% and 90%, respectively (Fig. 1).

Table 1

Responses of potato germplasms to Rhizoctonia solani inoculation at 0, 2, 3, 4, and 5 g/seed piece in 2009 in field conditions

Potato	0 g			2 g			3 g			4 g			5 g		
	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]
Desiree	0.17 g	0.99	HR	23.70 h	0.66	MR	29.00 h	0.58	MR	33.48 ef	0.55	MR	32.29 g	0.51	MR
Heimeiren	0.00 g	1.00	I	25.27 gh	0.63	MR	43.73 def	0.37	MS	31.55 efg	0.57	MR	44.25 ef	0.35	MS
Longshu 3	2.80 def	0.80	HR	30.98 fgh	0.55	MR	30.00 h	0.57	MR	45.53 cd	0.38	MS	48.72 de	0.28	MS
LK99	0.00 g	1.00	I	33.33 fgh	0.52	MR	48.33 cde	0.30	MS	23.98 g	0.67	MR	45.95 def	0.32	MS
Kexin 1	0.00 g	1.00	I	34.08 fgh	0.51	MR	32.38 gh	0.53	MR	35.01 ef	0.52	MR	46.25 def	0.32	HS
Zihuabai	1.05 fg	0.92	HR	36.63 efgh	0.47	MS	33.23 fgh	0.52	MR	28.26 fg	0.62	MR	39.08 fg	0.42	MS
favorite	2.00 efg	0.86	HR	48.15 cde	0.30	MS	47.58 cde	0.31	MS	53.77 c	0.27	MS	62.30 ab	0.08	HS
Longshu 6	7.58 b	0.45	MS	27.88 gh	0.60	MR	26.92 h	0.61	MR	39.35 de	0.47	MS	51.50 cde	0.24	MS
Qingshu168	9.52 b	0.31	MS	37.02 efg	0.46	MS	44.58 def	0.36	MS	36.63 ef	0.50	MR	46.94 def	0.31	MS
Longshu 5	13.89 a	0.00	HS	43.89 def	0.36	MS	34.36 fgh	0.50	MR	46.59 cd	0.37	MS	46.67 def	0.31	MS
Longshu 7	8.67 b	0.38	MS	31.67 fgh	0.54	MR	49.17 cde	0.29	MS	54.08 c	0.27	MS	44.08 ef	0.35	MS
J08-1	4.76 cd	0.66	MR	61.90 ab	0.10	HS	69.37 a	0.00	HS	48.08 c	0.35	MS	34.02 g	0.50	MR
J08-4	3.13 de	0.77	MR	51.37 bcd	0.26	MS	38.10 efgh	0.45	MS	51.71 c	0.30	MS	65.78 a	0.03	HS
Sepihong	0.00 g	1.00	I	38.06 efg	0.45	MS	63.61 ab	0.08	HS	53.17 c	0.28	MS	60.02 ab	0.11	HS
J07-2	7.58 b	0.45	MS	69.00 a	0.00	HS	58.33 bc	0.16	HS	73.70 a	0.00	HS	57.99 abc	0.14	HS
J08-2	0.00 g	1.00	I	41.67 def	0.40	MS	41.67 defg	0.40	MS	65.27 b	0.11	HS	50.22 cde	0.26	MS
Shepody	0.27 g	0.98	HR	58.89 abc	0.15	HS	46.36 de	0.33	MS	51.30 c	0.30	MS	54.21 bcd	0.20	MS
J07-5	0.00 g	1.00	I	38.33 efg	0.44	MS	50.00 cd	0.28	MS	66.34 ab	0.10	HS	60.30 ab	0.11	HS
Shuixihong	5.30 c	0.62	MR	49.09 cde	0.29	MS	44.44 def	0.36	MS	45.85 cd	0.38	MS	54.19 bcd	0.20	MS
Atlantic	4.76 cd	0.66	MR	53.33 bcd	0.23	MS	61.11 ab	0.12	HS	69.03 ab	0.06	HS	64.29 a	0.05	HS

[a] DI = Disease index. [b] RRI = Relative resistance index. [c] CR = Comprehensive resistance, including highly resistant (HR, 0.99 to 0.80), moderately resistant (MR, 0.79 to 0.50), and moderately susceptible (MS, 0.49 to 0.20). Mean values followed by different letters in each column were significantly different ($P < 0.05$).

In 2010 trial, disease indices of cultivars at 2 and 4 g wheat bran inoculums all showed significant differences ($P < 0.01$) and consistent proportions of resistant and susceptible cultivars with 2009 (Table 2). In addition, the average disease index of potato cultivars or lines at 2, 3, and 4 g inoculum levels in 2009 and at 2 and 4 g inoculum levels in 2010 showed a highly positive correlation ($r = 0.719$, $P < 0.01$, Table 3).

Table 2

Evaluation of stem canker resistance of potato cultivars and lines to Rhizoctonia solani inocula at 0, 2, 4 g/seed piece in 2010

Cultivars/lines	0 g			2 g			4 g		
	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]	DI[a]	RRI[b]	CR[c]
Desiree	0.09 d	0.99	HR	16.69 h	0.72	MR	32.64 fgh	0.50	MR
Heimeiren	0.00 d	1.00	I	35.09 cd	0.42	MS	22.06 h	0.66	MR
Longshu 3	2.5 c	0.63	MR	29.29 defg	0.51	MR	45.00 de	0.31	MS
LK99	0.00 d	1.00	I	20.00 gh	0.67	MR	30.60 gh	0.53	MR
Kexin 1	0.00 d	1.00	I	33.19 de	0.45	MS	31.91 fgh	0.51	MR
Zihuabai	0.23 d	0.97	HR	30.08 def	0.50	MR	23.89 h	0.64	MR
favorite	0.07 d	0.99	HR	28.33 defg	0.53	MR	56.04 abc	0.15	HS
Longshu 6	0.7 d	0.90	HR	42.67 bc	0.29	MS	43.33 de	0.34	MS
Qingshu 168	0.10 d	0.99	HR	18.33 h	0.69	MR	38.46 efg	0.41	MS
Longshu 5	0.56 d	0.92	HR	24.00 efgh	0.60	MR	40.19 defg	0.39	MS
Longshu 7	0.00 d	1.00	I	48.57 b	0.19	HS	40.91 defg	0.38	MS
J08-1	0.00 d	1.00	I	34.56 cd	0.42	MS	55.90 abc	0.15	HS
J08-4	0.03 d	1.00	HR	49.79 b	0.17	HS	65.60 a	0.00	HS
Sepihong	1.98 c	0.70	MR	31.11 def	0.48	MS	42.14 def	0.36	MS
J07-2	0.21 d	0.97	HR	45.34 b	0.24	MS	56.90 abc	0.13	HS
J08-2	6.70 a	0.00	HS	48.06 b	0.20	MS	48.06 bcde	0.27	MS
Shepody	0.00 d	1.00	I	59.77 a	0.00	HS	58.00 ab	0.12	HS
J07-5	0.00 d	1.00	I	45.98 b	0.23	MS	46.72 cde	0.29	MS
Shuixihong	3.90 b	0.42	MS	23.00 fgh	0.62	MR	32.64 fgh	0.50	MR
Atlantic	0.00 d	1.00	I	60.00 a	0.00	HS	50.55 bcd	0.23	MS

[a] DI = Disease index. [b] RRI = Relative resistance index. [c] CR = Comprehensive resistance, including highly resistant (HR, 0.99 to 0.80), moderately resistant (MR, 0.79 to 0.50), and moderately susceptible (MS, 0.49 to 0.20). Mean values followed by different letters in each column were significantly different ($P < 0.05$).

Table 3
Field evaluation for stem canker of potato to Rhizoctonia solani inocula at 2, 3, and 4 g/seed piece

Cultivars/lines	2009			2010		Years combined	
	DI[a]	RRI[b]	CR[c]	DI[d]	RRI[e]	CR[c]	CR
Desiree	28.73	0.57	MR	24.67	0.58	MR	MR
Heimeiren	33.52	0.50	MR	28.58	0.51	MR	MR
Longshu 3	35.50	0.47	MS	37.15	0.37	MS	MS
LK99	35.21	0.47	MS	25.30	0.57	MR	MS- MR
Kexin 1	33.82	0.50	MR	32.55	0.45	MS	MR -MS
Zihuabai	32.71	0.51	MR	26.99	0.54	MR	MR
favorite	49.83	0.26	MS	42.19	0.28	MS	MS
Longshu 6	31.38	0.53	MR	43.00	0.27	MS	MR -MS
Qingshu 168	39.41	0.41	MS	28.40	0.52	MR	MS- MR
Longshu 5	41.61	0.38	MS	32.10	0.46	MS	MS
Longshu 7	44.97	0.33	MS	44.74	0.24	MS	MS
J08-1	59.78	0.11	HS	45.23	0.23	MS	HS -MS
J08-4	47.06	0.30	MS	57.70	0.02	HS	MS- HS
Sepihong	51.61	0.23	MS	36.63	0.38	MS	MS
J07-2	67.01	0.00	HS	51.12	0.13	HS	HS
J08-2	49.54	0.26	MS	48.06	0.18	HS	MS- HS
Shepody	52.18	0.22	MS	58.89	0.00	HS	HS
J07-5	51.56	0.23	MS	46.35	0.21	MS	MS
Shuixihong	46.46	0.31	MS	27.82	0.53	MR	MS- MR
Atlantic	61.16	0.09	HS	55.28	0.06	HS	HS

[a] Average disease index of potato cultivars or lines at 2, 3, and 4 g inocula levels per seed tuber. [b] Relative resistance index of potato cultivars or lines at 2, 3, and 4 g inocula levels per seed tuber. [c] Comprehensive resistance includes highly resistant (HR, 0.99 to 0.80), moderately resistant (MR, 0.79 to 0.50), and moderately susceptible (MS, 0.49 to 0.20). [d] Average disease index of potato cultivars or lines at 2 g and 4 g inocula levels per seed tuber. [e] Relative resistance index of potato cultivars or lines at 2, and 4 g inocula levels per seed tuber. Mean values followed by different letters in each column were significantly different at ($P < 0.05$).

There were significant differences among potato cultivars and lines ($P < 0.01$). No cultivars were immune or highly resistant to R. solani (Table 3). 'Desiree' and 'Heimeiren' showed moderate resistance. Susceptibility was relatively stable across years with few exceptions, with the proportion of stably susceptible cultivars reaching 60% including 'Shepody', 'Atlantic', and 'Favorita', the main cropping cultivars. Some moderately resistant and susceptible cultivars showed different results between 2 years, changing from moderate resistance to moderate susceptibility or from moderate susceptibility to moderate resistance (Table 3).

Toxin-based Resistance Evaluation

Efficacies of RS toxins prepared with two methods. RS toxins were prepared using heating or carbon absorption. Toxins from both methods resulted in same symptoms on stem sections. At 8 days post inoculation, stems became light brown and the necrotic spots on leaves were expressed. However, there was a difference when the toxins were applied to stem sections. Heat-derived toxin showed higher level of severity (Fig. 2), with growth inhibited by 94.2%, than adsorption-derived toxin that caused less severity with inhibition of 57.9% (Table 4).

Table 4
Inhibition of potato seedlings by Rhizoctonia solani toxins prepared in two methods

Treatments	Stem length (cm)	Inhibition(%)	Absorption at 258.8 nm
Toxin derived by heating	0.3 c	94.23 a	7.484
Toxin derived by carbon adsorbing	2.5 b	57.92 b	1.716
Non-treated	5.2 a		
Mean values followed by different letters in each column were significantly different ($P < 0.05$).			

Pathogenicity caused by R. solani and RS toxin. The symptoms were the same when potato stem sections and seedlings were treated with either R. solani inoculum or RS toxin. The stems were discolored, and leaves showed necrotic spots. Severe symptoms appeared at stems to shrink and girdle, leading to eventual death of the plants, while the control did not show diseased symptoms (Fig. 3). Both R. solani and RS toxin caused the same severity, and same level of inhibition on seedlings and stem sections, or showed no differences between the two methods (Table 5).

Table 5
Inhibition of seedlings and stem sections of potato treated with Rhizoctonia solani and its derivative toxin

Treatments	Seedling increased height (cm)	Inhibition of seedling(%)	Seedling height grown from stem section (cm)	Inhibition of stem section(%)
Toxin	0.32 b	91.33	0.46 b	82.90
R. solani	0.30 b	91.87	0.50 b	81.41
Non-treated	3.69 a		2.69 a	
Mean values followed by different letters in each column were significantly different (P < 0.05).				

Dose effects of RS toxin on disease severity. When treated with 2 or 4 times concentrated toxin, potato seedlings and stem sections barely grew, instead, quickly showed necrosis symptoms and died (Fig. 4). The inhibitory effect on potato seedlings and stem sections decreased as RS toxin which were 87.44%, 87.88%, and 77.27% and 58.33%, in MS medium and water agar as the control respectively (Table 6). In addition, leaves of seedlings treated with 1/4 toxin were greener and larger, which promoted the growth of seedlings (Fig. 4, Table 6). Potato cultivars had different responses to RS toxin, with the inhibition rates of stem sections and seedlings being 33–100% and 32–148% respectively. At 3/4 x diluted concentration of RS toxin, the inhibition was almost undetectable (Table 7).

Table 6
Growth inhibition of potato seedlings and stem segments by Rhizoctonia solani RS toxin

Toxin	Toxin concentrations	Seedling increased height (cm)	Inhibition of seedlings(%)	Seedling height grown from stem section (cm)	Inhibition of stem section(%)
Concentrated	4 x	0.00 e	100.00	0.00 e	100.00
	2 x	0.11 e	97.24	0.10 e	95.45
In distilled water	1 x	0.20 d	77.27	0.28 d	58.33
	1/2 x	0.63 c	28.98	1.82 b	-157.50
	1/4 x	3.98 a	-352.27	2.10 a	-214.17
	0	0.88 b		0.80 c	
In Murashige and Skoog liquid medium	1 x	0.20 c	87.44	0.28 c	87.88
	1/2 x	2.56 b	35.68	1.68 b	23.64
	1/4 x	3.04 a	23.62	2.03 a	7.73
	0	3.98 a		2.20 a	
Mean values followed by different letters in each column were significantly different (P < 0.05).					

Table 7
Growth inhibition of potato seedlings and stem segments treated with Rhizoctonia solani toxin at different concentrations

Potato germplasm	Inhibition of stem section (%)		Inhibition of seedling (%)	
	1x toxin	3/4 toxin	1x toxin	3/4 toxin
Longshu 6	86.36	-131.82	80.35	-31.56
Sepihong	82.14	-35.71	53.41	5.23
Shepody	100.00	8.70	40.91	1.69
Qingshu 168	87.50	-31.25	60.00	1.53
Shuixihong	33.33	-58.33	148.28	-8.43
Disiree	51.85	-7.41	53.41	-3.25
Atlantic	65.52	17.24	115.63	7.56
LK99	46.67	-366.67	116.02	-66.63
Longshu 7	56.52	-30.43	32.26	-3.43
Longshu 5	85.19	7.41	122.93	3.40
Longshu 3	62.30	27.87	107.04	7.89
Kexin 1	96.77	9.68	71.05	2..63

Resistance evaluation by RS toxin treatment. Treatments using RS toxin had similar results compared to field trials using R. solani inoculum (Table 8). The stem of seedlings began to turn brown and necrotic eight days after inoculation and girdled or died for the most severe symptoms. Controls of all cultivars did not show any disease symptoms (Fig. 5). The test potato materials had a significant difference in disease severity when treated with RS toxin for eight days ($P < 0.01$), and disease index was highly correlated with field data, with $r = 0.731$ ($P < 0.01$) between disease index of virus-free potato seedlings and the average disease index potato cultivars or lines at 2, 3, and 4 g inoculum levels per seed tuber in 2009, and $r = 0.600$ ($P < 0.01$)), the average disease index of potato cultivars or lines at 2- and 4-g inoculum levels per seed tuber in 2010.

Table 8
Responses of potato seedlings to Rhizoctonia solani toxin treatment

Potato germplasm	Disease index [z]	Relative resistance index	Comprehensive resistance
Disiree	23.08 h	0.64	MR
Heimeiren	25.59 h	0.60	MR
Longshu 3	26.11 h	0.59	MR
LK99	31.02 g	0.51	MR
Kexin 1	35.94 f	0.44	MS
Zihuabai	42.34 e	0.34	MS
favorite	43.18 e	0.32	MS
Longshu 6	43.75 e	0.31	MS
Qingshu 186	45.51 e	0.29	MS
Longshu 5	45.33 e	0.29	MS
Longshu 7	45.05 e	0.29	MS
J08-1	45.24 e	0.29	MS
J08-4	50.62 d	0.21	MS
Sepihong	51.72 d	0.19	HS
J07-2	52.31 cd	0.18	HS
J08-2	54.86 bcd	0.14	HS
Shepody	56.86 bc	0.11	HS
J07-5	58.82 ab	0.08	HS
Shuixihong	62.75 a	0.02	HS
Atlantic	63.73 a	0.00	HS

[z] Mean values followed by different letters in each column are significantly different ($P < 0.05$). Comprehensive resistance was grouped into high resistance (HR, 0.99 to 0.80), moderate resistance (MR, 0.79 to 0.50), and moderate susceptibility (MS, 0.49 to 0.20).

Discussion

The resistance of potato to R. solani is polygenically inherited [30], but the phenotype can be significantly affected by environmental conditions and pathogen pressure [31]. The infection by R. solani can be promoted when the growth vigor of potato plants is weakened by pathogens. These evidences support our results.

The severity of stem canker is usually positively correlated with the subsequent formation of black scurf on progeny tubers [27, 32, 33]. However, this is not always the case [19, 34, 35, 36, 37]. We also have found the correlation between stem canker and black scurf in 2009, but not so in 2010 [38]. Thus, both stem evaluation and tuber evaluation are important in the examination of R. solani resistance.

In this study, we have demonstrated that inoculation with wheat bran-mediated R. solani inocula was an effective method for the evaluation of potato resistance in field trials. For a successful infection, inoculum at 2 to 4 g per seed piece seemed optimal. Both inoculum- and toxin-based evaluation methods were effective to evaluate stem canker resistance. RS toxin is host-specific and responsible to typical symptoms and pathogenicity factor of black scurf and stem canker of potato [22–23]. In this study, stem sections and seedlings expressed similar necrotic symptoms using either RS toxin or R. solani inoculum. This was in agreement with others [24–26]. Interestingly, we observed that low concentration of toxin could promote the growth of potato seedling. Similar observations have been reported by others [26].

We have used both stem sections and seedlings of potato for RS toxin treatment, and found that seedlings were appropriate materials, because some stem sections poorly grew and did not produce leaves nor buds in some cases. In contrast, the RS toxin-treated seedlings showed distinguished levels of symptoms and severity, including girdled stem and wilted or dead leaves. This could help to better evaluate the resistance. In preparing RS toxin preparation, heat-derived toxin [28] showed a better result than carbon absorption [29] for the evaluation of symptom expression and plant inhibition.

The toxin-based method can be used as a fast and alternative procedure to field test in screening potato germplasm for stem canker resistance. Compared to using R. solani culture as an inoculum [13, 16, 39], the toxin-based method has advantages of simplicity, efficiency, less influenced by environmental factors, and less time consumption. It provides a rapid method for preliminary screen of potato germplasm for resistance before field testing. This will significantly enhance the capacity and speed of resistance screen without sacrificing the quality. Although toxin-based assay can help the resistance evaluation, we do not suggest it to completely replace field tests.

By using these inoculation methods, we have validated that ‘Desiree’ expressed the lowest disease severity, while ‘Atlantic’, ‘Shepody’, and some Japanese lines showed high levels of stem canker. The results were consistent between inoculum- and toxin-based analyses. The toxin could make seedling sufficient disease and distinguish differences among cultivars, can be used as inoculation for identifying resistance of potato cultivars. Therefore, we concluded that RS toxin can be used for fast assay in potato resistance evaluation. In field evaluation, 2 to 4 g per seeding site was optimal inoculum for a consistent and reliable result.

Conclusion

Inoculation with wheat bran-mediated R. solani was an effective method for the evaluation of potato resistance in field trials. Inoculum at 2, 3 or 4 g per seed piece was optimal. The RS toxin did not lose activity after heating, and showed higher level of severity. The symptoms and severity were the same when potato stem sections and seedlings were treated with either R. solani inoculum or RS toxin. The RS toxin was key pathogenic factor. RS toxin could distingusied resistance of potato cultivars or lines, neither diluted nor concentrated toxins could reflect resistance of potato. In addition, 1/4 toxin promoted the growth of seedlings. Treatments using RS toxin had similar results compared to field trials using R. solani inoculums. Both inoculum- and toxin-based evaluation methods were effective to evaluate stem canker resistance. Both assays showed that none of the 20 potato materials was completely resistant to R. solani. However, cultivar ‘Desiree’ had the lowest level of disease, whereas ‘Atlantic’, ‘Favorita’, and ‘Shepody’ showed the high susceptibility.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xiaoyu Zhang designed the pro- ject, collected and prepared samples and contextual data, conduct trials and writed manuscript. Dezhou Li and Zhuo Yu made comments and suggestions. Jianjun Hao made comments and suggestions, modifed the language. Xing Xing and Yayan Feng helped to complete the tests.

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Figures

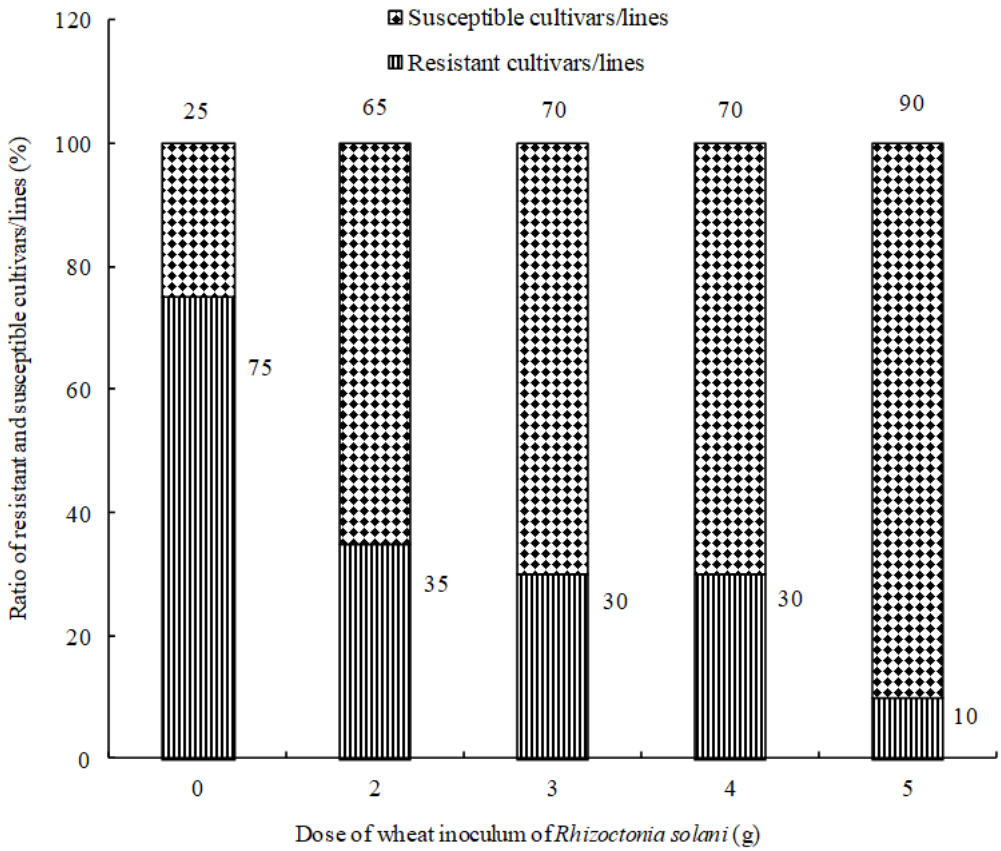

Figure 1

Ratio of resistant and susceptible materials in different inoculum densities.

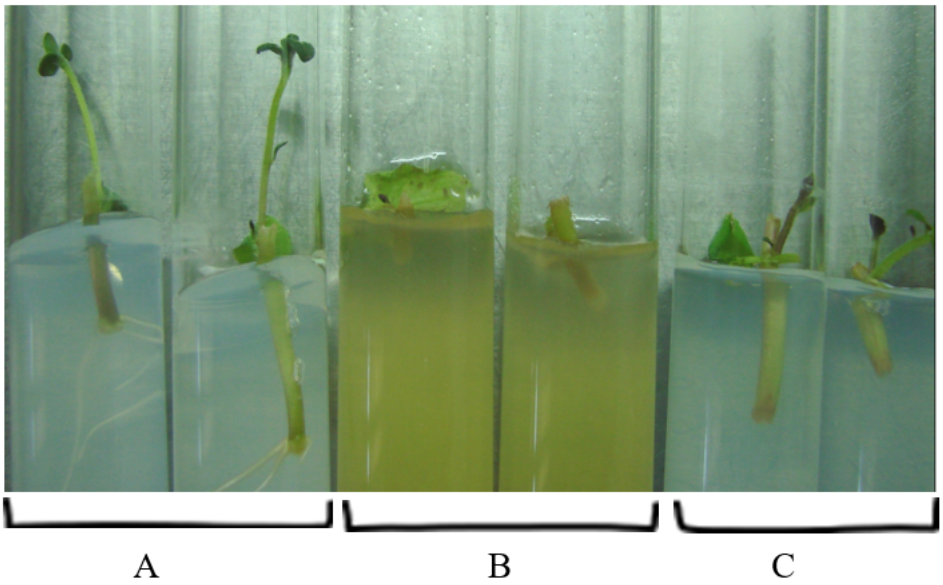

Figure 2

Symptoms on stem sections of potato treated with Rhizoctonia solani (RS) toxin. A: non-treated control; B: treatment with RS toxin derived by heating; C: treatment with RS toxin derived by carbon adsorption.

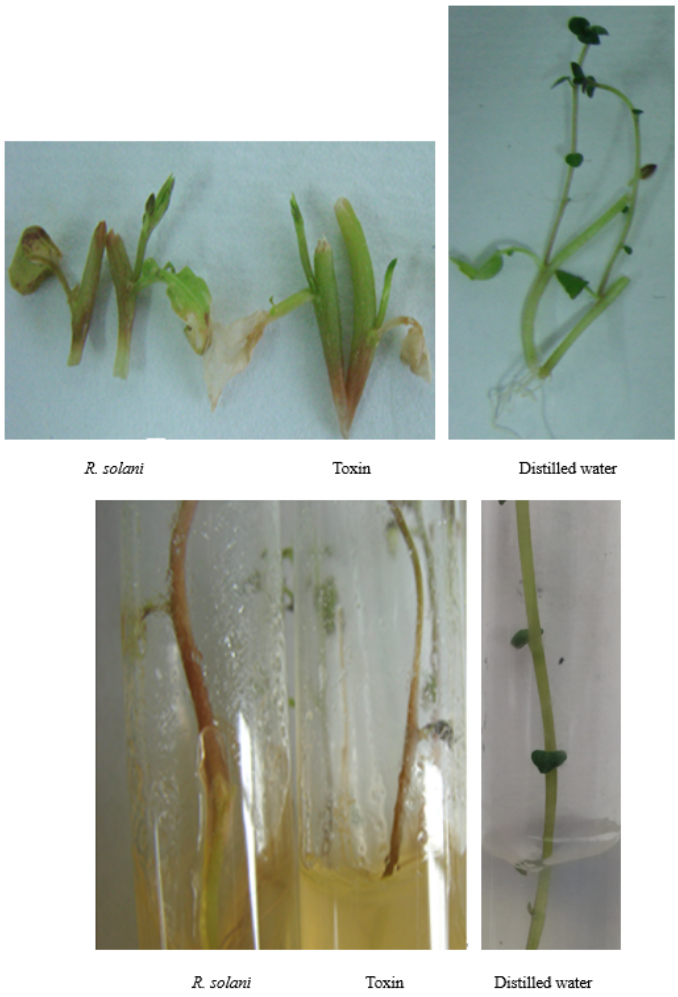

Figure 3

Symptoms caused by Rhizoctonia solani and its extracted toxin on stem sections and seedlings of tissue-cultured potato.

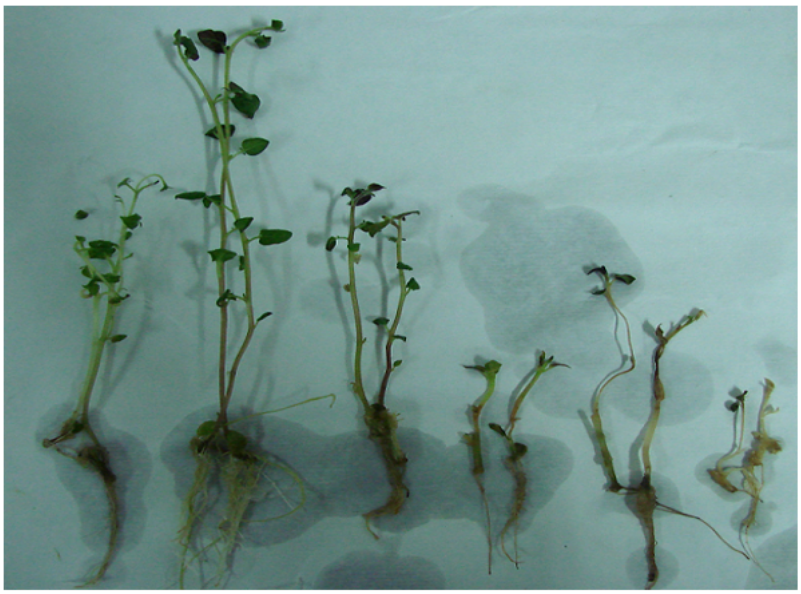

Figure 4

Growth inhibition of tissue-cultured potato seedlings by RS toxin at concentrations (From left to right) 0, 1/4, 1/2, 1, 2, and 4 times of original concentration.

Figure 5

Non-treated (A) and RS-toxin-treated (B) potato seedlings incubated for eight days of incubation after treatment.