Efficacy of different inoculum forms of *Rhizoctonia solani* AG 2-2IIIB for resistance screening of sugar beet varieties

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Abstract

Sugar beet (*Beta vulgaris* L.) is one of the major sugar sources in the world. *Rhizoctonia solani* causes damping-off and crown and root rot that can result in significant yield and economic losses. *R. solani* AG 2-2 IIIB is the most damaging anastomosis group in sugar beet production. In this study, we evaluated three different types of inoculums, namely barley grains colonized by fungal mycelium (CBG), agar plugs containing fungal mycelia (MAP), and sclerotia (SCL) for their ease of production and efficacy in inducing disease in sugar beet. First, the fungal growth rate and sclerotia production were compared on six types of media, clarified V8 [CV8], potato dextrose agar [PDA], metalaxyl benomyl vancomycin agar [MBV], yeast malt agar [YMA], corn meal agar [CMA], and oatmeal agar [OMA]. The fungus grew faster and produced more sclerotia in CV8 medium than in other media ($P<0.05$). The rate of fungal growth from CBG, MAP, and SCL was evaluated. The *in vitro* rate of growth of *R. solani* was faster when originated from MAP than from SCL ($P<0.05$) but equal to that from CBG. The different inoculum forms were then used to inoculate seeds at planting and 4-leaf stage sugar beet plants to evaluate the disease incidence and severity. *R. solani* on CBG caused greater severity. Overall, CBG was the best form of inoculum due to its ease of inoculum production, low cost, and ability to consistently cause severe disease symptoms on sugar beet plants.

Keywords Sugar beet - Soilborne pathogen – *Rhizoctonia solani* - Inoculum types - Resistance screening - Disease management

Introduction

Sugar beet (*Beta vulgaris* L.) is a leading sugar-yielding crop worldwide. Crown and root rot disease is a destructive fungal disease in all sugar beet growing regions of the world (Khan and Bolton 2021; Buhre et al. 2009; Anees et al. 2010a). This disease is caused by *Rhizoctonia solani* (Kühn), which is a ubiquitous, basidiomycetous, soil-borne necrotroph, infect many crop families, causing root rot, foliar blight, and pre- and post-emergence seedling damping-off, root and crown rot (Anderson 1982; Salazar et al. 2000). This fungus is generally inactive at temperatures below 15 ^{0}C but able to grow and infect hosts at 15 ^{0}C-35 ^{0}C (Neher and Gallian 2011). There are 13 anastomosis groups (AGs) of *R. solani* (Hanson and McGrath 2011; Arakawa and Inagaki 2014). In the United States (U.S.), *R. solani* AG 2-2IIIB, AG 2-2IV and AG 4 are the primary agent of Rhizoctonia crown and root rots, as well as seedling damping off and foliar blights (Windels and Brantner 2007; Kirk et al. 2008; Stachler 2009; Bolton et al. 2010; Strausbaugh et al. 2011). Although, the disease was mainly caused by *Rhizoctonia solani* (Kühn), recent reports showed that it can be also caused by several AG of binucleate Rhizoctonia (Yang and Li, 2012; Harveson and Bolton, 2013; Woodhall et al. 2020). Crop loss is estimated at around 30-60% or even the entire field may be lost to Rhizoctonia disease (Buttner et al. 2004; Neher and Gallian 2011).

The sexual stage of *R. solani* is *Thanatephorus cucumeris* [(Frank) Donk)] and is mostly cryptic in nature (Adam 1988). The fungus produces characteristic hyphae, which tend to branch

at right angles and bear a constriction at the base of parental hyphae but do not produce any asexual spores (Alexopoulos et al. 1996). Fungal growth passes through the initiation of mycelia, formation of sclerotia, and development and maturation of sclerotia. This fungus can survive in the soil and crop residues in mycelial or sclerotial forms to serve as overwintering infectious propagules (Naiki and Ui 1969). Sclerotia are light tan to dark brown, thick-walled, which are firm, irregularly globose to sub-globose, flat, or rounded on the top, and mature sclerotia consist of loosely interwoven hyphae without any well-defined zones (Agrios 2005). The sclerotia undergo direct myceliogenic germination (Ritchie et al. 2006). Nitrogen and carbon sources have a significant role in the formation of sclerotia, and the number and fresh weight of sclerotia are influenced by sucrose concentration (Kanwal and Reddy 2012). Besides, physical factors such as light, temperature, pH, and humidity influenced sclerotia formation and development (Rollins and Dickman 1998).

Currently, effective management of *R. solani* disease integrates multiple approaches, including cultivation of tolerant or resistant varieties, seed treatments, crop rotations with non-host crops, tillage operations, and timely application of proper fungicides (Khan et al. 2010; Carlson et al. 2012; Bartholomäus et al. 2017). However, resistant ratings in sugar beet varieties, an important component of disease management, may be affected by several factors, e.g., economical and durable production of inoculum, inoculum potentials i.e., the energy of growth of a parasite available at the infection court of various inoculum (Garrett 1970), their density and methods of inoculation, age of plants, soil properties, soil microbiome, and environmental conditions (Anees et al. 2010a; Grosch and Kofoet 2011; Behn et al. 2012; Liu et al. 2019; Brantner and Chanda 2020). In field conditions, disease rating is often inconsistent because disease incidence is patchy due to uneven distribution of overwintering propagules in organic debris. Artificial inoculation

under a controlled environment may be preferred to reduce variations in characteristics in field experiments (Pierson and Gaskill 1961; Scholten et al. 2001; Bolton et al. 2010; Kluth et al. 2010). Various inoculum like sclerotia, mycelial plugs and slurries, and pathogen-colonized grains of barley, millets, etc., were traditionally used in varietal screenings. Although sclerotia are natural overwintering propagules, it is cumbersome to recover sclerotia of *R. solani* from infested soils for large scale inoculation experiments. Hence there is a need to optimize conditions for sclerotia production *in vitro*. Also, it is quite inefficient to use mycelia and sclerotia for large-scale research in field conditions because of the inconvenience of *in vitro* preparation, handling, and storage. Moreover, no concerted efforts were made to compare those criteria and the inoculum potentials of various inoculum for maximum disease pressure in a controlled environment. Accurate evaluation of the reaction of sugar beet varieties to this pathogen depends on the selection, multiplication, and use of proper *R. solani* inocula, as well as artificial inoculation under uniform environmental conditions (Campbell and Bugbee 1993; Kluth et al. 2010).

Three types of experiments were conducted. The characterization of the features of the isolate grown on six growth media was carried out at the Sugar beet pathology laboratory at the NDSU campus in Fargo; the impact of inoculation with different types of *R. solani* inoculum on the emergence and damping off of seedlings of a single, susceptible cultivar, and the study of the impact of these inoculum on seven varieties with various levels of resistance to the disease were conducted at the Dalrymple Research Greenhouse at the NDSU campus in Fargo, ND.

Materials and Methods

Experiment location and condition and sugar beet varieties

For the first greenhouse study, an *R. solani* susceptible cultivar from SESVanderHave (Fargo, ND) was used. For the second study, seven commercial sugar beet varieties were chosen from four seed companies, Maribo (Holey, Denmark), Hilleshog (Longmont, CO), Crystal Beet Seed (Moorhead, MN), and Beta Seed (Moorhead, MN). These varieties have different levels of resistance to the disease. To protect their identities, the varieties were labeled and referred to in this manuscript, using the letters A-G. The greenhouse was set to allow 14-h photoperiod (600-W high-pressure sodium lamps; P.L. Light Systems, Inc., Beamsville, Ontario, Canada), maintained relative humidity around 70% and a temperature of 23 ± 2°C (Argus Control Systems Ltd.; British Columbia, Canada). These conditions were conducive to *R. solani* disease development (Parmeter 1970) during the experiment. The varieties are labeled as A (Maribo®; Holeby, Denmark), B (Hilleshog (Longmont, CO), C, D (Crystal Beet Seed, Moorhead, MN), and E, F, G (Beta Seed, Moorhead, MN) (Table 1). Plants were grown in plastic pots measuring 10 x 7 x 12 cm (T.O. Plastics Inc., Clearwater, MN, U.S.A.), filled with peat mix (Sunshine mix 1, Sun Gro Horticulture Ltd.; Alberta, Canada) added with slow-release fertilizer (N-P-K) (Osmocote 15-9-12; 20g/pot, Scotts-Sierra Horticultural Products Company, Marysville, OH). Plants were regularly watered and monitored until they reached a defined 4-leaf growth stage for root inoculation.

Preparation of *R solani* AG 2-2IIIB inoculum

We selected an AG2-2IIB isolate for the experiment on inoculum types because AG2-2IIB isolates are more virulent than AG4 isolates on sugar beets (Strausbaugh et al. 2011). *R. solani* AG 2-2IIIB isolate, RSKZ-1 (accession: OR185459) retrieved from a commercial field in Foxhome, MN, was

used for all trials. This isolate was selected for its high level of aggressiveness to sugar beet varieties. The isolate was grown in potato dextrose agar (PDA) and clarified V8 agar (CV8) media to obtain mycelial plug and sclerotia for inoculation. For preparing colonized-barely grain inoculum, sterilized barley grains were colonized by mycelial plugs of *R. solani* pure culture, which was grown in the PDA medium as described by Noor and Khan (2014).

***In vitro* evaluation of the fungal growth and development in various growing media**

Basic features of the isolate, such as radial growth, growth pattern, number of sclerotia produced, and biomass weight, were studied on six different growth media. The media were clarified V8 agar [CV8], potato dextrose agar [PDA], metalaxyl benomyl vancomycin [MBV], yeast malt agar [YMA], corn meal agar [CMA], and oatmeal agar [OMA] prepared on 9 cm Petri dishes (VWR®, Pennsylvania, USA) following the Manual of Microbiological Culture Media **(**Difco and BBL Manual**)**. Sclerotia were initially grouped into four categories: very large (>3 mm), large (≥1.9 but <3 mm), medium (≥1.4 but <1.9 mm), small (≥0.9 but <1.4 mm), and very small (<0.9 mm), respectively. A 5-mm diameter mycelial disc from a three-day-old culture or a large-sized sclerotium was placed onto the center of each different sterilized culture media and incubated for five days. Inoculated plates were kept in dark conditions at room temperature (25±2 ^{0}C), and the radial growth was recorded at 4 days post-inoculation (dpi) following Hendricks et al. (2017) with the help of Scienceware® Digi-Max™ slide calipers (Sigma Aldrich, USA). The number of sclerotia and biomass weight were recorded at 28 dpi. Three forms of inoculums (e.g., colonized barley grains, mycelial plugs, and sclerotia) were grown in CV8 medium, and the biomass and distribution of sclerotia were evaluated at 28 dpi, respectively. Fungal mycelial mat was harvested following Liu et al. (2003), and the wet weight was recorded in milligrams. Water content was

144 removed through oven drying to a constant weight at a temperature of 55 ^{0}C for an hour and half,
145 and the dry weights of the inoculums were weighed and recorded. The distribution of the sclerotia
146 was counted with the aid of ImageJ Software Version 1.8.0. The experiment was a randomized
147 complete block design with four replications, and it was carried out twice.
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150 **Effect of inoculums on seedling emergence and disease incidence**
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152 Sugar beet seeds (susceptible cultivar SESVanderHave cv. SV762) were inoculated in the
153 greenhouse experiments so that the infection was done together with sowing, and the seedling
154 emergence and disease incidence were evaluated at 14 days post inoculation/sowing (dpi). The
155 experiment was conducted twice with a completely randomized design (CRD) with four
156 replications and thirty seeds per replicate each time. The non-inoculated seeds were used as the
157 control. For each replication, trays measuring 35×25×5 cm (T.O. Plastics Inc., Clearwater, MN,
158 U.S.A.) were filled with FLX soilless mix (PRO-MIX, Quakertown, PA) amended with Osmocote
159 (N-P-K: 15-9-12) fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). In
160 each tray, two furrows were formed, and 15 seeds were planted in each of the two furrows.
161 Individual seeds were inoculated by placing the inoculum in direct contact and covered with the
162 soilless mix. One colonized barley grain or one mycelial plug or sclerotium was used in each
163 planting hole depending on the inoculum form. Inoculated trays were incubated at humidity
164 chamber for 5 days, maintained soil moisture level at around 75% (Bolton et al. 2010), and then
165 transferred to the greenhouse, then an evaluation of seedling emergence and disease incidence was
166 carried out.

Effect of inocula on root rot development in seven varieties, A - G

Three different forms of inoculum of *R. solani* AG 2-2IIIB, viz. colonized barley grains, mycelial plugs, and sclerotia were used. Sugar beet plants at the four-leaf stage were used and inoculated to the root by placing each type of inoculum at 2.5 cm depth from the soil surface and covered by soil in plastic pots measuring 8×8×9 cm (T.O. Plastics Inc., Clearwater, MN, U.S.A.) (Bhuiyan et al. 2023). A colonized barley grain, a 10-mm circular-shaped mycelial plug taken with an autoclaved cork-borer from 10 days old pure culture, and large-sized sclerotia obtained from 28-days old *R. solani* culture plates were used for root inoculations. The experiment was conducted twice with a completely randomized design (CRD) with four replications. For each inoculum type, four plants of each variety were inoculated, and four were left without any inoculation. The latter plants were considered as controls. Plants were lightly watered immediately after inoculations and watered regularly to ensure optimum moisture in the potting soil in the greenhouse during the experiment. Inoculated pots were incubated at humidity chamber for 5 days, maintained soil moisture level at around 75% (Bolton et al. 2010), and then transferred to the greenhouse. Disease severity was evaluated and scored at 28 dpi. Roots were carefully pulled by hand and washed under tap water. Disease severity was evaluated using a 0 to 7 root rot severity evaluation scale (Torres et al. 2016), where 0 = no visible disease symptoms, 1 = 1-5% root surface with visible lesions, 2 = 6-10 % root surface with visible lesions, 3 = 11- 25% root infection, 4 = 26 - 50% root infection,

188 5 = 51 - 75% root infection, 6 = > 75% root infection, and 7 = entire root completely deteriorated
189 or dead plant.
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191 **Statistical Analyses**
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193 For the *in-vitro* study and the greenhouse study on seedling emergence, Levene's test for
194 homogeneity of variance was conducted to determine whether a combined analysis could be
195 performed. Analysis of variance was conducted on these data sets using the generalized linear
196 mixed model (GLIMMIX) procedure SAS 9.4 software. Treatment means separation was
197 conducted using the Tukey-Kramer post-hoc test at a probability *P=0.05*. For the study on
198 inoculation of resistant varieties, the non-parametric Levene's test (Nordstokke et al. 2011) was
199 done to determine whether the variances of both trials were homogeneous and could be combined
200 for analysis. Data collected from plants inoculated with non-colonized barley grains were not
201 included in the analyses since they were all healthy and free from infection. In addition, the relative
202 treatment effects of root rot disease severity for each treatment and their 95% confidence intervals
203 were estimated and compared using Brunner's LD_CI SAS macro as described by Shah and
204 Madden (2004). Briefly, if R_{ik} is the rank (R) among all observations (N), the mean rank for the i^{th}
205 treatment combination was determined as:
206 $$R_{i.} = \frac{1}{n_i}\sum_{k}^{n_i} R_{ik}$$
207 The dot subscript indicates the mean rank over all four replications (n_i) for the i^{th} treatment
208 combination with the rank position (k). The relative treatment effect of the i^{th} treatment
209 combination was estimated from the mean rank as:
210 $$RE_i = \frac{1}{N}\left\{R_{i.} - \frac{1}{2}\right\}$$

For the *in vitro* study, a linear regression model was used to evaluate the radial growth of fungus from different inoculums at 95% confidence interval. For sclerotial counts from the different inoculums and different growth media, glimmix with adaptive quadrature method and Poisson distribution was used. Treatment's least square mean (lsmeans) ranks for radial growth, biomass accumulation, sclerotia development from different media, and inoculums were separated using the Tukey-Kramer test ($P = 0.05$).

Results

In vitro evaluation of the morphology of *R. solani* AG 2-2IIIB

Levene'ts test for homogeneity of variances was not significant for radial growth ($P=0.7915$), wet weight ($P=0.9918$), dry weight ($P=0.3006$), or the number of sclerotia ($P=0.8960$). Among the six different media used to evaluate the radial growth from mycelial plug, CV8 medium produced the largest colony areas followed by PDA medium ($P<0.001$), whereas OMA medium produced the lowest colony growth (Supplementary Fig. S1 and S2). The colony area (21566 mm^2) produced in CV8 media was 3.2 times more than the colony areas produced in the OMA medium. Biomass accumulation (wet weight: 823 mg) was highest in the CV8 medium, followed by PDA medium (641 mg) ($P<0.001$), and the lowest amount of biomass weight (wet) was recorded in the YMA medium (340 mg). CV8 medium generated 2.4- and 1.2 times higher biomass weight (wet) than the YMA and PDA medium, respectively. There were no significant variations in the dry weight of biomass produced in all media ($P>0.05$) (Fig. 1). In the case of sclerotia development, CV8 growth medium produced the highest number of sclerotia (409) followed by PDA, MBV, and

OMA medium respectively (*P<0.001*) (Fig. 1). YMA medium produced the lowest number of sclerotia (117). CV8 medium produced 3.4 and 2-fold more sclerotia than the YMA and PDA media (Fig. 1). Radial growth of mycelia developed from three different types of inoculum showed significant variation (*P<0.001*). The radial growth of mycelia represented a linear trend in the culture media (Fig. 2). *R. solani* AG 2-2IIIB grew faster (*P<0.05*) from mycelia than from sclerotia but at a similar rate from colonized barley. Radial growth of mycelia grown from barley (using area of colonies) was significantly larger (*P<0.05*) than for mycelia or sclerotia, and areas of colonies for mycelia were significantly larger than that of sclerotia (Fig. 2). The colony area from colonized barley inoculum (62801 mm^2) was 3-times and 1.5 times more than the colony areas generated from sclerotia (20611 mm^2) and mycelial plugs (40843 mm^2), and mycelial plugs produced about 2 times more colony areas than the sclerotia, respectively. The rate of growth of radial mycelia from colonized barley was 1.2- and 2-times faster than the growth from mycelial plugs and sclerotia inoculated plates, respectively, whereas the growth from mycelial plug growth was 1.7 times faster than the growth from sclerotia. Biomass growth (wet and dry weight) developed from three forms of inoculum showed significant variations (*P<0.001*). Colonized barley inoculum produced the highest amount of biomass weight (wet weight = 815 mg) which was 1.5 times more than the biomass produced by sclerotia (537 mg), whereas the dry weight of biomass grown from colonized barley (217 mg) was 2.5 folds higher than the sclerotia inoculum. The development of sclerotia from different inoculums was significantly different in the growth media (*P<0.001*). Colonized barley inoculated plates in CV8 medium produced significantly higher number of sclerotia (521) compared to other inoculums *in vitro* (*P<0.001*) (Fig. 2).

Fig. 1 *R. solani* AG 2-2IIIB biomass accumulation (wet and dry weight) and the number of sclerotia developed in different growth media. Bars within each measurement category that share the same letter are not significantly different at $P = 0.05$. Here, CV8=clarified V8 juice, PDA = potato dextrose agar, MBV = metalaxyl benomyl vancomycin, OMA = oatmeal agar, CMA = corn meal agar, YMA = yeast malt agar, respectively.

Fig. 2 The fitted simple linear regression lines of mycelial growth of *R. solani* with three different inoculums, colonized barley grains, mycelial plugs and sclerotia. In the fitted line equation y = bx+a, the parameter b is the radial growth rate per day, and a is the intercept. The R^2 is the coefficient of determination.

Effect of different inoculums on seedling emergence

Inoculated seeds developed various symptoms, including post-emergence damping-off, root lesions, death of seedlings, and brown to black discolored hypocotyl. Seedlings that emerged from non-inoculated seeds were symptomless at 14 dpi. Seedling emergence was significantly different from the non-inoculated control ($P<0.001$) (Fig. 3). Seedling emergence was 73% for colonized barley grains and 77% and 79% for mycelial plug and sclerotia-inoculated seeds, respectively. Consequently, seedling emergence was reduced by 27, 23, and 21% for colonized barley grains, mycelial plug, and sclerotia due to pre-emergence damping-off (Fig. 3). No statistical differences were observed in seedling emergence among the three inoculum types, but their effects on disease

incidence were significantly different ($P<0.05$). The average incidence of symptomatic seedlings was 59, 33, and 20% for colonized barley grains, mycelial plugs, and sclerotia, respectively.

Fig. 3 The emergence of sugar beet seedlings and disease incidence inoculated with three different types of *R. solani* AG 2-2IIIB inoculums were evaluated at 14 dpi. Non-inoculated seeds were healthy and free from infection. Bars within each data measurement variable that do not share the same letter are not significantly different at $P = 0.05$.

Root rot severity in seven sugar beet varieties

In the greenhouse experiments, root inoculation of seven sugar beet varieties inoculated with three forms of inoculum produced typical root rot symptoms (Supplementary Fig. S3), indicating that all types of inoculums were effective. The effect of different inoculums was statistically significant ($P<0.05$). Varieties responded significantly differently ($P<0.001$) when different forms of inoculums were used. The interaction between the varieties and inoculum types was significant ($P<0.001$). As expected, the mock-inoculated plants were symptomless. The colonized barley grains inoculum consistently produced higher median root rot severity ($P<0.05$) than the other two inoculums (Table 1; Fig. 4). Sugar beet varieties were inoculated with sclerotia, developed lower disease severity ($P<0.05$) compared to colonized barley grains and sclerotia (Fig. 4). However,

301 the varieties A (Maribo seed), C (Crystal), and F (Beta seed) had higher disease severity than others
302 in colonized barley grains inoculated plants whereas no significant differences were recorded
303 among varieties when roots were inoculated with mycelial plugs. Mycelial plug inoculation to all
304 sugar beet varieties showed comparatively lowered disease severity compared to colonized barley
305 grains inoculum except for D and E varieties, but there were no significant differences in root rot
306 severity among the varieties.

307

308

309 **Fig. 4** The effect of colonized barely barley seeds, mycelial plug, and sclerotia on the disease
310 severity of seven sugar beet varieties was measured by the relative effect (solid circles). The
311 vertical bars represent a 95% confidence interval of the estimated relative effect. The relative
312 treatment effects for each treatment and their confidence intervals were estimated and compared
313 using Brunner's LD_CI SAS macro. Here, A = Maribo®; B = Hilleshog®; C, D = Crystal® and E,
314 F, G = Beta® seed proprietary materials, respectively. Values relative effects closer to 1 are more
315 susceptible, and those closer to 0 are more resistant.

316

317 In the case of sclerotia inoculation, no significance ($P>0.05$) was observed in disease
318 severity among the varieties except for the E variety, which had higher root rot severity (Fig. 4).
319 The disease severity of sclerotia-inoculated plants of all varieties was significantly lowered than
320 the colonized barley grains inoculated plants ($P<0.001$). Among the response of the disease
321 severity scores within each inoculum type, the median severity was measured higher (5 to 6) in
322 colonized barley grain inoculated plants, which corresponds to more than 75% of the root rot

infection, whereas mycelial plug inoculated plants represented median severity of 2 to 3.5, which is equivalent to no more than 25% root rot area except for variety G which showed the highest median severity 4.5. In the case of sclerotia, the median severity ranges from 1 to 2, with the root infection no more than 10 % root area (Table 1).

Comparing the inoculum efficacy study evaluated in the greenhouse condition and the existing resistance ratings of the varieties by the American Crystal Sugar Company (ACSC), it showed variations in the disease severity score of the varieties. The colonized barley grain inoculated plants showed higher disease ratings compared to the current ratings determined by the ACSC whereas mycelial plug inoculation showed that all the varieties were resistant (median severity 2 to 3.5) except variety G (median severity: 4.5), which showed moderate resistance to root rot disease. All varieties were found to be resistant through sclerotia inoculation, where the median severity ranges between 1 to 2 (Table 1). However, the disease severity scores arrived at by the colonized barley grains inoculum were better correlated with the designated disease ratings by ACSC compared with those scores derived either from the mycelial plug or sclerotia inoculum. Kendall rank correlation coefficient was 0.5164 ($p<0.05$) between the colonized barley grains derived and ACSC-designated disease severity scores, whereas Kendall rank correlation coefficient was not significant between mycelial plug (-0.3162 ($p>0.81$)) or sclerotia-derived (0.3586 ($p>0.11$)) and the ACSC-designated disease severity scores was not significant

Discussion

Evaluation of damping-off and root rot caused by *R. solani* in commercial sugar beet varieties is an important consideration for this important disease management. In this regard, we analyzed the vegetative growth and sclerotia generation of *R. solani* AG2-2IIIB isolate on six different culture media and found that the CV8 medium is most suitable for both vegetative growth and producing sclerotia. Our finding that the clarified V8 was most suitable for fungal growth and sclerotia production agreed with that in the report by Haque and Parvin (2022). Moreover, we evaluated three different types of *R. solani* inoculums (namely, mycelial plugs, colonized barley grains, and laboratory-grown sclerotia) for (1) ease of large-scale production, (2) maximum disease severity, and (3) uniform disease-causing ability on sugar beet seeds and seedlings under controlled conditions in the greenhouse. *R. solani*-colonized barley grains as inoculum was found to be the most suitable inoculum satisfying the above three advantages.

Researchers have attempted to define the ability of soilborne inoculum to quickly proliferate upon germination, colonize hosts, and cause maximum disease by introducing concepts like inoculum potential, inoculum density, biological potential, proliferation potentials, etc. (Walter et al. 2004; Schmidt et al. 2005). Various definitions of inoculum potentials were also presented by some of those workers. Although the reliable quantification of such attributes remains elusive, the knowledge of inoculum potentials of plant pathogenic soil microorganisms is necessary to prevent soilborne diseases in efficient and economical ways (Geypens, 1974). Leštan et al. (1996) defined biological potentials as a measure of fungal biomass, or fungal biomass-associated metabolic activity per weight or volume of inoculum. Leŝtan et al. (1996) also defined the proliferation potential as the ability of the inoculum to proliferate from several different carriers and the effect of medium concentration and incubation temperature on its proliferation. Garrett (1970) defined the inoculum potential as the energy of growth of a parasite available for infection

of a host at the surface of the host organ to be infected; we consider this definition of Garrett (1970) most suitable to compare our *in vitro* and *in vivo* results (e.g., rate of mycelial growth, total biomass in terms of wet weight and dry weight, the number of sclerotia produced per unit surface, and disease generation) for explaining the differences of the three inoculum types to cause maximum and uniform disease in our experiments. According to Garret (1970), the inoculum potential can be defined by the growth energy in three aspects: (1) directly proportional to the number of infectious units or propagules of the pathogenic agent in contact with the unit of surface area of the host; (2) function of fungal hyphae vigor, which is dependent on the amount of nutrients that the fungus is able to extract from a substrate and translocate to the apical zone; (3) the joint effect of soil environmental factors, which may vary from optimal values to completely inhibitory pathogenic activity, highlighting the interaction of two elements of a virulent pathogen, and a favourable environment in the host-pathogen-environment disease triangle.

Our *in vitro* studies demonstrated that the rate of mycelial growth, total biomass in terms of wet weight and dry weight, and the number of sclerotia produced per unit surface is higher when *R. solani*-colonized barley grains were used as inoculum; the trend was followed by vegetative mycelia in agar plugs, and *in vitro* generated sclerotia as inoculum, in that order. Similarly, the colonized barley grains caused the highest seedling mortality (e.g., lowest seedling stands); this was followed by the mycelial plug inoculum and sclerotia, in that order. Moreover, the severity of the disease in terms of disease scores matched the trend with the respective inoculums and confirmed that the colonized barley grains have the highest inoculum potential (Garrett, 1970), followed by mycelial plugs and sclerotia. However, this finding was in marked contrast to the finding in the recent report by Haque and Parvin (2022), in which they found that the inoculum

form sclerotia caused the most severe root rot severity followed by mycelial plugs and colonized barley grains.

Actively growing mycelia produced more disease than sclerotia. It has been previously reported that the colonized barley grains and mycelia are effective inoculums for disease rating in greenhouse conditions (Liu et al. 2019; Noor and Khan 2014; Behn et al. 2012, Rajabi et al. 2012; Wigg and Goldman 2020). The lowest score generated by sclerotia for inoculum potential is worth noting. Sclerotia development undergoes several steps, e.g., initiation of mycelia, formation of sclerotia, and maturation and development of sclerotia. In a dormant state, sclerotia are not actively causing disease in the host. Growth and development of sclerotia in the artificial culture media responded differently (Naiki and Ui. 1969). In nature, sclerotia germinate under humid conditions and are often attracted by the root exudates of the germinated seedlings (Flentje et al. 1963) and the mycelia in contact with the host tissue, penetrate the root cortex and thus result in infections to the tissue (Sneh et al. 1996). Sherwood (1969) also reported a similar observation on sclerotia morphology on growth media. We reported that sclerotia were capable of causing seedling damping-off in greenhouse conditions at 10 days post-inoculation, but their effect was significantly lower than inoculation with colonized barley seeds and mycelial plug. This observation is consistent with the fact that sclerotia bear a lag phase of germination and an initial slow growth of emerging mycelia (Liang et al. 2010; Pandey et al. 2007; Aliferis and Jabaji 2010).

We observed that sugar beet varieties developed characteristic root rot and seedling damping-off symptoms upon inoculation in greenhouse conditions. Buttner et al. (2004) reported that inoculated sugar beet produced severe and uniform disease infection if effective inoculum was used in the controlled conditions, whereas field trials may produce variable pressure due to the

patchy patterns of this disease and unpredictable environmental conditions (Van Bruggen et al. 1996) which can hamper the disease severity rating of breeding materials (Dhingra and Sinclair 1995).

Inconsistencies were noted between the Rhizoctonia disease resistance ratings of varieties reported by the commercial entities from experiments conducted in the natural environment with our present observations from experiments conducted under greenhouse conditions. We observed that sugar beet varieties represented characteristics of root rot and seedling damping-off symptoms in greenhouse conditions. Moreover, we compared the disease development utilizing natural (sclerotia) as well as artificial (mycelial plugs and colonized barley grains) inoculums. Thus, we consider that the evaluation of inoculum types and accurate disease ratings in the greenhouse in our experiments reflected the innate susceptibility of the respective sugar beet varieties.

Conclusions and recommendations

In summary, *R. solani* AG 2-2 IIIB is a major concern for the economic crop production of sugar beet worldwide, and screening and utilization of resistant varieties are important strategies for Rhizoctonia disease management. Experiments with various culture media favored CV8 medium for maximum growth of vegetative mycelia and sclerotia production. Disease severity trials under greenhouse conditions demonstrated uniformity of disease development in replicated experiments, and trials with three types of inoculums (i.e., Rhizoctonia mycelial plugs, colonized barley grains, and sclerotia) also demonstrated that the inoculum in the form of colonized barley grains was the most effective (i.e., having inoculum potentials) for accurately screening sugar beet varieties for

assessing disease resistance. This was because colonized barley grains had the highest ability to cause damping off incidence and casued the expected root rot severity that better matched the known rating evaluated in the field by seed companies across the seven cultivars.

Therefore, we recommend that the *R. solani*-colonized barley grains could be used for generating maximum disease pressure for germplasm resistance screening. Moreover, we documented that resting spore secrotia were slow-growing in media, and less effective in causing disease symptoms when inoculated on seeds and 4-leaf stage plants compared with the other two forms of inoculum. These findings contrasted those reported by Haque and Parvin (2022) that the sclerotia had the most effecacy follwed by mycelial plugs and colonized barley grains. Our findings will aid in the development of implementing effective disease management practices to control Rhizoctonia disease in sugar beet.

Supplementary information The online version contains supplementary material available at https://doi.org/...

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

M. Z. R. Bhuiyan: Investigation, Data curation, Formal analysis. Writing – original draft, **Luis E. Del Rio Mendoza:** Writing and data analyses– review & editing, **Dilip K. Lakshman:** Writing – review & editing. **Aiming Qi:** Writing and data analyses – review & editing, **M. F. R. Khan:** Conceptualization, Supervision, and Resources.

References

Adam GC (1988) *Thanatephorus cucumeris* (*Rhizoctonia solani*) a species of wide host range. In Advances in Plant Pathology, Vol. 6. Genetics of Plant Pathogenic Fungi. (G.S. Sidhu, ed.), pp. 535-552. Academic Press, New York. https://doi.org/10.1016/B978-0-12-033706-4.50040-2

Agrios GN (2005) Plant Pathology. 5th Ed., Elsevier Academic Press, Amsterdam, 26: 398-401.

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory Mycology. 4th Ed., 869 pp., John Wiley and Sons, New York

Aliferis KA, Jabaji S (2010) Metabolite composition and bioactivity of Rhizoctonia solani sclerotial exudates. Journal of Agricultural and Food Chemistry 58:7604-7615. https://doi.org/10.1016/j.apsoil.2011.03.006

478 Anderson NA (1982) The genetics and pathology of *Rhizoctonia solani*. Annu Rev Phytopathol
479 20:329-347. https://doi.org/10.1146/annurev.py.20.090182.001553

480 Anees M, Edel-Hermann V, Steinberg C (2010a) Build up of patches caused by *Rhizoctonia solani*.
481 Soil Biol Biochem 42:1661-1672. https://doi.org/10.1146/annurev.py.20.090182.001553

482 Arakawa M, Inagaki K (2014) Molecular markers for genotyping anastomosis groups and
483 understanding the population biology of Rhizoctonia species. J Gen Plant Pathol 80:401-
484 407. https://doi.org/10.1007/s10327-014-0536-0

485 Bartholomäus A, Mittler S, Märländer B, Varrelmann M (2017) Control of *Rhizoctonia solani* in
486 sugar beet and effect of fungicide application & plant cultivar on inoculum potential in the
487 soil. Plant Dis 101:941-947. https://doi.org/10.1094/PDIS-09-16-1221-RE

488 Behn A, Ladewig E, Manthey R, Varrelmann M (2012) Resistance testing of sugar beet varieties
489 against *Rhizoctonia solani*. Sugar Industry-Zuckerindustrie. 137:49-57

490 Bhuiyan MZR, Lakshman DK, del Rio Mendoza LE, Khan MFR. (2023) Comparison of crown
491 and root Inoculation methods for evaluating resistance response of sugar beet cultivars to
492 *Rhizoctonia solani* AG 2-2IIIB. Crop Prot. 163:106120.
493 https://doi.org/10.1016/j.cropro.2022.106120

494 Buttner G, Pfahler B, Marlander B (2004) Greenhouse and field techniques for testing sugar beet
495 for resistance to Rhizoctonia root and crown rot. Plant Breed 123:158-166.
496 https://doi.org/10.1046/j.1439-0523.2003.00967.x

497 Bolton MD, Panella L, Campbell L, Khan MFR (2010) Temperature, moisture, and fungicide

498 effects in managing Rhizoctonia root and crown rot of sugar beet. Phytopathology 100:689-
499 697. https://doi.org/10.1094/PHYTO-100-7-0689

500 Brantner JR, Chanda AK (2021) Effects of Inoculum Density and Cultivar Susceptibility on
501 Rhizoctonia Damping-Off and Crown and Root Rot in Sugar Beet. Plant
502 Disease 105:1019-1025

503 Buddemeyer J, Märländer B (2004) Integrierte kontrolle der späten rübenfäule (*Rhizoctonia solani*
504 Kühn) in Zuckerrüben—Einfluss von anbaumaßnahmen und fruchtfolgegestaltung sowie
505 sortenwahl unter beson-derer berücksichtigung des maises. Zuckerin-dustrie 129:676-686

506 Büttner G, Pfahler B, Marlander B (2004) Greenhouse and field techniques for testing sugar beet
507 for resistance to Rhizoctonia root and crown rot. Plant Breed 123:158-166.
508 https://doi.org/10.1046/j.1439-0523.2003.00967.x

509 Campbell CL, Altman J (1976) Rapid laboratory screening of sugar beet cultivars for resistance to
510 *Rhizoctonia solani*. Phytopathology 66:1373-1374

511 Campbell LG, Bugbee WM (1993) Pre-breeding for root-rot resistance. Journal of Sugar Beet
512 Research 30:241-251

513 Carlson AL, Khan MFR, Stachler JM, Boetel MA (2012) Survey of fungicide use in sugarbeet in
514 Minnesota and eastern North Dakota in 2012. Sugar Beet Res Ext Rep 42:181-182

515 Cubeta MA, Vilgalys R (1997) Population biology of the *Rhizoctonia solani* complex.
516 Phytopathology 87:480-484. https://doi.org/10.1094/PHYTO.1997.87.4.480

517 Dhingra OB, Sinclair J.B (1995) Basic Plant Pathology Methods. 2nd Edition, CRC Press, Boca

Raton

Flentje NT, Kerr A, Dodman RL (1963) Mechanism of Host Penetration by *Thanatephorus Cucumeris*. Aus J Biol Sci 16:784. https://doi.org/10.1071/BI9630784

Garrett, SD (1970). Pathogenic root-infecting fungi. Cambridge: Cambridge University Press, Cambridge, England. pp.xi + 294 pp.

Geypens M (1974) Inoculum potential of soil-borne plant pathogenic fungi: Problems encountered in analysis and significance in epidemiology. Agro-Ecosystems 1:177-192. https://doi.org/10.1016/0304-3746(74)90025-0

Grosch R, Kofoet A (2011) Influence of temperature, pH and inoculum density on bottom rot on lettuce caused by *Rhizoctonia solani*. Z Pfl Krankheiten Pfl Schutz 110:366-378

Hanson LE, McGrath JM (2011) Rhizoctonia seedling damping-off in sugar beet in Michigan. In: Proceedings of the American Society of Sugar Beet Technologists. 36th Biennial Meeting, March 2-5, Albuquerque, New Mexico, USA.

Haque, ME, Parvin, MS (2022) Evaluation of three forms of *Rhizoctonia solani* mediated pathogenicity to sugar beet cultivars in greenhouse studies. bioRxiv

Harveson RM, Bolton MD (2013) First Evidence of a Binucleate Rhizoctonia as the Causal Agent of Dry Rot Canker of Sugar Beet in Nebraska. Plant Disease 97(11):1508. doi: 10.1094/PDIS-04-13-0375-PDN

536 Hendricks KE, Christman MC, Roberts PD (2017) A Statistical Evaluation of Methods of In-Vitro
537 Growth Assessment for *Phyllosticta citricarpa*: Average Colony Diameter vs. Area. PLoS
538 One. 26:12(1):e0170755. doi: 10.1371/journal.pone.0170755

539 Kanwal HK, Reddy MS (2012) The effect of carbon and nitrogen sources on the formation of
540 sclerotia in *Morchella* spp. Ann Microbiol 62:165-168

541 Khan MFR, Khan J, Bradley CA (2010) Effect of azoxystrobin applications based on soil
542 temperature on Rhizoctonia root and crown rot of sugar beet. Int Sugar J 112:557-560

543 Khan MFR, Bolton MD (2021). Management of Rhizoctonia Root and Crown Rot of Sugarbeet.
544 Plant Disease Management. North Dakota State University Extension. PP1495.

545 Kirk WW, Wharton PS, Schafer RL, Tumbalam P, Poindexter S, Guza C, Fogg R, Schlatter T,
546 Stewart J, Hubbell L, Ruppal D (2008) Optimizing fungicide timing for the control of
547 Rhizoctonia crown and root rot of sugar beet using soil temperature and plant growth stage.
548 Plant Dis 92:1091-1098. https://doi.org/10.1094/PDIS-92-7-1091

549 Kluth C, Buhre C, Varrelmann M (2010) Susceptibility of intercrops to infection with *Rhizoctonia*
550 *solani* AG 2-2 IIIB and influence on subsequently cultivated sugar beet. Plant Pathol
551 59:683-692. https://doi.org/10.1111/j.1365-3059.2010.02274.x

552 Leŝtan D, Leŝtan M, Chapelle JA, Lamar RT (1996) Biological potential of fungal inocula for
553 bioaugmentation of contaminated soils. J Ind Microbiol 16:286-294.
554 https://doi.org/10.1007/BF01570036

555 Liang Y, Strelkov SE, Kav NNV (2010) The proteome of liquid Sclerotial exudates from

556 *Sclerotinia sclerotiorum*. J Proteome Res 9:3290-3298. https://doi.org/10.1021/pr900942w

557 Liu C, Lakshman, DK, Tavantzis SM (2003) Quinic acid induces hypovirulence and expression of
558 a hypovirulence-associated double-stranded RNA in *Rhizoctonia solani*. Current Genetics
559 43:103-111. https://doi: 10.1007/s00294-003-0375-6.

560 Liu Y, Qi A, Khan MFR (2019) Age-Dependent Resistance to *Rhizoctonia solani* in Sugar beet.
561 Plant Dis 103:2322-2329. https://doi.org/10.1094/PDIS-11-18-2001-RE

562 Naiki T, Ui T (1969) On the survival of the sclerotia of *Rhizoctonia solani* Kuhn in soil. Men Fac
563 Agric Hokkaido Univ 6:430-436

564 Neher OT, Gallian JJ (2011) *Rhizoctonia* on sugar beet. Importance, identification, and control in
565 the Northwest. A Pacific Northwest Extension Publication, University of Idaho, USA.

566 Noor A, Khan MFR (2014) Efficacy and safety of mixing azoxystrobin and starter fertilizers for
567 controlling *Rhizoctonia solani* in sugar beet. Phytoparasitica 43:51-55.
568 https://doi.org/10.1007/s12600-014-0416-3

569 Nordstokke W., Zumbo BD, Cairns SL, Saklofske DH (2011) The operating characteristics of the
570 nonparametric Levene test for equal variances with assessment and evaluation data.
571 Practical Assessment, Research, and Evaluation. 16:9. https://doi.org/10.7275/5t99-zv93

572 Pandey MK, Sarma BK, Singh DP, Singh UP (2007) Biochemical Investigations of Sclerotial
573 Exudates of *Sclerotium rolfsii* and their Antifungal Activity. J Phytopathol 155:84-
574 89. https://doi.org/10.1111/j.1439-0434.2007.01194.x

575 Parmeter JR (1970) *Rhizoctoniasolani*: Biology and Pathology. University of California Press.

576 Berkeley

577 Pierson VG, Gaskill JO (1961) Artificial exposure of sugar beets to *Rhizoctonia solani*. J. Am.
578 Soc. Sugar beet Technol 11:574-590

579 Rajabi A, Shahbazi H, Pedram A., Darabi S, Javaheri MA, Soltani J, Aminiaie M, Khorsshid A,
580 Babaie B, Shahmoradi Sh, Katal S (2012) Evaluation of resistance of sugar beet varieties
581 to Rhizoctonia root rot disease. Sugar beet seed research institute (SBSI). Pp.57

582 Ritchie F, McQuilken MP, Bain RA (2006) Effects of water potential on mycelial growth,
583 sclerotial production, and germination of *Rhizoctonia solani* from potato. Mycol Res
584 110:725-33. pmid:16765034. https://doi.org/10.1016/j.mycres.2006.04.008

585 Rollins JA, Dickman MB (1998) Increase in endogenous and exogenous cyclic AMP levels
586 inhibits sclerotial development in *Sclerotinia sclerotiorum*. Applied and Environmental
587 Microbiology 64:2539-2544. https://doi.org/10.1128/AEM.64.7.2539-2544

588 Salazar O, Julian MC, Hyakumachi M, Rubio V (2000) Phylogenetic grouping of cultural types of
589 *Rhizoctonia solani* AG2-2 based on ribosomal ITS sequences. Mycologia 92:505-509.
590 https://doi.org/10.2307/3761509

591 Schmidt KR, Chand S, Gostomski PA, Boyd-Wilson KSH, Ford C, Walter M (2005)
592 Fungal inoculum properties and its effect on growth and enzyme activity of *Trametes*
593 *versicolor* in soil. Biotechnol Prog 21:377. https://doi.org/10.1021/bp049675m

594 Scholten O, Lee E, Panella W, Theo S, De Bock M, Lange W (2001) A Greenhouse Test for
595 Screening Sugar Beet (*Beta Vulgaris* L.) for Resistance to *Rhizoctonia Solani*. Eur J Plant

596 Pathol 107:161-166. https://doi.org/10.1023/A:1011208903344

597 Shah DA, Madden LV (2004) Nonparametric Analysis of Ordinal Data in Designed Factorial
598 Experiments. Phytopathol 94:33-43. https://doi.org/10.1094/PHYTO.2004.94.1.33

599 Sherwood RT (1969) Morphology and physiology in four anastomosis groups of *Thanatephorus*
600 *cucumeris*. Phytopathology 59:1924-1929

601 Sneh B, Jabaji-Hare S, Neate S, Dijst G (1996) Rhizoctonia species: Taxonomy, Molecular
602 Biology, Ecology, Pathology, and Control, 578 pp. Kluwer Academic Publishers,
603 Dordrecht, The Netherlands

604 Stachler JM, Carlson AL, Luecke JL, Boetel MA, Khan MFR (2009) Survey of weed control and
605 production practices on sugar beet in Minnesota and eastern North Dakota. SBREB. 40:39-
606 60

607 Strausbaugh CA, Eujayl IA, Panella LW, Hanson LE (2011) Virulence, distribution and diversity
608 of *Rhizoctonia solani* from sugar beet in Idaho and Oregon. Can J Plant Pathol 33:210-226

609 Torres SV, Vargas MM, Godoy-Lutz G, Porch TG, Beaver JB (2016) Isolates of *Rhizoctonia*
610 *solani* can produce both web blight and root rot symptoms in common bean (*Phaseolus*
611 *vulgaris* L.). Plant Dis 100:1351-1357

612 Van Brüggen AV, Grünwald N, Bolda M (1996) Cultural methods and soil nutrient status inlow
613 and high input cropping systems as they affect *Rhizoctonia* species. In: Sneh, B., S. Jabaji-
614 Hare, S. Neate, G. Dijst (eds.): *Rhizoctonia* Species: Taxonomy, Molecular Biology,
615 Ecology, Pathology and Disease Control. Second International Symposium on

Rhizoctonia. Wageningen, Netherlands1995. Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp. 407-421

Walter M, Boul L, Chong R, Ford C (2004) Growth substrate selection and biodegradation of PCP by New Zealand white rot fungi. J Environ Manage 71:361-369

Wigg KS, Goldman IL (2020) Variability in Reaction to Root and Crown Rot Caused by *Rhizoctonia solani* Among Table Beet Cultivars, Breeding Lines, and Plant Introductions in Controlled Environment Conditions. HortScience 55:1482-1494

Windels CE, Brantner JR (2007) *Rhizoctonia* inoculum and rotation effects on a following sugar beet crop. Sugar beet Res Ext Rept 37:182-191

Woodhall JW, Brown L, Harrington M, Herbert K, Keith S, Webb KM, Neher O (2020) First Report of Binucleate Rhizoctonia (*Ceratobasidium*) AG F Causing Dry Rot Canker of Sugar Beet in Idaho. Plant Disease 104:9. https://doi.org/10.1094/PDIS-06-19-1328-PDN

Yang G Li C (2012) General Description of Rhizoctonia Species Complex. Chapter 2. Plant Pathology, Eds. Cumagun, C.J.R., ISBN 978-953-51-0489-6, 374 pages, Publisher: In: Tech, www.intechopen.com.

636 **Table 1** Reaction on the response of the seven varieties to *R. solani* AG 2-2IIIB inoculated with
637 three different types of inocula (colonized barley grains, mycelial plugs, and sclerotia) under
638 greenhouse conditions and compared with seed companies assigned disease score ratings.

Variety code	Disease severity (1-7)[1]				Cultivar appraisal by seed companies	
	Median					
	Colonized Barley	Mycelia	Sclerotia	Varietal Effect[2]	Rating	Reaction[3]
A	6	2.5	1	3	4.5	S
B	5	2.5	1.5	3	3.6	R
C	6	3	2	3	4.8	S
D	5	3.5	1	4	4.4	MR
E	5	2	2	3	4.7	S
F	6	2.5	1	3	4.5	S
G	5	4.5	1	4	4.3	MR
Inoculum Effect[2]	5	2.5	1		-[4]	-

639

640 [1]Plants inoculated at 4-leaf growth stage with barley grains colonized with *R. solani* AG 2-2IIIB
641 mycelia. Disease severity was assessed at 28-dpi following Torres et al. (2016), where 0 = no
642 visible disease symptoms, 1 =1-5% root surface with visible lesions, 2 = 6-10 % root surface
643 with visible lesions, 3 = 11-25% root infection, 4 = 26-50% root infection, 5 = 51-75% root
644 infection, 6 = greater than 75% root infection, and 7 = root completely deteriorated or dead plant.

645 [2]Main effects of variety and inoculum type are median severity across two trials and four
646 replications per trial.

647 [3]S = susceptible, MR = moderately resistant, and R = resistant. Ratings and reactions based on the
648 field evaluation conducted by American Crystal Sugar Company (ACSC).

649 [4] "-" indicates no analysis was applied.

650

651

652 **Supplementary Figures**

653 **Fig. S1** Growth and development mycelia and sclerotia of *Rhizoctonia solani* in different growth
654 media (A) radial growth of *R. solani* evaluated at 4 dpi (days post inoculation). Here, CV8 =
655 clarified V8 juice, PDA = potato dextrose agar, MBV = metalaxyl benomyl vancomycin, OMA =
656 oatmeal agar, CMA = corn meal agar, YMA = yeast malt agar, respectively.

657

658 **Fig. S2** Development and distribution of sclerotia was grown in different growth media recorded
659 at 28 dpi (days post inoculation); Here, CV8 = clarified V8 juice, PDA = potato dextrose agar,
660 MBV = metalaxyl benomyl vancomycin, OMA = oatmeal agar, CMA = corn meal agar, YMA =
661 yeast malt agar, respectively.

662

663 **Fig. S3** Typical root rot symptoms observed in the *R. solani* AG 2-2IIIB inoculation experiments
664 are presented. Root rot scoring scale (0-7 scale) adapted from Torres et al., (2016) in this
665 experiment where 0= no visible disease symptoms, 1 = 1-5% root surface with visible lesions, 2 =
666 6-10 % root surface with visible lesions, 3 = 11-25% root infection, 4 = 26-50% root infection, 5
667 = 51-75% root infection, 6 = greater than 75% root infection, and 7 = the root completely
668 deteriorated or dead plant), respectively.

669