A longitudinal study on morpho-genetic diversity of pathogenic Rhizoctonia solani from sugar beet and dry beans of western Nebraska

CURRENT STATUS: POSTED

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DOI:
10.21203/rs.2.13499/v1

SUBJECT AREAS
General Microbiology

KEYWORDS
Rhizoctonia solani, Sugar beet, Dry bean, DNA Marker, Nebraska, USA
Abstract

Root and stem rot caused by *Rhizoctonia solani* is a serious fungal disease of sugar beet and dry bean production in Nebraska. The objective of this study was to analyze morphogenetic diversity of 38 *Rhizoctonia solani* isolates from sugar beet and dry bean fields in western Nebraska over 10 years. Morphological features and ISSR-based DNA markers were used to study the morphogenetic diversity. Fungal colonies were morphologically diverse in shapes, aerial hyphae formation, colony, and sclerotia color. Marker analysis using nineteen polymorphic ISSR markers showed polymorphic bands ranged from 15 - 28 with molecular weight of 100bp to 3kb. Polymorphic loci ranged from 43.26 – 92.88%. Nei genetic distance within the population ranged from 0.03 – 0.09 and Shannon diversity index varied from 0.24 – 0.28. AMOVA analysis based on ΦPT values showed 87% variation within and 13% among the population with statistical significance (p<0.05). Majority of the isolates from sugar beet showed nearby association within the population. A significant number of isolates showed similarity with isolates of both the crops suggesting their broad pathogenicity. Isolates were grouped into three different clusters in UPGMA based cluster analysis using marker information. Interestingly, there was no geographical correlation between the isolates. Principle component analysis showed randomized distribution of isolates from the same geographical origin. This information may help in molecular pathotyping of the pathogen for better disease management.

Background

*Rhizoctonia solani* is a polyphagous plant pathogen with worldwide distribution. It is a soil-borne pathogen and known for severe plant diseases like collar rot, root rot, damping off and wire stem [1]. The fungus survives on the infected plant debris and act as a inoculum for the susceptible crops like sugar beet (*Beta vulgaris* subsp. *vulgaris*) [2] dry beans
(Phaseolus vulgaris) [2], potato (Solanum tuberosum) [3], and soybean (Glycine max) [4]. It is a major problem for the sugar beet and dry bean producers of western Nebraska. Total production acreage of dry bean and sugar beet crops in Nebraska are 45,500 and 140,000 – 200,00 acres respectively. However, every year Rhizoctonia root rot and crown rot in sugar beet and dry bean have reduced the yield significantly and has also created problems in storage. It has been estimated that on average 2% of annual sugar beet yield loss is due to the Rhizoctonia rot, and even in some rare scenarios 30% - 60% to complete loss of the field had also been observed [5]. In Nebraska, a total of 52% and 42% of yield reduction can be observed in case of dry bean variety viz. Great Northern Beans and Pinto beans respectively due to rhizoctonia rot [6].

*R. solani* occurs in varying degree of morphogenetic diversity. Cultural appearance, anastomosis, virulence, and physiology are different among different strains. Many scientific attempts have been made to categorize the *R. solani* isolates based on morphological, physiological and pathological differences. The most accepted grouping of *R. solani* is based on the formation of anastomosis or hyphal fusion [7, 8]. There are now 14 anastomosis groups (AG), several of which are divided into subgroups [9]. But the presence of more than one AG and occasional loss of anastomosis ability always complicated the identification and characterization of *Rhizoctonia sp*. Further, several studies have also indicated distinct pathogenesis even within the same AG groups [7, 10]. Morphological characteristics are further influenced by the culture conditions, which makes it more difficult to characterize and categorize the isolates. The problem associated with characterization can be better addressed with the DNA-based molecular studies [10–13]. Several DNA-based markers were used to analyze the genetic diversity of *R. solani* viz. genome sequence complementary analysis [14], Random Amplified Polymorphic DNA (RAPD) [10, 13, 15], amplified fragment length polymorphism (AFLP) [16], simple
sequence repeats (SSR) [17] and inter-simple sequence repeats (ISSR) [13, 18–21]. ISSR markers were developed in 1994 and since then it is widely used for rapid differentiation among the closely related species. The technique involves the amplification of inter-region between two SSR regions with a primer of 16 – 18 bp long and with a flanking region of nucleotides at the 3’ or 5’ end. ISSR analysis is simple and less expensive than RAPD, and AFLP. It can be used to assess the genetic diversity of a large number of phytopathogen within relatively less time and with high reproducibility [15]. Several researchers used dominant nature of the ISSR to establish the genetic variations and relationships among the \textit{R. solani} isolates of different geographic province and within the same anastomosis group [10, 13, 15].

Rhizoctonia root, stem, and crown rot are common in sugar beet and dry bean fields every year across western Nebraska. Our hypothesis was that isolates collected from two different crops could be different and also isolates collected from the same crop across the years and geographic region could be different. Therefore, pathogen isolates were collected from both crops over years and were stored. The objective of this study was to determine the morpho-genetic diversity among \textit{R. solani} isolates of sugar beets and dry beans from western Nebraska. The genetic diversity was assessed with 19 polymorphic ISSR markers, from a collection of isolates from the central great plains.

**Methods**

**Fungal isolates**

Infected plants were collected from different regions of western Nebraska (Fig. 1a - b). A total of 38 \textit{R. solani} were isolated from the collected samples and used for this study. Twenty-nine were from sugar beet and nine isolates were from dry bean. Isolates were recovered from symptomatic diseased sugar beets and dry bean roots. For isolation, surface sterilized plant material was platted on potato dextrose agar (PDA) medium
containing streptomycin antibiotic to reduce the opportunistic bacterial growth. Culture plates were incubated at 26 °C. Isolates were identified microscopically, and morphological features were recorded (Table 1). Three control strains of *R. solani* of sugar beet (RZ_SBC23, RZ_SBC28, and RZ-SBC51) from Nebraska, Montana and Colorado were collected from Dr. Linda E. Hanson (USDA – ARS Sugarbeet and Bean Research, East Lansing, Michigan) (Table 1). These strains were used for ISSR marker analysis to compare the genetic relatedness among the isolates and with known AG grouping.

Population 1, 2, and 3, were used in this report to indicate the group of 29 sugar beet isolates, 9 dry bean isolates, and 3 control strains.

**DNA Extraction**

For DNA extraction fresh fungal culture was used. Five ¼ x ¼ inch plugs of agar were placed into 50 ml of sterile PDB and grown for 5 days at 26 °C. After the incubation period, mycelia were harvested by filtration through cheesecloth. The collected mycelia were lyophilized with liquid nitrogen and grounded into a fine powder with mortar and pestle. The powder was transferred to 50 ml conical tubes containing 15 ml of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 0.1 M Tris, pH 8.0, 0.4% B-mercaptoethanol) preheated to 65 °C. The samples were then incubated in a 65 °C water bath for one hour, with mixing at an interval of every 10 minutes. Samples were cooled for 10 minutes and 20 ml of chloroform/isoamyl alcohol (24:1) was added and mixed with each tube. The tubes were centrifuged at 3500 rpm for 20 minutes at 15 °C. The aqueous layer was transferred to a new tube and a double volume of chilled 95% ethanol was added to precipitate the DNA. The tubes were centrifuged at 3500 rpm for one minute and the supernatant was discarded. Pellets were washed with chilled 70% ethanol. After all, ethanol was removed the dry DNA pellet was suspended in 1 ml of TE buffer (10 mM
Tris-HCl and 1 mM EDTA, pH 8.0) and treated with 5 ul of RNAse (10mg/ml) at room temperature for one hour. DNA was quantified using the mini-gel method [30] that compares band intensities with a standard lambda/HindIII DNA marker (Gibco BRL, Betheseda, MD) in a 0.8% agarose gel. DNA was diluted to a concentration of 25-50 ng/ul for use in a polymerase chain reaction (PCR).

**PCR amplification of ISSR**

The 50 UBC primers screened in this study were obtained from Eurofins Genomics (Huntsville, Alabama). Nineteen (Table 2) were selected for analysis based on amplification profile (band intensity, quality, and reproducibility in at least two independent replications) on two random isolates. PCR amplification was performed in a 25 µl reaction mixture containing Promega 5x Green GoTaq Flexi Buffer, 2 mM MgCl2, 100 µm of dNTPs, 0.24 µM primer, 50-100 ng DNA, and 1 unit of Taq polymerase. The cyclic reaction was set at initial denaturation at 94 °C for 30 s, annealing at 50°C for 45 s, and an elongation at 72°C for 2 min (45 cycles). Final elongation was performed for 10 min. The PCR was completed on an Applied Biosystems Thermocycler 2720. PCR products were separated electrophoretically in 2% agarose gels including 0.5 µg/ml ethidium bromide and bands were visualized in the gel-doc system (Biorad, USA). ISSR gels were photographed using FOTO Analyst Express Electronic Imaging System (Fotodyne, Inc). Marker size range was determined by comparison with a 100bp DNA ladder (New England BioLabs). When scoring the gels, a marker locus was considered polymorphic if the band was not present in every isolate. Only clear DNA bands that were reproducible were scored. ISSR marker loci were designated by primer name.

**Data Analysis**

A correlation matrix and dendrogram were prepared with unweighted pair group method with arithmetic mean (UPGMA) method to distinguish the isolates based on morphological
characteristics. The categorical data of morphological traits were converted into a 0, 1 matrix based on presence and absence of that. The dendrogram (method = UPGMA) and correlation plot (method = Pearson correlation) were prepared using R statistical software (package: pvclust and corrplot). Dominant polymorphic ISSR markers were scored based on presence and absence with 0 (absent) - 1 (present) matrix. Cluster analysis was carried out using UPGMA method and dendrogram was created by using R statistical software with a bootstrap value of 1000 with package pvclust [31]. The package was used to compute two values: an approximately unbiased (AU) p-value based on multi-step multiscale bootstrap resampling procedure [32] and a bootstrap probability (BP) p-value from ordinary bootstrap resampling [33]. A significance threshold of $\alpha = 0.05$ (95% confidence interval) was used in this approach. For a cluster with AU p-value > 0.95 (95%), the hypothesis that "the cluster does not exist" is rejected with significance level 0.05. The percentage of polymorphic loci (P), Shannon’s diversity index ($I$) and Nei’s gene diversity were calculated to estimate the genetic variation among the isolates using GenAlex 6.5 [34]. Genetic differentiation among populations was estimated by pairwise values of $\phi_{PT}$.

Analysis of molecular variance (AMOVA) was used to compute the genetic variation among and within the population. AMOVA calculations were performed in GenAlex 6.5. Principle component analysis was used to determined genetic differentiation among the isolates of different region using R statistical software (Package: FactoMiner, factoextra, and ggplot2). Morphological and genetic traits were correlated with R-stat to determine the diversity among and within the isolates of sugar beets and dry beans (Package: corrplot).

**Results**

**Morphological Diversity**

All the 38 isolates showed distinctive morphological variation in their appearance. Colony
morphology varied from dark brown to light brown and light tan colored colonies (Fig. 1c), sclerotia and presence of aerial hyphae (Table 1). Correlation analysis showed a positive correlation within the isolates of same crops. The isolates from sugar beet showed a positive correlation with sugar beet isolates and dry beans with dry bean isolates (Fig. 2). There was high positive correlation within the isolates of sugar beets, RZ_SB16, RZ_SB56, RZ_SB373, RZ_SB387, RZ_SB389, RZ_SB364, RZ_391, RZ_332 (r = 1.00 / 1.00, p <0.0001). Similarly, the isolates from dry beans R_DB10, RZ-DB305, RZ_DB386, RZ_DB116, RZ_DB336, RZ_DB360, RZ_DB379 showed significantly high correlation with each other (r = 1.00 / 1.00, p < 0.0001). Though most of the isolates showed morphological correlation within the isolates of same crop there was also positive cross crop correlation with statistical significance but at a lower degree. Isolates RZ_DB22 showed correlation with RZ_SB358 (r = 0.77/1.00, p < 0.01) and RZ_SB359 (r = 0.63/1.00, p < 0.05), RZ_DB305 with RZ-SB375 (r = 0.67/1.00, p < 0.05) (Fig. 2) (Supplementary file: Table: T1). There was no correlation between the isolates from the same geographical origin and year of origin.

Genetic Diversity

A total of 50 UBC primers were screened and 19 primers were selected based on their 100% polymorphism index. A total of 396 alleles were identified from 41 isolates (representative gel image at Fig. 3). An average number of loci per primer was 20.84 and band size ranged from 1 – 3 kb. The primer UBC 889 produced the highest number of polymorphic loci (29) followed by UBC 808 (27), UBC 809 (26) and UBC 812(25) (Table 2). The Shannon information index (I) varied from 0.235 – 0.280 with an average of 0.251. The percentage of polymorphic loci (% P) ranged from 43.26% - 92.88%. The highest % polymorphic loci (92.88%) was observed within the dry bean isolates (population 2) (Table
The Nei genetic distance within the population ranged from 0.033 - 0.083 with an average of 0.51.

Cluster analysis based on the UPGMA method had produced three distinct clusters. The first clusters majorly represented the isolates from the dry beans (RZ_DB 116, RZ_386, RZ_DB336, RZ_DB10, RZ_DB305, and RZ_DB360) showing their genetic similarity. However, four sugar beets isolates (RZ_SB373, RZ_374, RZ_375, and RZ_SB38) also showed significant similarities with the dry bean isolates in the first cluster. The second cluster included only sugar beet the isolates viz. RZ_SBC51, RZ_SB349, RZ_B358, RZ_SB37, RZ_SB1, RZ_SB389, RZ_SB391, RZ_SB16, RZ_SB56, RZ_SB338, RZ_SBC23, RZ_SB359, RZ_SB387, RZ_SB332, RZ_SB364. While the third cluster showed cross relation among the dry bean and sugar beet isolates (Fig. 4). Dry bean isolates like RZ_DB22, RZ_DB222, and RZ_DB379 showed genetic relatedness with RZ_SB330, RZ_SB188, and RZ_SB54 in the third cluster. Isolates like RZ_SB374 and RZ_SB375 (with au = 99% and bp = 97%) which were isolated in the year of 2008 from Scottsbluff showed similarity in genetic makeup. Isolates like RZ_SB332 (2005) showed similar genetics with isolates RZ_SB364 (2006) (au = 99% and bp = 96%) (#22).

The AMOVA analysis based on $\Phi$PT values indicated that most of the genetic diversity occurred within the populations (87%, $P < 0.023$) while variability among the population only contributed 13% ($P < 0.023$) (Table 4). Statistically significant genetic differentiation was observed among the isolates.

Discussion

The genus *Rhizoctonia* is a diverse group of fungi which causes stem and root rot, foliar blights in many crops [1]. *Rhizoctonia solani* Kuhn, a ubiquitous soil borne basidiomycetes, which causes diseases in many economically important crops like rice, potato, soybean,
corn, sugar beet and dry beans [22]. In the classical identification method, *R. solani* were
characterized based on differences in pathogenicity, morphology, and physiology [18]. In
this study, a total of 38 *R. solani* was isolated from sugar beets and dry beans in a time
span of 10 years from different location of western Nebraska, USA (*Table 1*). Studies on
cultural characteristics revealed that the colony color of the different *R. solani* isolates
varied from cream-colored to brown, dark tan to light tan in PDA culture plates with the
production of areal hyphae and sclerotia with dark to light brown color (*Table 1*). The
results showed a close agreement with other works [23–25].
Results of the genetic analysis indicated a high degree of genetic diversity within the
population (87%). Both the population of sugar beet and dry beans have unique genetic
makeup which can be observed from the marker genotypes. The sugar beet and dry bean
isolates are mostly conserved within each crop and formed distinct and different clusters
in dendrogram analysis with few variations. Some of the sugar beet isolates also showed
cross correlation with the isolates of dry bean (cluster 1 and cluster 3) (*Fig. 4*). This
suggests relatedness among the population and wide pathogenicity spectrum of the group.
AMOVA analysis also showed low variation among population (13%) compared to within
the population (87%). PCA analysis and grouping based on marker genotypes also showed
similar grouping patterns (Supplementary File: *Fig. S1*). It indicates, however, the origin
of *R. solani* for sugar beet and dry beans are same but there is a certain degree of
differentiation. The difference may have originated during the evolution and selection
over pathogenicity. Similar results can be observed form the studies of Dubey et al.,
(2012), where the *R. solani* isolates were independent or did not corresponds to crops of
origin [10, 26].
Morphological classification of the isolates showed high degree of differentiation among
the isolates from sugar beet and dry beans. Grouping based on only morphological traits
showed correlation within the isolates of same populations (Fig. 2). On differing, marker-based analysis showed certain degree of cross-correlations among the populations. Grouping of the isolates based on location and isolation year over the marker data highlighted stimulating facts. The high bootstrap (97%) and p-value (99%) between the isolates RZ_SB374 and RZ-375 indicate the same genetic origin based on the fact they were collected in same year (2008) and from same place (Scottsbluff) (Fig. 4). It also indicates the chances of them being in the same pathovar group which couldn't be morphologically differentiated. Isolates from consecutive years also showed a high degree of genetic similarity defining the chances of same pathogens infecting the fields in the successive year. This also indicates their inoculum may have been present in the crop residues or soil from previous year, which are left untreated and produced the infection. Isolates like RZ_SB194 showed 100% bootstrap identity with control strain RZ_SBC28 of sugar beet (au =100%). This suggests genetic similarity and possibility of RZ_194 belonging to AG-Group 2-2 IIIB, which is a major anastomosis group responsible for sugar beet crown and root rot [27]. Isolates RZ_SB388 and control strain RZ_SBC23 also showed genetic resemblance but with low bootstrap probability value (56%) and was not statistically significant (84%) (#13) (Fig. 4). It can be concluded, though there is some degree of similarity, they are totally different pathovars.

The location-based grouping of the isolates based on marker profile showed random distribution. Scottsbluff with the highest number of isolates showed a correlation with all the isolates from other places and among themselves. Isolates from the Scottsbluff showed a high degree of similarity with the isolates of Box Butte, Morril, Imperial. Although sampling of this study was uneven with respect to crop and colaction. But, there was a mixed genetic population as noticed in cluster analysis. Isolates distributed independently of their geographical locations. Among the 31 isolates from Scottsbluff,
there are four distinct divisions (Fig. 5). Several studies report this uneven relationship or no correlation between the place of origin and isolates of *R. solani* [10, 26, 28, 29].

Relying only on morphological characteristics often may results in misidentification as the pathogens belonging to same group with similar morphological feature may differ in pathogenicity. In this study, we observed morphological correlation showed a distinct difference between the isolates of sugar beet and dry beans, while genetic diversity showed certain degree of cross correlation between the isolates of sugar beet and drybeans. Therefore, using morphological traits as sole identification method can results in biased grouping when the population are distinct or unique in their genetic makeup. Thus, this study proved that marker information in conjunction with morphological traits give a better identification and characterization of intra and intergenic genetic variations. For differentiation and characterizations of the *R. solani* from sugar beet and dry beans, ISSR marker was found suitable with the morphological features. This method can give a comprehensive estimation of genetic diversity of polyphyletic isolates where anastomosis or morphological features were not sufficient. Further studies based on ITS profiling and sequencing will be needed to provide more vivid knowledge of the genetic identity and variations with respect to crops and geographical origin. Identification and proper categorization of the pathogen will be helpful in designing integrated disease management guidelines for sugar beet and dry beans of mid western America.

Declarations

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and material:** All the data are represented in the manuscript

**Competing interests:** The authors declare that they have no competing interests

**Funding:** There was no grant fund for this project. The project was partially supported by ‘Research State Aided’ internal fund (21-6243-1001 to D. Santra) and 21-6243-1006 to R. Harveson.

**Authors’ contribution:** Santra and R. Harveson conceived the idea and design of the study. S. Das and T. Plyler-Harveson performed experiment, data analysis and wrote the manuscript. D. Santra and R. Harveson reviewed and prepared the final draft. All authors have read and approved the final
Acknowledgments: Authors would like to acknowledge Dr. Lind E. Hanson, USDA Research Scientist, for providing the three control strains for solani with known AG group.

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Tables

Table 1: Details of 41 *Rhizoctonia solani* isolates (38 test isolates and three control isolates with known AG group) with source, origin, year of isolation and morphological attributes.
| Isolates* | Date of Isolation | Isolate source | Geographical origin | State     | Morphological description                                      |
|-----------|-------------------|----------------|---------------------|-----------|---------------------------------------------------------------|
| RZ_DB22   | 2001              | DB             | Box Butte           | Nebraska  | Light tan, sclerotia throughout agar                         |
| RZ_DB10   | 2002              | DB             | Box Butte           | Nebraska  | Brown                                                        |
| RZ_DB116  | 2003              | DB             | Scotts Bluff        | Nebraska  | Tan                                                          |
| RZ_DB222  | 2004              | DB             | Scotts Bluff        | Nebraska  | Light tan, aerial hyphae                                     |
| RZ_DB305  | 2005              | DB             | Scotts Bluff        | Nebraska  | Brown                                                        |
| RZ_DB336  | 2006              | DB             | Scotts Bluff        | Nebraska  | Light brown                                                  |
| RZ_DB360  | 2007              | DB             | Scotts Bluff        | Nebraska  | Light brown                                                  |
| RZ_DB379  | 2008              | DB             | Scotts Bluff        | Nebraska  | Light brown                                                  |
| RZ_DB386  | 2009              | DB             | Scotts Bluff        | Nebraska  | Brown                                                        |
| RZ_SB 37  | 2000              | SB             | Scotts Bluff        | Nebraska  | Dark tan, aerial hyphae, light brown sclerotia               |
| RZ_SB1    | 2001              | SB             | Scotts Bluff        | Nebraska  | Tan, aerial hyphae                                           |
| RZ_SB 12  | 2001              | SB             | Scotts Bluff        | Nebraska  | Smooth, cream colored                                        |
| RZ_SB 38  | 2001              | SB             | Scotts Bluff        | Nebraska  | Dark tan                                                      |
| RZ_SB 39  | 2001              | SB             | Scotts Bluff        | Nebraska  | Light tan, aerial hyphae                                     |
| RZ_SB 16  | 2002              | SB             | Morrill             | Nebraska  | Dark tan, aerial hyphae                                      |
| RZ_SB 23  | 2002              | SB             | Scotts Bluff        | Nebraska  | Tan, sclerotia throughout agar, flat                         |
| RZ_SB 31  | 2002              | SB             | Colorado            | Colorado  | Light tan, aerial hyphae                                     |
| RZ_SB 54  | 2003              | SB             | Scotts Bluff        | Nebraska  | Tan, sclerotia throughout agar, flat                         |
| RZ_SB 56  | 2003              | SB             | Box Butte           | Nebraska  | Dark tan, aerial hyphae                                      |
| RZ_SB 59  | 2003              | SB             | Scotts Bluff        | Nebraska  | Smooth, cream-colored, brown sclerotia                       |
| RZ_SB 188 | 2004              | SB             | Scotts Bluff        | Nebraska  | Tan, sclerotia throughout agar, flat                         |
| RZ_SB 194 | 2004              | SB             | Scotts Bluff        | Nebraska  | Cream colored, aerial hyphae, brown sclerotia                |
| RZ_SB 202 | 2004              | SB             | Scotts Bluff        | Nebraska  | Smooth, light tan, aerial hyphae                             |
| RZ_SB 308 | 2005              | SB             | Scotts Bluff        | Nebraska  | Brown, aerial hyphae                                         |
| RZ_SB 310 | 2005              | SB             | Scotts Bluff        | Nebraska  | Brown, aerial hyphae, sclerotia                              |
| RZ_SB 330 | 2006              | SB             | Scotts Bluff        | Nebraska  | Light tan, smooth                                            |
| RZ_SB 332 | 2006              | SB             | Scotts Bluff        | Nebraska  | Dark tan, sclerotia                                          |
| RZ_SB 349 | 2006              | SB             | Scotts Bluff        | Nebraska  | Dark tan, aerial hyphae, brown sclerotia                     |
| RZ_SB 358 | 2007              | SB             | Box Butte           | Nebraska  | Light tan, sclerotia, aerial hyphae                          |
| RZ_SB 359 | 2007              | SB             | Box Butte           | Nebraska  | Light tan, sclerotia, aerial hyphae, brown                   |
| RZ_SB 364 | 2007              | SB             | Scotts Bluff        | Nebraska  | Dark tan, sclerotia                                          |
| RZ_SB 373 | 2008              | SB             | Scotts Bluff        | Nebraska  | Dark tan, aerial hyphae                                      |
| RZ_SB 374 | 2008              | SB             | Scotts Bluff        | Nebraska  | Cream colored                                                |
| RZ_SB 375 | 2008              | SB             | Scotts Bluff        | Nebraska  | Brown, aerial hyphae                                         |
| RZ_SB 387 | 2009              | SB             | Scotts Bluff        | Nebraska  | Dark tan, aerial hyphae                                      |
| RZ_SB 388 | 2009              | SB             | Scotts Bluff        | Nebraska  | Brown, aerial hyphae, sclerotia                             |
| RZ_SB 389 | 2009              | SB             | Scotts Bluff        | Nebraska  | Dark tan, aerial hyphae                                      |
| RZ_SB 391 | 2010              | SB             | Wyoming             | Wyoming    | Dark tan, sclerotia                                          |
| RZ_SBC23  | Unknown           | SB             | Imperial            | Nebraska  | AG group 2-2, ISG-IIIB                                      |
| RZ_SBC28  | Unknown           | SB             | Swink               | Colorado   | AG group 4                                                   |
| R_Z_SBC51 | Unknown           | SB             | Montana             | Montana    | AG group 2-2, ISG-IV                                         |

*SB = Sugar beet; DB = Dry bean; SBC = Sugar beet control strain

Table 2: List of 19 ISSR markers used for the study
**Table 3 Allele diversity within the population**

| Pop   | N     | Na   | Ne   | I     | He   | uHe  | %I  |
|-------|-------|------|------|-------|------|------|-----|
| Pop1  | Mean  | 8.000| 0.980| 1.269 | 0.240| 0.159| 0.169| 48  |
|       | SE    | 0.000| 0.050| 0.018 | 0.014| 0.010| 0.010|     |
| Pop2  | Mean  | 30.000| 1.858| 1.249 | 0.280| 0.167| 0.170| 92  |
|       | SE    | 0.000| 0.026| 0.014 | 0.010| 0.008| 0.008|     |
| Pop3  | Mean  | 3.000| 0.891| 1.259 | 0.235| 0.156| 0.187| 43  |
|       | SE    | 0.000| 0.050| 0.017 | 0.014| 0.009| 0.011|     |

*Na = No. of Different Alleles; Ne = No. of Effective Alleles = 1 / (p^2 + q^2); I = Shannon's Information Index = -1 * (p * Ln(p) + q * Ln(q)); He = Expected Heterozygosity = 2 * p * q; uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He [for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 - Band Freq.)^{0.5} and p = 1 - q]*

Table 4 Hierarchical distribution of genetic diversity among the population of *R. solani* from sugar beet and dry beans
| Source           | df | SS     | MS    | Estimated Variation | % of variation | Φ   |
|------------------|----|--------|-------|---------------------|----------------|-----|
| Among Pops       | 2  | 219.93 | 109.97| 7.29                | 13%            | 0   |
| Within Pops      | 38 | 1787.53| 47.04 | 47.04               | 87%            |     |
| Total            | 40 | 2007.46| 54.33 | 54.33               | 100%           |     |

*Φ PT = AP / (WP + AP) = AP / TOT (Key: AP = Est. Var. Among Pops, WP = Est. Var. Within Pops)*

**Figures**

![Figure 1](image)

(a) Dry bean root rot (b) Sugar beet root rot (c) PDA culture plate (d) microscopic hyphal structure
Figure 2

Correlation of the isolates based on their morphological characteristics. The isolates showed crop specific crop correlation. Sugar beet isolates were correlated with sugar beets and dry beans were correlated with dry beans with one exception. Correlation between sugar beets was highlighted with green, orange shades which define correlation among sugarbeet and dry bean isolates respectively. Blue shades represent inter-correlation between the sugarbeet and dry bean isolates.
Figure 3

DNA marker profiles of Rhizoctonia solani isolates from sugar beets and dry beans with ISSR primer UBC809. Sugar beet and dry bean isolates are designated by RZ_SB and RZ_DB followed by a number.
Dendrogram derived from the combined analysis of 19 ISSR primers for 41 Rhizoctonia solani with UPGMA method. In the figure the values are represented as (%); where au = approximately unbiased p-value (red colored), bp = bootstrap probability (bootstrap value = 1000) (green colored) and #edge = number of the sub clusters (39 total clades) (yellow colored). Clusters with Au larger than 95% are strongly supported by the data. Clades (#clade number or edge#) were grouped based on the bootstrap identity and their characteristics. Blue highlighted clades are with high bootstrap identity (≥ 80%) and statistically significant (≥ 95%). Blue highlighted (#3, #7, #22, #24) clades are with high bootstrap identity (≥ 80%) and statistically significant (≥ 95%). Orange color highlighted clade is with high bootstrap value but statistically non-significant (#13).
Figure 5

Location-based distribution of the *R. solani* isolates based on marker data.

**Supplementary Files**

This is a list of supplementary files associated with the primary manuscript. Click to download.

Supplementary Table 1 - Correlation Matrix_Morphology.xlsx
Supplementary Figure S1_Morphology and Molecular_PCA.jpg