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Variability among the potato sclerotial isolates of *Rhizoctonia solani* of Mountainous Region, Gilgit-Baltistan, Pakistan

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An experiment was carried out to find the variability among twenty sclerotial isolates of *Rhizoctonia solani* collected from potato growing areas of Gilgit-Baltistan. These isolates were grown in different culture media, mycelial compatibility and fungus protein profile were investigated. Culture media were used to study radial colony growth and sclerotial production of isolates. Radial colony growth (RCG) and sclerotial production (SP) of isolates against culture media ranged from 12.31-21.55; 3.66-22.66 in potato dextrose agar, 12.67-18.56; 4.66-10.66 in czpedox agar, 12.02-20.42; 2.00-8.66 in corn meal agar and 10.54-14.16; 0.00-3.00 in water agar, respectively. These isolates were further classified into three categories on the basis of RCG and SP. Result revealed that out of total isolates, 60% showed medium RCG and 40% fast growth, while 10, 60 and 30% isolates showed low, medium and high SP. Furthermore, sclerotial characteristic such as size, shape and distribution pattern were also recorded. Mycelial compatibility and incompatibility among the *R. solani* isolates was also studied. The results indicated that out of 190 combinations, 72.10% were compatible, whereas 27.90% were incompatible. Fungus protein profile of twenty isolates of *R. solani* by sodium dodecyl suplphate gel electrophoresis (SDS-PAGE) recovered sixty one bands with different frequencies among the isolates. Cluster analysis of twenty isolates divided them into two major lineage groups, A and B. Lineage A contained 65% isolates whereas lineage B contained 35% isolates. These lineages were further divided into thirteen clusters (C_1-C_{13}); A was comprised of eight and B five clusters, respectively.

Key words: *Rhizoctonia solani*, sclerotial isolates, radial colony growth, sclerotial feature, mycelial compatibility, sodium dodecyl suplphate gel electrophoresis (SDS-PAGE), Gilgit-Baltistan.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the important vegetable crops in the world. In Pakistan, its production increases year by year. According to Agricultural statistics, potato is grown on an area of 145000 ha during

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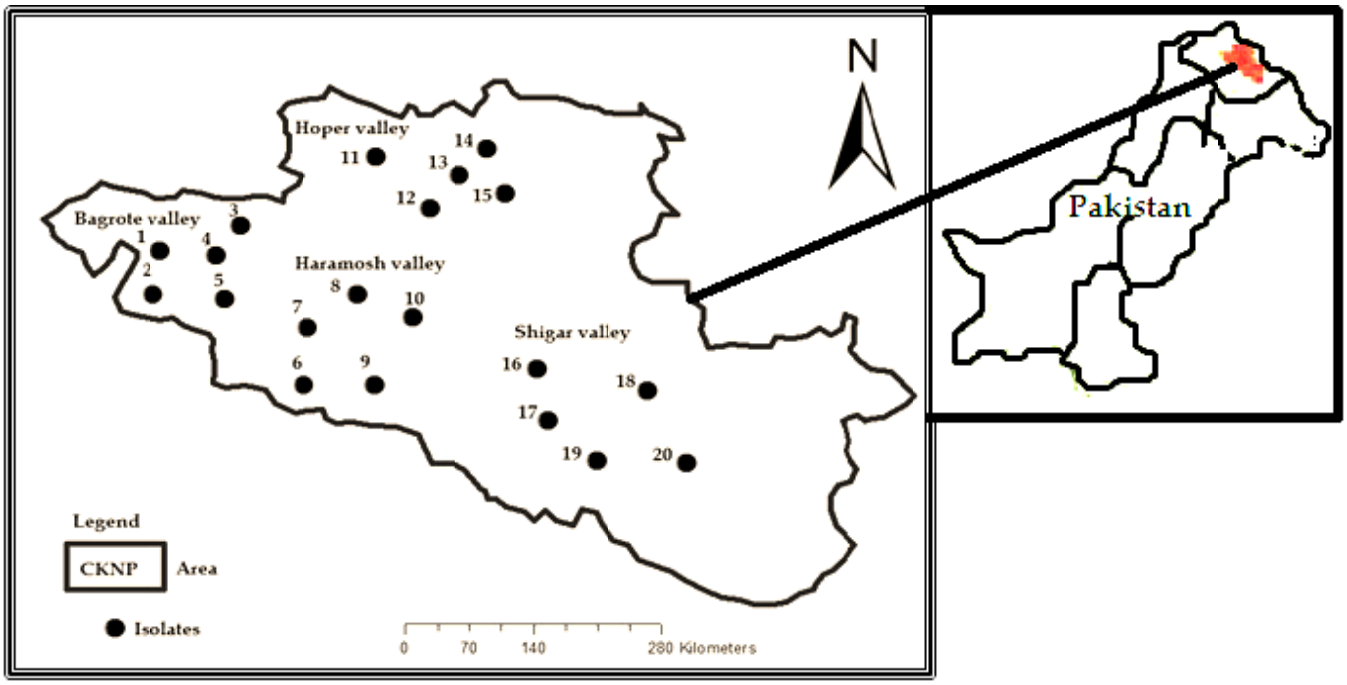


Figre 1. Map of the study area and dot showing collection site of isolates.

the year 2008-2009, with a production of 2941300 tons (Agricultural Statistics of Pakistan, 2008-2009) while in Gilgit-Baltistan, it is cultivated on an area of 8526 h with a production of 134031 Mt/ha (Agricultural Statistics Gilgit-Baltistan, 2009). In Gilgit-Baltistan, the production of potato is low as compared to its potential. Many biotic (fungal, bacterial and viral) and abiotic (temperature, pH, humidity and soil nutrients) constrains are involved. Many diseases especially black scurf caused by *Rhizoctonia solani* are the important biotic constraints (Bhutta et al., 2004). *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) was reported nearly 150 years ago as destructive pathogen of potato crop by Kuhn (1858). It is a widely distributed pathogen affecting different economically important crops, especially potato, where the tuber quality and production are highly decreased by the action of this fungus (Krechel et al., 2002).

Infection starts when mycelia or hyphae from a germinating sclerotium starts to grow towards a suitable host as a result of attracting chemical exudates, e.g., amino acids, sugars, organic acids and phenols, from the plants (Keijer, 1996). Inoculum sources of *R. solani* include seed tubers and soil, both of which can harbour mycelium and sclerotia (Tsror and Peretz-Alon, 2005). *R. solani* infects subterranean stems and stolons, and severe lesions can have a negative effect on plant growth and tuber development (Banville et al., 1996). Mycelia and sclerotia can grow and develop on plant debris as well as tubers, allowing inoculum to survive in the soil as well as on seed from season to season (Dijst, 1988; Gudmestad et al., 1979).

Several attempts have been made to group the isolates of this pathogen taxonomically. So far, anastomosis of mycelia is the criterion most widely accepted and used to group the isolates of this fungus (Anguiz, 1989). But grouping by anastomosis does not always correspond to grouping by colony morphology, pathogenicity or other physiological features (Carling, 1996).

R. solani is highly diverse, comprises of a number of genetically different groups, often varying in their cultural, morphological, pathological and physiological characters (Ou, 1985). The variation in the fungus *R. solani* has been reported from different parts of the world and can affect management of the disease (Basu et al., 2004). It occurs as an aggregate of strains varying in cultural appearance, anastomosis grouping and physiology (Parmeter and Whitney, 1970).

MATERIALS AND METHODS

Collection of potato tuber

Twenty isolates of *R. solani* used in the current study were isolated from potato tuber showing typical symptoms of black scurf. All samples were collected during the harvesting stage from four valleys and key potato growing villages of Central Karakoram Nation Park of Gilgit-Baltistan, Pakistan (Figure 1). The collected samples were packed in polythene bags and transferred to the laboratory for variability study among the isolates of *R. solani* designated as RS_1-RS_{20}. Samples were surface sterilized by 0.1% mercuric chloride for 2-3 min and extensively washed with sterile distilled water. Sclerotial spot on tuber surface were scratched and placed on Petri plates containing potato-dextrose-agar (PDA). Each isolates was further purified by hyphal tip method (Mundkur, 1959) and maintained as pure culture and stored at 4°C for further study.

Morphological and sclerotial variability of *R. solani* isolates

Morphological and sclerotial variability of *R. solani* isolates were studied on four solid media viz, PDA, CZPA, CMA and WA. Twenty millilitres of each medium was poured into sterilized Petri-dishes and 5 mm actively growing mycelial plug were inoculated. Experiment was repeated twice and maintained three replicates in each isolate. Radial colony growth was recorded every 24 h during 4 days at 25 ± 2°C. Mean radial colony growth rate per day was determined by dividing colony growth recorded at 96 h by 4 days. Radial colony growth rate (mmd^{-1}) was calculated by using following formula as described by Guleria et al. (2007).

$$\text{RCG d}^{-1} = \frac{\text{RCG 96h}}{4}$$

RCG = Radial colony growth.

Sclerotial variability was studied after three weeks and also number of sclerotia was counted under binocular microscope (Goswami et al., 2011). Based on radial colony growth rate (mmd^{-1}) and sclerotial production (cm^2), isolates were categorized into three groups as: RCG = low (<10), medium (10-20) and fast (>20) and SP = low (<5), medium (5-10) and fast (>10), respectively.

Size, shape and distribution pattern among the isolates were also recorded as described by Jayaprakashvel and Mathivanan (2012).

Mycelial compatibility

Mycelial discs (5 mm in diameter) taken from the edge of an actively growing colony (4^{th} day old) of each isolate were placed 40 mm apart on opposite sides of Petri dishes (90 mm in diameter) and incubated at 25 ± 2°C. Two isolates were paired on one Petri dish and the test was repeated twice. The pairings were examined macroscopically for presence of an antagonistic (barrage or aversion) zone in the region of mycelial contact as described by Punja and Grogan (1983).

Extraction of proteins from *R. solani* isolates

Protein profile of *R. solani* isolates was carried out using SDS-PAGE as described by Laemmli (1970). Fungus protein was extracted from the mycelium mat of each isolate grown on potato dextrose agar plate for 6 days at 27± 2°C. Mycelium of each isolate was harvested then dried. A dry mycelium of each isolate was ground to make a fine powder with mortar and pestle.

Mycelium flour 0.01 g was added to 400 µl extraction buffer (0.5 M Tris-HCL (pH 6.8), 2.5 SDS, 10% glycerol and 5% 2-mercaptoethanol) mixed 400 µl in Eppendorf tube and vortexed (Automatic lab Mixer DH-10). Then, the samples were centrifuged at 15,000 rpm at least ten minutes at ambient temperature. The clear supernatant was transferred into 1.5 ml Eppendorf tubes and stored at 2°C until they were run on the polyacrylamide gel.

Electrophoretic procedure

Fungus protein was analyzed through slab type SDS-PAGE using 12.25% polyacrylamide gel PAGE (Model: AE-6530M, Japan), resolving gel (3.0M Tris-HCl) pH9, 0.4% SDS and 4.5% stacking gel (0.4M Tris-HCl pH 7.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125 % SDS) was loaded top pool of apparatus. A 15 µl of the supernatant of isolates along with marker were loaded into the wells of the gel. Apparatus was connected with uninterrupted electric supply (100 V) until the bromophenol blue (BPB) reached the bottom of the gel plate.

Gel staining

Gel were placed in staining solution (0.2% Commassie Brilliant Blue dissolved in 10% glacial acetic acid, 40% methanol and water in the ratio of 10:40:50) for one hour then placed in destaining solution (5% acetic acid and 20% methanol). Destained gels were analyzed directly using photographic method or drying gel by gel-drying processor for about 2-4 h.

Statistical analysis

The design of *in vitro* experiment was a randomized complete block and analysis of variance (ANOVA) of the data was performed using the statistical package STATISTICA 8.1 and SPSS Version 16.0 for Windows 2007.

RESULTS

Effect of different culture media on colony growth rate and sclerotium production

In this study, different culture media and twenty isolates of *R. solani* were used to determine suitable medium and variability of radial colony growth, sclerotium production, size, shape and distribution of sclerotia. Four culture media viz., PDA, CZPA, CMA and WA were used. During the studies, it was observed that the colony growth rate and sclerotium production in the PDA ranged from 12.31-21.55; 3.66-22.66 in CZPA, 12.67-18.56; 4.66-10.66 in CMA 12.02-20.42; 2.00-8.66 and in WA (10.54-14.16; 0.00-3.00). Mean radial colony growth and sclerotial production were recorded in PDA (18.29, 9.94), CZPA (16.76, 6.83), CMA (15.80, 4.79) and WA (12.70, 1.33). The result observed that potato dextrose agar were suitable medium for culture of *R. solani* (Table 1 and Figure 5). Table 2 showed that the *R. solani* isolates grown in potato dextrose agar were further categorized on the basis of slow, medium and high. Result indicated that 60% of isolates had medium and 40% had fast growth on the basis of radial colony growth (Figure 2) while on the basis of sclerotium production, 10% isolate had low, 60% medium and 30% high (Figure 3). Besides these, significant variations among sclerotial size, shape and distribution pattern of *R. solani* isolates were observed (Table 2, Figure 4 and Figure 6).

Mycelial compatibility group

There were one hundred and ninety combinations of the twenty isolates of *R. solani.* Amongst only 53 showed incompatibility reaction and 137 showed compatibility (Table 3). For combinations which showed antagonistic reactions with each other, a thin band of living or dead mycelia was formed (Figure 7). Based on mycelial compatibility, 72.10% mycelial compatibility and 27.90% non compatible among the tested isolates were shown.

Table 1. Colony mycelial growth, rate/day and sclerotium production of twenty isolates of *R. solani* on different media culture at 25±2°C.

Isolates	Media							
	Colony growth rate (mmd^{-1})				Sclerotium production(cm^2)			
	PDA	CZPA	CMA	WA	PDA	CZPA	CMA	WA
RS_1	14.55^{K}	12.67^{L}	12.02^{O}	10.54^{I}	15.33^{D}	10.66^{A}	8.66^{A}	2.00^{ABC}
RS_2	21.12^{C}	14.66^{K}	13.86^{N}	11.04^{HI}	18.33^{C}	7.00^{BCD}	5.00^{BCDEF}	2.00^{ABC}
RS_3	21.36^{B}	17.08^{F}	14.56^{M}	11.32^{H}	21.00^{AB}	5.00^{EF}	5.00^{BCDEF}	2.66^{AB}
RS_4	21.55^{A}	18.56^{A}	15.64^{GH}	12.56^{FG}	22.66^{A}	8.00^{B}	7.33^{AB}	2.00^{ABC}
RS_5	21.09^{C}	18.20^{B}	16.12^{E}	12.80^{EF}	18.66^{BC}	6.00^{CDEF}	6.00^{ABCDE}	0.00^{D}
RS_6	14.58^{K}	15.52^{H}	16.64^{D}	12.58^{EFG}	9.00^{EFG}	4.66^{F}	5.33^{BCDEF}	2.66^{AB}
RS_7	13.12^{M}	17.58^{D}	17.12^{C}	12.92^{DEF}	7.00^{FGH}	7.00^{BCD}	4.00^{CDEFG}	1.66^{ABCD}
RS_8	14.02^{L}	15.06^{J}	17.26^{BC}	12.74^{EF}	6.00^{HIJ}	7.00^{BCD}	3.66^{DEFG}	0.00^{D}
RS_9	12.31^{N}	17.38^{E}	14.84^{KL}	13.04^{CDEF}	4.00^{IJ}	6.00^{CDEF}	6.33^{ABCD}	2.00^{ABC}
RS_{10}	21.09^{C}	16.83^{G}	20.42^{I}	13.56^{ABCD}	10.00^{E}	8.33^{B}	4.33^{CDEFG}	0.00^{D}
RS_{11}	18.14^{H}	18.08^{B}	17.54^{A}	14.16^{A}	7.00^{FGH}	6.00^{CDEF}	4.00^{CDEFG}	3.00^{A}
RS_{12}	20.52^{D}	18.56^{A}	17.44^{AB}	13.82^{AB}	6.33^{HI}	7.33^{BCD}	6.66^{ABC}	1.00^{BCD}
RS_{13}	16.10^{J}	16.66^{H}	15.82^{F}	13.60^{ABC}	3.66^{J}	7.66^{BC}	4.33^{CDEFG}	0.00^{D}
RS_{14}	19.58^{F}	15.28^{I}	15.08^{J}	12.66^{EFG}	6.66^{GH}	6.66^{BCDE}	3.33^{EFG}	1.00^{BCD}
RS_{15}	21.26^{B}	17.38^{E}	14.72^{L}	10.92^{HI}	9.33^{EF}	6.00^{CDEF}	3.00^{FG}	1.00^{BCD}
RS_{16}	21.05^{B}	14.69^{K}	15.08^{J}	13.21^{BCDEF}	11.00^{E}	5.00^{EF}	3.00^{FG}	1.00^{BCD}
RS_{17}	18.96^{G}	17.64^{D}	15.80^{FG}	13.68^{ABC}	7.00^{FGH}	7.33^{BCD}	2.00^{G}	2.00^{ABC}
RS_{18}	19.98^{E}	17.86^{C}	15.56^{H}	13.64^{ABC}	6.00^{HIJ}	7.33^{BCD}	5.00^{BCDEF}	0.33^{CD}
RS_{19}	17.53^{I}	18.04^{B}	14.98^{JK}	12.06^{G}	5.00^{HIJ}	5.66^{DEF}	4.66^{BCDEFG}	0.66^{CD}
RS_{20}	18.05^{H}	17.52^{DE}	15.52^{H}	13.22^{BCDE}	5.00^{HIJ}	8.00^{B}	4.33^{CDEFG}	1.66^{ABCD}
Mean	18.29	16.76	15.80	12.70	9.94	6.83	4.79	1.33
Minimum	12.31	12.67	12.02	10.54	3.66	4.66	2.00	0.00
Maximum	21.55	18.56	20.42	14.16	22.66	10.66	8.66	3.00
SD	3.11	1.59	1.70	1.03	5.92	1.39	1.60	0.96
CV	17.00	9.49	10.79	8.16	59.52	20.35	33.36	72.58

All the values are means of three replications and values with same letters are not statistically different at LSD 0.05%. PDA: Potato dextrose agar; MYE: malt yeast agar; CMA: corn meal agar; WA: water agar. RS = isolate of *Rhizoctonia solani.*

SDS-PAG gel electrophoresis

Twenty isolates of *R. solani* were subjected to SDS-PAGE gel electrophoresis producing sixty one fungal protein bands. On the basis of presence or absence of protein bands in individual isolates, RS_{17} exhibited highest number of protein bands followed by RS_6, RS_8, RS_{10}, RS_{13}, RS_{19} and RS_{20} whereas on the basis of molecular weight, highest number of bands were produced at 18 kDa and lowest were observed in 45 kDa (Figures 8 and 9). Cluster analysis divided twenty isolates into two major lineage groups, groups A and B. Lineage A contains 65% of isolates whereas lineage B contains 35% isolates (Figure 10). These lineages were further divided into thirteen clusters (C_1-C_{13}). Lineage A consists of eight cluster while lineage B comprises five clusters. Among the lineage A, C_1 contains three isolates, C_3, C_4, and C_6 has two isolates, while C_2, C_5, C_7 and C_8 comprises single isolate each. Similarly, in the lineage B, C_9 consists of three isolates while C_{10}, C_{11}, C_{12} and C_{13} contain single isolate each (Table 4).

DISCUSSION

Different culture media, mycelial compatibility and protein profile through SDS-PAGE were used to study variability of *R. solani* isolates. All isolates of *R. solani* showed variation in radial colony growth and sclerotial production against culture media. Maximum radial colony growth and sclerotial production was found in PDA which was followed by CZPA and CMA, while least radial colony growth and sclerotial production was recorded in WA. The results were in agreement with that of Lalan et al. (2013) who studied colony diameter, growth, colour and sclerotia formation of six isolates of *R. solani* of soybean and concluded that PDA was best for growth and development among the tested culture media. It has been further observed that PDA is a frequently used medium, due to its simple formulation and supportive nature of different plant pathogenic fungi (Maheshwari et al., 1999; Saha et al., 2008). Numerous researchers have confirmed that PDA is most excellent for colony growth of different fungi (Xu et al., 1984; Meera et al., 2012;

Table 2. Classification of *R. solani* isolates on the basis of radial colony growth, sclerotial production, size, shape and distribution pattern.

S/N	Category	Number	Isolates
Radial colony growth			
1	Low < 10	0	None
2	Medium 10-20	12	RS_1, RS_6, RS_7, RS_8, RS_9, RS_{11}, RS_{13}, RS_{14}, RS_{17}, RS_{18}, RS_{19} and RS_{20}
3	High >20	8	RS_2, RS_3, RS_4, RS_5, RS_{10}, RS_{12}, RS_{15} and RS_{16}
Sclerotial production		**Number**	**Isolates**
1	Low <5	2	RS_1 and RS_9
2	Medium 5-10	12	RS_6, RS_7, RS_8, RS_{10}, RS_{11}, RS_{12}, RS_{13}, RS_{14}, RS_{17}, RS_{18}, RS_{19} and RS_{20}
3	High >10	6	RS_2, RS_3, RS_4 RS_5 , RS_{15} and RS_{16}
Sclerotial characteristics		**Number**	**Isolates**
	Macro	10	RS_1, RS_6, RS_7, RS_8, RS_9, RS_{10}, RS_{11}, RS_{12}, RS_{13}, RS_{14},
Size	Micro	6	RS_2, RS_3, RS_{17}, RS_{18}, RS_{19} and RS_{20}
	Mixed	4	RS_3, RS_4, RS_5, RS_{15} and RS_{16}
	Spherical	3	RS_1, RS_6 and RS_8
Shape	irregular	12	RS_2, RS_3, RS_5, RS_7, RS_{12}, RS_{13}, RS_{14}, RS_{17}, RS_{18}, RS_{19}, RS_{20}, and RS_{15}
	Mixed	5	RS_4, RS_6, RS_9, RS_{10} and RS_{11}
	Scatter	7	RS_1, RS_5, RS_6, RS_{11}, RS_{12}, RS_{14} and RS_{15}
Distribution	Concentrated	9	RS_4, RS_7, RS_8, RS_{13}, RS_{17}, RS_{18} RS_{16}, RS_{19} and RS_{20}
	Mixed	4	RS_2, RS_3, RS_9 and RS_{10}

RS = isolate of *Rhizoctonia solani.*

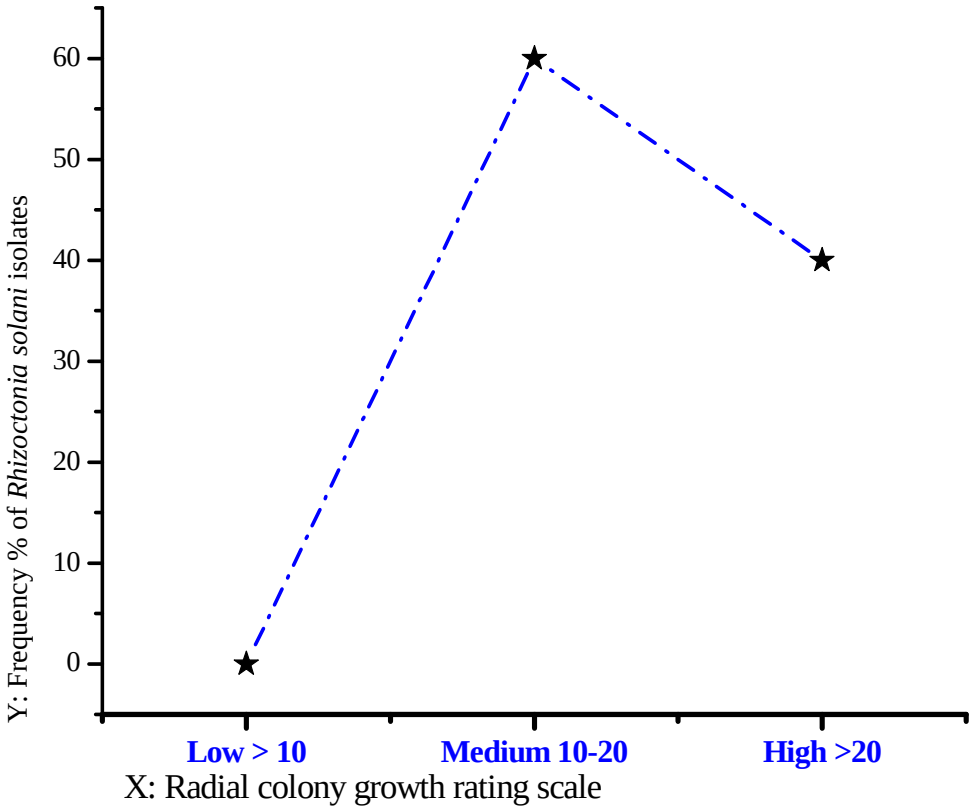


Figure 2. *Rhizoctonia solani* isolates classified into three groups on the basis of colony growth rate mmd^{-1} on PDA medium.

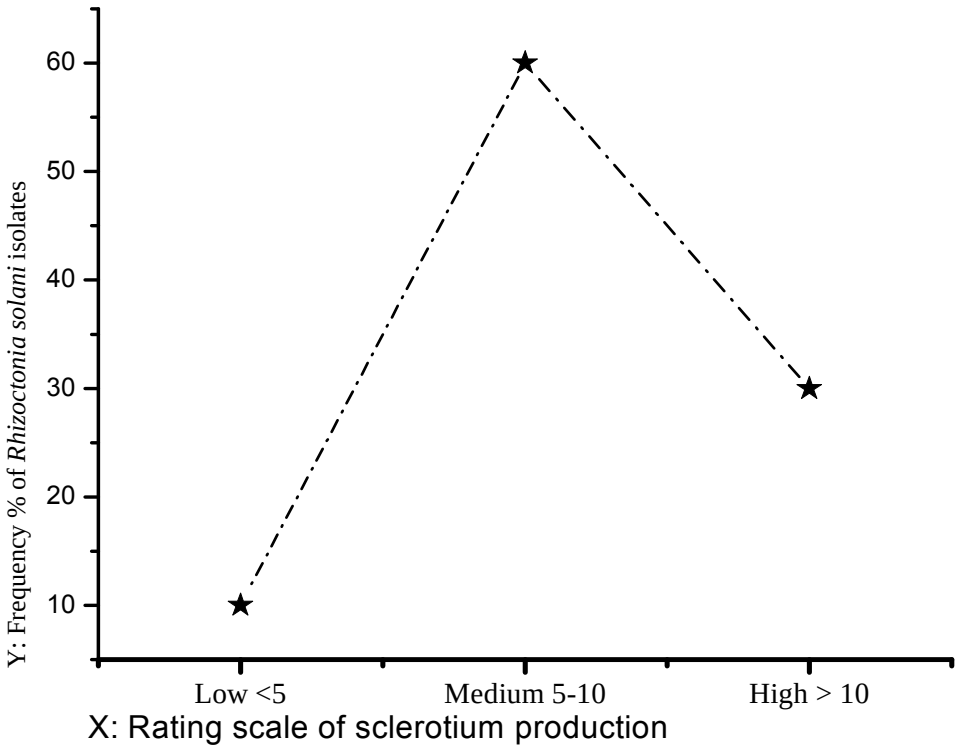


Figure 3. *Rhizoctonia solani* isolates classified into three groups on the basis of sclerotium production cm^2 on PDA medium.

Saha et al., 2008). *R. solani* is a complex pathogen with wide host range. Due to their ill-define taxonomy and poor understanding of natural history, its identification and study is always a challenging tasks (Cubeta and Vilgalys, 1997). Variability within the isolates of *R. solani* has been reported by many researchers (Sherwood, 1969; Neeraja et al., 2002; Linde et al., 2005; Guleria et al., 2007; Thind and Aggarwal, 2008). In our current study, different isolates of *R. solani* showed considerable variation in terms of redial colony growth, number, size, shape, and distribution pattern of sclerotia which is in agreement with report of Thind and Aggarwal (2008) who studied morphological and sclerotial characteristics of potato *R. solani* isolate. Yadav and Anamika (2005) studied morphological and culture variation of different isolates of *R. solani* that caused damping off fenugreek vegetable and concluded that all isolates differ in colony growth, colour, width and sclerotial production.

The finding of the current study revealed that majority of the isolates showed compatibility reaction by pairing. Mycelial compatibility and incompatibility reactions are a useful way of categorizing intraspecific heterogenecity. It is a self and non-self-recognition system controlled by multiple loci, but knowledge of the underlying genetic mechanisms is limited in most filamentous fungi (Glass and Kaneko, 2003). In contrast, isolates that are different at one or some or more of these loci will not anastomose. Rosa et al. (2012) studied compatibility/incompatibility of 433 *R. solani* isolates and found that about 91% isolates were incompatible, while 9% of the pairing were compatible. Majority of isolates showed compatibility in the same county except few isolates.

In the current study, different fungus protein band were recovered from mountaneous isolates of *R. solani*. The results also agree with previous finding of Monica et al. (1983) who studied protein pattern of different anastomosis group of *R. solani* and showed that there was sufficient variability among the isolates particularly AG3 having distinct protein band regardless of isolates sources. Zuber and Manibushanrao (1982) also reported that polyacrylamide gel electrophoresis showed marked variation among five virulent isolates of *R. solani*. Similarly, El-Akkad (1997) also recorded fungus protein band heterogeneity among the *R. solani* isolates of AG-4. Hussein et al. (2000) reported that cluster analysis of protein band recoverd by SDS-PAGE form seventeen isolates of multinucleate and binucleate *R. solani* showed clear-cut differentiating features between *Rhizoctonia* spp. The results of the current study showed variation in fungus protein banding. However, some isolates gave similar binding pattern, they could be differentiated by some components. Iqbal et al. (2005) reported that similarity might be due to comigration of different peptides on the SDS-PAGE and closely related ancestry.

The grouping on the basis of morphological characters was quite different from that which was on the basis of

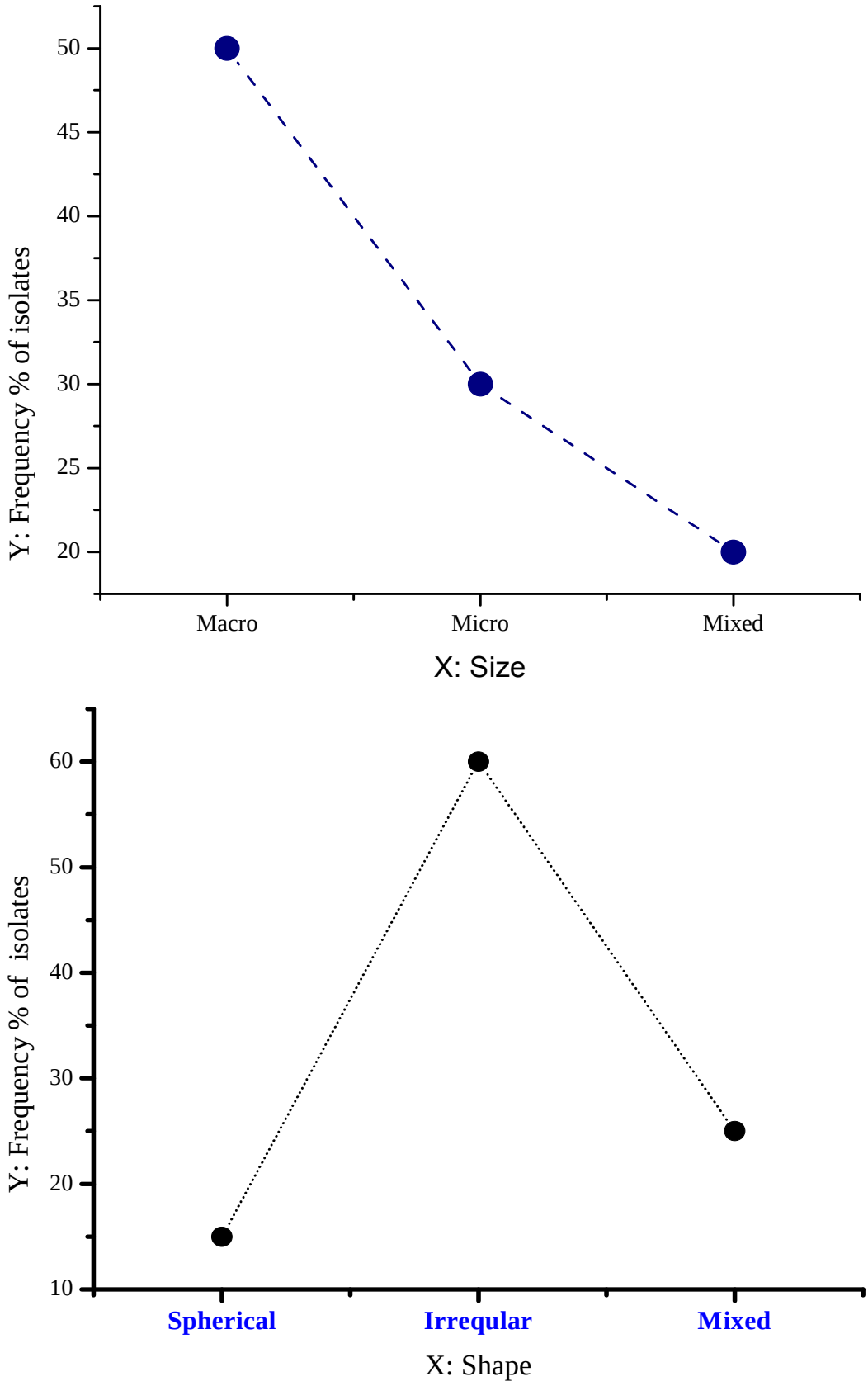
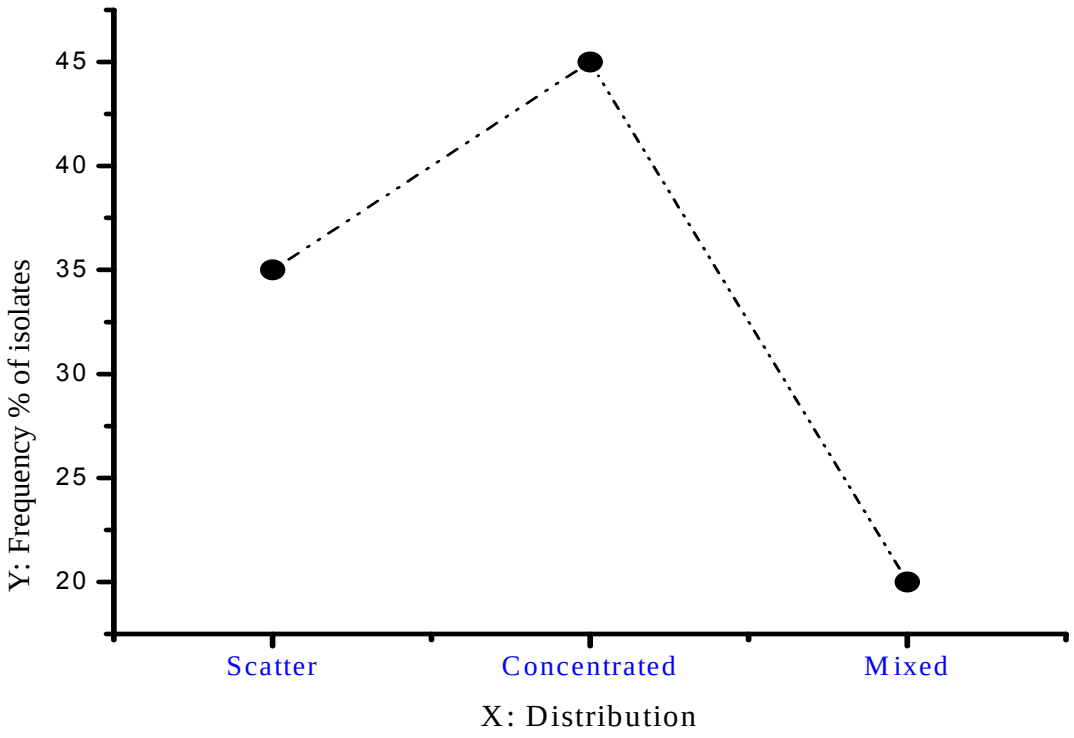


Figure 4. Sclerotial variability (size, shape and distribution pattern) of *Rhizoctonia solani* isolates.



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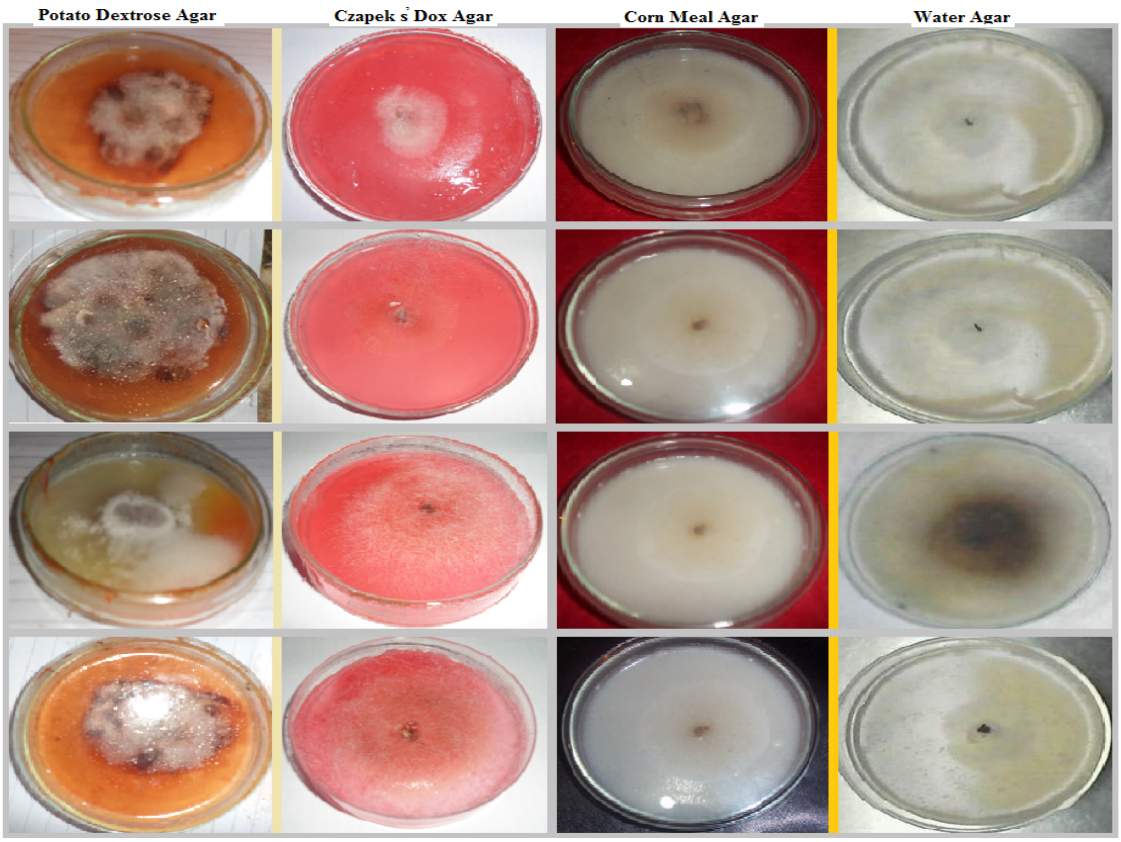

Figure 5. Radial colony growth of different isolates of R. *solani* on different culture media.

Table 3. Mycelial compatibility (MCGs) of different isolates of *Rhizoctonia solani* collected in CKNP region, Gilgit-Baltistan.

Isolates	RS_2	RS_3	RS_4	RS_5	RS_6	RS_7	RS_8	RS_9	RS_{10}	RS_{11}	RS12	RS_{13}	RS_{14}	RS_{15}	RS_{16}	RS_{17}	RS_{18}	RS_{19}	RS_{20}
RS_1	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
RS_2		-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
RS_3			+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve
RS_4				+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
RS_5					-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
RS_6						-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
RS_7							+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
RS_8								+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
RS_9									+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
RS_{10}										-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
RS_{11}											+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
RS_{12}												+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
RS_{13}													-ve	+ve	+ve	+ve	+ve	+ve	+ve
RS_{14}														+ve	+ve	-ve	+ve	+ve	+ve
RS_{15}															+ve	+ve	+ve	+ve	+ve
RS_{16}																-ve	+ve	-ve	+ve
RS_{17}																	+ve	+ve	-ve
RS_{18}																		+ve	+ve
RS_{19}																			+ve

+Ve = Mycelial compatible; -Ve = mycelial incompatible.

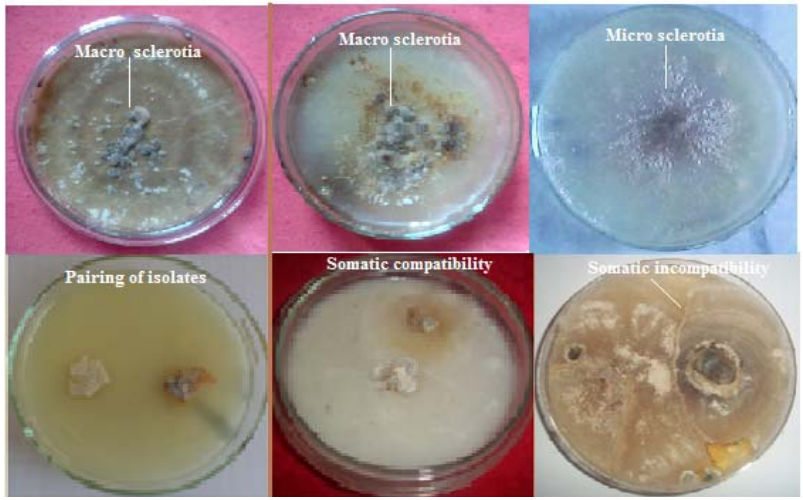

Figure 6. Sclerotial growth of different *R. solani* isolates.

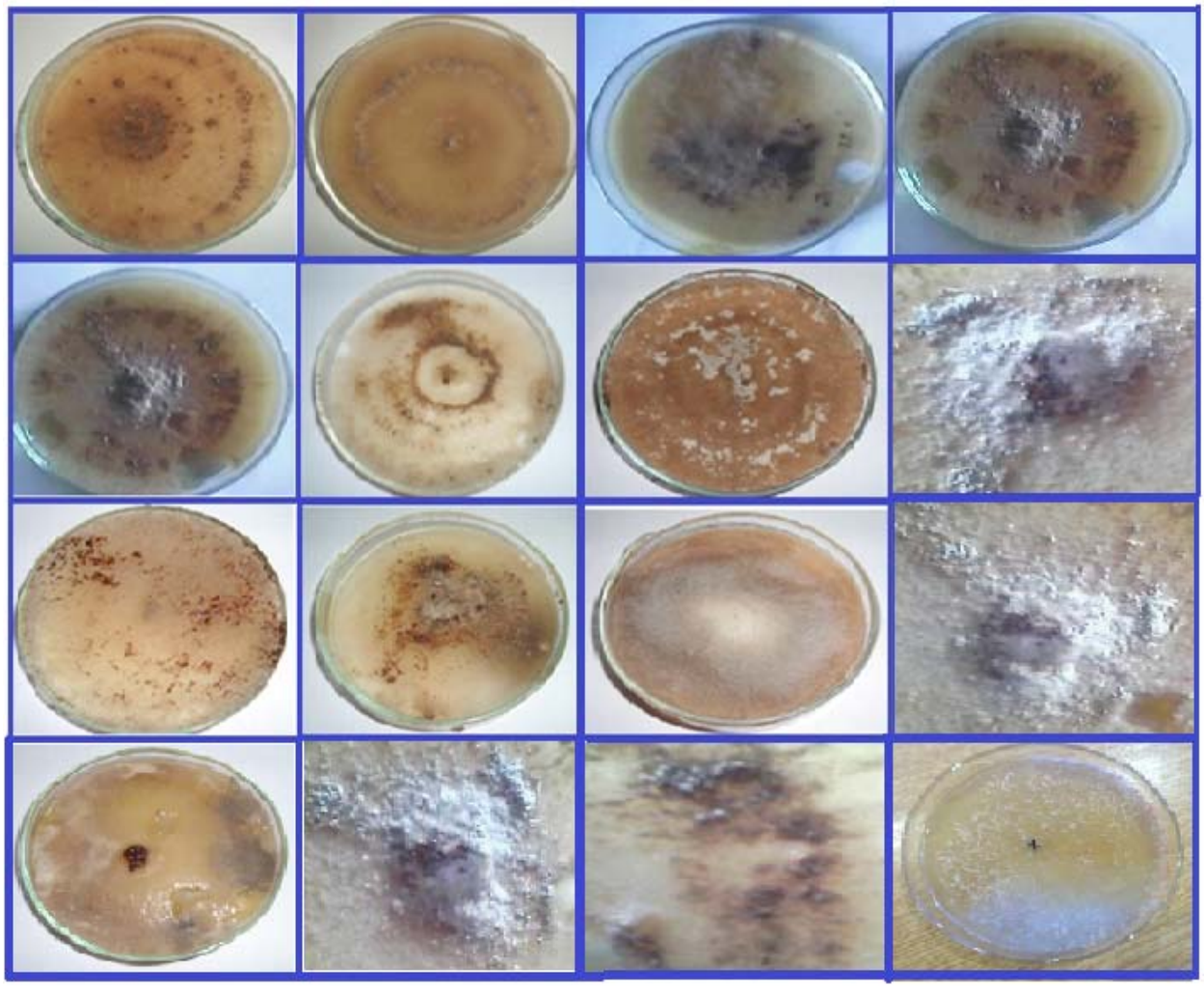

Figure 7. Macro, micro sclerotia, pairing, mycelial compatibility/incompatibility.

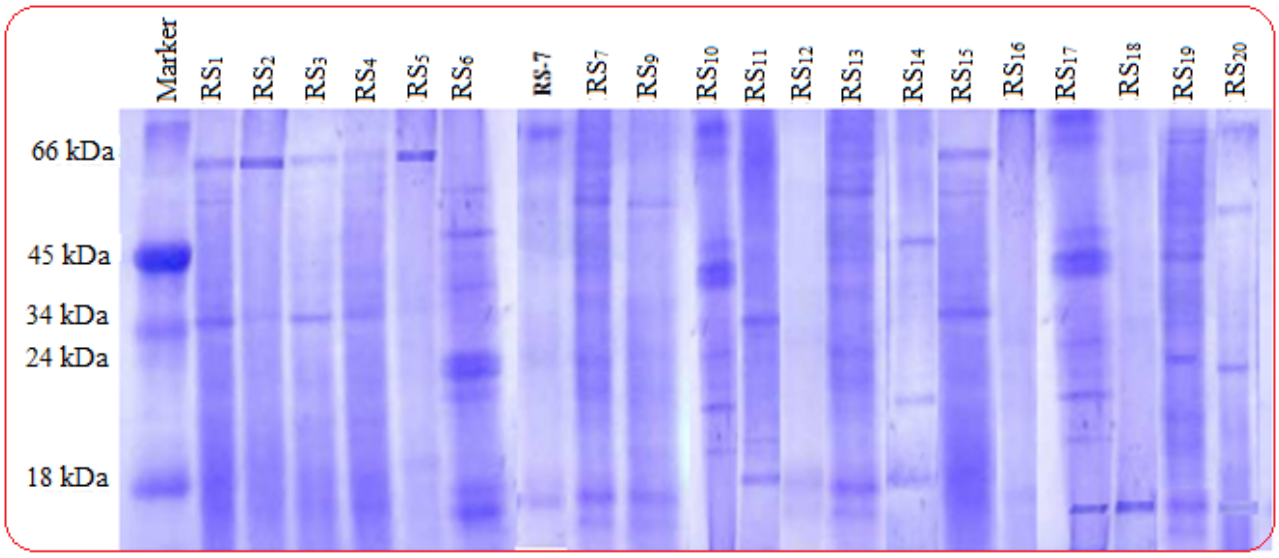

Figure 8. Electrophoregram of 20 isolates of *Rhizoctonia solani* showing polymorphic protein bands.

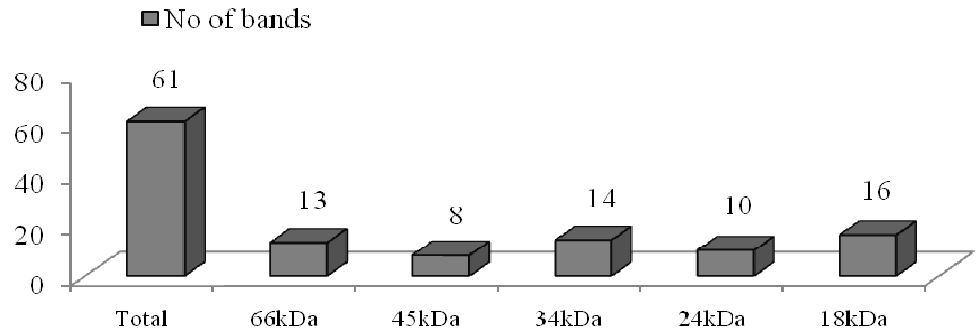

Figure 9. Number of protein bands present on the basis of molecular weight.

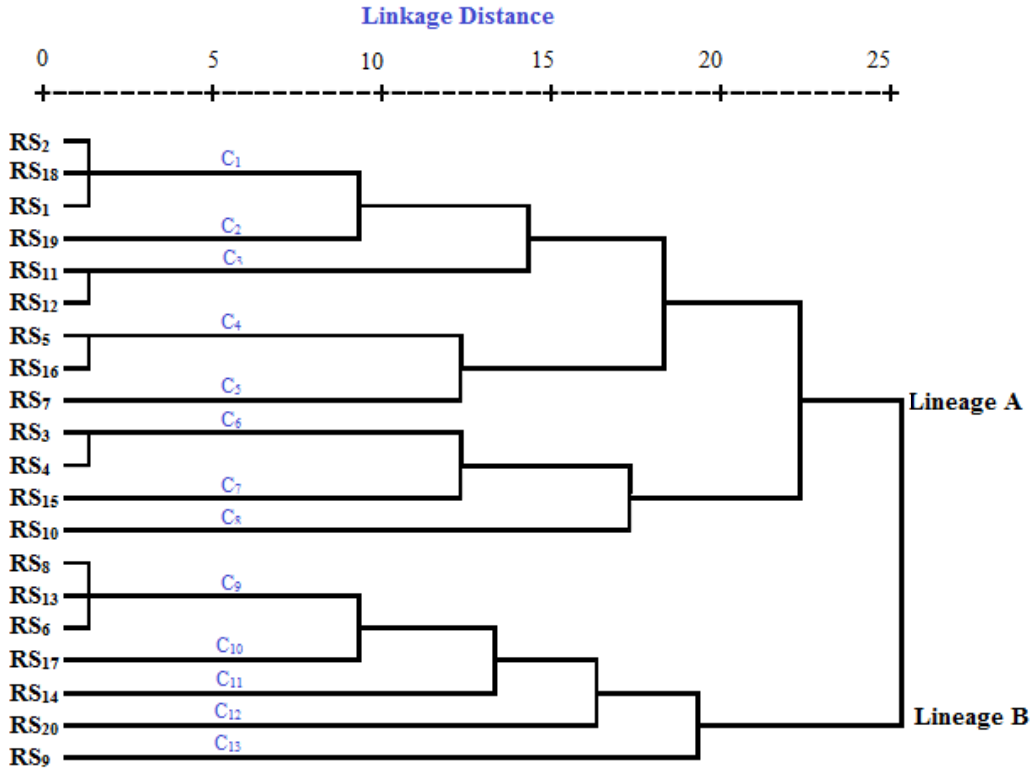

Figure 10. Dendrogram of twenty isolates of *Rhizoctonia solani* on the basis of SDS-PAGE.

Table 4. Cluster analysis of *R. solani* isolates based on SDS-PAGE electrophoresis.

	Cluster	Isolates		Cluster	Isolates
Lineage A	C_1	RS_2, RS_{18}, RS_1	Lineage B	C_9	RS_8, RS_{13}, RS_6
	C_2	RS_{19}		C_{10}	RS_{17}
	C_3	RS_{11}, RS_{12}		C_{11}	RS_{14}
	C_4	RS_5, RS_{16}		C_{12}	RS_{20}
	C_5	RS_7		C_{13}	RS_9
	C_6	RS_3, RS_4			
	C_7	RS_{15}			
	C_8	RS_{10}			

SDS-PAGE. This is because of the fact that all the genes are not expressed in a particular environment. However, variability among the isolates was observed. The result of the current study indicates that *R. solani* of mountainous region Gilgit-Baltistan is composed of pathotype and morphological variability and genetic diversity exists. More studies are needed by using different molecular techniques for understanding of diversity among the isolates.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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