JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. 7(3), p. 1789-1798

Molecular Characterization of Anastomosis Groups of *Rhizoctonia solani* Associated with Potato Tubers in Saudi Arabia

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(Received: 28 February 2013; accepted: 20 March 2013)

The study was initiated to collect potato-borne isolates of *Rhizoctonia solani* from different regions of Saudi Arabia and determine their anastomosis groups (AGs). Group categorization was done on the basis of hyphal fusion; and their pathogenicity and virulence behavior was also studied. An attempt was made to corroborate the observed anastomosis grouping with PCR analysis of rDNA-ITS and microsatellite regions. Twenty three isolates paired with the tester isolates representing six anastomosis groups: AG 2-1, AG 2-2, AG 3, AG 4, AG 5, and AG 8. Eleven isolates belonged to AG 3. Three belonged to AG 5; while two each belonged to AG 2-1, AG 2-2, AG 4HGI, and AG 4HGII. Only one isolate was found to be from AG 8. Many isolates from uni-, bi-, and multi-nucleate populations did not anastomose with any of the tester isolates and remained unidentified. These isolates may probably belong to AGs other than tested in this study and need to be further characterized. Ample variability was recorded in pathogenicity on potato cv. 'Amb-3' and in virulence on popular cultivars. Isolates belonging to AG3 were found to be most pathogenic and virulent. PCR analysis of rDNA-ITS and microsatellite regions showed genetic diversity in the isolates belonging to different anastomosis groups.

Key words: *Rhizoctonia solani*; Potato; Anastomosis groups; ITS; Microsatellite sequences.

Rhizoctonia solani is a fungal pathogen associated with several diseases in many crops including potato (*Solanum tuberosum*). Potato is one of the most important crops cultivated for human foods and trade worldwide[1]. Known strains of *R. solani* can be classified into 13 anastomosis groups (AGs), but the classification is not strictly demarcated because some bridging strains are able to anastomose with strains of at least two different AGs[2, 3]. The phenomenon of anastomosis (hyphal fusion) can also be applied to identify and categorize similar isolates of *R. solani* into different groups that are more homogeneous than the species as a whole. For instance, *R. solani* contains several isolates that vary from one another in terms of virulence, colony morphology, and other characteristics[4]. Some AGs in potato are found to have subsets that are identified on the basis of characteristics such as morphology, virulence, host range, molecular characteristics and DNA sequence[5, 6]. Subsets of AGs identified from potato isolates based on one of these criteria are not always identical to subsets identified from other AGs (*Raphanus sativus*, *Zea mays* etc) with the same criteria. Therefore, recognition of the subsets of an AG and the membership of each subset depends on a combination of criteria used for characterization.

In addition to hyphal interaction, biological characteristics of anostomosis groups like, vitamin requirement analysis[7], serological reaction[8], fatty acid analyses[9], isozyme analyses[10], total soluble protein pattern[11], and GC content analysis and DNA-DNA hybridization[12] have also been studied to characterize different isolates of *R. solani*.

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In previous studies, isolates of AGs 6, 9, 10 and BI were found to be weak pathogens in Saudi Arabian potato cultivars, whereas AG 3 isolates were more aggressive than the rest of the AGs[13]. Among these isolates, AG 6 was found to have much lower level of pathogenicity and virulence than AG 9.

The present study was initiated to determine whether isolates of *Rhizoctonia* spp. recovered from black scurf-affected potato tubers collected from fields and nurseries in Saudi Arabia are of the same AGs as those found elsewhere, or isolates of additional AGs exist in this region. An attempt was made to corroborate the observed anastomosis grouping with PCR analysis of rDNA-ITS and microsatellite sequences.

MATERIALS AND METHODS

Potato tubers of unknown cultivars with black scurf symptoms were collected from different potato-growing regions of Saudi Arabia and over eighty isolates of *R. solani* were recovered from these tubers.

For isolation of fungus, potato tubers with black scurf were washed in running tap water, surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 1 min, washed twice in sterile water, and blotted on paper towels. Scurf depositions were sliced from the tuber surface and were placed on water agar supplemented with 250 µg /ml chloramphenicol. Emerging hyphal tips were transferred to potato dextrose agar plates supplemented with 250µg/ml chloramphenicol as described by Ichielevich-Auster *et al.*[14]. Having been derived from potato, the isolates were numbered serially with prefix 'Rsp'. Water-soaked and air-dried wheat grains colonized with isolates were used for sustenance of inoculum, and were prepared as described by Sneh and Adams[15] and Sneh *et al.*[16].

Anastomosis group of isolates was determined on the basis of frequency of hyphal fusion reactions with tester isolates[4, 17, 18]. Tester strains representing 12 AGs of *R. solani* namely, AG 1, AG 2-1, AG 2-2, AG 3, AG 4HGI, AG 4HGII, AG 5, AG 6, AG 8, AG 9, AG 10, and AG 12 were procured from International Potato Center (CIP), Kafr El-Zayat, Egypt. To allow mycelial fusion, a lab isolate and a tester isolate were grown, one at each end of a PDA-coated slide. The slide was incubated in a 9cm petri dish, sealed with parafilm, at 25ºC in the dark for a minimum of 48hrs, until advancing mycelia met. The agar was then allowed to dry in a laminar flow cabinet for 2 or more hrs and mycelia were stained with lactophenol-trypan blue for observation under the microscope (Nikon, Dxm 1200). Nature of hyphal fusion was assigned to different categories according to the scheme of Carling *et al.*[19]:

C_0: No interaction between hyphae observed = Isolates belonging to different AGs.

C_1: Contact between hyphae, fusion of walls but not membranes, occasionally cells die = distantly related isolates or different AGs.

C_2: Obvious wall fusion, uncertain membrane fusion, diameter of anastomosis point less than that of hyphal diameter, anastomosing and adjacent cells always die = closely related isolates.

C_3: Cell wall and membrane fuse, diameter of anastomosis point equal to hyphal diameter, adjacent cells generally do not die = Isolates from the same AG or may be the same isolates.

AG 2-1 and AG 2-2 isolates were differentiated as described by [19]Carling et al. (1988), where unknown isolates were paired independently with tester isolates for both AG 2 sub-groups. When the C_3 reaction was observed in more than 50% interactions with one sub group (and C_1 or C_0 reactions with the other sub-group), the unknown isolate was assigned to that same AG. If C_2 fusion occurred with tester isolates of both sub-groups the isolate remained unclassified.

Isolates along with their AGs were further categorized as uninucleate, binucleate, or multinucleate. For nuclear counts, the isolates were grown on PDA at 25ºC for 3-4 days, after which small piece of mycelial mat was loosened in a drop of lactophenol on a microscopic slide and was stained with acetocarmine. The mounts were observed under the microscope and number of nuclei per cell was enumerated in each isolate.

Pathogenicity of the isolates, deemed as ability to cause infection, was assessed on plants of cv. 'Amb-3'. Sclerotial suspension of each isolate was inoculated onto five plants in a pot grown in greenhouse following the method of Balali et al.[20]. Three replicate were maintained per treatment and

same number of uninoculated plants were retained as controls. The potting mix comprised 2 parts of sandy loam soil and one part of leaf compost. The potting mix was fumigated with methyl bromide. Potato tubers were surface sterilized with 1% (w/v) sodium hypochlorite for 2 min, then cut into several pieces with at least one eye per piece, and left at room temperature over night to cure the cut surface. Five pieces were evenly placed at a depth of 3 cm in potting mix in each pot. The pots were kept in the green house at 25ºC in natural light and were watered to maintain appropriate moisture. After 80 days, frequency of plants showing symptoms of stem canker and black scurf was recorded as functions of pathogenicity. Means of frequency of infected plants in a sample were differentiated on the basis of standard deviations.

Virulence of the isolates, a function of plant-pathogen interaction, was quantified as severity of disease symptoms on the plant. In all 15 isolates namely, Rsp -1,- 2,- 3, -4 ,-5, -7, -8, -9, -11, -13, -14, -15, -18, -19, and -27b were tested against 9 cultivars: 'Atlas', 'Atlantic', 'Cara', 'Desiree', 'Hermes', 'Lady', 'Rosette', 'Spunta' and 'Valor' in permuted combinations. Disease reaction in three replicates comprising five plants in each cultivar was recorded as susceptible, highly susceptible, resistant, and highly resistant.

For extraction of DNA, isolates were grown in potato dextrose broth supplemented with 250µg /ml chloramphenicol on shaker for 10 days. Mycelium was collected by filtration and lyophilized to dryness. DNA was extracted using DNeasy plant mini kit (Qiagen) following the instructions of the manufacturer. The DNA was suspended in TE buffer and stored at 4ºC until use.

The rDNA-ITS segment (ribosomal ITS 1, ITS2 and 5.8 S gene) was amplified using primers ITS4 and ITS5 with sequences: 5′-TCCTCCGCTTATTGATATGC- 3′ and 5′-GGAAGTAAAAGTCGT AACAAGG-3′ respectively[21]. For SSR (microsatellite) analysis, primers CAG_5 (5′-CAGCAGCAGCAGCAG-3′) and AGG_5 (5′-AGGAGGAGGAGGAGG-3′) were used. Polymerase chain reaction was performed in 25µl volume in BioRad C1000 thermal cycler. The reaction mix consisted of 2.5 µl of 10x PCR buffer, 2.5 µl of 10 µM primers each, 0.5 µl of 10mM dNTPs, 1.0 µl of 1U μl^{-1} Taq DNA polymerase, 2.0 µl of 1ng μl^{-1} template DNA, 0.5 µl of 25 mM $MgCl_2$, and water to make 25 µl. Thermal parameters of the PCR were as follows: initial denaturation at 94ºC for 4 min followed by 30 cycles of 94ºC for 1 min, 52 ºC for 30 sec, 72ºC for 40 sec,; and a final extension at 72 ºC for 8 min. PCR-amplified r-DNA products were electrophoresed on 1.2% agarose gel and were visualized under UV in UVIBandmap (UVTec, Cambridge).

RESULTS AND DISCUSSION

Twenty three isolates paired with the tester isolates representing six anastomosis groups: AG 2-1, AG 2-2, AG 3, AG 4, AG 5, and AG 8 (Table-1). On the basis of paring reactions it was concluded that highest number of isolates, i.e. 11 belonged to AG 3. Three isolates belonged to AG 5; while two each belonged to AG 2-1, AG 2-2, AG 4HGI, and AG 4HGII (Table-3). Only one isolate was found to be from AG 8. Isolates Rsp-21, Rsp-23 and Rsp-26 did not grow properly and degenerated. Bridging reactions (C_1) were observed in several isolates falling in each category of nuclear number. On the other hand, many isolates from uni-, bi-, and multi-nucleate populations did not anastomose with any of the tester isolates and remained unidentified. These isolates may probably belong to AGs other than tested in this study and need to be further characterized. It is conspicuous that majority of the isolates were multinucleate and belonged to AG 3.

At least 14 anastomosis groups (AGs) have been reported[4, 18, 22], although the mechanisms of anastomosis behavior are not fully understood[7, 23]. The predominant isolates found to affect potato plants belong to anastomosis group AG 3[24, 25, 26]. Other studies have also indicated that AG 3 and AG 5 caused tuber symptom in India[27]. Previously, *R. solani* AG 7 was recorded on tubers in potato-growing areas in different countries[13, 28, 29].

In all self pairing experiments, the colonies merged and showed a perfect hyphal fusion in microscopic studies, indicating somatic compatibility (Table-2). This reaction was characterized visually by the presence of a 1-2mm wide demarcation line with sparse aerial hyphae between the confronting colonies; and verified microscopically by the killing reaction. Somatic incompatibility occurrence showed no distinct

Table 1. Hyphal interaction between recovered and tester isolates of *R. solani*

Tester isolates	Anastomosis behavior																							
	Pot 09	Pot 10	Pot 11	Pot 12	Pot 13	Pot 14	Pot 16	Pot 17	Pot 19	Pot 20	Pot 22	Pot 27b	Pot 30	Pot 38	Pot 48	Pot 57	Pot 60	Pot 64	Pot 65	Pot 71	Pot 78	Pot 81	Pot 85
AG 1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
AG 2-1	C0	C1	C0	C1	C1	C1	C0	C0	C2	C0	C0	C0	C3	C1	C1	C1	C3	C0	C1	C1	C0	C0	C2
AG 2-2	C1	C1	C2	C1	C2	C1	C1	C3	C3	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
AG 3	C0	C0	C3	C3	C0	C1	C1	C2	C2	C1	C2	C1	C1	C3	C3	C3	C0	C3	C3	C3	C3	C3	C3
AG 4HGI	C3	C3	C0	C0	C1	C0	C1	C1	C0	C0	C0	C0	C0	C1	C1	C1	C0	C0	C0	C0	C1	C1	C0
AG 4HGII	C0	C0	C1	C1	C1	C1	C0	C1	C0	C3	C3	C0	C1	C1	C0	C0	C0	C1	C0	C1	C0	C2	C0
AG 5	C1	C1	C1	C1	C3	C3	C3	C0	C0	C0	C0	C1	C1	C1	C1	C1	C1	C1	C1	C1	C0	C0	C0
AG 6	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
AG 8	C0	C0	C1	C2	C0	C0	C0	C0	C0	C1	C0	C3	C0	C0	C0	C1	C0	C1	C2	C2	C1	C0	C0
AG 9	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
AG 10	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
AG 12	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0

pattern, a clear demarcation line was produced in pairings between the parental isolate and its progeny lines.

Somatic incompatibility may vary in degree and its visual intensity is dependent in part on the medium used[30, 31]. For basidiomycetes, somatic compatibility is defined as 'rejection of genetically dissimilar secondary mycelia'[30]. For two isolates to be somatically compatible, all vegetative compatible loci, which are allelic in some basidiomycetes, must be the same. In a few cases for which there is evidence, somatic compatibility in basidiomycetes may be controlled by nuclear genes that are not linked to sexual compatibility loci[32, 33]. Differences at one locus may lead to incompatibility, but in most cases somatic incompatibility is more intense with greater genetic differences[33, 34].

Nuclear condition of isolates in different AGs is presented in Table-3. Among the uninucleate isolates, two each belonged to AGs 2-1 and 2-2; three isolates were from AG 5 and only one belonged to AG 8 (Table-3). All the four binucleate isolates belonged to AG 4, two each to AG 4HGI and 4HGII; while eleven multinucleate isolates were all from AG3. Isolates of *Rhizoctonia solani* have been previously divided into uninucleate, binucleate and multinucleate groups based on the number of nuclei per cell of young vegetative hyphae[35]. For a fungus to be recognized as *R. solani* it must possess a *T. cucumeris* perfect state. The other groups of *Rhizoctonia* isolates that are characterized by multinucleate cells are *R. zeae*, *R. oryzae* and a type culture *Waitea circinata var. circinata*[36], that all have teleomorphs in the genus *Waitea*. The anamorphic name of *W. circinata var. circinata* has not yet been assigned, but the teleomorphic name may be useful, although the teleomorph is rarely observed[36, 37]. *Rhizoctonia* isolates that are characterized by binucleate cells have a *Ceratobasidium* teleomorph[36].

Infection tests showed pathogenicity of all the 20 tested isolates manifesting in development of typical brown colored veins on the leaves and stems of the potato plants within 3-4 weeks after inoculation (Table-4). This shows that the sclerotia developed on to potato seed play major part in etiology of the disease.

AG 3 isolates proved to be more aggressive than the rest of the isolates. In our study

Table 2. Pairing behavior of the selected isolates of *R. solani**

Isolates	Rsp 30	Rsp 60	Rsp 57	Rsp 85	Rsp 81	Rsp 78	Rsp 38	Rsp 65	Rsp 64	Rsp 71	Rsp 48	Rsp 09	Rsp 10	Rsp 11	Rsp 12	Rsp 13	Rsp 14	Rsp 16	Rsp 17	Rsp 41
Rsp-30	C3	C2	C2	C2	C2	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
Rsp-60		C3	C2	C1	C2	C2	C0	C1	C0	C1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
Rsp-57			C3	C2	C1	C2	C1	C2	C2	C1	C0	C1	C0	C0	C0	C0	C0	C0	C0	C0
Rsp-85				C3	C2	C1	C2	C2	C1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C1
Rsp-81					C3	C2	C2	C1	C1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
Rsp-78						C3	C2	C2	C1	C2	C0	C0	C0	C0	C0	C0	C0	C0	C0	C1
Rsp-38							C3	C0	C0	C0	C1	C0	C0	C0	C0	C0	C0	C0	C0	C1
Rsp-65								C3	C2	C2	C3	C2	C2	C0	C0	C0	C0	C0	C0	C0
Rsp-64									C3	C2	C3	C2	C2	C2	C0	C0	C0	C0	C0	C1
Rsp-71										C3	C2	C3	C2	C2	C0	C0	C0	C0	C0	C1
Rsp-48											C3	C0	C0	C0	C0	C0	C0	C0	C0	C0
Rsp-09												C3	C2	C0	C0	C0	C0	C0	C0	C1
Rsp-10													C3	C1	C2	C0	C0	C0	C0	C0
Rsp-11														C3	C0	C0	C0	C0	C0	C1
Rsp-12															C3	C3	C2	C0	C0	C0
Rsp-13																C3	C3	C3	C3	C0
Rsp-14																	C3	C3	C3	C0
Rsp-16																		C3	C3	C0
Rsp-17																			C3	C0
Rsp-41																				C3

* C0 - C3: following Carling (1988)

AG 4 isolates were also found to be pathogenic to potato. AG 4 is known to be pathogenic to potato but it causes serious damage only in warm temperatures[38]. Both AG 5 and AG 3 attack the root, stems and stolons of potato; but AG 5 is less aggressive than AG 3[39]. This test also indicated that all isolates caused different levels of disease on potato cultivars tested. The highest disease severity mean on all potato cultivars was caused by AG 3 isolates followed by AG 4, AG 2 and AG 5 isolates. The highest percentage of infection for stem canker was found to be 31.8% and the highest percentage for black scurf was found to be 16.8%; whereas the lowest percentage was found to be 11.20% for stem canker and 5.3% for black scurf.

Infection could be induced with much lower concentrations of the inoculum than used in this study with pre-establishment of the pathogen

Table 3. Nuclear status of *R. solani* isolates belonging to different AGs

Nuclear condition	AG	Isolates
Uninucleate	AG 2-1	Rsp-30, Rsp-60
	AG 2-2	Rsp-17, Rsp-19
	AG 5	Rsp-13, Rsp-14, Rsp-16
	AG 8	Rsp-27b
Binucleate	AG 4 HGI	Rsp-9, Rsp-10
	AG 4HGII	Rsp-20, Rsp-22
Multinucleate		
2-4 nuclei	AG 3	Rsp-11, Rsp-12, Rsp-57, Rsp-78, Rsp-81, Rsp-85
2-5 nuclei		Rsp-48, Rsp-64
2-7 nuclei		Rsp-38, Rsp-71
2-8 nuclei		Rsp-65

Table 4. Pathogenicity of *R. solani* isolates on potato cv. 'Amb-3' plants under greenhouse conditions

Isolates	AGs	Frequency of infected plats (%)	
		Stem canker	Black scurf
Rsp-30	AG 2-1	15.50 ± 0.87	10.30 ± 0.00
Rsp-60	AG 2-1	18.40 ± 0.53	11.10 ± 1.15
Rsp-17	AG 2-2	17.00 ± 0.56	10.50 ± 0.69
Rsp-19	AG 2-2	16.90 ± 4.25	8.80 ± 0.17
Rsp-11	AG 3	13.30 ± 5.37	8.90 ± 0.69
Rsp-12	AG 3	20.80 ± 5.89	7.30 ± 0.20
Rsp-38	AG 3	16.30 ± 1.84	7.00 ± 0.00
Rsp-48	AG 3	31.80 ± 3.56	16.80 ± 2.70
Rsp-57	AG 3	21.2 ± 1.060	13.20 ± 0.10
Rsp-64	AG 3	18.50 ± 1.91	8.80 ± 0.26
Rsp-65	AG 3	11.60 ± 0.87	10.30 ± 0.44
Rsp-71	AG 3	23.50 ± 5.37	7.50 ± 0.26
Rsp-78	AG 3	20.30 ± 0.98	8.60 ± 0.20
Rsp-81	AG 3	16.60 ± 1.22	7.30 ± 0.44
Rsp-85	AG 3	20.10 ± 0.53	13.10 ± 0.10
Rsp-09	AG 4HGI	28.50 ± 1.91	13.70 ± 1.13
Rsp-10	AG 4HGI	16.90 ± 4.51	12.10 ± 0.20
Rsp-13	AG 5	13.20 ± 4.50	8.30 ± 0.26
Rsp-14	AG 5	11.20 ± 1.73	5.30 ± 1.30
Rsp-16	AG 5	23.70 ± 5.89	13.60 ± 0.61

in the planting mix[35, 40, 41]. However, without pre-conditioning of the soil, higher levels of the inoculum were required to produce disease symptoms[42, 43, 44].

In virulence tests almost all cultivars proved to be susceptible to isolates of AG 3, with conspicuous disease damage (Table 5). This indicates that these isolates were highly virulent on the tested cultivars. On the other hand, isolates of AG 4 produced weak disease response in a few combinations only, showing ample resistance of the cultivars or lower virulence of the fungal genotypes.

Differences in virulence have been widely used as phenotypic and genotypic markers[45, 46], but have not been fully investigated on all anastomosis groups of *R. solani*. Perhaps the most serious disadvantage of relying on virulence data to infer population structure is that genes involved in host-specificity represent a very small fraction of genes in the pathogen and may be subjected to strong selection by the host[47].

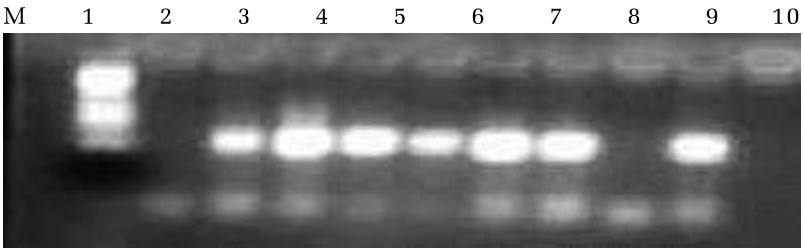
DNA amplification of selected isolates by using primers ITS1 and ITS4 is shown in Figure-1. Bright bands of approximately 250 bp represent the amplicons which show very little degree of polymorphism between the isolates of non-virulent AG 4 and moderately virulent AG5. When the same primers were used for amplifying more virulent isolates like, AG 3, AG 2-1, and AG 1, marker bands were generated in the size of around 200 bp (Fig.2). This indicates that virulent genotypes have common sequences among themselves; while non-virulent or less virulent strains have a different set of sequences. It is well known that 5.8s rDNA segment is highly conserved across AGs, whereas the ITS1 and ITS2 rDNA sequences show significant differences between AGs[48].

Using the microsatellite primers (CAG)5 and (AGG)5, polymorphism was generated between and within different isolates of *R. solani* (Fig. 3). AG 5 isolates are known to be moderately virulent[49]. Molecular approaches based on the analysis of ribosomal DNA (r DNA gene) sequences have added genetic support to the AG classification system and allowed the investigation of their evolutionary relationships[6]. Sequence data may support genetic groups within *Rhizoctonia* species better than other characters used in the past such as number of nuclei, plant host or morphology[5].

Table 5. Virulence of *R. solani* isolates on potato cultivars

Cultivar	Disease reaction* to isolates of different AGs														
	AG 3				AG 2-1			AG 5			AG 4				
	Rsp-4	Rsp-5	Rsp-8	Rsp-9	Rsp-3	Rsp-7	Rsp-1	Rsp-14	Rsp-2	Rsp-11	Rsp-13	Rsp-15	Rsp-18	Rsp-19	Rsp-27b
'Atlas'	S	R++	S	S	S	S	R	R	R	R	R	R	R	R	R
'Atlantic'	S	R	S	R	S	S	R	R	R	R	R	R	R	R	R
'Cara'	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R
'Desire'	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R
'Hermes'	S++	R	S	S	R	S	R	R	R	R	R	R	R	R	R+
'Lady'	S	R	S	S	R	R	R	R	R	R	R	S	S	R	R
''Rosette'	R	R	S	S	S	R	S	S	R	R	R	R	S	S	R+
'Spunta'	R	S	S	S	S	R	S	R	S	R	R	R	R	R	R
'Valor'	R	S	S	S	S	R	S	R	S	R	R	R	R	S	R

* S = susceptible; S++ = highly susceptible; R = resistant; R+ = highly resistant.



(M: marker; lanes 2, 3, 4 non-virulent AG 4; lanes 5, 6, 7, 9 moderately virulent AG 5)

Fig. 1. ITS-PCR of *R. solani* isolates using primers ITS1 and ITS4

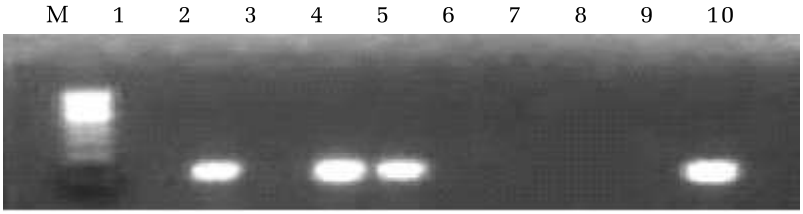


Fig. 2. ITS-PCR of *R. solani* isolates using primers ITS1 and ITS4 (lanes 2, 4, 5, 10 virulent isolates)

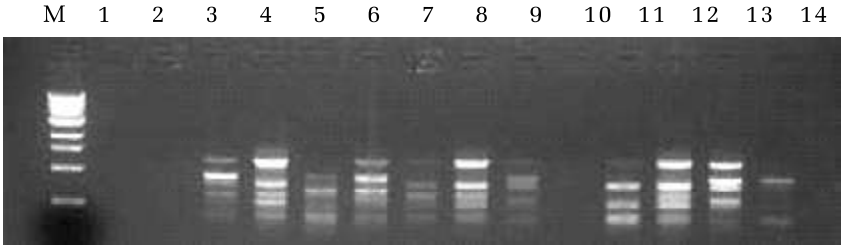


Fig. 3. Microsatellite-primed PCR of *R. solani* virulent isolates (AG 3) using primer AGG_5

Typically microsatellites display high mutation rate generating high levels of allelic diversity[50, 51]. The mutation rate of SSR markers varies depending on the locus, the length of the repeated motif, the species and sometimes the allele[52]. Microsatellites gain and lose repeat units by DNA-replication slippage and by proofreading errors during DNA replication. Both mechanisms primarily change the number of repeats and thus the length of the repeat string[50, 51, 52].

The study has brought to notice some AGs of *R. solani* other than those which anastomosed with the tester isolates; indicating the possibility of presence of some novel AGs in this region. There seems to be a need to design and test a wider range of primers for greater precision in identification of an isolate.

ACKNOWLEDGMENTS

This study was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

REFERENCES

1. DeFauw SL, He Z, Larkin RP, and Mansour SA Sustainable potato production and global food security. In: *Sustainable Potato Production: Global Case Studies* (He Z, Larkin R, and Honeycutt W eds). Springer Science + Business Media BV; 2012; 3-19.
2. Sharon M, Kuninaga S, Hyakumachi M, Naito S, and Sneh B., Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycosci* 2008; **49**: 93-114.

3. Carling DE, Kuninaga S, Brainard KA., Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among sunsets of *R. solani* anastomosis group 2 and AG BI. *Phytopathol.* 2002; **92**: 43-50.
4. Carling DE, Grouping in *Rhizoctonia solani* by the anastomosis reaction. In: *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh B, Jabaji-Hare S, Neate S, and Dijst G eds). Netherlands: Kluwer Academic Publishers; 1996; 37-47.
5. Gonzalez D, Cubeta MA, and Vilgalys R., Phylogenetic utility of indels within ribosomal DNA and ß-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. *Molecular Phylogenetics Evolution* 2006; **40**: 459-470.
6. Guillemaut C, Edel-Herman V, Camporota P, Alabouvette C, Richard-Molard M. and Steinberg C., Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. *Canadian Journal of Microbiology* 2003; **49**: 556-568.
7. Ogoshi A., Ecology and pathogenicity of anastomosis and intraspecific groups of *R. solani* Kuhn. *Phytopathol* 1987; **25**: 125-143.
8. Adams GC Jr. and Butler EE., Serological relationships among anastomosis groups of *R. solani*. *Phytopathol* 1979; **69**: 629-633.
9. Stevens JJ and Jones RK, Shew HD, and Carling DE., Characterization of populations of *Rhizoctonia solani* AG-3 from potato and tobacco. *Phytopathology* 1993; **83**: 854-858.
10. Reynolds M, Weinhold AR, and Moris TJ., Comparison of anastomosis groups of *Rhizoctonia solani* by polyacrylamide gel electrophoresis of soluble proteins. *Phytopathology* 1983; **73**: 903-906.
11. Liu ZL and Ge QX.,Studies on soluble protein patterns of *R. solani* by polyacrylamide gel electrophoresis. I. The influence of different source of isolates on protein patterns of anastomosis group. *Acta Mycol. Sin*. 1988; **7**: 175-182.
12. Kuninaga S and Yokosawa R., A comparison of DNA base compositions among anastomosis groups in *Rhizoctonia solani* Khn. *Ann. Phytopathol. Soc. Jpn*. 1980; **46**: 150-158.
13. Zakria M, Rauf CA, Minhas NM, and Khan MSA., Pathogenicity of *R. solani* isolates from potato. Integrated plant disease management. Proceedings of 3rd National Conference of Plant Pathology, NARC. Islamabad, Pakistan; 2002; 90-93.
14. Ichielevich-Auster M, Sneh B, Barash I, and Koltin Y., Pathogenicity, host specificity and anastomosis groups of *Rhizoctonia* spp. isolated from soils in Israel. *Phytoparasitica* 1985; **13**: 103-112.
15. Sneh B and Adams GC., Culture preservation methods for maintaining genetic integrity of *Rhizoctonia* spp isolates. In: *Rhizoctonia* sp. Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. (Sneh B, Neate S, and Dust G eds). Dordrecht: Kluwer Academic Publishers; 1996; 473-483.
16. Sneh B, Zeidan M, Ichielevich-Auster M, Barash I, and Koltin Y., Incresed growth responses induced by a non-pathogenic Rhizoctonia solani. *Canadian J. Botany* 1986; **64**: 2372-2378.
17. Martin FN., *Rhizoctonia* spp recovered from strawberry roots in central coastal California. *Phytopathology* 2000; **90**: 345-353.
18. Sneh B, Burpee L, and Ogoshi A (eds). Identification of *Rhizoctonia* species. St. Paul, MN: APS Press, 1991; pp. 133.
19. Carling DE, Kuninaga S, and Leiner RH., Relatedness within and among intraspecific groups of *R. solani* a comparison of grouping by anastomosis and by DNA hybridization. *Phytoparasitica* 1988; **16**: 209-210.
20. Balali GR, Neate SM, Scott ES, Whisson DL, and Wcks TJ., Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. *Plant Pathol.* 1995; **44**: 1050-1057.
21. White TJ, Bruns TD, Lee S, and Taylor JW., Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. (Innis MA, Gelfand DH, Sinsky JJ, and White TJ eds). New York: Academic Press; 1990; 315-322.
22. Carling DE, Baird RE, Kuninaga S, and Gitaitis RD., Characterization of anastomosis group (AG) 13 of *Rhizoctonia solani* (abstract). *Phytopathology* 1999; **89**: S11.
23. Anderson NA., The genetics and pathology of *R. solani*. *Ann. Rev. Phytopathol* 1982; **20**: 329-347.
24. Lehtonen MJ, Ahvenniemi P, Wilson PS, German-Kinnari M, and Valkonen JPT., Biological diversity of *Rhizoctonia solani* (AG-3) in a northern potato-cultivation environment in Finland. *Plant Pathol*. 2008; **57**: 141-151.
25. Carling DE and Leiner RH., Virulence of isolated *R. solani*. AG-3 collected from potato plant organs and soil. *Plant Dis* 1990; **74**: 901-903.
26. Ritchie F, McQuilken MP, and Bain RA., Effects of water potential on mycelial growth, sclerotial production, and germination of *Rhizoctonia solani* from potato. *Mycological Research* 2006; **110**: 725-733.

27. Thind TS and Aggarwal R., Characterization and pathogenic relationships of *R. solani* isolates in a potato-rice system and their sensitivity to fungicides. *J Phytopathol* 2008; **156**: 615-621.
28. Truter M and Wehner FC., Anastomosis grouping of *Rhizoctonia solani* associated with black scurf and stem canker of potato in South Africa. *Plant Dis* 2004; **88**: 83-98.
29. Carling DE, Brainard KA, Virgen-Calleros G, and Olalde-Portugal V., First report of *Rhizoctonia solani* AG-7 on potato in Mexico. *Plant Dis* 1998; **82**: 127.
30. Worrall JJ., Somatic incompatibility in basidiomycetes. *Mycologia* 1997; **89**: 24-36.
31. Yokoyama K and Ogoshi A., Studies on hyphal anastomosis of *Rhizoctonia solani*. Nutritional conditions for anastomosis. *Trans. Mycol. Soc. Japn.* 1988; **29**: 125-132.
32. Rizzo DM, Rentmeester RM, and Burdsall HH Jr., Sexuality and somatic incompatibility in *Phellinus gilvus*. *Mycologia* 1995; **87**: 805-820.
33. Hansen EM, Stenlid J, and Johansson M., Genetic control of somatic incompatibility in the root-rotting basidiomycete *Heterobasidion annosum*. *Mycolo Res* 1993; **97**: 1229-1233.
34. Malik M and Vilgalys R., Somatic incompatibility in fungi. In: Structure and Dynamics of Fungal Populations (Worral JJ ed). Dordrecht. The Netherlands: Kluwer Academic Publishers; 1999; 123-138.
35. Yang HA, Sivasithamparam K, Alemohammad J, Barton JE, and O' Brien PA., Association of *Rhizoctonia* strains with bare patch disease of wheat in Western Australia. *Plant Pathol* 1994; **43**: 878-884.
36. Carling DE and Sumner DR., *Rhizoctonia* Methods *for Research on Soil borne Phytopathogenic Fungi*. (Singleton LL, Mihail JD, and Rush CM eds). St. Paul, MN: Am. Phytopathological Society; 1992; 157-165.
37. Leiner RH and Carling DE., Characterization of *Waitea circinata* (*Rhizoctonia)* isolated from agricultural soils in Alaska. *Plant Dis* 1994; **78**: 385-8.
38. Anguize R and Martin C., Anastomosis groups, pathogenicity, and other characteristics of *Rhizoctonia solani* isolated from potato in Peru. *Plant Dis* 1989; **73**: 199-201.
39. Bandy BP, Leach SS, and Tavantzis SM., Anastomosis group 3 is the major cause of *Rhizoctonia* disease of potato in Maine. *Plant Dis* 1988; **72**: 596-598.
40. MacLeod WJ and Sweetingham MW., A root disease of *Lupinus angustifolius* caused by a new species of binucleate *Rhizoctonia*. *Aust J Agricul Res* 1997; **48**: 21-30.
41. Sweetingham MW, Cruickshank RH, and Wong DH., Pectic zymograms and taxonomy and pathogenicity of ceratobasidiaceae. *Trans. British Mycol. Soc.* 1986; **86**: 305-311.
42. Botha A, Denman S, Lamprecht SC, Mazzola M, and Crous PW., Characterisation and pathogenicity of *Rhizoctonia* isolates associated with black root rot of strawberries in the Western Cape province of South Africa. *Aust Plant Pathol* 2003; **32**: 195-201.
43. Demirci E., *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. *Plant Pathol* 1998; **47**:10-15.
44. Ogoshi A, Cook RJ, and Bassett EN., *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathol* 1990; **80**: 784-788.
45. Al-Kherb SM, Roelfs AP, and Groth JV., Diversity for virulence in asexually reproducing population of *Puccinia coronate*. *Can J Bot* 1987; **65**: 994-998.
46. Linde DC, Groth JV, and Roelfs AP., Comparison of isozyme and virulence diversity patterns in the bean rust fungus *Uromyces appendiculatus*. *Phytopathol* 1990; **80**: 141-147.
47. Leung H, Nelson RJ, and Leach JE., Population structure of plants pathogenic fungi and bacteria. *Plant Pathology* 1993; **10**: 157-250.
48. Fenille RC, Ciampi MB, Kuramae EE, and Souza NI., Identification of *Rhizoctonia solani* associated with soybean in Brazil by rDNA-ITS Sequences, *Fitopatologia Brasileira* 2003 **28**: 413-419.
49. Karaca *et al,* Karaca GH, Ozkocl and Erperl., Determination of the anastomosis grouping and virulence of *Rhizoctonia solani* Kuhn isolates associated with bean plants grown in Samsun, Turkey. *Pak J Biol Sci* 2002; **5**: 434-437.
50. Schlotterer C., Opinion: The evolution of molecular markers just a matter of fashion? *Nature Reviews Genetics* 2004; **5**: 63-69.
51. Karaoglu H, Lee CMY, and Meyer W., Survey of simple sequence repeats in completed fungal genomes. *Mol. Biol. Evol.* 2004; **22**: 639-649.
52. Benali S, Mohamed B, Eddine H, and Neema C., Advances of molecular markers application in plant pathology research. *Eur. Jour. Sci. Res*. 2011; **50**: 110-123.
53. Ellegren H., Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* 2004; **5**: 435-445.