Anastomosis Groups, Pathogenicity, and Other Characteristics of *Rhizoctonia solani* Isolated from Potatoes in Peru

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ABSTRACT

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Rhizoctonia solani isolates were recovered from stem lesions and sclerotia on tubers of potato plants at three agroecological zones of Peru: coastal valleys (150–270 m elevation), highland valleys (2,460–3,600 m elevation), and eastern slopes of the Andes (850 m elevation). Most *R. solani* isolates recovered belonged to anastomosis group (AG)-3 and AG-4, the first in the highlands (cool environment) and the second in the coast and eastern slopes of the Andes (warm environment). Pathogenicity of AG isolates was determined by planting true potato seed and tubers in soil infested with *R. solani* isolates at low (9–18 C) and high (18–24 C) temperatures. The highest percentage of damping-off occurred among seedlings in soil infested with AG-4 isolates at both temperature regimes. However, percent of seedling damping-off in soil infested with AG-4 isolates was approximately 50% higher at high temperature than at low temperature. A similar response was obtained with AG-3 isolates.

Additional keywords: *Solanum tuberosum*

Rhizoctonia stem canker and black scurf of tubers is caused by *Rhizoctonia solani* Kühn (asexual stage), a pathogen not only of potatoes but also of a large number of plant species around the world. On potatoes, *R. solani* retards or impedes plant emergence, damages older plants established in the field, and the tubers lose value at harvest (1). *R. solani* also causes extensive damage to potato seedlings grown from true potato seed (10,11). *Thanatephorus cucumeris* (Frank) Donk is the sexual stage of this pathogen. Single basidiospore progeny are considered to have low genetic variability. In contrast, the asexual stage (*R. solani*) is generally considered to have great genetic variability between pathogenicity groups (1,12). Several attempts have been made to group isolates of this pathogen taxonomically. So far, anastomosis of mycelia is the criterion most widely accepted and used to group isolates of this fungus (1,12,14). Nine anastomosis groups (AG) and two subgroups have been recognized and accepted so far (7,8,13). Studies have shown a host specificity in relation to anastomosis groups (i.e., AG-1 on leguminaceae and graminaceae, AG-2 on brassicae and cruciferae, and AG-3 on solanaceae). In potatoes, AG-3, AG-4, and AG-5 have been reported, although AG-3 is the most common (1,4–6,8,12,15,16). According to these observations, Ogoshi (13) has concluded that AGs are distributed worldwide, but their distribution is highly dependent on the crop cultivated in that area. In Peru, research on *R. solani* and on AG occurrence and distribution is scarce and only AG-4 has been reported on potatoes so far (10,11).

This paper reports the results of identification, characterization, and pathogenicity of some isolates of *R. solani* in potatoes collected from three important agroecological zones of Peru. A preliminary report has been published (2).

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MATERIALS AND METHODS

Collection, isolation, and identification. Isolates of *Rhizoctonia* were obtained from potato plants in different potato-growing areas of Peru, representing the three major agroecological zones of the country: irrigated valleys along the coastal desert, Andean highland valleys, and a tropical rain forest location (jungle). Along the coast, plant samples were collected at La Molina (Lima, 240 m elevation), at Cañete (200 km south of Lima, 200 m elevation), and at Tacna (1,300 km south of Lima, 200 m elevation). At La Molina, stem and root samples with Rhizoctonia lesions were collected, whereas at Cañete and Tacna only diseased stems were collected. Highland samples were collected at Huancayo in the Mantaro Valley from the International Potato Center's (CIP) experimental station (3,280 m elevation), at Cuzco (3,400 m elevation), and at Huanuco (2,450 m elevation). At the highland locations, samples consisted mostly of sclerotia collected from infested tubers, although stems were also collected at Huancayo. In those cases where sclerotia were present, 20 tubers were collected per location. At the jungle location, samples were collected from stems and roots of potato plants grown at CIP's experimental station at San Ramon (800 m elevation, eastern slopes of the Andes). At each location, 40–50 samples of plants showing Rhizoctonia stem or root lesions were collected. Stem and root sections with Rhizoctonia lesions were washed in tap water, disinfected in 1% sodium hypochlorite for 5 min, and rinsed three times in

distilled sterilized water. Small pieces (2–3 mm) of infected tissue were plated on acidified potato-dextrose agar (PDA) and kept at 23–25 C for 48 hr. Fungal colonies were then transferred to 2% water agar and observed with a dissecting microscope. Hyphal tips were sectioned and transferred to acidified PDA where they grew at 25 C for 10–14 days. These isolates were then stored at 4 C and served as parent cultures for further research experiments carried out in this study.

To identify the Rhizoctonia isolates that were *R. solani*, they were characterized individually as: a presence of multinucleate cells (3), branching near the distal septum of cells, constriction of the branch near the point of origin, and rapid growth in culture (the colony covers a petri dish in 2 days) (1,12,15). Ten isolates of *R. solani* per location and per plant part were sampled at random and used for anastomosis grouping.

Anastomosis group identification. Anastomosis was determined by the method described by Parmeter et al (14). Each isolate was paired with a representative of each of five available anastomosis group tester isolates (AG-1, AG-2, AG-3, AG-4, and AG-5). Hyphae and agar (3–6 mm diameter plugs) from tester and unknown isolates were placed 2 cm apart on a sterile microscope slide coated with a thin layer of 2% water agar. Anastomosis was determined microscopically (200×), without staining, after the cultures were incubated for 48–72 hr at 25 C. The fusion and death of anastomose cells were considered an anastomosis-positive reaction. Additionally, isolates were plated on Stewart's medium to identify those belonging to AG-3, according to the method proposed by Castro et al (9).

Mycelial growth at different temperatures. Ten isolate sets from four selected locations (La Molina, AG-4; Cañete, AG-4; San Ramon, AG-4; and Huancayo, AG-3) were plated on PDA and kept at 20, 25, and 28 C for 7 days. Isolates were initially grown in PDA for 7 days and then 5-mm-diameter plugs were removed and placed in the center of a 9-cm petri plate. Colony growth was recorded every 24 hr and 10 days after plating. Other colony characteristics, such as color and appearance of the mycelial mat, were also recorded. Three replicate plates at each temperature regime per location were evaluated. The experiment was repeated twice.

Pathogenicity tests. Pathogenicity of *R. solani* isolates from Huancayo, La Molina, and San Ramon was compared. Ten isolates per location were selected at random, representing five isolates obtained from stems and roots and five isolates obtained from sclerotia. For these comparative studies, 1 kg of pasteurized mixture of sandy loam soil/peat moss (1:2, v/v) was amended with 7 g of dried wheat kernels colonized with each of the isolates. The same pasteurized mixture without colonized wheat kernels was used as an uninoculated control.

Percent of seedling damping-off was calculated 30 days after 100 true potato seed of clone DTO-33 were sown in a plastic tray containing soil infested with each of the isolates. Tubers of cultivar Revolucion were planted in the same infested soil as before, and 90–100 days later roots were collected, washed, and the percentage area with Rhizoctonia lesions was recorded. A preliminary pathogenicity test was conducted following the same procedure as indicated above to determine the most virulent isolates. Five isolates from each location that produced the highest percentage of seedling damping-off were selected for these two experiments. Both experiments had three replications and were carried out in a screenhouse at La Molina (18–24 C) and at CIP's facilities in Huancayo (9–18 C). A factorial experiment in a completely random design was used for data analysis.

Table 1. Anastomosis groups (AG) of *Rhizoctonia solani* isolated from different potato plant parts at seven locations in Peru[a]

Location	Elevation (m)	Plant part	Anastomosis group		
			AG-3	AG-4	AG unknown
Huanuco	2,450	Tuber[b]	10/10[c]	0/10	0/10
Huancayo	3,280	Stem	4/10	0/10	6/10
		Tuber	10/10	0/10	0/10
Cuzco	3,400	Tuber	10/10	0/10	0/10
Cañete	200	Stem	0/10	5/10	5/10
Tacna	200	Stem	0/10	10/10	0/10
La Molina	240	Stem	0/10	5/10	5/10
		Root	0/10	6/10	4/10
San Ramon	800	Stem	0/10	8/10	2/10
		Root	0/10	6/10	4/10

[a] Isolates tested against AG-1 through AG-5 tester isolates.
[b] Sclerotia on tubers.
[c] Number of isolates identified in respective anastomosis groups over total number of isolates tested.

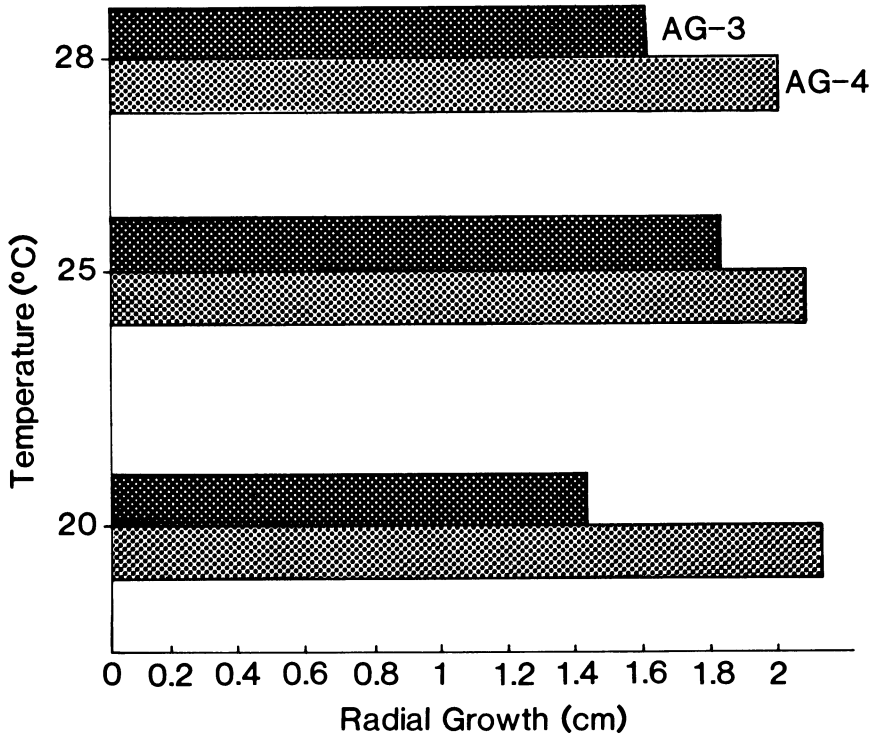

Fig. 1. Daily radial growth of *Rhizoctonia solani* AG-3 and AG-4 isolates from potato on PDA plates at three different temperatures (average of 4 days).

RESULTS

Forty-eight hours after plating tissue sections, *Rhizoctonia* colonies were clearly distinguished from other colonies, when present. Only *R. solani* was identified from among the more than 220 isolates studied. No binucleate or Rhizoctonialike isolates were found. Most *R. solani* isolates belonged to AG-3 and AG-4. However, approximately 26% of the isolates did not anastomose with the available AG tester cultures (Table 1).

All AG-3 isolates tested on Stewart's medium were brown after 6–8 days at 28 C. In contrast, AG-4 isolates were consistently white and developed much faster than AG-3 isolates. Undetermined AGs were all white on Stewart's medium.

Isolates of *R. solani* from stem lesions and from sclerotia on tubers obtained in the highlands (Cusco, Huancayo, Huanuco) were identified as AG-3. AG-3 was more readily isolated from sclerotia than from stem lesions. *R. solani* isolates from stem and root lesions collected at the lower elevations, either from the coastal valleys (Cañete, Tacna, La Molina) or from the jungle at San Ramon, were identified as AG-4. Sclerotia were never observed on tubers of potato crops sampled from these lower elevations.

Mycelial growth on PDA was faster among the AG-4 isolates than among the AG-3 isolates at all three temperatures (Fig. 1). Growth rate at 20 C of the AG-4 isolates was 2 cm/day compared with 1.6 cm/day for AG-3. After 4 days, the growth rate at 25 C was 2.0–2.1 cm/day for AG-4 and 1.8 cm/day for AG-3. Growth rate at 28 C was 2.1 cm/day for AG-4 compared with 1.4 cm/day for AG-3. The optimum temperature for the development of the AG-3 isolates was between 20 and 25 C as compared with 25–28 C for the AG-4 isolates. Large numbers of sclerotia were produced by AG-3 isolates at 20 and 25 C, whereas only a few sclerotia were produced at 28 C, and they took much longer. The AG-4 isolates produced only a few sclerotia at 25 C, and they took over 12 days to develop. Significant differences in seedling survival were observed among Huancayo, La Molina, and San Ramon isolates under La Molina conditions (Table 2). The lowest seedling survival was obtained from La Molina AG-4 isolates (22.8%), followed by San Ramon AG-4 (37.3%) and Huancayo AG-3 isolates (44.3%). The same trend in response was observed for the percent of root area affected where the highest value was obtained from La Molina AG-4 isolates (28.3%) and the lowest value from Huancayo AG-3 isolates (13.0%). Seedling survival under Huancayo conditions (9–18 C), including the control seedlings, was generally lower than those obtained under La Molina conditions (18–24 C) (Table 2). This indicates that temperature also affects seedling survival. Inoculation with Huancayo AG-3 isolates and La Molina AG-4 isolates resulted in a lower seedling survival and a lower percent of root area affected than inoculation with the San Ramon AG-4 isolates under Huancayo conditions.

Table 2. Percent of seedling survival of true potato seed of clone DTO-33 (considering the survival of uninoculated seedlings as 100%) and percent of root area affected by *Rhizoctonia solani* isolates from Huancayo (AG-3), and from La Molina and San Ramon (AG-4)[a]

	Testing site			
	La Molina		Huancayo	
Source of isolates[b]	Seedling survival[c] (%)	Root area affected[d] (%)	Seedling survival (%)	Root area affected (%)
Huancayo (AG-3)	44.3	13.0	57.0	12.7
La Molina (AG-4)	22.8	28.3	60.1	12.4
San Ramon (AG-4)	37.3	26.3	64.8	15.0
LSD (0.05)	1.7	9.7	4.5	2.0+

[a] Test conducted at La Molina (18–24 C, 240 m elevation) and Huancayo (9–18 C, 3,280 m elevation).
[b] Average of 10 isolates of each anastomosis group per location.
[c] Average of three replications and 100 true potato seed per replication.
[d] Average of three replications and five plants per replication.

DISCUSSION

Most *R. solani* isolates recovered from infected potato plants or tubers belonged to either AG-3 or AG-4. The geographical distribution of these two AGs are quite distinct. AG-3 isolates are found at high elevation and a cool environment and AG-4 isolates are found at a lower elevation and a warm environment (Table 1). Temperature effect may have influenced the agroecological distribution on PDA medium. AG-3 growth is optimum between 20 and 25 C, compared with 25–28 C for AG-4 (Fig. 1). Isolates of AG-3 developed satisfactorily at 25 C, and after 10 days under these conditions they produced a large number of sclerotia and dark brown mycelium. In the field, sclerotia were only observed on tubers of plants grown in the cooler environments. Similar results were obtained by Sherwood (15), who found that the optimum temperature for AG-3 development was between 20 and 24 C, while AG-4 isolates reached their optimum growth at a temperature between 25 and 28 C without sclerotia formation. Similar results were presented by Bolkan and Ribeiro in 1985 (5).

Approximately 2% of the isolates studied do not belong to anastomosis groups 1–5. They did not anastomose with the type isolates, they were white in the Stewart's medium, and they were not pathogenic. Additional testing is needed to determine the taxonomic and pathological relationships.

Isolates of *R. solani* of both AG-3 and AG-4 were pathogenic to seedlings grown from true potato seed, although pathogenicity of AG isolates varied according to location (Table 2). In general, AG-4 isolates were more pathogenic than AG-3 isolates. This difference was at a warm lowland site (La Molina) rather than in a cool upland (Huancayo) environment.

Pathogenicity of AG-4 on potatoes has not been fully studied. However, these studies add information about the role of AG-4 in causing disease in potatoes to earlier general reports (4,10,16). The ability of AG-4 isolates to cause damping-off on potato seedlings (70% of seedling mortality) and lesions on roots (28% of affected root area) was demonstrated.

LITERATURE CITED

1. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
2. Anguiz, R., and Martin, C. 1986. Identification of anastomosis groups (AG) of *Rhizoctonia solani* of potato from three agroecological zones of Peru (Abstr.) Fitopatologia 21:3.
3. Bandoni, R. J. 1979. Safranin O as a rapid nuclear stain for fungi. Mycologia 71:873-874.
4. Bandy, B. P., Zanzinger, D. H., and Tavantzis, S. M. 1984. Isolation of anastomosis group 5 of *Rhizoctonia solani* from potato field soils in Maine. Phytopathology 74:1220-1224.
5. Bolkan, H. A., and Ribeiro, W. R. C. 1985. Anastomosis groups and pathogenicity of *Rhizoctonia solani* isolates from Brazil. Plant Dis. 69:599-601.
6. Carling, D. E., Kebler, K. M., and Leiner, R. H. 1986. Interactions between *Rhizoctonia solani* AG-3 and 27 plant species. Plant Dis. 70:577-578.
7. Carling, D. E., and Leiner, R. H. 1986. Isolation and characterization of *Rhizoctonia solani* and binucleate *R. solani*-like fungi from aerial stems and subterranean organs of potato plants. Phytopathology 76:725-729.
8. Carling, D. E., Leiner, R. H., and Kebler, K. M. 1986. Characterization of *Rhizoctonia solani* and binucleate rhizoctonia-like fungi collected from Alaskan soils with varied crop histories. Can. J. Plant Pathol. 8:305-310.
9. Castro, C., Davis, J. R., and Wiese, M. V. 1983. Differential medium for identification of *Rhizoctonia solani* AG-3. Plant Dis. 67:1069-1071.
10. International Potato Center. 1983. Annual Report CIP, 1982. Lima, Peru. 148 pp.
11. Martin, C., and Torres, H. 1986. Fungicides for the control of *Rhizoctonia solani* damping-off in seedlings derived from true potato seed. Fitopatologia 21:74-80.
12. Ogoshi, A. 1985. Anastomosis and intraspecific groups of *Rhizoctonia solani* and binucleate Rhizoctonia. Fitopatol. Bras. 10:371-390.
13. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.
14. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
15. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups of *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
16. Suresh, K., and Mall, S. 1982. Anastomosis groups of potato isolates of *Rhizoctonia solani* in India. Mycologia 74:337-338.