Differentiation of Two Anastomosis Groups of *Rhizoctonia solani* by Isozyme Analysis

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This research was supported in part by NSERC operating grants to the second and third authors.

We thank Jean Bousquet for critically reviewing a previous draft of the manuscript and A. Goulet for his technical assistance.

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Accepted for publication 29 July 1992.

ABSTRACT

Laroche, J. P., Jabaji-Hare, S. H., and Charest, P.-M. 1992. Differentiation of two anastomosis groups of *Rhizoctonia solani* by isozyme analysis. Phytopathology 82:1387-1393.

Isozyme electrophoresis was applied to study the genetic relationship between anastomosis groups (AGs) 3 and 9 of *Rhizoctonia solani*. The banding patterns of seven enzymes were determined by protein polyacrylamide gel electrophoresis. A total of 76 phenotypes were detected for all the enzymes. Diaphorase gave the most polymorphic banding patterns, followed by esterase and malate dehydrogenase. On the basis of principal component analysis and cluster analysis, the isolates were subsequently divided into two dissimilar and genetically distant groups (I and II). These groups are in agreement with previous anastomosis groups 3 and 9. Group I represented all isolates belonging to AG-9. Group II represented all isolates belonging to AG-3 and was subdivided into three subgroups (IIA, IIB, and IIC). Subgroup IIA was made up of two isolates originating from Canada; IIB represented all isolates originating from Japan and Britain; and IIC represented the majority of the North American isolates. No specific relation was found between isozyme phenotype and biogeographical origins of AG-9 isolates. The isozyme results indicate that the anastomosis grouping concept is genetically based.

Additional keywords: genetic relatedness, multivariate analysis, potato, Rhizoctonia disease.

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk) is a destructive pathogen with an almost unlimited host range, causing damping-off of seedlings; root, crown and stem rots; and sheath blight (2,36). The *R. solani* complex is a taxonomic entity composed of morphologically similar groups that share the following characteristics: multinucleate cells with dolipores, production of sclerotia, and lack of conidia (41). The identification of *R. solani* isolates is based on the ability of their hyphae to anastomose (35,40) when grown on solid media. Three different anastomosis reactions can occur: complete, intermediate, and no reaction (11). On the basis of these reactions, 11 anastomosis groups (AGs) are now recognized (14,36,37). Each AG appears to have different physiological characteristics and is genetically isolated and diverse (2,22,36,48).

The markers often used to study pathogen diversity are virulence, restriction fragment length polymorphisms (RFLPs), and isozymes. Differences in virulence have been widely used as phenotypic and genotypic markers (1,25) but have not been fully investigated in all anastomosis groups of *R. solani* (3,7,42). Isozymes and RFLPs in the mitochondrial and ribosomal genomes have been used increasingly in the study of fungal pathogen diversity and in addressing taxonomic problems (8,10,20,27,39). In the *R. solani* complex, RFLPs of ribosomal DNA revealed a high degree of intergroup variation in some AGs, supporting the evidence that the AG concept is genetically based (22,48).

Polyacrylamide and starch gel electrophoresis of enzymes, as detected by specific stains, have been widely used to classify organisms at various taxonomic levels (30). The techniques rely on the existence of genetic polymorphisms resulting in amino acid substitutions that cause differences in relative mobilities among allelic forms on an electrophoretic gel (24). Apart from few studies of electrophoresis of soluble proteins (43), pectic and esterase enzymes (28,34,50), and biochemical studies involving detection of enzyme activity (33), isozyme analysis has not been extensively applied to the study of genetic relationships of *R. solani*. Recently, Liu and co-workers (26) clearly demonstrated by isozyme analysis that AG-2 isolates of *R. solani* were differentiated into three distinct groups that were congruent with those determined by anastomosis (AG-2-1, AG-2-2 IIB, and AG-2-2 IV).

Black scurf, or Rhizoctonia disease, is known to occur wherever potatoes are grown and is an important disease of potatoes in Québec, Canada (6). The etiological agents for this disease are generally isolates of *R. solani* belonging to AG-3 (4,11,16), although AG-1, AG-2-1, AG-2-2, AG-4, and AG-5 isolates have been occasionally isolated from various parts of the potato plant as well as from the soils in which potatoes were recently cultivated (4,5,15,16). In addition to the above mentioned AGs, a newly designated anastomosis group, AG-9, which is indigenous to the soils of Alaska, has been found to induce lesions on stems and stolons of field-grown potato plants (13,14). To date, in Québec, *R. solani* isolated from infested tubers with sclerotia have been identified as belonging only to AG-3 (38). In this study, we examine the genetic relationships and diversity among and within isolates belonging to AG-3 and AG-9, using isozyme phenotypes. Our interest in these groups is justified by the importance of AG-3 isolates as the principal causal agent of Rhizoctonia disease of potato and the recent association of AG-9 isolates with potato plants (13).

MATERIALS AND METHODS

Storage and culture of *R. solani*. A total of 48 *R. solani* isolates belonging to anastomosis groups (AG) 3 and 9 are presented in Table 1. All of AG-9 isolates were from Alaska, whereas those of AG-3 were isolated from different geographical regions. Within AG-9, isolates BS24, KHP15, S21, and V12M are thiamine prototrophs, and isolates S9R1 and 86-1 are thiamine auxotrophs (11). All *R. solani* isolates were cultured on potato-dextrose agar for 5 days in the dark at room temperature. To obtain fresh mycelium for enzyme extraction, two agar culture plugs (7 mm in diameter) per isolate were transferred to an Erlenmeyer flask (250 ml) containing 100 ml of potato-dextrose broth. Isolates were grown without agitation at room temperature for 7 days. For long-term storage and maintenance, 1-wk-old culture plugs

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were transferred to freezing ampules, covered with 10% sterile glycerol, and stored in liquid nitrogen.

Sample preparation and protein extraction. Mycelial mats were collected by filtration through filter paper (Whatman No.1) and ground in liquid nitrogen to a fine powder. The frozen fungal powder (1.5 ml) was mixed with 500 μl of extraction buffer (1 M Tris-HCl, pH 6.8, supplemented with 6 mM cysteine, 6 mM ascorbic acid, 1 mM EDTA, and 10% glycerol [v/v] supplemented with 0.1% β-mercaptoethanol and 1 μl of the protease inhibitor mix). The protease inhibitor mix consisted of 5.0 μg/ml each of pepstatin A, antipain, and leupeptin and 1.0 mM phenylmethylsulfonyl fluoride. Samples were vortexed and centrifuged at 13,000 *g* in a Beckmann microfuge for 15 min. The precipitate was discarded, and the supernatant was transferred into a new microfuge tube and centrifuged at 100,000 *g* in a Beckmann Airfuge for 15 min. Protein extracts were kept at −80 C until further use.

Electrophoresis. The seven systems used in this study (Table 2) were resolved on 7.5% polyacrylamide gel electrophoresis at 4 C according to Davis (19), and their activities were revealed according to well-established staining protocols (17). The α-esterase activity was detected with the protocol of Harris and Hopkinson (21). Gels were dried between Bio-Gel wrap TM cellulose membranes (Bio-Design, Canton, MA). Reference isolates were loaded on each gel, and the relative mobility (R_m) values were calculated to determine the position of the bands for all isolates. Two separate electrophoresis runs were performed for all isolates. No intra-isolate variation was detected.

TABLE 1. Anatomosis group, origin, and host of 48 *Rhizoctonia solani* isolates used for isozyme analysis

Isolate designation	Anastomosis group	Origin	Source[a]	Host
M33	3	Alaska	1	Potato
BS29	3	Alaska	1	Beet seed
KHP30	3	Alaska	1	Soil
DP216	3	Alaska	1	Soil
F101	3	Alaska	1	Soil
L67	3	Alaska	1	Potato
B37	3	Alaska	1	Soil
SD39	3	Alaska	1	Unknown
SCL8	3	Alaska	1	Potato
SCL65	3	Alaska	1	Potato
SCL28	3	Alaska	1	Potato
L13	3	Alaska	1	Potato
L71	3	Alaska	1	Potato
BS24	9	Alaska	1	Beet seed
BS35	9	Alaska	1	Beet seed
KHP15	9	Alaska	1	Soil
KHP26	9	Alaska	1	Soil
M6	9	Alaska	1	Potato
S9R1	9	Alaska	1	Beet seed
S21	9	Alaska	1	Unknown
V12M	9	Alaska	1	Potato
86-1	9	Alaska	1	Lettuce root
1-6B-9	3	Washington	2	Unknown
ST9	3	Japan	3	Unknown
ST41	3	Japan	3	Unknown
QSU-02-8	3	Québec	4	Potato
QSU-07-6	3	Québec	4	Potato
QSU-04-2	3	Québec	4	Potato
QSU-04-8	3	Québec	4	Potato
QN-04-1	3	Québec	4	Potato
PSB-01-7	3	Prince Edward Island	4	Potato
PSB-03-8	3	Prince Edward Island	4	Potato
PSB-08-5	3	Prince Edward Island	4	Potato
ORB-10-2	3	Ontario	4	Potato
ORB-06-9	3	Ontario	4	Potato
ORB-03-9	3	Ontario	4	Potato
ORB-02-3	3	Ontario	4	Potato
ORB-01-12	3	Ontario	4	Potato
PSU-10-7	3	Prince Edward Island	4	Potato
AN-04-1	3	Alberta	4	Potato
AN-02-5	3	Alberta	4	Potato
AN-06-12	3	Alberta	4	Potato
AN-05-7	3	Alberta	4	Potato
NK-01-4	3	New Brunswick	4	Potato
NK-04-7	3	New Brunswick	4	Potato
NK-03-10	3	New Brunswick	4	Potato
RH-76	3	Great Britain	5	Unknown
100-T	3	Japan	5	Unknown

[a] 1 = D. Carling, University of Alaska, Agricultural and Forestry Experiment Station, Palmer; 2 = J. Cook, USDA, Root Disease and Biological Control Research Unit, Pullman, Washington; 3 = A. Ogoshi, Faculty of Agriculture, Hokkaido University, Kita-Ku, Sapporo, Japan; 4 = B. Otrysko, Station de Recherche Les Buissons, Québec, Canada; and 5 = L. L. Burpee, Department of Plant Pathology, University of Georgia, Griffin.

TABLE 2. Enzyme systems, enzyme commission (EC) numbers, and subunit numbers used for phenotypic analysis of *Rhizoctonia solani*

Enzyme[a]	EC number	Subunit numbers[b]
α-Esterase (EST)	3.1.1.1	1
Diaphorase (DIA)	1.6.4.3	1
Fumarase (FUM)	4.2.1.2	4
Hexokinase (HK)	2.7.1.1	1
Leucine aminopeptidase (LAP)	3.4.11.1	1
Malate dehydrogenase (MDH)	1.1.1.37	2
Mannose-6-phosphate isomerase (MPI)	5.3.1.8	1

[a] Staining recipes for polyacrylamide gel electrophoresis are according to the protocols of Cheliak and Pital (17), except for α-esterase (21).
[b] After Harris and Hopkinson (21) and Darnall and Klotz (18).

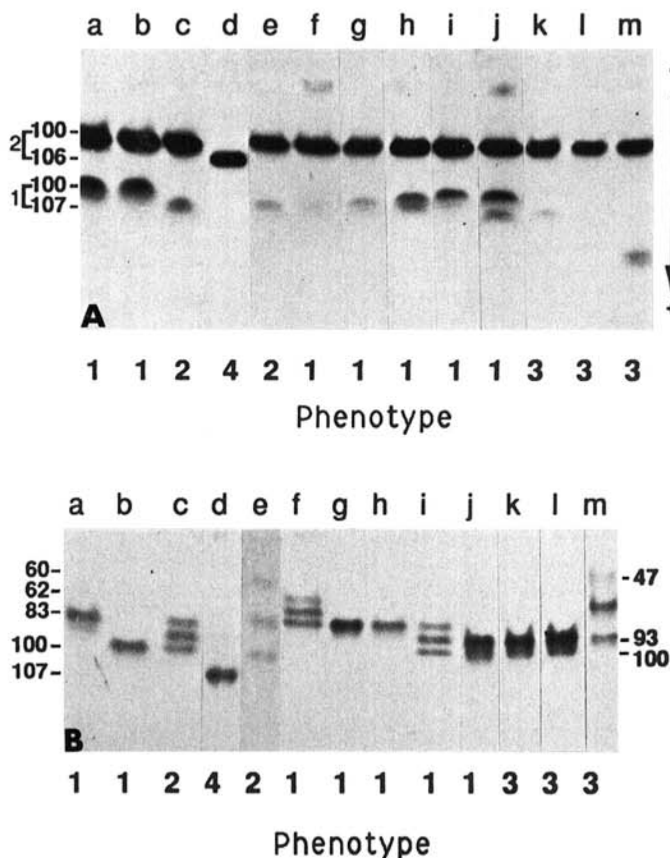

Fig. 1. Example for scoring isozyme gels. Scores were determined by relative enzyme mobility in the electric field. **A,** Hexokinase. Letters represent isolate designation: a = SCL28; b = L13; c = L17; d = 1-6B-9; e = QSU-02-8; f = QSU-07-6; g = PSB-08-5; h = ST9; i = 100-T; j = RH-76; k = M6; l = S9R1; m = S21. **B,** Malate dehydrogenase. Letters represent isolate designation: a = BS29; b = KHP30; c = DP216; d = 1-6B-9; e = ORB-03-9; f = QSU-02-8; g = ST9; h = 100-T; i = RH-76; j = KHP26; k = M6; l = S9R1; m = S21.

Because the teleomorph of *R. solani* does not form readily in pure culture, and no crosses between monokaryotic isolates were attempted, the genetic basis of the isozyme bands, or genotype, cannot be determined. Therefore, we chose to take a conservative approach to the interpretation of the observed isozyme phenotypes by assuming that mobility differences, or electromorphs, are allelic variants within a given isozyme locus.

Genic nomenclature. The enzyme nomenclature was that used by Yeh and Layton (49) and May et al (29). Abbreviations with capitalized letters represent enzymes (e.g., FUM). Presumed loci were recorded, an integer value of 1 was assigned for the most anodal enzyme locus, and successive cathodal loci were designated loci 2 and 3, respectively. The most common allele was given the arbitrary value of 100, and the other alleles of the same locus were numbered according to their relative migration to the 100-allele (Fig. 1). Isozyme loci were interpreted in accordance with known subunit composition of the enzymes (18).

Data analysis. To present the data structure and relationship among individual isolates, principal component analysis (PCA) and cluster analysis were carried out. For the PCA, the SAS program based on the variance-covariance matrix was used. Prior to the analysis, the allozyme of each isolate was coded according to its genotype, using the algorithm of Smouse and Neel (44). The percent contribution of each locus was calculated according to the method of Bousquet et al (9). For the cluster analysis, a matrix of Jaccard's similarity coefficients was generated from the presence or absence of isozyme bands (45). The unweighted pair-group method with arithmetic averages was performed on the matrix of Jaccard's similarity coefficients using the "R" software (46).

RESULTS

Seven enzymes were selected and resolved for each of the 48 isolates examined. Banding patterns of the enzymes did not vary in replicate experiments. All enzymes tested behaved as monomers, except for MDH, which revealed a dimeric, three-banded pattern. Phenotypic banding patterns or electromorphs were identified on the basis of relative position of isozymes and interpreted in terms of presumed alleles of the genetic loci coding for the isozymes. From three to 31 distinct electromorphs occurred per enzyme, with a total of 76 for all enzymes (Table 3 and Fig. 2). The esterase (EST) and diaphorase (DIA) produced complex banding patterns that were interpreted as each being coded by four loci (Fig. 2). Presumed null alleles were common among certain isolates.

α-Esterase (α-EST). Some variation was noted in EST patterns among isolates within a group (Fig. 2), and a total of 19 phenotypes had been detected (Table 3). The isolates showed a minimum of three bands per phenotype (Fig. 2). Four putative loci with 14 alleles that were variable between groups were identified (Fig. 2). The three most anodal alleles of locus 1 (95,97,100) were detected in AG-3 isolates, and the two cathodal alleles were observed in AG-9 isolates. Locus 2 was particular to AG-9 isolates, with heterozygosity detected in isolates BS35 (phenotype 7 and presumed genotype 100/105) and S21 (phenotype 10 and presumed genotype 96/100). Locus 3 had four alleles, one of which (allele 83) was specific to AG-9 isolates. The remaining alleles were shared among AG-3 isolates, with all of the Alaskan isolates being homozygous at the most common allele (100/100). The most common allele (100) of locus 4 was observed only in AG-3, except for isolate 1-6B-9 (presumed null allele). Allele 30 of the same locus was detected in all AG-9 isolates.

Diaphorase (DIA). When DIA activity was analyzed, each of the isolates had several isozymes that were variable in both mobility and number (Fig. 2). A total of 31 phenotypes were detected (Table 3). The isolates showed one, two, or multiple bands (up to six) (Fig. 2). Four zones of enzymatic activity that were interpreted as the products of four loci were observed consistently (Fig. 2). Loci 1 and 2 were observed only in the majority of AG-3 isolates, whereas loci 3 and 4 were common among isolates of both groups.

Fumarase (FUM). Each isolate was characterized by a single band pattern of FUM, and three different electromorphs were identified (Fig. 2). AG-3 isolates were characterized by having phenotype 1, and all those belonging to AG-9 expressed phenotype 2. Isolate 1-6B-9 (AG-3) was separated from all other isolates of AG-3 by the expression of a unique third phenotype (Table 3). No fumarase activity was detected in ORB-03-9 (AG-3).

Leucine aminopeptidase (LAP). Three phenotypes were observed in this enzyme. From these, phenotypes 1 and 2 were the most frequent and were shared among the majority of isolates of both groups (Table 3). Phenotype 2 had two bands, and those isolates expressing this phenotype were interpreted as heterozygous genotypes (106/100) (Fig. 2). Phenotype 3 was detected only in B37 and PSU-10-7 (AG-3) and in S9R1 and V12M (AG-9).

Hexokinase (HK). Four phenotypes or electromorphs were

TABLE 3. Distribution of phenotypes by isolates of *Rhizoctonia solani*[a]

Isolation designation	Enzyme						
	α-EST[b]	DIA	MDH	FUM	LAP	HK	MPI
M33	1	1	1	1	1	1	1
BS29	2	2	2	1	1	2	1
KHP30	2	3	3	1	1	1	1
DP216	2	4	1	1	2	1	1
F101	2	5	1	1	1	2	1
L67	3	6	3	1	2	1	1
B37	4	5	1	1	3	2	2
SD39	5	4	1	1	2	1	2
SCL8	2	6	1	1	2	2	1
SCL65	2	5	1	1	1	1	1
SCL28	2	4	3	1	2	1	1
L13	2	7	1	1	2	1	1
L71	2	8	3	1	1	2	1
BS24	6	9	4	2	1	3	3
BS35	7	10	4	2	1	3	4
KHP15	8	11	5	2	2	3	5
KHP26	9	12	4	2	2	3	6
M6	7	11	4	2	2	3	5
S9R1	8	13	4	2	3	3	...[c]
S21	10	14	5	2	2	3	5
V12M	8	15	4	2	3	3	5
86-1	8	16	4	2	1	3	5
1-6B-9	11	17	6	3	1	4	7
ST9	12	18	2	1	1	1	1
ST41	13	18	2	1	2	1	1
QSU-02-8	14	19	7	1	2	2	1
QSU-07-6	15	1	1	1	2	1	1
QSU-04-2	15	20	1	1	2	1	1
QSU-04-8	15	20	1	1	2	1	1
QN-04-1	15	21	1	1	2	1	1
PSB-01-7	15	21	1	1	2	3	1
PSB-03-8	15	22	2	1	2	2	1
PSB-08-5	16	23	3	1	2	1	1
ORB-10-2	17	24	1	1	2	2	...
ORB-06-9	15	25	1	1	2	1	1
ORB-03-9	15	26	8	...	2	1	1
ORB-02-3	17	27	1	1	2	1	...
ORB-01-12	18	28	3	1	2	1	1
PSU-10-7	16	21	1	1	3	1	...
AN-04-1	15	21	1	1	1	1	1
AN-02-5	15	29	2	1	2	1	8
AN-06-12	15	3	2	1	2	1	8
AN-05-7	15	5	1	1	1	1	1
NK-01-4	2	21	3	1	1	1	1
NK-04-7	2	30	3	1	1	1	1
NK-03-10	2	18	3	1	2	2	...
RH-76	19	31	1	1	2	1	...
100-T	13	18	2	1	2	1	1
Total	19	31	8	3	3	4	8

[a] Phenotype number represents that depicted in Figure 2.
[b] α-EST = α-esterase, DIA = diaphorase, MDH = malate dehydrogenase, FUM = fumarase, LAP = leucine aminopeptidase, HK = hexokinase, MPI = mannose-6-phosphate isomerase.
[c] Phenotype not observed.

identified (Fig. 2). A double band pattern was detected for phenotypes 1 and 2 (Fig. 1) All AG-3 isolates, except for isolates 1-6B-9 (from Washington) and PSB-01-7 (from Prince Edward Island), exhibited phenotypes 1 and 2 (Table 3). Electromorph 3 was specific for AG-9 isolates. Phenotype 4 was detected only in isolate 1-6B-9.

Malate dehydrogenase (MDH). Phenotype 1 was the most common phenotype detected in AG-3 isolates (Table 3). The rest of AG-3 isolates exhibited either phenotype 2 or 3. Isolates QSU-02-8 and ORB-03-9 showed phenotypes 7 (presumed genotype 62/83) and 8 (presumed genotype 60/100) respectively (Fig. 1). Only isolate 1-6B-9 exhibited phenotype 6 and was assumed to be homozygous for the putative allele 107 (Table 3). Phenotypes 4 and 5 were specific for AG-9 isolates. Phenotype 5 (presumed genotype 47/93) was observed in only two isolates of AG-9 isolates (KHP15 and S21), and the rest of AG-9 isolates were assumed to be homozygous for allele 93.

Mannose-6-phosphate isomerase (MPI). Five different phenotypes (1, 2, 5, 6, and 8) were characterized by a single band pattern, whereas phenotypes 3, 4 and 7 had a double band pattern (Fig. 2). The majority of AG-3 isolates expressed phenotype 1 and were presumed to be homozygous for allele 100. Phenotype 2 was detected in isolates B37 and SD39, phenotype 7 was specific to isolate 1-6B-9, and phenotype 8 was specific to AN-02-05 and AN-06-12 (Table 3). Phenotypes 3, 4, 5, and 6 were only detected in AG-9 isolates. Heterozygosity was detected in some isolates of both AGs. These were isolate 1-6B-9 (phenotype 7), with putative alleles 103/105, and isolates BS24 (phenotype 3) and BS35 (phenotype 4), with putative alleles 69/76 and 76/87, respectively. Presumed null alleles were common in certain isolates of each group (Table 3).

The pattern of genetic variation generated by cluster analysis was further demonstrated by PCA of putative polymorphic loci (Fig. 3). A total of 50.4% of the total variance was attributed to the first two principal components. Component I accounted for 41.9% of the variation, and component II accounted for 8.5%. Component I was heavily weighted by α-Est-4, Fum-1, α-Est-3, α-Est-1, Dia-3, and Mpi-1, and clearly separated the isolates of AG-3 from those of AG-9 (Fig. 3). Component II was dominated by Lap-1, Dia-4, α-Est-1, Dia-2, and α-Est-3. This component separated the Japanese and British AG-3 isolates and isolate 1-6B-9 (Washington) from the rest.

The calculation of a similarity matrix, with a cophenetic correlation coefficient of 0.96, allowed for the construction of a dendrogram that summarized the phenetic relationships among the 48 isolates (Fig. 4). Two major groups (I and II) were formed, and they join at 56% similarity. Each cluster represents isolates from a different AG. Group I is made up of all AG-9 isolates clustering at 84%, and all isolates of AG-3, except two, formed group II at a similarity of 79%. The two isolates (1-6B-9 and ORB-01-12), being the most dissimilar, joined with group II at 68 and 73%, respectively. In general, within group I there were no recognizable trends reflecting the relationship of geographical origin of isolates or thiamine requirement of certain isolates to isozyme subgrouping. Group II could be split into three subgroups, which will be referred to as subgroups IIA (isolates QSU-02-8 and PSB-03-8, which were grouped at 89% similarity), IIB (the Japanese isolates ST9, ST41, 100-T, and the British isolate RH-76, which were grouped at 85% similarity) and IIC (the rest of AG-3 isolates that originate from North America, which were grouped at 83% similarity). Subgroups IIA and IIB were first linked to each other at 81% before they joined subgroup IIC at 79%. Within subgroup IIC, two Québec isolates, QSU-04-2 and QSU-04-8, were grouped at 100% similarity.

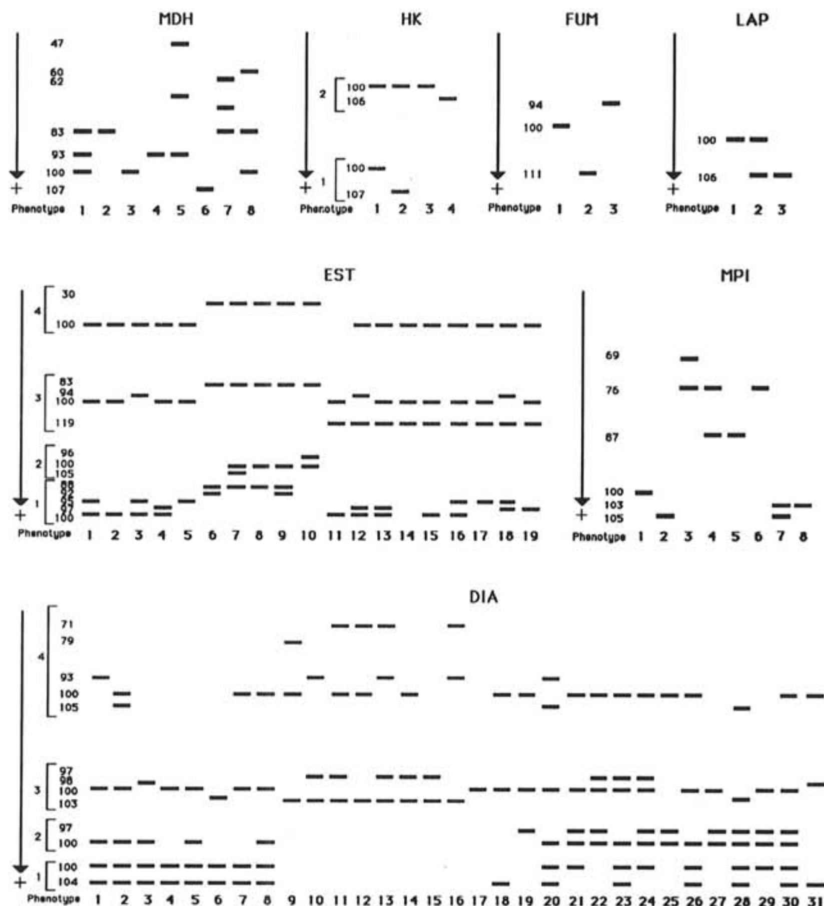

Fig. 2. Electrophoretic phenotypes of seven enzymes with interpretable genetic bases for 14 presumed loci. Phenotypic groups are indicated by numbers from 1 to 31. Numbers at left represent presumed loci and alleles. Alleles are represented by relative distance from the protein coded by the most common allele (designated as 100). Direction of migration is toward the anode (+).

DISCUSSION

Isozyme electrophoresis was used to elucidate genetic relationships between two AGs of *R. solani* that are associated with potato (12,38). The isolates used in this study were collected by several investigators and do not represent a methodical attempt to generate a collection of isolates representative of this species. However, in selecting the 48 isolates used, we attempted to represent both anastomosis groups of *R. solani* that are associated with potato and collected over a wide geographic range.

The seven enzyme systems were selected because of their high resolution and reproducibility on polyacrylamide gel electrophoresis. Phenotypic banding patterns were identified and interpreted in terms of presumed alleles of the genetic loci coding for the isozymes. Although we have considered the absence of bands in certain isolates as the expression of presumed null alleles, it would be necessary to perform crosses experiments to determine whether null alleles or other alternative mechanisms are involved. Micales and co-workers (30,31) had listed some plausible explanations that may account for the absence of bands. These are 1) low levels of detection of certain enzymes, 2) denaturation of enzymes during sample preparations, 3) lack of expression of inducible enzymes in culture medium, and 4) inability of isolates to produce certain enzymes, because of different selection pressures that they face in culture medium.

The presence of multiple bands for certain enzymes might represent the expression of mixed genomes. However, multiple bands can also be explained by the expression of different loci coding for the same enzyme and the production of secondary isozymes (21,30). In this study, multiple bands were observed for α-EST, DIA (monomers), and MDH (dimer). Some of the additional bands were probably the product of posttranslational processes, or the existence of different transformational states of the enzyme (21). However, others are likely the expression of alleles at different loci, since they were almost always found in different regions of the gel or, in the case of MDH, are the expression of heteromeric bands caused by the random combination of polypeptide chains to produce hybrid enzyme molecules.

The cluster analysis derived from the electrophoretic data summarized the intraspecific genetic variation among the 48 isolates and demonstrated that two genetically distinct groups, designated I and II, were formed. These groups were in agreement with previous groupings of AG-3 and AG-9 isolates, based on studies of anastomosis and pathogenicity tests (14,38). Although the isolates of both groups have been isolated from the same host species or from soils in which potatoes have been cultivated, our results reconfirm what has been previously documented, that anastomosis groups are genetically isolated, and that each represents a noninterbreeding population (2,11). In addition, the results of the present study are consistent with our recent knowledge of the genetics of *R. solani* (22,23,47,48). On the basis of DNA/DNA hybridizations and RFLPs, these investigators have also suggested that several groups exist which are congruent with those determined by anastomosis, as has also been suggested by the results of this study. A positive correlation between RFLPs and isozyme electrophoresis has also been demonstrated with other plant pathogenic fungi, such as *Phytophthora cryptogea* and *P. drechsleri* (32).

Compared to cluster analysis, PCA provided more information about the contribution of each locus to the genetic variation of all the isolates. Certain loci of some enzymes (α-EST, DIA, FUM, LAP, and MPI) tested were more responsible for the genetic variation among and within groups, since they exhibited the greatest dissimilarity between AG-3 and AG-9. Considering this and the fact that EST and DIA were the most polymorphic enzymes in the two groups, it is not surprising that component I represented much greater variation within the two groups. Genetic variation in AG-2 of *R. solani* has also been demonstrated by isozyme analysis (26).

In the case of group II (AG-3 isolates), attempts to correlate intragroup variation within the North American isolates to their geographical origins were unsuccessful. To our knowledge, there is no restriction on the movement of potatoes infested with AG-3 sclerotia across North America. This is clearly demonstrated in the dendrogram (Fig. 4); the Canadian and American isolates (subgroup IIC) were grouped together and were genetically distant from those originating from Asia and Europe (subgroup IIB).

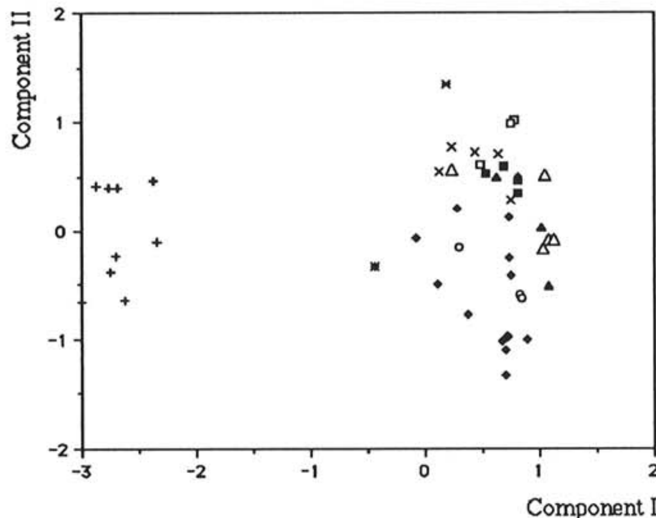

Fig. 3. Principal component analysis scatterplot of 48 isolates of *Rhizoctonia solani* belonging to AG-3 and AG-9. Symbols used for AG-3 isolates are: Alaska, ◆ ; Japan, □; Québec, △; Prince Edward Island, ■; Alberta, ▲; New Brunswick, ○; Ontario, ×; Great Britain, ⋇; Washington, ✱. Symbol used for AG-9 isolates: +.

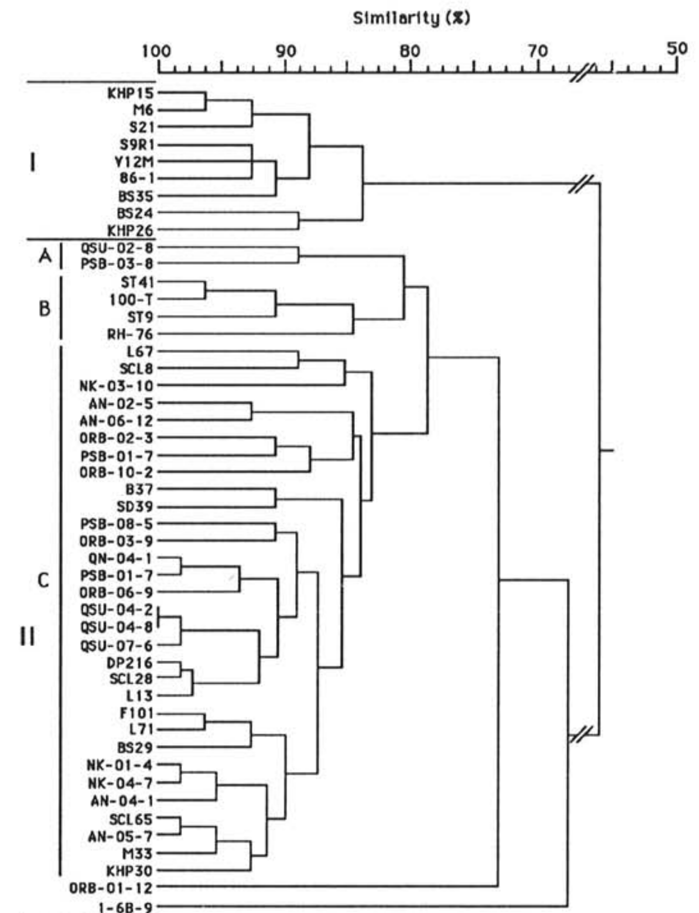

Fig. 4. Dendrogram based on isozyme analysis showing the groupings of 48 isolates of *Rhizoctonia solani*. The dendrogram was constructed using the unweighted pair-group method with arithmetic averages with Jaccard's similarity values.

Of interest was the complete relatedness found between isolates QSU-04-2 and QSU-04-8, indicating that these could be the same. This is justified by the evidence that both isolates were obtained from two different sclerotia found on the same potato tuber (38).

Our results also demonstrated that isolates 1-6B-9 (Washington) and ORB-01-12 (Ontario) were genetically less related to group II, which is represented by all of AG-3 isolates. It is not clear why these particular isolates are grouped at a lower level of similarity. They might have been accidentally misidentified and do not belong to their respective AG.

Carling and Kuninaga (11) defined AG-9 as a heterogeneous group and subdivided it into two subgroups (AG-9 TP and AG-9 TX) on the basis of their thiamine requirement as well as on DNA–DNA reassociation studies. In this study, a relationship between thiamine requirement of AG-9 isolates and isozyme electrophoretic data was not observed. It is not known why isozymes did not relate closely to subgroups of AG-9. The level of diversity detected with isozymes is a function of the number of loci detected and the size of population sampled. It is likely that a different level of diversity could have been detected within AG-9 if the total isolate sample was larger. Nevertheless, our results agree with the RFLP studies of Jabaji-Hare et al (21) and Vilgalys and Gonzalez (48), who did not observe a relationship between RFLP patterns in AG-9 isolates and their thiamine requirement. It is apparent from this study and from the work of Liu et al (26) that isozyme polymorphisms can be used with confidence for the differentiation of certain AGs of *R. solani*.

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