Tropical Plant Pathology, vol. 33, 1, 020-026 (2008)

www.sbfito.com.br

RESEARCH ARTICLE / *ARTIGO*

Characterization of powdery mildews strains from soybean, bean, sunflower, and weeds in Brazil using rDNA-ITS sequences

Álvaro M.R. Almeida[1], Eliseu Binneck[1], Fernanda F. Piuga[2], Silvana R.R. Marin[1], Paula R.Z. Ribeiro do Valle[3] & Cesar A. Silveira[1]

[1]Embrapa Soja, Cx. Postal 231, 86001-970, Londrina, PR, Brazil; [2]Departamento de Farmácia e Bioquímica, Universidade Norte do Paraná, Londrina, PR, Brazil; [3]Departamento de Química, Universidade Estadual de Londrina UEL, 86051-990, Londrina, PR, Brazil

Corresponding author: Álvaro M.R. Almeida, e-mail: amra@cnpso.embrapa.br

ABSTRACT

Soybean powdery mildew (*Erysiphe diffusa*) was considered a minor disease in Brazil in the decades immediately after its identification. However, since the outbreak in 1996/97 in all cultivated areas the disease has become a constant threat to farmers and losses of up to 25% have been reported. The report of a new species, *E. glycines*, infecting soybean in Japan, and the occurrence of the disease in other plant species (*Phaseolus vulgaris*, *Helianthus annuus*, *Sonchus oleraceus*, *Hypochaeris brasiliensis,* and *Bidens pilosa*) commonly found growing nearby soybean fields, raised questions in relation to the taxonomy of the powdery mildew strains found in or around soybean fields in Brazil. Analysis of the internal transcribed sequence (ITS) of the rDNA was undertaken to ascertain the pathogen species associated to each of the hosts. Powdery mildew strains isolated from *Glycine max* were identified as *E. diffusa*. Strains from *P. vulgaris* were very similar to *E. diffusa,* with 4 nt differences, and differed from *Erysiphe poligony* by 11 nt. Strains from *H. annuus* and *S. oleraceus* grouped with the species *Golovinomyces cichoracearum,* while strains from *H. brasiliensis* and *B. pilosa* were similar to *Podosphaera fusca* and *Neoerysiphe cumminsiana*, respectively. To our knowledge this is the first molecular identification of powdery mildew in Brazil based on rDNA sequence comparison. In addition, this study presented evidence for the occurrence of *N. cumminsiana* in America.
Keywords: PCR, sequencing.

RESUMO

Caracterização de isolados de oídio de soja, feijão, girassol e plantas daninhas no Brasil usando sequências de rDNA-ITS

O oídio da soja, causado por *Erysiphe diffusa*, foi considerado uma doença de menor importância, desde a sua identificação no Brasil. Entretanto, após o surto na safra de 1996/97, em todas as áreas cultivadas, a doença tornou-se uma ameaça constante para os agricultores, com perdas relatadas de até 25%. A identificação de uma nova espécie (*E. glycines*) no Japão e a ocorrência em outras espécies de plantas comumente encontradas próximas a campos de soja (*Phaseolus vulgaris*, *Helianthus annuus*, *Sonchus oleraceus*, *Hypochaeris brasiliensis* e *Bidens pilosa*) levantou questões em relação à taxonomia dos oídios encontrados nas lavouras. Análises moleculares da região espaçadora interna transcrita (ITS) do rDNA foi utilizada para a identificação molecular das espécies presentes em cada planta hospedeira. Oídios isolados de soja (*Glycine max*) foram identificados como *E. diffusa*. Isolados de feijão (*P. vulgaris*) foram similares a *E. diffusa*, com 4 nt diferentes, e diferiram de *E. poligony* em 11 nt. Isolados de *H. annuus* e *S. oleraceus* agruparam-se com a espécie *Golovinomyces cichoracearum*, enquanto isolados de *H. brasiliensis* e *B. pilosa* foram similares a *Podosphaera fusca* e *Neoerysiphe cumminsiana*, respectivamente. Ao nosso conhecimento esta é a primeira identificação molecular de oídios no Brasil, através da comparação de seqüências de rDNA. Esta também é uma evidência da ocorrência de *N. cumminsiana* na América.
Palavras-chaves: PCR, sequenciamento.

INTRODUCTION

Powdery mildew is an obligate plant parasite that is very common in cultivated crops such as soybean, sunflower and bean, and also on weeds growing in or around cropped fields. Some species are host-specific, while others can infect a wide range of plant species. Soybean powdery mildew was first observed in Germany in 1921 (Wahl, 1921) and is currently distributed worldwide (Grau, 1984). In Brazil, the disease was considered of secondary importance (Ferreira *et al.,* 1979). However, an outbreak in the 1996/97 growing season and the countrywide occurrence of the disease resulted in high losses in susceptible cultivars, such as 'BR-16', the most widely grown variety in Brazil at that time. Currently, the disease is not as severe as in 1996/97. A similar fact was reported for soybean powdery mildew epidemics in USA (Grau, 1984). Nevertheless, the disease continues to be a threat and requires usage of resistant cultivars.

Powdery mildew is commonly found in crops such as bean, soybean and sunflower, as well as in weeds such as *Sonchus oleraceus* L., *Hypochaeris brasiliensis* (Less) Griseb, and *Bidens pilosa* L. The main questions that triggered this study were: 1- which species were infecting those plants; 2- which species was really infecting soybean, since the report by Takamatsu *et al.* (2002) describes the occurrence of two species on this crop.

The present work was undertaken to determine whether strains of powdery mildew from soybean and other crops and weeds growing close to the commercial fields in Brazil were similar to either of the groups previously identified in Japan (Takamatsu *et al.,* 2002), by comparison of nucleotide sequences of the internal transcribed spacer region (ITS) DNA.

MATERIAL AND METHODS

Sample sources and DNA extraction. Infected leaves with dense mycelia were collected from soybean (*Glycine max* L. Merr.), sunflower (*Helianthus annuus* L.), common bean (*Phaseolus vulgaris* L.), *Sonchus oleraceus* L., *Hypochaeris brasiliensis* (Less) Griseb., and *Bidens pilosa* L. at different locations in Brazil (Table 1) and used to infect the original hosts cultivated in greenhouse. Only ten out of 65 soybean strains, randomly chosen from among the strains from each collection region, were used in this work. Mycelia and conidia were transferred to healthy leaves with a small brush and the inoculated plants kept inside a PVC transparent cage to avoid contamination. A single colony from each pathosystem was used for successive inoculations. The new colonies were scraped from the leaf surface with a small brush and water and used for DNA extraction. After centrifugation, the pellet containing mycelia and conidia was crushed on liquid nitrogen and treated with CTAB extraction buffer (50mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA, 2% hexadecyltrimethyl-ammonium bromide-CTAB), followed by phenol/chloroform purification, precipitated with ethanol and stored at -80ºC (Almeida *et al*., 2003). DNA concentration was determined through spectrophotometer and stored at –20ºC.

PCR of rDNA ITS region and sequencing. The nuclear rDNA region including the internal transcribed spacer regions (ITS 1, 5.8S rRNA gene and ITS 2) was amplified with primers ITS5 and ITS4 (White *et al.,* 1990) in a Perkin Elmer 9600 thermocycler. Amplification reactions were performed in 50 µL-volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM each deoxynucleoside triphosphate, 0.5 µM each primer (ITS5 and ITS4), 10 ng of genomic DNA and 2.5 U Taq DNA polymerase. Temperature parameters were 94º C for DNA denaturation, 3 min for the first cycle and 1 min for the remaining cycles, 45º C for 1 min for primer annealing, and 72º C for 2 min for primer extension with a total of 35 cycles. Amplified products were analyzed by electrophoresis in 1.2% agarose gel and visualized after staining with ethidium bromide. The amplified fragment was excised from the gel and cloned using TOPO TA kit (Invitrogen®). Sequencing was performed by the chain-termination method using the ABI Big Dye Terminator Cycle sequencing kit v 2.0 (Applied Biosystems®) on an ABI PRISM model 3100 DNA sequencer.

Data analysis. Homologous sequences from the NCBI GenBank database, from relevant species, were included in the analysis: *Erysiphe cumminsiana* (U. Braun) U. Braun, *Golovinomyces cichoracearum* DC., *Golovinomyces cichoracearum* DC., *Oidium* sp., *Podosphaera fusca* (Fr.) U. Braun & N. Shishkoff, *Erysiphe glycines* Tai, *Erysiphe betae* and *Erysiphe polygoni* DC (Accession Numbers in Table 1). DNA fragments were aligned by means of ClustalW (Thompson *et al.,* 1994) and a neighbor-joining phylogenetic tree was constructed from Kimura 2-parameter pairwise distances using the Molecular Evolutionary Genetics Analysis software MEGA 3.1 (Kumar *et al.,* 2004). The consistency of phylogenetic resolution was supported by a bootstrap analysis using 1,000 replicates. Pairwise number of differences were calculated for ITS 1 and ITS 2. Indels and gaps were treated as missing data.

RESULTS

There were differences in PCR fragment sizes among the samples. Single bands were consistently amplified for all strains. Sequences ranged from 587 bp (strain from *H. brasiliensis*) to 671 bp (strain from *G. max*). Sequences from all ten soybean strains were identical. The numbers of pairwise differences, with regard to the ITS-rDNA region, among the 16 strains (ten from soybean and one from each of the six weed species) were compared together and with other homologous sequences from the NCBI GenBank database (Table 2).

The sequences of all strains from soybean were identical to that of the species *Oidium* sp., which is likely to be *Erysiphe diffusa* (Cooke & Peck) U. Braun & S. Takamatsu, formerly known as *Microsphaera diffusa* (Cooke & Perk) (Takamatsu *et al*., 2002). All major clades in the phylogenetic tree have high bootstrap values, suggesting that the results are strongly supported (Fig. 1). A major clade I (bootstrap value of 100%) grouped all the strains from soybean, *Oidium* sp. (several strains) and the strain from *Lupinus albus* with 100% of identity. This clade also grouped the *Erysiphe* sp. found in common bean (*P. vulgaris*), *E. glycines* (AB015923), *E. betae* (DQ164440) and *E. polygoni* (AF011308); the last is reported as a causal agent of powdery mildew of common bean.

The second major clade (II) grouped the strain from *B. pilosa* (AY739108) and *E. cumminsiana* (AF011299) also with a bootstrap value of 100%. The third clade grouped the strain from *H. annuus* (AY739110) and *S.*

TABLE 1 - Powdery mildews used in this study with respective host plants and collection locations. Accession number of rDNA ITS sequence are specified. Sequence data of powdery mildews entries collected outside of Brazil were obtained form NCBI GenBank database

GenBank accession No.	Species	Host	Location (City/State)	Country of origin
EF196668	*Erysiphe diffusa* strain OIGma 3	*Glycine max*	Londrina, Paraná	Brazil
AY739112	*Erysiphe diffusa*	*Glycine max*	Floresta, Paraná	Brazil
EF196675	*Erysiphe diffusa* strain OIGma 10	*Glycine max*	Passo Fundo, Rio Grande do Sul	Brazil
EF196667	*Erysiphe diffusa* strain OIGma 2	*Glycine max*	Cambé, Paraná	Brazil
EF196674	*Erysiphe diffusa* strain OIGma 9	*Glycine max*	Uberaba, Minas Gerais	Brazil
EF196673	*Erysiphe diffusa* strain OIGma 8	*Glycine max*	Guarapuava, Paraná	Brazil
EF196672	*Erysiphe diffusa* strain OIGma 7	*Glycine max*	Coxilha, Rio Grande do Sul	Brazil
EF196671	*Erysiphe diffusa* strain OIGma 6	*Glycine max*	Campo Mourão, Paraná	Brazil
EF196670	*Erysiphe diffusa* strain OIGma 5	*Glycine max*	Uberlândia, Minas Gerais	Brazil
EF196669	*Erysiphe diffusa* strain OIGma 4	*Glycine max*	Rio Verde, Goiás	Brazil
EF196666	*Erysiphe diffusa* strain OILal	*Lupinus albus*	Londrina, Paraná	Brazil
AY739109	*Erysiphe* sp.	*Phaseolus vulgaris*	Londrina, Paraná	Brazil
AY739110	*Golovinomyces cichoracearum*	*Helianthus annuus*	Londrina, Paraná	Brazil
AY739111	*Golovinomyces cichoracearum*	*Sonchus oleraceous*	Londrina, Paraná	Brazil
AY739113	*Podosphaera fusca*	*Hypochaeris brasiliensis*	Londrina, Paraná	Brazil
AY739108	*Neoerysiphe cumminsiana*	*Bidens pilosa*	Londrina, Paraná	Brazil
AB078813	*Oidium* sp MUMH1162	*Glycine soja* (Wild soybean)	Gifu	Japan
AB015923	*Erysiphe glycines* var. glycines	*Lespedeza thunbergii*		Japan
AF011308	*Erysiphe polygoni*	*Rumex crispus*		USA
AB026148	*Podosphaera fusca*	*Taraxacum officinale*		Japan
AB077642	*Golovinomyces cichoracearum* MUMH345	*Aster subulatus*	Nara, Yamato-koriyama	Japan
AB077673	*Golovinomyces cichoracearum* MUMH683	*Sonchus arvensis*	Budapest	Hungary
AF011299	*Erysiphe cumminsiana*	*Eupatorium rugosum*		USA
EU159425	*Podosphaera fusca*	*Calendula officinalis*		Slovenia
AB046989	*Podosphaera fusca*	*Euryops pectinatus*	CA,Salinas	USA
AB040332	*Podosphaera fusca*	*Melampyrum nemorosum*		
AF011292	*Golovinomyces cichoracearum*	*Ambrosia trifida*		
AB000934	*Golovinomyces cichoracearum*	*Eupatorium japonicum*		
AB015921	*Erysiphe glycines* var. lespedezae	*Lespedeza cuneata*		
AB078812	*Oidium* sp. SMK15414	*Glycine soja* (Wild soybean)	Chunchon	South Korea
AB078811	*Oidium* sp. MUMH1464	*Glycine max*	Illinois	USA
AB078810	*Oidium* sp. MUMH1463	*Glycine max*	Minnesota	USA
AB078803	*Oidium* sp. MUMH878	*Glycine max*	Nara	Japan
AB078808	*Oidium* sp. SMK17078	*Glycine max*	Chongju	South Korea
AB078804	*Oidium* sp. MUMH1452	*Glycine max*	Shizuoka	Japan
AB078802	*Oidium* sp. MUMH793	*Glycine max*	Fukushima	Japan
AB078805	*Oidium* sp. MUMH1453	*Glycine max*	Okayama	Japan
AB078800	*Oidium* sp. MUMH791	*Glycine max*	Oita	Japan
AB078801	*Oidium* sp. MUMH789	*Glycine max*	Kumamoto	Japan
AB078806	*Oidium* sp. MUMH1462	*Glycine max*	Mie	Japan
AB237809	*Oidium* sp. MUMH1183	*Acacia mangium*		Japan
DQ164440	*Erysiphe betae*	Sugar beet cv. Hilleshg 2984Rz	Ontario, Oregon	USA

oleraceus (AY739111) with *Golovinomyces cichoracearum* (previously found in *Aster subulatus*, *Sonchus arvensis, Ambrosia trifida, Eupatorium faponicum*), supported by a bootstrap value of 91%, and the fourth clade was formed by *Podosphaera fusca* (AY739113) and the strains from *H. brasiliensis*, also with a bootstrap value of 100%.

TABLE 2 - Kimura 2-parameter pairwise distances (lower left) and number of nucleotide differences (upper rigth) for ITS1 and ITS2, considering 426 sites, showing the relationships among the powdery mildews. Indels and gaps/missing data were rejected. Sequences from all ten soybean strains were identical, thus just one was used for comparison. Asterisk(*) indicates Brazilian strains

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	EF196673	*E. diffusa* OIGma 8*		0	0	0	4	11	13	8	10	74	74	72	73	72	79	77	66	65	65	65	61	61
2	AB078803	*Oidium* sp MUMH878	0.000		0	0	4	11	13	8	10	74	74	72	73	72	79	77	66	65	65	65	61	61
3	EF196666	*E. diffusa* OILal*	0.000	0.000		0	4	11	13	8	10	74	74	72	73	72	79	77	66	65	65	65	61	61
4	AY739112	*E. diffusa**	0.000	0.000	0.000		4	11	13	8	10	74	74	72	73	72	79	77	66	65	65	65	61	61
5	AY739109	*E.* sp.*	0.009	0.009	0.009	0.009		12	14	9	11	72	72	70	71	70	81	79	66	65	65	65	61	61
6	AB015923	*E. glycines*	0.026	0.026	0.026	0.026	0.029		3	7	11	77	77	75	76	75	80	78	67	66	66	66	62	62
7	AB015921	*E. glycines* var. lesp	0.031	0.031	0.031	0.031	0.034	0.007		9	13	79	79	77	78	77	80	78	68	67	67	67	64	64
8	DQ164440	*E. betae*	0.019	0.019	0.019	0.019	0.021	0.017	0.021		4	71	71	69	70	69	79	77	65	64	64	64	58	58
9	AF011308	*E. polygoni*	0.024	0.024	0.024	0.024	0.026	0.026	0.031	0.009		72	72	70	71	70	80	78	68	67	67	67	61	61
10	EU159425	*P. fusca*	0.200	0.200	0.200	0.200	0.194	0.209	0.216	0.191	0.194		0	2	3	2	81	79	73	72	72	72	72	72
11	AB046989	*P. fusca* MUMH813	0.200	0.200	0.200	0.200	0.194	0.209	0.216	0.191	0.194	0.000		2	3	2	81	79	73	72	72	72	72	72
12	AY739113	*P. fusca* *	0.194	0.194	0.194	0.194	0.188	0.203	0.210	0.185	0.188	0.005	0.005		1	0	80	78	72	71	71	71	71	71
13	AB026148	*P. fusca*	0.197	0.197	0.197	0.197	0.191	0.206	0.213	0.188	0.191	0.007	0.007	0.002		1	81	79	73	72	72	72	72	72
14	AB040332	*P. fusca* MUMH774	0.194	0.194	0.194	0.194	0.188	0.203	0.210	0.185	0.188	0.005	0.005	0.000	0.002		80	78	72	71	71	71	71	71
15	AY739108	*N. cumminsiana*	0.216	0.216	0.216	0.216	0.222	0.218	0.218	0.215	0.218	0.221	0.221	0.218	0.221	0.218		2	55	54	54	54	59	59
16	AF011299	*E. cumminsiana*	0.210	0.210	0.210	0.210	0.216	0.212	0.212	0.209	0.212	0.215	0.215	0.212	0.215	0.212	0.005		53	52	52	52	57	57
17	AY739110	*G. cichoracearum**	0.174	0.174	0.174	0.174	0.174	0.177	0.180	0.171	0.180	0.195	0.195	0.192	0.195	0.192	0.143	0.137		1	1	1	24	24
18	AF011292	*G. cichoracearum*	0.171	0.171	0.171	0.171	0.171	0.174	0.177	0.168	0.177	0.192	0.192	0.189	0.192	0.189	0.140	0.135	0.002		0	0	23	23
19	AB077642	*G. cichoracearum*	0.171	0.171	0.171	0.171	0.171	0.174	0.177	0.168	0.177	0.192	0.192	0.189	0.192	0.189	0.140	0.135	0.002	0.000		0	23	23
20	AB000934	*G. cichoracearum*	0.171	0.171	0.171	0.171	0.171	0.174	0.177	0.168	0.177	0.192	0.192	0.189	0.192	0.189	0.140	0.135	0.002	0.000	0.000		23	23
21	AY739111	*G. cichoracearum**	0.160	0.160	0.160	0.160	0.160	0.162	0.169	0.151	0.159	0.193	0.193	0.190	0.194	0.190	0.155	0.149	0.059	0.056	0.056	0.056		0
22	AB077673	*G. cichoracearum*	0.160	0.160	0.160	0.160	0.160	0.162	0.169	0.151	0.159	0.193	0.193	0.190	0.194	0.190	0.155	0.149	0.059	0.056	0.056	0.056	0.000	

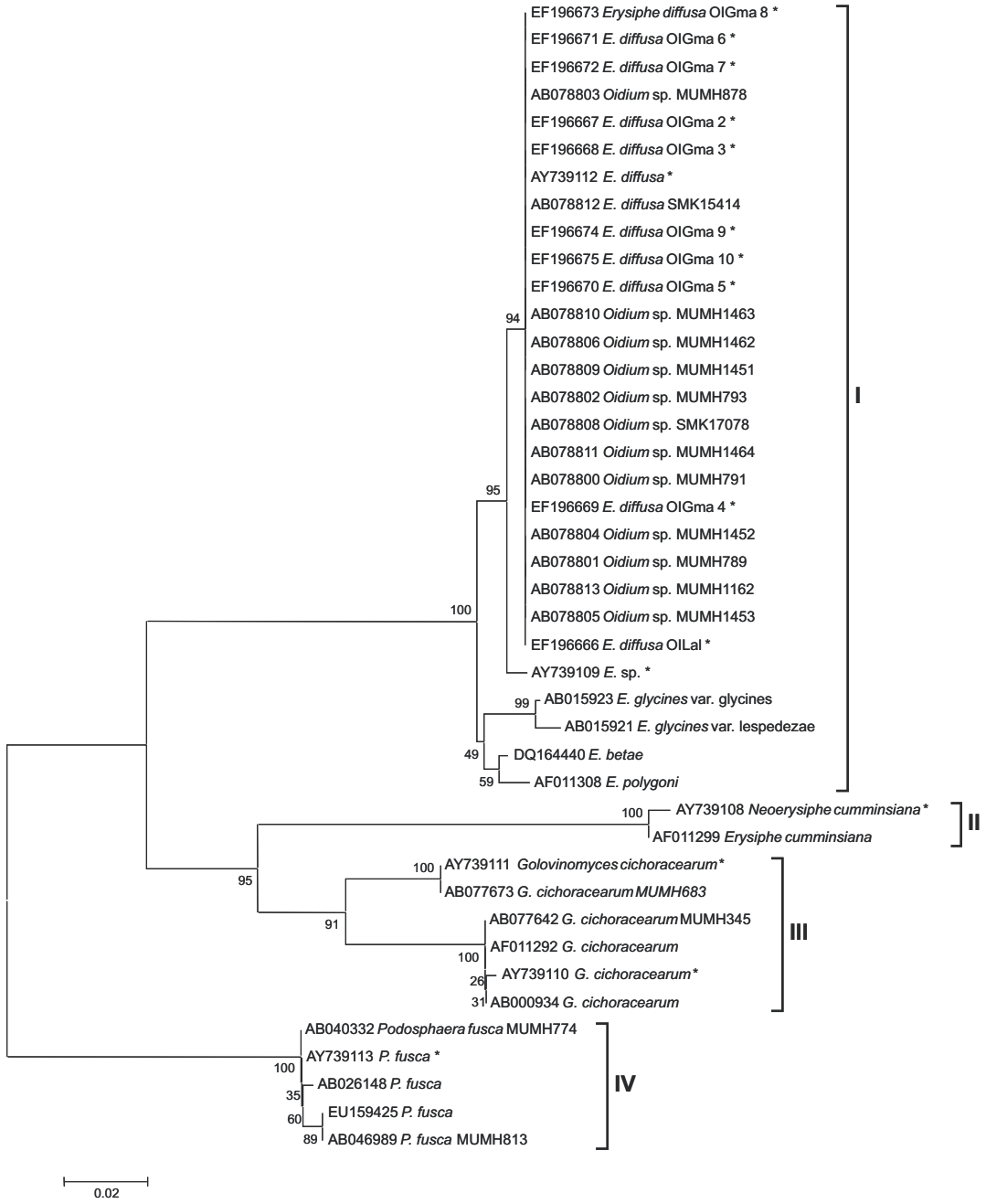

FIG. 1 - Unrooted phylogenetic tree based on rDNA ITS1 and ITS2 sequences showing the relationships among strains of powdery mildew. The tree was constructed by the Neighbor-joining method, using the Kimura 2-parameter pairwise distances. Topology was evaluated by bootstrap analysis (MEGA program, 1000 replications). The numerical values in the tree represent bootstrap results. The distance between two strains is the sum of the branch lengths between them. Asterisk(*) indicates Brazilian strains. Brackets indicate the clusters referred to in the text.

DISCUSSION

This study describes the first molecular identification, based on rDNA-ITS sequences, of four species of powdery mildew in Brazil (*E. diffusa, G. cichoracearum*, *P. fusca* and *N. cumminsiana*) naturally infecting cultivated plants and weed species commonly found in and around soybean fields. Although the number of soybean strains used in this work may be considered low (ten out of 65 strains) they were collected in different geographical regions including those where severe outbreaks occurred and also from traditional areas. This study, based on ITS-rDNA sequence analysis,

showed that the only species so far identified on soybean in Brazil was *E. diffusa*. An additional phylogeographic research has been planned to evaluate the genetic diversity through RAPD or AFLP, using a larger number of strains. This research may help to provide an explanation for the outbreak observed in 1996/97 and why a marginal disease became important after that event. An acceptable hypothesis is that the pathogen population changed substantially over the years with the occurrence of more virulent strains capable of infecting resistant cultivars. Unfortunately, the strains from the 1996/97 season were not available for comparison in this study.

As mentioned by Takamatsu *et al*. (2002), in Japan the outbreak was caused by two species of powdery mildew (*E. diffusa* and *E. glycines*), but the present study shows that in Brazil the outbreak was caused by only one species. No nucleotide difference was verified between the rDNA-ITS sequences of all Brazilian strains from soybean and the *Oidium* sp. sequence (AB078803) isolated from soybean in Japan (Takamatsu *et al.,* 2002). As mentioned by Takamatsu *et al*. (1999), *Oidium* sp. may well be *E. diffusa*, since the authors did not find the teleomorphic stage of the fungus (S. Takamatsu, personal communication).

Erysiphe diffusa should be used as the causal species of soybean powdery mildew in Brazil instead of *Microsphaera diffusa*. According to Braun & Takamatsu (2000), *Microsphaera* and *Erysiphe* are grouped together in a clade and cannot be separated from one another in phylogeny. They proposed to combine the genera *Erysiphe*, *Microsphaera*, and *Uncinula* into a single genus, based on molecular phylogenetic studies and scanning electron microscopy of conidia (Takamatsu *et al.,* 1999; Cook *et al.,* 1997). Based on this information and the evidence provided by those authors, the name *E. diffusa* was used instead *M. diffusa*.

Although *E. polygoni* is the causal agent of powdery mildew in beans, in this study the infection was probably caused by *E. diffusa*. Comparing rDNA-ITS sequence of the strain from *P. vulgaris* (AY739109) with sequences of *E. diffusa* and *E. polygoni* (AF011308) resulted in 4 and 11 nt differences, respectively. It is possible that inoculum from soybean fields contributed to powdery mildew epidemics on beans since *E. diffusa*, formerly known as *M. diffusa*, was mentioned as a pathogen of common bean (http://www.hort.purdue.edu/newcrop/duke_energy/Phaseolus_vulgaris.html). The same inoculum could explain the infection in lupins, used as cover crop in southern regions of Brazil, by *E. diffusa*.

The sequences of the sunflower strain were similar (99.8 %) to the sequences of *G. cichoracearum* (DC.) V. P. Geljuta, while the strain from *Hypochaeris brasiliensis* (Less) Griseb was similar (99.8%) to *P. fusca* (Fr.) U. Braun & N. Shishkoff. The powdery mildew species, *G. cichoracearum*, is reported to infect more than 300 hosts among eight plant families. Evidence suggests, however, that the species is a complex of host-specialized biotypes (Matsuda & Takamatsu, 2003).

The sequence of the rDNA-ITS for strains from *B. pilosa* was identified as *E. cumminsiana* based on a high similiarity (only 2 nt differences) with AF011299. So far there is no report of occurrence of *Neoerysiphe* spp. on *B. pilosa* except an invalid record in Cuba (Amano, 1986). To our knowledge, this is the first analysis on rDNA-ITS sequences of powdery mildews associated with soybean, bean, sunflower and weeds in Brazil. It also describes for the first time *H. brasiliensis* as a host of *P. fusca*.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S. Takamatsu (Mie University, Japan), Dr. T. Harrington (Iowa State University, USA) and Dr. E. Mizubuti (Universidade Federal de Viçosa, Brazil) for critical reading of the manuscript. We also thank L.C. Benato and Mauro Caetano for technical assistance.

REFERENCES

Almeida AMR, Abdelnoor RV, Arias CAA, Carvalho VP, Jacoud Filho DS, Marin SRR, Benato L, Pinto MC, Carvalho CGP (2003) Genotypic diversity among Brazilian isolates of *Macrophomina phaseolina* revealed by RAPD. Fitopatologia Brasileira 28:279-285.

Amano K (1986) Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Society Press. Tokyo.

Braun U, Takamatsu S (2000) Phylogeny of *Erysiphe, Microsphaera, Uncinula* (Erysipheae) and *Cystotheca, Podosphaera, Sphaerotheca* (*Cystotheceae*) inferred from rDNA ITS sequences: some taxonomic consequences. Schlechtendalia 42:1-33.

Cook RTA, Inmana J, Billings C (1997) Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range. Mycological Research 101:975-1002.

Ferreira LP, Lehman PS, Almeida AMR (1979) Doenças da soja no Brasil. Londrina PR. Embrapa.

Grau CR (1984) Powdery mildew, a sporadic but damaging disease of soybean. Proceedings of the III World Soybean Research Conference. Shiebles R (Ed.) Iowa, USA. Westview Press, Boulder. pp. 568-574.

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Briefings in Bioinformatics 5:150-163.

Matsuda S, Takamatsu S (2003) Evolution of host–parasite relationships of *Golovinomyces* (Ascomycete: Erysiphaceae) inferred from nuclear rDNA sequences. Molecular Phylogenetics and Evolution 27:314-327.

Takamatsu S, Hirata S, Sato Y, Nomura Y (1999) Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildew) inferred from the rDNA ITS sequences. Mycoscience 40:259-268.

Takamatsu S, Shin HD, Paksiri U, Limkaisang S, Taguchi Y, Binh N (2002) Two *Erysiphe* species associated with recent outbreak

of soybean powdery mildew: results of molecular phylogenetic analysis based on nuclear rDNA sequences. Mycoscience 43:333-341.

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Wahl CV (1921) Schädlinge an der Sojabohne. Zeitschrift für Planzenkrankheiten 31:194-196.

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninskey JJ, White TJ (Eds.) PCR protocols: A guide to methods and applications. San Diego CA. Academic Press. pp. 315-322.

Received 22 December 2006 - Accepted 25 February 2008 - TPP 6146
Associate Editor: Marcos P.S. Câmara