GENETIC VARIABILITY AND VEGETATIVE COMPATIBILITY OF *Erythricium salmonicolor* ISOLATES

Fernanda Luiza de Souza Sebastianes[1]; Cristina Sayuri Maki[1]; Fernando Dini Andreote[1]; Welington Luiz Araújo[2]; Aline Aparecida Pizzirani-Kleiner[2]*

[1]*USP/ESALQ - Programa de Pós-Graduação em Genética e Melhoramento de Plantas.*
[2]*USP/ESALQ - Depto. de Genética, Av. Pádua Dias, 11, C.P. 83 - 13418-900 - Piracicaba, SP - Brasil.*
**Corresponding author <aapklein@esalq.usp.br>*

ABSTRACT: The Pink Disease is caused by *Erythricium salmonicolor*, which attacks broad hosts, such as citrus, coffee, rubber, *Eucalyptus* spp. and *Acacia* spp., infecting mainly branches. This disease became a serious problem in Brazil, reducing the citrus production up to 10%. However the genetic diversity and compatibility of the fungus *E. salmonicolor* from Brazilian citrus plants is not yet evaluated. Therefore, the aims of this study were to evaluate: *i*) the genetic variability of *E. salmonicolor* in the São Paulo and Minas Gerais States by the RAPD technique, and *ii*) the vegetative compatibility between these isolates. After RAPD analysis, six distinct groups were observed without correlation between the isolation site or host species. In the vegetative compatibility test, the contact of fungal hyphae between all evaluated crosses was observed, of which 84% presented hyphal fusion. Although the compatibility between strains was observed, no correlation between RAPD haplotypes and hyphal anastomosis was verified. These results show the importance of future studies on the sexual cycle of *E. salmonicolor,* since hyphal fusion, which precedes the formation of heterokaryons (sexual and parasexual reproduction) that could be responsible for the genetic variability in this species.
Key words: *Corticium salmonicolor*, RAPD, Pink Disease, hyphal fusion, heterokariosis

VARIABILIDADE GENÉTICA E COMPATIBILIDADE VEGETATIVA DE ISOLADOS DE *Erythricium salmonicolor*

RESUMO: A rubelose é uma doença causada pelo fungo *Erythricium salmonicolor* que atinge muitos hospedeiros, como citros, café, seringueira, eucalipto, *Acacia* sp., infectando principalmente os galhos. Rubelose é um sério problema para o Brasil, reduzindo a produção de citros em valores próximos de 10%. A diversidade do fungo *E. salmonicolor* em cultivares brasileiras ainda não foi avaliada. Este trabalho teve como objetivos: *i)* avaliar a variabilidade genética, por meio de RAPD, de 19 isolados de *E. salmonicolor* provenientes de diferentes regiões citrícolas de São Paulo e Minas Gerais, *ii)* avaliar a compatibilidade vegetativa e fusão de hifas do fungo *E. salmonicolor*. Após a análise por RAPD, foram observados 6 grupos distintos, os quais não apresentaram correlação com o local de origem e espécie hospedeira. No teste de compatibilidade vegetativa, houve encontro de hifas em todos os cruzamentos e 84% destes apresentaram fusão entre elas. Foi verificada compatibilidade entre linhagens, embora não tenha sido observada correlação com os haplótipos. Os resultados observados neste trabalho indicam a importância de futuros estudos sobre a fase sexual do fungo *E. salmonicolor*, uma vez que a anastomose de hifas precede a formação de heterocário, onde ocorrem os processos de recombinação sexual e parassexual responsáveis pela variabilidade genética em fungos filamentosos.
Palavras-chave: *Corticium salmonicolor*, RAPD, rubelose, fusão de hifas, heterocariose

INTRODUCTION

Brazil is the largest citrus fruit producer in the world, dominating 80% of the international trade of concentrated orange juice, an activity that trades about US$ 4 billion per year. Brazilian citriculture has many phytossanitary problems, including more than 50 fungal diseases - among them, the Pink Disease, caused by fungus *E. salmonicolor* (Feichtenberger & Spósito, 2004). The symptoms include production of a salmon pink mycelium on branches and stems of the tree, causing twig and branch injuries, stem canker, and

eventually host plant death. Initial infections are characterized by a gum production and white mycelial growth on the surface of infected branches and stems. Host trees with severe infection present salmon color of the mycelium, crank and dry branches and fall of fruits, causing a decrease of plant growth, resulting in a reduction of citrus production (Gezahgne et al., 2003; Ploetz, 2003; Old et al., 2000).

RAPD, described by Williams et al. (1990) and Welsh & McClelland (1990), has been used to evaluate the genetic diversity (Lacava et al., 2001; Iran & Ahmad, 2005); population studies (Jamil et al., 2000); taxonomic identification (Guzmán et al., 1999); characterization of fungal strains (Fungaro, 2000), and production of genomic fingerprints of many microorganism species (Anderbrhan & Furtek, 1994; Fenille et al., 2005; Welsh & McClelland, 1990).

The most filamentous fungi form an intraspecific heterokaryons in a single hyphal talus with two or more genetically distinct nuclei. Therefore, some of the studied fungi do not form a heterokaryon with other genetically similar organisms due to the system of vegetative incompatibility (Glass et al., 2000; Di Primo et al., 2001). Also, in some basidiomycetes, such as *Rhizoctonia solani*, the pathogenicity is correlated with anastomosis grouping (Fenille et al., 2005), which have also been used for identification and classification purposes (Sneth et al., 1996). Therefore, the aims of this study were to evaluate the genetic diversity of *E. salmonicolor* and to analyze the vegetative compatibility of different strains.

MATERIAL AND METHODS

Fungal DNA extraction

Nineteen isolates (Table 1) were grown on PDA medium for 7 days at 28ºC and total DNA was extracted according to Raeder & Broda (1985). RAPD analysis was carried out on 25 mL samples, containing 10ng of template DNA, 0.28 mM of each dNTP (Invitrogen), 3.2 mM of $MgCl_2$, 2U of Taq DNA polymerase (Invitrogen) and 0.45 µM of primer. The amplification was carried out as follows: 5 min at 94ºC, followed by 40 cycles of 1 min at 92ºC, 1 min at 35ºC, 2 min at 72ºC with a final extension at 72ºC for 5 min. PCR products were analyzed in 1.5% agarose gel stained with ethidium bromide. Primers AX17, C08, W04, AX10, G13, A02 and P12 (*Operon Technologies*) were selected by reproducibility patterns and further used in separated reactions.

Dendrogram based on the Jaccard coefficient was constructed using the NTSYS-pc program (Copyright C - 1986-1997 - Applied Biostatistics Inc) considering the Unweighted Pair Group Method with Arithmetical Average (UPGMA) cluster analysis. A consensus tree was obtained using the WinBoot software (Yap & Nelson, 1996) with the bootstrap replicate number test set at 100.

Compatibility analysis

Isolates belonging to different RAPD clusters were used to study the vegetative compatibility. The crossing method was performed as follows: agar blocks cut from the growing edge of a colony of each strain were placed on opposite sides of 5 cm Petri plates containing potato dextrose agar (PDA). Cultures were incubated at 28ºC for 7 to 30 days or until hyphae of both isolates overlapped. The area of hyphal overlapping was stained with 0.04% cotton blue in lactophenol, and examined under a light microscope.

RESULTS AND DISCUSSION

RAPD analysis was conducted with seven primers and only the well-defined and reproducible bands were considered. The evaluated primers resulted in 79 polymorphic and scorable bands, which allowed differentiating the isolates (Figure 1). The confidence of these bands was evaluated in at least two independent experiments. The obtained dendrogram (Figure 2) shows the genetic variability among isolates, clustering the strains in two major groups, whose genetic similarity is around 35%. This variability suggests that future studies about the genetic recombination in this pathogenic fungus are necessary for a better understanding of the genetic variability of this species.

In a more detailed view of the dendrogram, six minor groups could be identified sharing approximately 88% of genetic similarity. Groups A, B and D include only strains from citrus (CS4, IB14/02, CS8, Cs12A, Cs12B. CS7, Cs15, CsBH and CS3) and the group C from citrus and fig trees (Cs13, Cs14, Cs16, CS6, CsL, CsBR e IB16/02). Group E comprises one strain from apple (CsSC) and group F strains isolated from *Psidium cattleianum* and citrus (CsPA and RWB190). Analyses of minor groups suggest no correlation between groups and hosts, revealing an absence of specificity of fungi in colonize a single host. In a similar study, Tigano & Aljanabi (2000) verified a correlation between the RAPD profile of *Nomuraea rileyi* and the insect hosts. Correlation analysis of strain characteristics and RAPD profile are commonly used in studies of microbial ecology and genetic variability. Some authors describe a low genetic variability of species in a single region (Délye et al., 1995; Gomes et al., 2001). However, this was not noticed in this study, whose results revealed the absence of specific clusters in RAPD analysis for each local of isolation - strains from Bebedouro were found in groups

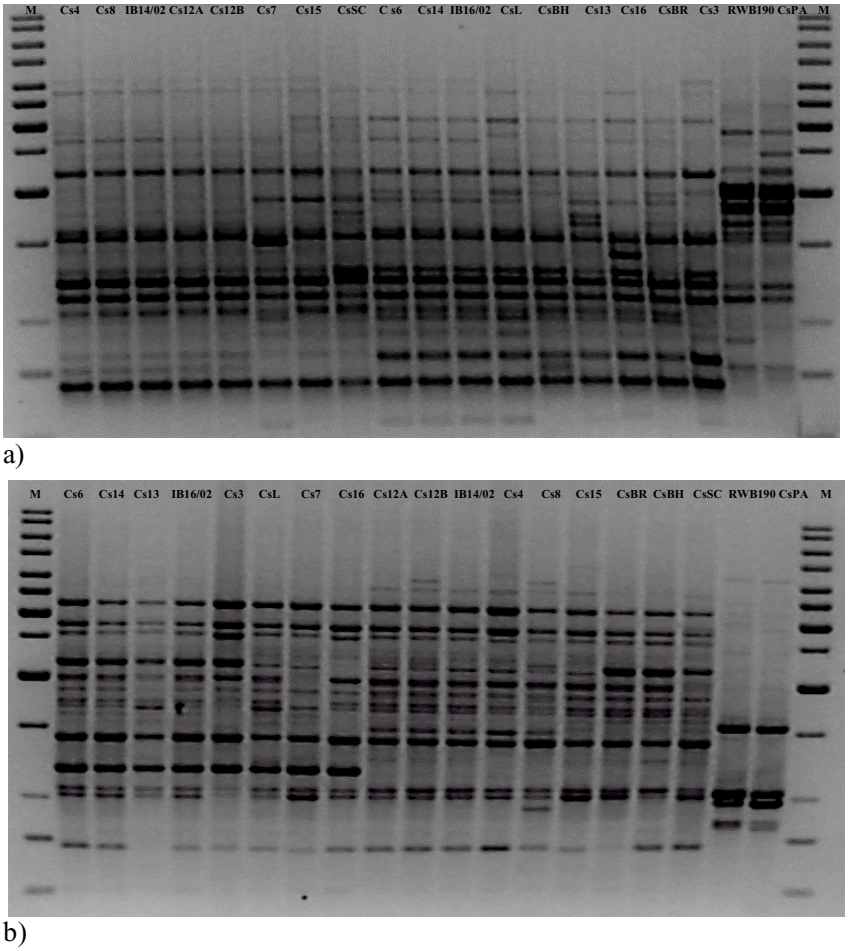

Figure 1 - RAPD analysis of genomic DNA. Lanes correspond to individual *Erythricium salmonicolor*. The primers used were: a)AX10 and b)C08 *(Operon Technologies*).

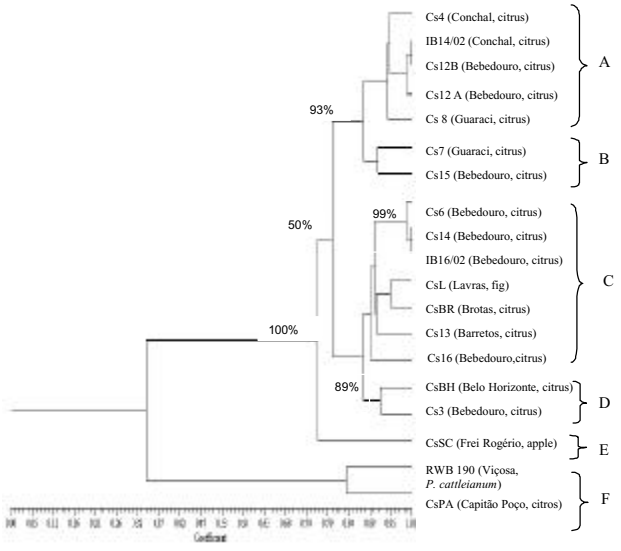

Figure 2 - Dendrogram of the genetic relationship among *Erythricium salmonicolor* strains based on RAPD analyses. The name of the strains, haplotypes, local and hosts are indicated in the dendrogram. RAPD analyses using seven primers revealed a total of 101 bands, which were used to construct the dendrogram.

A, B, C and D, while strains from Guaraci were allocated to groups A and B. Remarkable is also the variability intra orchard, whose strains Cs16, Cs14, Cs15 and Cs6 (isolated from the same infected orchard in Bebedouro, SP) revealed different band patterns. Some isolates, such as IB14/02 and Cs12B; IB16/02 and Cs14, presented 100% of similarity. Taking in mind that these isolates were obtained at different times, IB14/02 and Cs12B or orchard IB16/02 and Cs14, we suggest that some genotypes may have been spread in this citrus growing area for a long period.

RAPD analysis showed that most *Crinipellis perniciosa* isolates from solanaceous hosts in Bahia were distinguishable from cocoa isolates (Anderbrhan & Furtek, 1994). Tigano & Aljanabi (2000) did not reveal a correlation between fingerprints of *N. riley* strains and the geographical area, presenting a clustering of strains from Oliveiros (Argentina), Sete Lagoas, Goiânia and Brasília (Brazil). However, Vaillancourt & Hanau (1992) demonstrated a correlation between RAPD patterns of *Colletotrichum* populations from maize and sorghum and their isolation regions.

Table 1 - Strains of *Erythricium salmonicolor* used in this study.

Strain	Host	Location	Source
CS3	Citrus	Bebedouro - SP	(Assis, 2003)
CS4	Citrus	Conchal - SP	(Assis, 2003)
CS6	Citrus	Bebedouro - SP	(Assis, 2003)
CS7	Citrus	Guaraci - SP	(Assis, 2003)
CS8	Citrus	Guaraci - SP	(Assis, 2003)
Cs12A	Citrus	Bebedouro - SP	(Assis, 2003)
Cs12B	Citrus	Bebedouro - SP	(Assis, 2003)
Cs13	Citrus	Barretos - SP	(Assis, 2003)
Cs14	Citrus	Bebedouro - SP	(Assis, 2003)
Cs15	Citrus	Bebedouro - SP	(Assis, 2003)
Cs16	Citrus	Bebedouro - SP	(Assis, 2003)
CsPA	Citrus	Capitão Poço - PA	(Assis, 2003)
RWB190	*Psidium cattleianum*	Viçosa - MG	(Assis, 2003)
CsSC	Apple	Frei Rogério - SC	(Assis, 2003)
CsL	Fig	Lavras - MG	(Assis, 2003)
CsBR	Citrus	Brotas - SP	Dr. Nelson Massola
CsBH	Citrus	Belo Horizonte - MG	FUNDECITRUS
IB16/02	Citrus	Bebedouro - SP	Mário Figueiredo
IB14/02	Citrus	Conchal - SP	Mário Figueiredo

Table 2 - Isolates crossed in the vegetative compatibility and hyphal fusion tests.

Crossed strain	Hyphal interaction*	RAPD groups crossed
IB14/02 - Cs4	a+	A X A
IB14/02 - Cs15	a+	A X B
IB14/02 - CsL	a+	A X C
IB14/02 - Cs BH	a+	A X D
IB14/02 - CsSC	a+	A X E
IB14/02 - CsPA	b-	A X F
IB14/02 - Cs12B	a+	A X A
IB14/02 - IB16/02	a+	A X C
Cs15 - Cs7	a+	B X B
Cs15 - CsL	b-	B X C
Cs15 - CsBH	a+	B X D
Cs15 - CsSC	a+	B X E
Cs15 - CsPA	b-	B X F
CsL - CsBH	a+	C X D
CsL - CsSC	a+	C X E
CsL - CsPA	a+	C X F
CsBH - CsSC	a+	D X E
CsBH - CsPA	a+	D X F
CsSC - CsPA	a+	E X F
IB16/02 - Cs14	b-	C X C
IB16/02 - CsSC	a+	C X E
IB16/02 - CsBH	a+	C X D
IB16/02 - Cs15	a+	C X B
IB16/02 - CsL	a+	C X C
IB16/02 - CsPA	a+	C X F

* a+ = present of hiphal contact and fusion; b- = absent of hiphal fusion.

Similar *Erythricium salmonicolor* strains may also colonize citrus plants from different regions - this may indicate a clonal dissemination. As an example strains IB14/02 and CS12B, which were respectively isolated from Conchal and Bebedouro, had identical RAPD profile. Colauto et al. (2002) characterized genotypically 5 isolates from the basidiomicete *Agaricus blazei* by RAPD. Although these isolates presented distinct origins, there was no genetic variability among them. These results indicate that these isolates may have a common origin.

There are no previous reports about the genetic variability of the fungus *E. salmonicolor*. The high genetic variability and the coexistence of different genotypically fungal strains in a same region may predict a greater difficulty of the control of this disease.

The increasing incidence of the Pink Disease reveals the importance of studies on this fungus species, aiming to obtain more information on the disease etiology. In the present study, vegetative compatibility was evaluated between isolates from the same or different RAPD haplotypes. Crossed isolates are indicated in Table 2. Crosses between the incompatible strains must form a blocking reaction, observed by a zone separating strains, but crossing between compatible strains, the inhibition zone is not observed (Nauta & Hoekstra, 1996; James et al., 2004; Aimi et al., 2002; 2005; McCabe et al., 1999).

Macroscopic observation of crossings indicated that all evaluated crossings presented hyphae contact 7 to 30 days after inoculation (Figure 3). The

Figure 3 - Contact between hyphae of *Erythricium salmonicolor* isolates. Crossing between isolates a) Cs4-IB1402, b) IB1402-CsSC and c) CsBH-IB1402

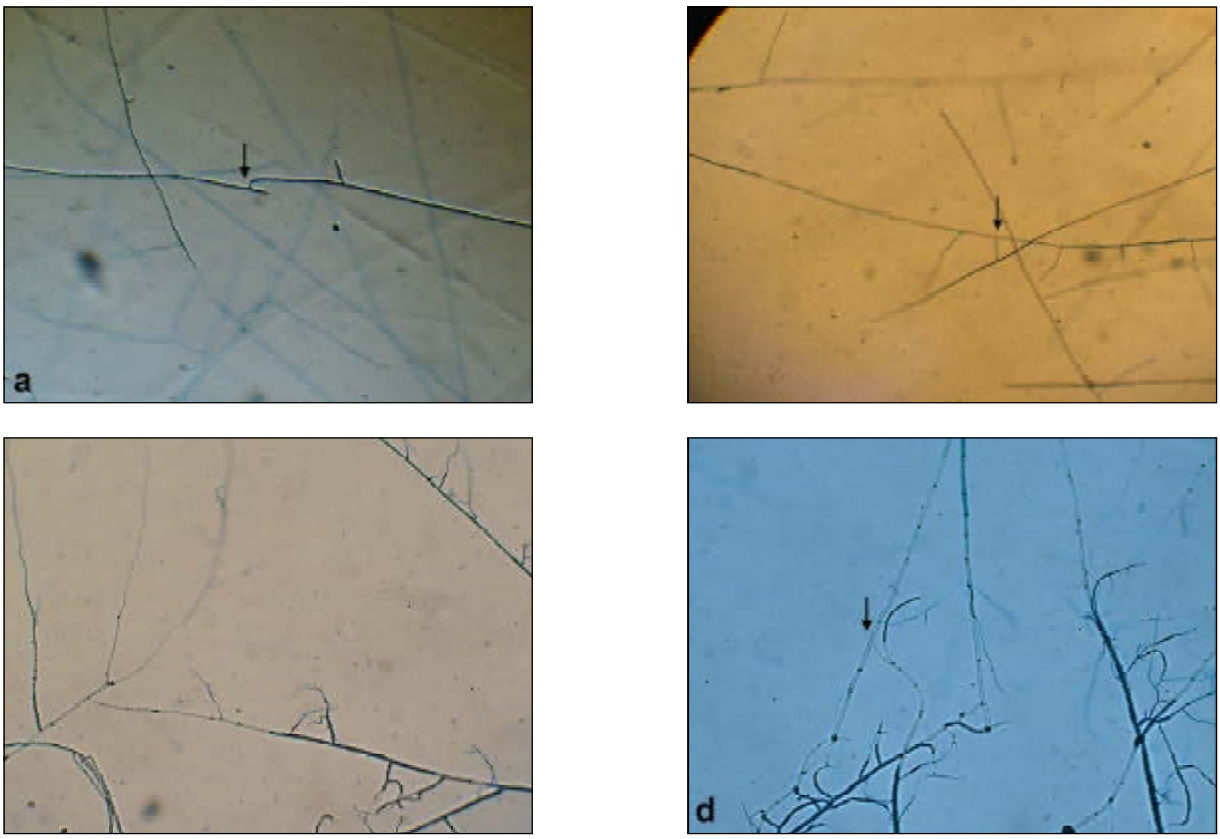

Figure 4 - Hyphal fusion in crossing a) IB16/02-Cs15 b)IB14/02-IB16/02, c) CsSC-CsBH and d)IB16/02-CsBH. The arrows indicate points of fusion between hyphae of fungus *Erythricium salmonicolor*

vegetative compatibility of hyphae was also evaluated under light microscopy (Figure 4), presenting a hyphal fusion in 84% of the crossings. Assis (2003) using some of these isolates observed mycelial contact, but not hyphal fusion. Although crossings IB14/02-CsPA, Cs15-CsL, Cs15-CsPA, and IB16/02-Cs14 presented hyphal contact, they did not present hyphal anastomosis, which suggests an incompatibility reaction. The strains with greater number of incompatibility crosses were CsPA and Cs15, which presented absence of hyphal fusion in most of the evaluated crossings.

Many researchers suggest that a filamentous fungus cannot form heterokaryons with genetically similar ones (Worral, 1997; Debets, 1998; Jacobson et al., 1998). In the present study, crossing between strains IB16/02 and Cs14 was observed, which have similar genetic constitution according to RAPD analysis. This crossing presented hyphal contact, but not hyphal fusion, and therefore there was no heterokaryon formation. However, one complete crossing with hyphal fusion was obtained from crossing IB14/02 and Cs12B that had also the same band profile.

The compatible and incompatible crossings occurred between strains belonging to different and to the same RAPD haplotypes. Therefore, correlations between mycelium compatibility groups (MCG) and haplotypes were not observed. These results suggest that either there is no *mating-type* process in *E. salmonicolor* or the RAPD technique was not able to detect the genetic divergence among evaluated strains.

In previous studies, Amazonian isolates of *C. perniciosa* from solanaceous hosts and isolates from cocoa did not cross-infect and are somatically incompatible (Bastos & Evans 1985, Bastos et al., 1988). However, in the present study isolates obtained from different plant hosts, e.p. IB14/02 and CsL; IB14/02 and CsSC, had hyphal anastomosis, suggesting that a gene flow could occur between isolates from different plant hosts.

Such data represent an important component in the selection of resistant or tolerant genotypes within breeding programmes designed to establish more effective and durable resistance in relation to the Pink Disease. Results obtained in this work show the importance of future studies concerning the sexual phase of *E. salmonicolor*, since the genetic variability seems to be high. The hyphal fusion, which precedes the formation of heterokaryons (sexual and parassexual reproduction), could be responsible for the genetic variability of this species.

ACKNOWLEDGMENT

This research was supported by Fundo de Defesa da Citricultura (FUNDECITRUS) and CNPq. The authors thank Dr. Marcel Bellato Spósito (FUNDECITRUS, Araraquara, Brazil) for discussions. Strains of *Erythricium salmonicolor*, except for CsBH, were kindly supplied by Dra. Neusa de Lima Nogueira (Laboratory of Vegetal Histopathology, CENA/USP, Piracicaba, SP, Brazil), Dr. Mário Figueiredo (Biological Institute, Laboratory of Mycology, Campinas, SP, Brazil) and Dr. Nelson Sidnei Massola Jr. (Department of Zoology, Phytopatology and Entomology, ESALQ/USP, Piracicaba, SP, Brazil).

REFERENCES

AIMI, T.; YOTSUTANI, Y.; MORINAGA, T. Cytological analysis of anastomosis and vegetative incompatibility reactions in *Helicobasidium monpa*. **Current Microbiology**, v.44, p.148-152, 2002.

AIMI, T.; YOSHIDA, R.; BAO, M.I.D.; KITAMOTO, Y. Identification and linkage mapping of the genes for the putative homeodomain protein (*hox1*) and the putative pheromone receptor protein homologue (*rcb1*) in a bipolar basidiomycete, *Pholiota nameko*. **Current Genetics**, v.48, p.184-194, 2005.

ANDEBRHAN, T.; FURTEK, D.B. Randon amplified polymorphic DNA (RAPD) analysis of *Crinipellis perniciosa* isolates from different hosts. **Plant Pathology**, v.43, p.1020–1027, 1994.

ASSIS, R. Estudos da rubelose em citros e do seu agente causal *Erythricium salmonicolor (Corticium salmonicolor) (Berk. & Br.) Burdsall*. Piracicaba: USP/CENA, 2003. 67p. (Tese - Doutorado).

BASTOS, C.N.; ANDEBRHAN, T.; DE ALMEIDA, L.C. Comparações morfológica e patológica de *Crinipellis perniciosa*. **Fitopatologia Brasileira**, v.13, p.202–206, 1988.

BASTOS, C.N.; EVANS, H.C. A new pathotype of *Crinipellis perniciosa* (witches' broom disease) on solanaceous hosts. **Plant Pathology**, v.34, p.306–312, 1985.

COLAUTO, N.B.; DIAS, E.S.; GIMENES, M.A.; EIRA, A.F. Genetic characterization of isolates of the basidiomycete *Agaricus blazei* by RAPD. **Brazilian Journal of Microbiology**, v.33 p.131-133, 2002.

DEBETS, A.J.M. Parasexuality in fungi: mechanisms and significance in wild populations. In: COUTEAUDIER, P.B.Y.; CLARKSON, J. **Molecular variability of fungal pathogens.** New York: CAB International, 1998. p.41-53.

DÉLYE, C.; CORIO-COSTET, M.F.; LAIGRET, F. A RAPD assay for strain typing of the biotrophic grape powdery mildew fungus *Uncinula necator* using DNA extracted from the mycelium. **Experimental Mycology**, v.19, p.234-237, 1995.

DI PRIMO, P.; CARTIA, G.; KATAN, T. Vegetative compatibility and heterokaryon stability in *Fusarium oxysporum* f.sp. radicis-lycopersici from Italy. **Plant Pathology**, v.50, p.371-382, 2001.

FEICHTENBERGER, E.; SPÓSITO, M.B. Doenças fúngicas dos citros: manejo integrado. **Visão Agrícola**, v.2, p.44-47, 2004.

FENILLE, R.C.; CIAMPI, M.B.; SOUZA, N.L.; NAKATANI, A.K.; KURAMAE, E.E. Binucleate Rhizoctonia sp. AG G causing root rot in yacon (*Smallanthus sonchifolius*) in Brazil. **Plant Pathology**, v.54, p.325-330, 2005.

FUNGARO, M.H.P. PCR na micologia. **Biotecnologia Ciência & Desenvolvimento**, v.14, p.12-16, 2000.

GEZAHGNE, A.; ROUX, J.; WINGFIELD, M.J. Diseases of exotic *Eucalyptus* and *Pinus* species *in* Ethiopian plantations. **South African Journal of Science**, v.99, p.29-33, 2003.

GLASS, N.L.; JACOBSON, D.J.; PATRICK, K.T.S. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. **Annual Review of Genetics**, v.34, p.165-186, 2000.

GOMES, R.C.; SÊMEDO, L.T.A.S.; SOARES, R.M.A.; LINHARES, L.F.; ULHOA, C.J.; ALVIANO, C.S.; COELHO, R.R.R. Purification of thermostable endochitinase from *Streptomyces* RC1071 isolated from a cerrado soil and its antagonism against phytopathogenic fungi. **Journal of Applied Microbiology**, v.90, p.653-661, 2001.

GUZMÁN, P.; GEPTS, P.; TEMPLE, S.; MKANDAWIRE, A.B.C.; GILBERTSON, R.L. Detection and differentiation of *Phaeoisariopsis griseola* isolates with the polymerase chain reaction and group-specific primers. **Plant Disease**, v.83, p.37-42, 1999.

IRAM, S.; AHMAD, I. Analysis of variation in *Alternaria alternata* by pathogenicity and RAPD study. **Polish Journal of Microbiology**, v.54, p.13-19, 2005.

JACOBSON, D.J.; BEURKENS, K.; KLOMPARENS, K.L. Microscopic and ultrastructural examination of vegetative incompatibility in partial diploids heterozygous at *het* loci in *Neurospora crassa*. **Fungal Genetics and Biology**, v.23, p.45-56, 1998.

JAMES, T.Y.; LIOU, S.R.; VILGALYS, R. The genetic structure and diversity of the A and B mating-type genes from the tropical oyster mushroom, *Pleurotus djamor*. **Fungal Genetics and Biology**, v.41, p.813-825, 2004.

JAMIL, F.F.; SARWAR, N.; SARWAR, M.; KHAN, J.A.; GEISTLINGER, J.; KAHAL, G. Genetic and pathogenic diversity with in *Ascochyta rabiei* (Pass.) Lab. Populations in Pakistan causing blight of chickpea (*Cicer arientinum L.*). **Physiological and Molecular Plant Pathology**, v.57, p.243-254, 2000.

LACAVA, P.T.; ARAUJO, W.L.; MACCHERONI JR., W.; AZEVEDO, J.L. RAPD profile and antibiotic susceptibility of *Xylella fastidiosa*, causal agent of citrus variegated chlorosis. **Letters in Applied Microbiology**, v.33, p.302-306, 2001.

McCABE, P.M.; GALLAGHER, M.P.; DEACON, J.W. Microscopic observation of perfect hyphal fusion in *Rhizoctonia solani*. **Mycological Research**, v.103, p.487-490, 1999.

NAUTA, M.J.; HOEKSTRA, R.F. Vegetative incompatibility in Ascomycetes: highly polymorphic but selectively neutral? **Journal of Theoretical Biology**, v.183, p.67-73, 1996.

OLD, K.M.; DAVISON, E.M. Canker diseases of eucalypts. In: OLD, K.M.; DAVISON, E.M.; KEANE, P.J.; KILE, G.A.; PODGER, F.D.; BROWN, B.N. **Disease and pathogens of eucalypts**. Melbourne: CSIRO Publishing, 2000. p.241-257.

PLOETZ, R.C. Diseases of atemoya, cherimoya, sour sop, sugar apple and relates fruit crops. In: PLOETZ, R.C. **Disease of tropical fruit crops**. Wallingford: CABI Publishing, 2003. p.21-34.

RAEDER, U.; BRODA, P. Rapid preparation of DNA from filamentous fungi. **Letters in Applied Microbiology**, v.1, p.17-20, 1985.

SNETH, S.; JABAJI-HARE, S.; NEATE, S.; DIJST, G. **Rhizoctonia species**: taxonomy, molecular biology, ecology, pathology and disease control. 1.ed. Dordrecht: Kluwer Academic Publishers, 1996. 559p.

TIGANO, M.S.; ALJANABI, S. RAPD analysis of *Nomuraea rileyi*. **Journal of Invertebrate Pathology**, v.75, p.240-242, 2000.

VAILLANCOURT, L.J.; HANAU, R.M. Genetic and morphological comparisons of *Glomerella* (*Colletotrichum*) isolates from maize and from sorghum. **Experimental Mycology**, v.16, p.219-229, 1992.

WELSH, J.; McCLELLAND, M. Fingerprinting genomes using PCR with arbitrary primers. **Nucleic Acids Research**, v.18, p.7213-7218, 1990.

WILLIAMS, J.G.K.; KUBELIC, A.R.; LIVAK, K.J.; RAFALSKI, J.A.; TINGEY, S.V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research**, v.18, p.6531-6535, 1990.

WORRALL, J.J. Somatic incompatibility in basidiomycetes. **Mycologia**, v.89, p.24-36, 1997.

YAP, I.; NELSON, R.J. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. Manila: IRRI, 1996. (Discussion Paper Series, 14).

Received March 07, 2006
Accepted February 09, 2007