ABSTRACT: 407

ASSESSMENT OF THE GENETIC DIVERSITY OF THE CAUSAL AGENT OF ASIAN SOYBEAN RUST IN THE MERCOSUR COUNTRIES USING AFLP

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Asian soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi* Syd. & Syd, is regarded as one of the most destructive diseases of soybean [*Glycine max* (L.) Merr.], as it results in yield losses of up to 80% under conditions that favour disease development. SBR was first reported in Japan in 1902, and afterwards it spread to other parts of the world. As for the Mercosur countries, it was reported in Paraguay and Brazil in 2001, in Argentina in 2002, and in Uruguay in 2004.

The objective of this study was to assess the genetic diversity of *P. pachyrhizi* isolates from Mercosur countries using a direct methodology based on AFLP technique. Isolates from Argentina, Brazil, Paraguay and Uruguay were collected from soybean fields. Genus and species identification was accomplished by PCR. From 23 *P. pachyrhizi* isolates, a total of 3,014 alleles were amplified with 33 pairs of AFLP primers.

From a total of 1,550 loci, 1,545 were polymorphic (99.7%). Analysis of molecular variance (AMOVA) showed high genetic differentiation of *P. pachyrhizi* within countries, with 87.6% variation. Variance among countries represented only 12.4% of the total variation, suggesting the existence of a gene flow among countries. Such flow was confirmed by a Phi_{ST} value of 0.12. Phi_{ST} values of 0.15 and 0.14 were obtained for the 2007-2008 and 2008-2009 seasons, respectively, while comparison between seasons resulted in a Phi_{ST} value of 0.22, which implies that each year genetic diversity changes. A dendogram analysis, based on the Jaccard coefficient, showed similar results: two separated clusters grouped according to the collection season (2007-2008 / 2008-2009). The pathogen's capability to break down soybean resistance genes successively in this important production area may be explained by the high genetic diversity described in the present study.

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Assessment of the genetic diversity of the causal agent of Asian soybean rust in the Mercosur countries using AFLP

BiotecSojaSur: Soybean Virtual Lab in Mercosur

C. M. L. Rocha, **G. R. Vellicce**, M. G. García, E. M. Pardo, A. De Lucía, J. Gilli, C. Ghione, N. Bogado, S. German, V. Bonnecarrere, M. Rodríguez, F. Correa Marcelino, L. D. Ploper and A. P. Castagnaro

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ESTACION EXPERIMENTAL AGROINDUSTRIAL OBISPO COLOMBRES
Tucumán | Argentina

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- The host range of *P. pachyrhizi* includes more than 90 legume species.
- The full life cycle of *P. pachyrhizi* has not been reported so far, thus, it is thought that reproduction is predominantly asexual.
- The urediniospores can be dispersed thousands of kilometres by wind.

- SBR was first reported in Japan in 1902 and afterwards it spread to other parts of the world. In the Mercosur, it was reported in Paraguay and Brazil in 2001, in Argentina in 2002, and in Uruguay in 2004*.

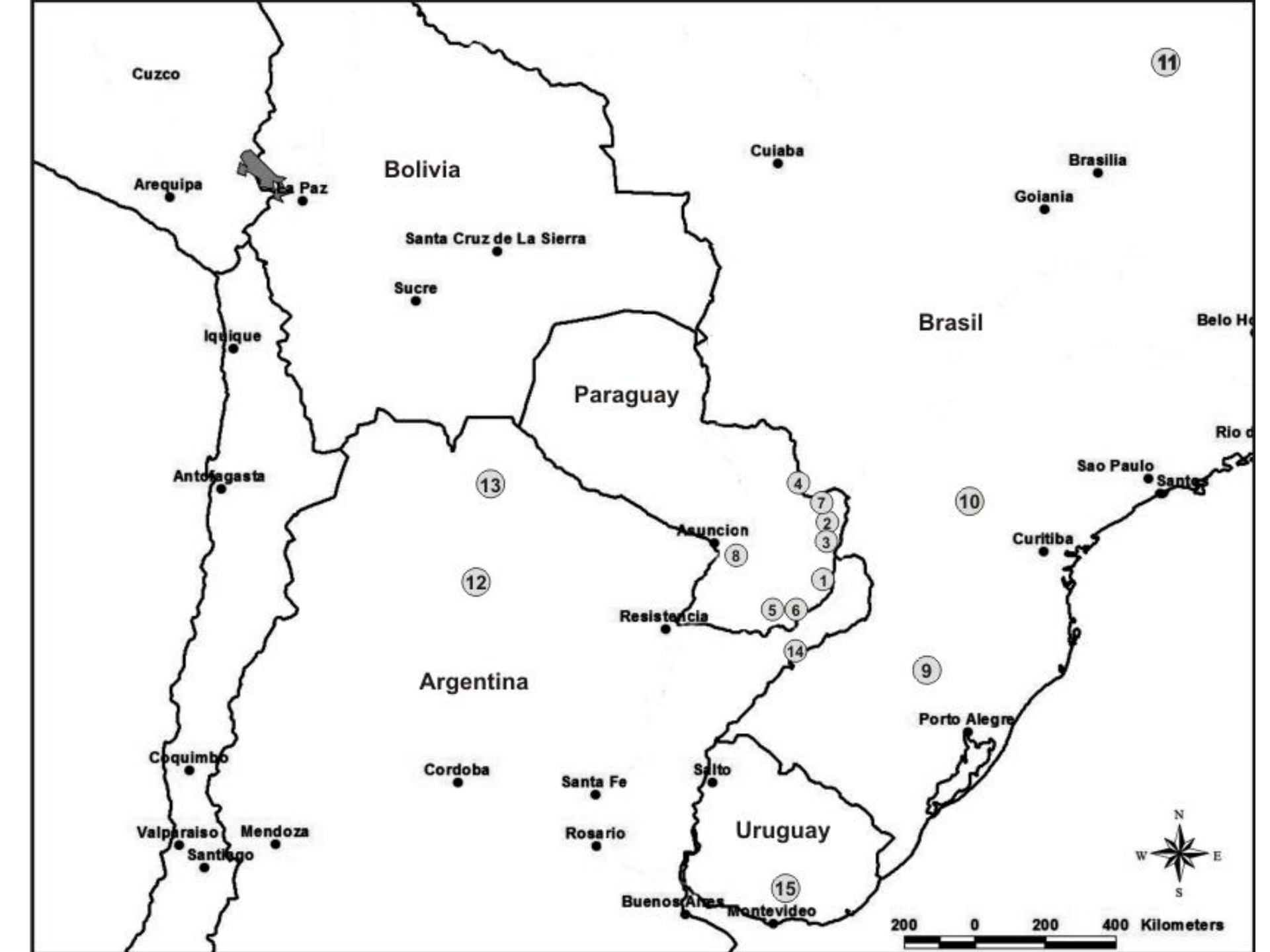
- *Stewart et al., 2005, Plant Disease 89 (8), 909.

- We were able to estimate the genetic diversity based on AFLP analysis in one important soybean production area. Argentina, Brazil, Paraguay and Uruguay gather 45% of the world's surface in soybean production with 46.24 million hectares.

Objetive

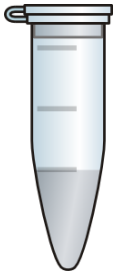
- The objective of this work was to determine the genetic diversity of *P. pachyrhizi* field samples from South American countries using a direct method based on AFLP technology.

Isolate	Identification number	Geographic origin	Year of sampling
1p	1	Santa Rita, Paraguay.	2008
2p	2	San Alberto Paraguay.	2008
3p	3	Troncal Paraguay.	2008
4p	4	Pindoty, Paraguay.	2008
5p	5	Capitán Miranda, Paraguay.	2008
CRIA	6	CRIA, Paraguay.	2009
CC	7	Corpus Christi, Paraguay.	2009
SA	8	San Antonio, Paraguay.	2009
PL2	6	CRIA, Paraguay.	2009
PL5	6	CRIA, Paraguay.	2009
PV	6	CRIA, Paraguay.	2009
VP	6	CRIA, Paraguay.	2009
RS	9	Rio Grande do Sul, Brasil.	2009
PR	10	Paraná Mama da Serra, Brasil.	2009
BA	11	Bahia, Brasil.	2009
UNC	12	Tucuman, Argentina.	2008
Mo	13	Salta, Argentina.	2008
KUD	14	Misiones, Argentina.	2008
MS	14	Misiones, Argentina.	2008
RU1	15	INIA, Las Brujas, Uruguay.	2009
RU2	15	INIA, Las Brujas, Uruguay.	2009
RU3	15	INIA, Las Brujas, Uruguay.	2009
RU6	15	INIA, Las Brujas, Uruguay.	2009


Cuzco
Arequipa
La Paz
Bolivia
Santa Cruz de La Sierra
Sucre
Iquique
Antofagasta
Cuiaba
Brasilia
Goiania
Brasil
Belo Ho
Paraguay
Rio d
Sao Paulo
Santos
Asuncion
Curitiba
Resistencia
Argentina
Porto Alegre
Coquimbo
Cordoba
Santa Fe
Salto
Valparaiso
Mendoza
Santiago
Rosario
Uruguay
Buenos Aires
Montevideo
11
13
12
4
7
2
3
8
1
5
6
14
10
9
15
N
W
E
S
200
0
200
400
Kilometers

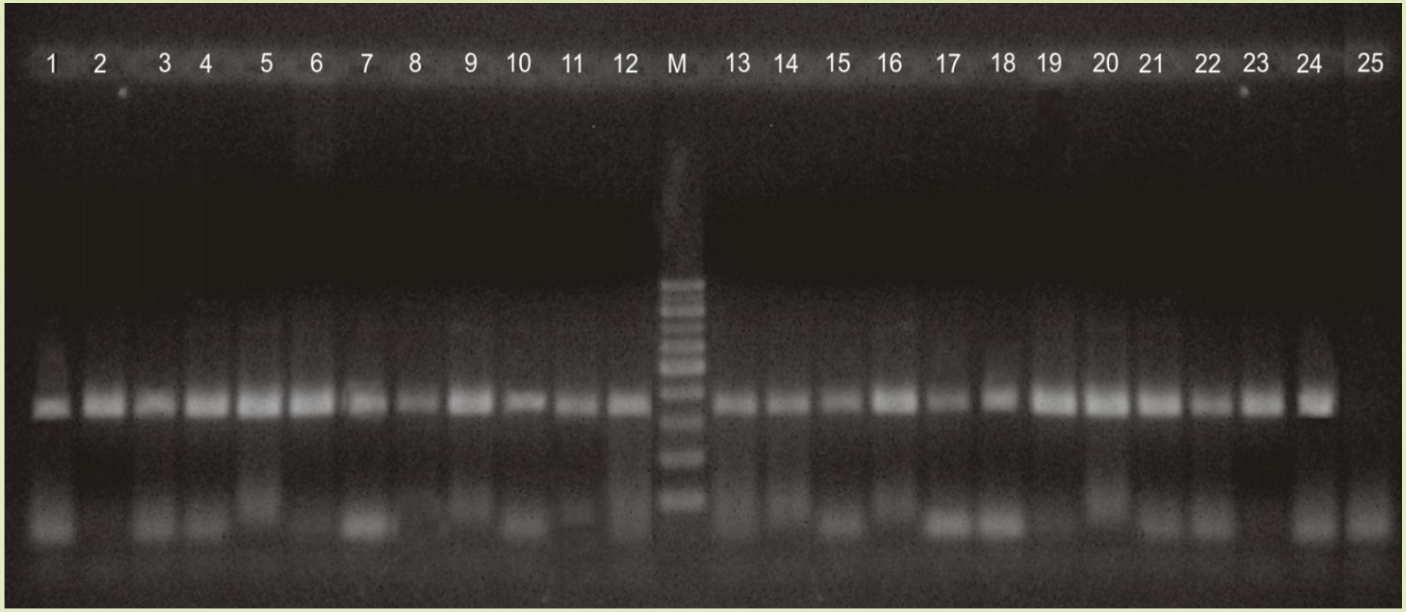
Total genomic DNA was extracted from 20 mg of uredeniospores using the CTAB protocol.

spore suspension



Villavicencio et al, 2007 *Radiation Physics and Chemistry* 76, 1878–81.

Results

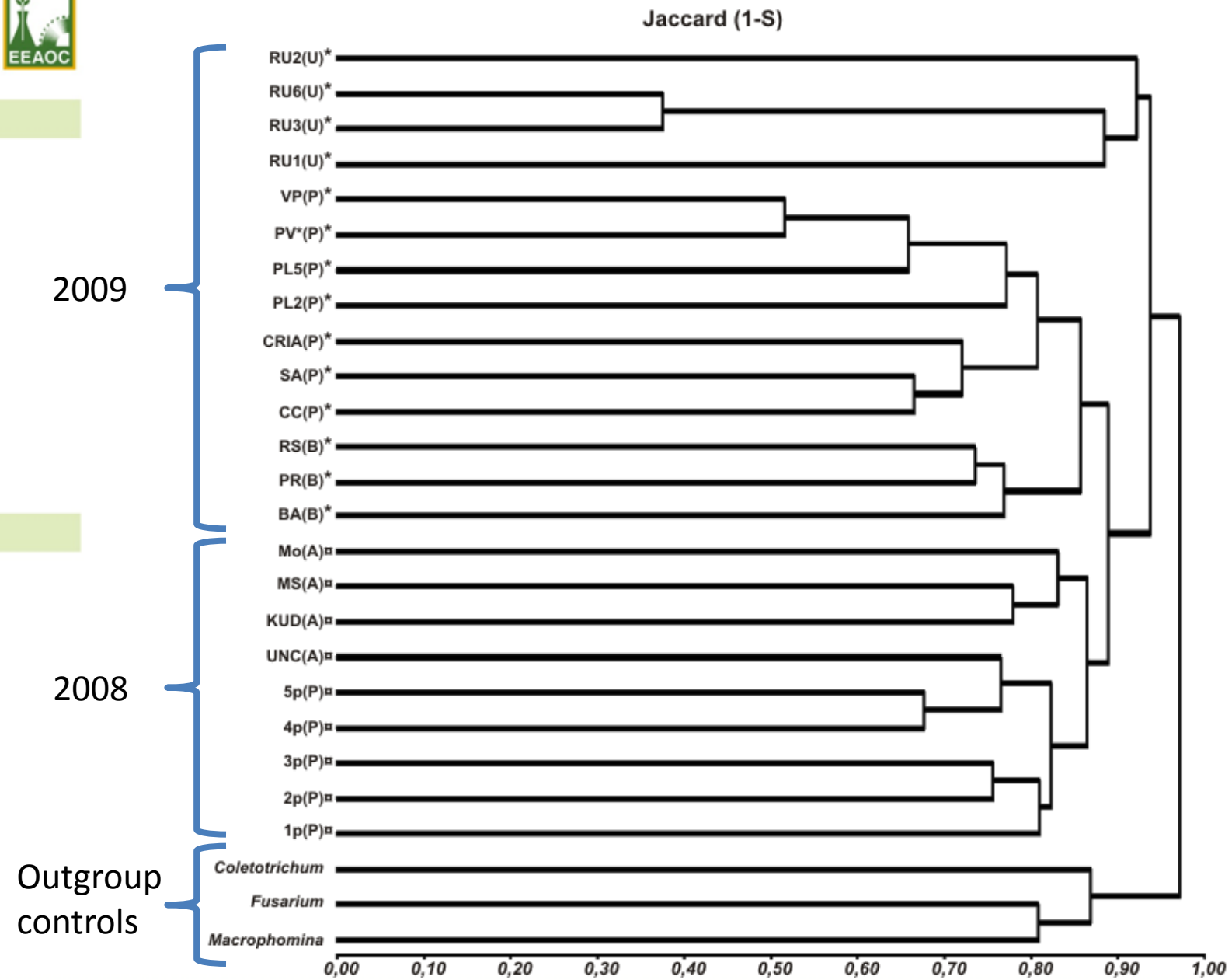


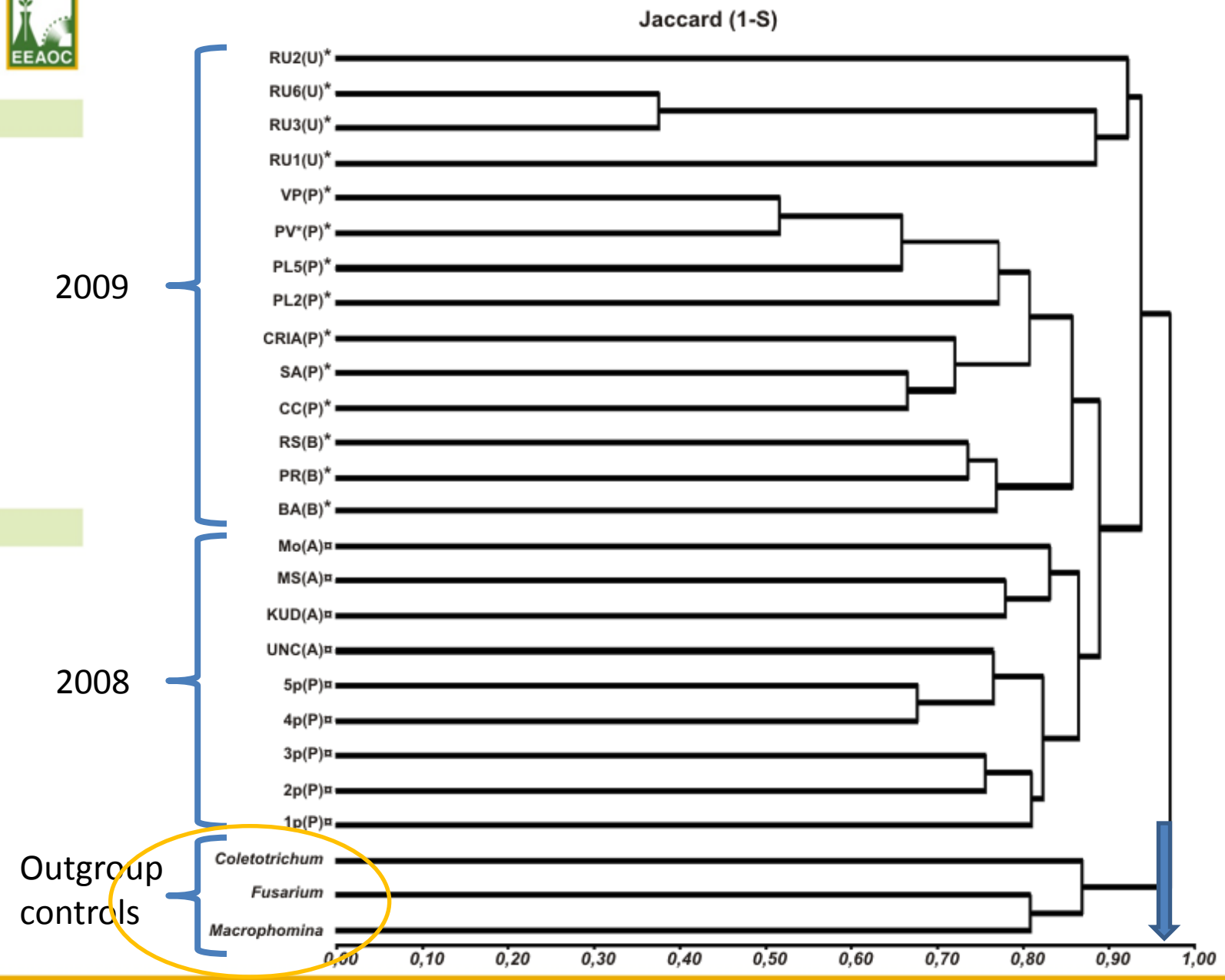
Agarose gel electrophoresis of PCR amplifications with specific primers for *Phakopsora pachyrhizi* (Ppa1/Ppa2) of the samples collected in Argentina, Brazil, Paraguay and Uruguay. 1) 1p, 2) 2p, 3) 3p, 4) 4p, 5) 5p, 6) CRIA, 7) CC, 8) SA, 9) PL2, 10) PL5, 11) PV, 12) VP, 13) RS, 14) PR, 15) BA, 16) UNC, 17) Mo, 18) KUD, 19) MS, 20) RU1, 21) RU2, 22) RU3, 23) RU6, 24) Positive control, 25) Negative control: Soybean DNA.

Checking reproducibility of AFLP patterns by using duplicate DNA purified from the same sample.

Number of samples collected in Argentina, Brazil, Paraguay and Uruguay in 2008 and 2009, and percentage of polymorphic bands.

Samples	23
Replicate samples	0
Bands (number)	1550
Duplicate bands pattern	696
Monomorphic bands	5
Polymorphic bands	1545
Primers	33


Jaccard (1-S)
RU2(U)*
RU6(U)*
RU3(U)*
RU1(U)*
VP(P)*
PV*(P)*
PL5(P)*
PL2(P)*
CRIA(P)*
SA(P)*
CC(P)*
RS(B)*
PR(B)*
BA(B)*
Mo(A)¤
MS(A)¤
KUD(A)¤
UNC(A)¤
5p(P)¤
4p(P)¤
3p(P)¤
2p(P)¤
1p(P)¤
Coletotrichum
Fusarium
Macrophomina
0,00
0,10
0,20
0,30
0,40
0,50
0,60
0,70
0,80
0,90
1,00
2009
2008
Outgroup controls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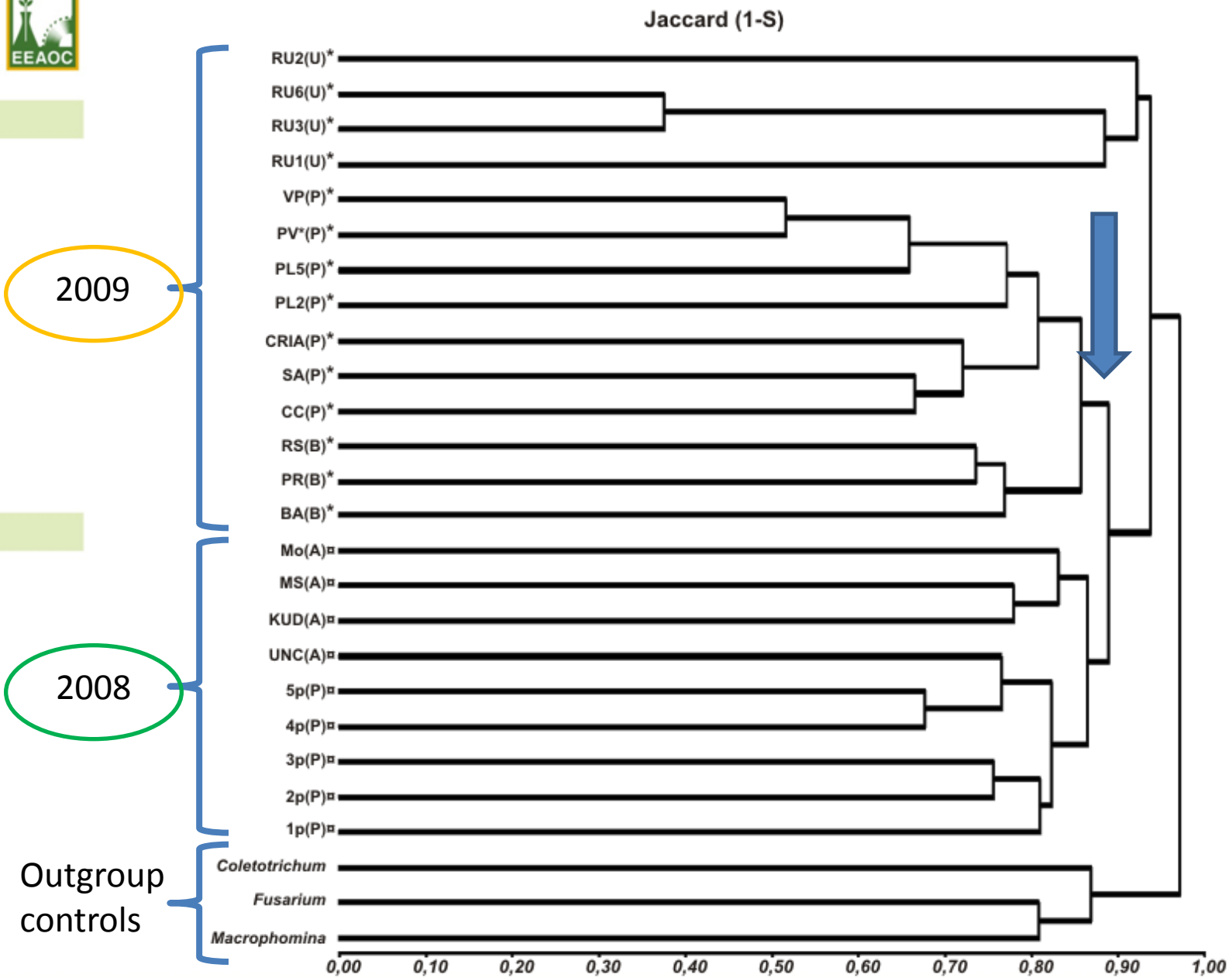
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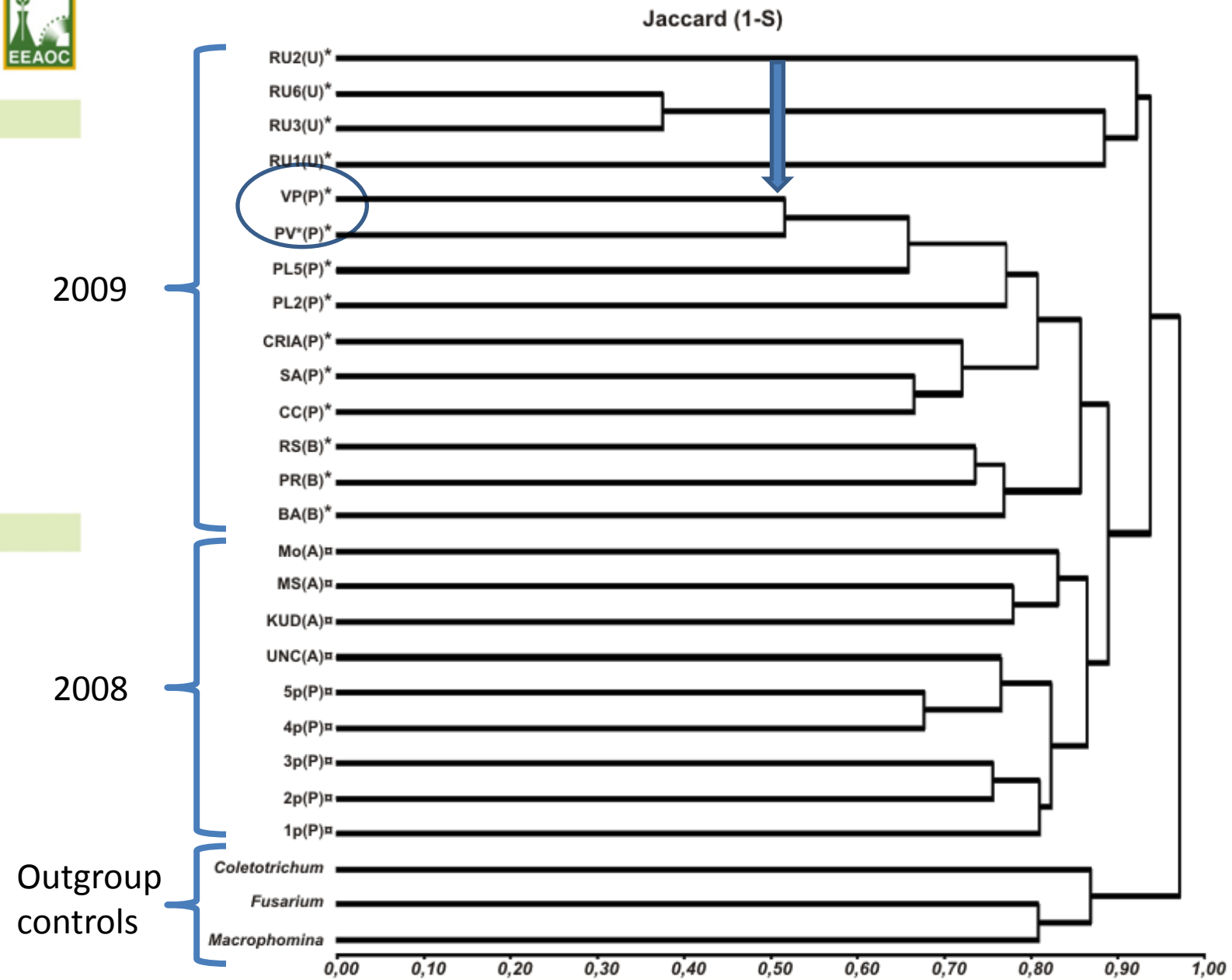
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Outgroup controls

AMOVA 1

Source of variation	Sum of squares	D.f.	Mean squares	p-value	Iter.#	Variance components.	Percentage of variation
Among countries	399216,06	3	133072,02	0,03	400	11953,72	12,36
Within countries	1102248,76	13	84788,37	0,01	400	84788,37	87,64
Total	1501464,82	16	93841,55			96742,09	100

Phi_ST= 0.12
Phi_population= 0.12

Analysis of molecular variance (AMOVA) and percentage of variation among and within samples of *P. pachyrhizi* from Argentina, Brazil, Paraguay and Uruguay.

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Phi_population= 0.12

Analysis of molecular variance (AMOVA) and percentage of variation among and within groups of isolates of *P. pachyrhizi* from Argentina, Brazil, Paraguay and Uruguay.

AMOVA 2

Analysis of molecular variance (AMOVA) and percentage of variation of *P. pachyrhizi* samples collected in 2008 vs 2009.

Source of variation	Sum of squares	D.f.	Mean squares	p-value	Iter.#	Variance components.	Percentage of variation
Year	265123,73	1,00	265123,73	0	400	23529,67	22,21
Within	1236341,09	15,00	82422,74	0	400	82422,74	77,79
Total	1501464,82	16,00	93841,55			105952,41	100

Phi_ST=0,22

Phi_year=0,22

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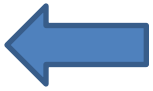
Phi_year=0,22 

Conclusions

- In our study, we observed a high genetic diversity of samples collected in South America using AFLP markers.

Conclusions

- We observed changes between samples collected in 2008 and 2009.

Conclusions

- This area comprises several agro ecological zones with different soybean cultivars and a high genetic diversity of the pathogen, where the plant-pathogen co-evolution could be responsible for the loss of effectiveness of Mendelian resistance genes.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