

Identification by PCR-RFLP of *Phomopsis/Diaporthe* species on Italian soybean seeds

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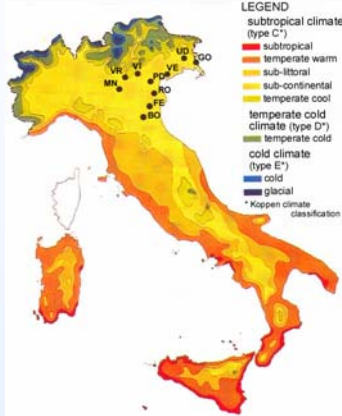
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No.	Cultivar	Growing area	Infection %	% PL	% DPC	% DPS
1	Susan	Mantova (MN)	2	0.5	1	0.5
2	Susan	Padova (PD)	2	2		
3	Susan	Padova (PD)	2.5	2.5		
4	Stephany	Padova (PD)	7.5	16.5	0.5	0.5
5	Stephany	Padova (PD)	8	7		
6	Pacific	Vicenza (VI)	1	0.5		0.5
7	Pacific	Verona (VR)	0			
8	Pacific	Vicenza (VI)	5	5		
9	Susan	Venezia (VE)	8	3	3.5	1.5
10	Sissi	Venezia (VE)	10	2.5	4.5	3
11	Patty	Ferrara (FE)	7	2	4	1
12	Patty	Ferrara (FE)	10	5	4	1
13	Patty	Bologna (BO)	2			2
14	Dekabig	Ferrara (FE)	2			2
15	Dekabig	Gorizia (GO)	27	14	6	7
16	Dekabig	Rovigo (RO)	4			2
17	Dekabig	Gorizia (GO)	22	15	3	4
18	Dekabig	Ferrara (FE)	3			1
19	Dekabig	Ferrara (FE)	2			2
20	Dekabig	Ferrara (FE)	10	1	8	1
21	Dekabig	Ferrara (FE)	9	1	6	2
22	Sapporo	Ferrara (FE)	15	11	3	1
23	Sapporo	Ferrara (FE)	12	9	3	
24	Sapporo	Ferrara (FE)	19	11	7	1
25	Sapporo	Ferrara (FE)	11	2	7	2
26	Sakai	Udine (UD)	6	1	2	3
27	Sakai	Udine (UD)	10	2	3	5
28	Pekino	Bologna (BO)	7	2	1	4
29	Sapporo	Ferrara (FE)	15	8	3	4
30	Sapporo	Ferrara (FE)	10	4	5	1
Total incidence				47%	32%	21%

DIAPORTHE/PHOMOPSIS complex (D/P) on soybean in Italy

Phomopsis longicolla (PL) (Seed decay)

Diaporthe phaseolorum var. *caulivora* (DPC) (Stem canker)

D. phaseolorum var. *sojiae* (DPS) (Seed and pod decay)

-The morphological identification is time consuming and not totally reliable.

-The European soybean seeds are certified for *Diaporthe phaseolorum* infection (tolerance for infection ≤ 15%).

Objectives

-Know the incidence of each species of the D/P complex on the infection level of Italian certified soybean seeds.

-Know the correlation between the incidence and soybean growing area. **Activity**

Fig. 1 – Seed sample locations on the climate map of Italy.

Tab.1 – Soybean seed varieties analysed and infection incidence of *Diaporthe/Phomopsis* species (PI=*Phomopsis longicolla*; DPC=*Diaporthe phaseolorum* var. *caulivora*; DPS=*Diaporthe phaseolorum* var. *sojiae*).

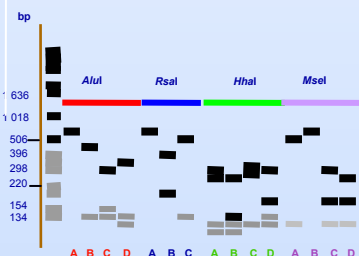


Fig. 2 – Schematic representation of the electrophoretic profiles (profile A, B, ...) for each restriction enzyme obtained by PCR-RFLP of the ITS region of the ribosomal DNA.

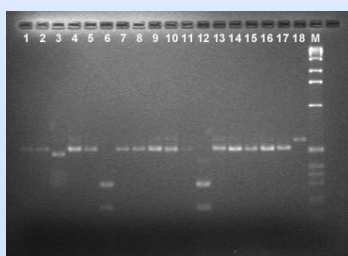


Fig. 3 – Agarose gel of ITS region amplified with ITS4 and ITS5 primers and digested with *MseI*, of 17 D/P isolates: 1, 2, 4, 5, 7-11, 13-17 = profile B; 3 = profile A; 6, 12 = profile C; 18 = control not digested; M = marker 0.07-12.2 kbp.

Species	<i>AluI</i>	<i>RseI</i>	<i>HhaI</i>	<i>MseI</i>
<i>P. longicolla</i>	B	B	A	B
<i>D.p. caulivora</i>	C	A	A/B	A
<i>D.p. sojiae</i> (56.0)	B	A	C	B
<i>D.p. sojiae</i> (10.1)	D	A	B	A
<i>D.p. sojiae</i> (15.2)	B	A	A	B
<i>D.p. sojiae</i> (11.9)	B	B	A	C
<i>D.p. sojiae</i> (5.1)	B	A	A	C
<i>D.p. sojiae</i> (1.7)	B	A	B	A

Tab. 2 – Identification of the D/P species based on electrophoretic profile combinations obtained by PCR-RFLP. In brackets the % of frequency of DPS profile combination. In red the profile(s) enabling to identify the single species.

Thirty seed samples (2001 production), with D/P infection level ranging from 1 to 27%, were analysed (100-200 seeds) (Tab. 1 and Fig. 1).

PCR – RFLP (polymerase chain reaction – restriction fragment length polymorphisms) were used. After incubation of the seeds on PDA Petri dishes, from the D/P isolates the DNA was extracted and amplified with ITS4 and ITS5 primers. The amplification product was digested with *AluI*, *RseI*, *HhaI* and *MseI* restriction enzymes (Fig. 3). The banding patterns obtained were compared with known banding pattern profiles (Tab. 2 and Fig. 2) (Zang *et al.*, 1998). The morphological characters were also observed after 25-30 days, to confirm the molecular identification.

Results

The banding patterns obtained with each enzyme allowed the identification of all the 295 isolates analyzed (Tab. 1).

Morphological confirmation allowed the identification of the species of only 80 % of the isolates.

The total incidence of each species was PL=47%, DPC=32% and DPS=21%; the distribution of the three species in the different growing areas was not significantly different.

Fifty-nine isolates were identified as DPS, and among them the molecular profile - B A C B – was the most frequent (56%). The morphological characteristics of those isolates, and in particular the constant presence in culture of perithecia, suggest that they could be the “true” *D. phaseolorum* var. *sojiae*. The other isolates never produced perithecia.

The method showed high reliability and therefore it could be a powerful method to identify the D/P species, and possibly to identify the different taxa among “DPS isolates”.

Reference

Zhang A.W., L. Riccioni, W.L. Pedersen, K. P. Kollipara, G. L. Hartman, 1998. Molecular identification and phylogenetic grouping of *Diaporthe phaseolorum* and *Phomopsis longicolla* isolates from soybean. *Phytopathology*, 88 (12), 1306-1314.