

*Pythium aphanidermatum*



( : : )

*P. aphanidermatum*

*P. aphanidermatum*

8P

*P. aphanidermatum*

OPB-08 (bp)

OPA-15

*P. aphanidermatum*

*P. aphanidermatum*

(bp)

*Phytophthora drechsleri* *P. deliense* *P. oligandrum* *P. ultimum*

*P. aphanidermatum*

OPB-08 OPA-15

*Rhizoctonia solani* *Fusarium solani*

*P. aphanidermatum*

.PCR

:

**Pathogenic variability of *Pythium aphanidermatum* isolates the causal agent of sugar beet root rot in Iran**

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**Abstract**

During 2007-2010, diseased samples of sugar beet were collected from fields in major sugar beet production areas of Iran in different growth stages. From 71 diseased samples, 23 isolates of *Pythium aphanidermatum* were isolated. On the basis of pathogenic variability in seed, seedling and mature stages of sugar beet, the results showed that *P. aphanidermatum* isolates have significant differences in their pathogenicity. All of the tested isolates were classified in 3 groups with high, moderate and low pathogenicity. 8P isolate from West Azarbaijan province had the highest pathogenicity among investigated isolates in each 3 stages. PCR fingerprinting was used to confirm identification of *P. aphanidermatum* isolates. The results showed an individual band approximately 2000 bp using OPA-15 and two bands (1000 and 1850 bp) using OPB-08 decamer primers. The bands were observed through all *P. aphanidermatum* isolates. The banding patterns of *P. aphanidermatum* was not similar to other species including *P. ultimum*, *P. oligandrum*, *P. deliense*, *Phytophthora drechsleri*, *Fusarium solani* and *Rhizoctonia solani*. It seems that OPA-15 and OPB-08 primers are capable to identify *P. aphanidermatum* from other root rot agents of sugar beet as well as other *pythium* species.

**Key words:** sugar beet, root rot, pathogenic variability, PCR.

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*Pythium aphanidermatum*

:

*P. paroecandrum* *P. osteracodes* *P. oliganderum* *P. deliense*

*Pythium* *Pythium* group "HS" *P. ultimum* var *ultimum*

group "T"

.(Gallian, 2001)

*P. deliense* Meurs

Mahmoudi and Soltani (2005) .(Behdad, 1996)

*P. aphanidermatum*

(Van der Plaasts-Niterink, 1981)

DNA

*P. aphanidermatum* Ahmadinejad and Okhovat (1976)

Herreo and Klemsdal (1998) .(Olive and Bean, 1999)

OPB-08 OPA-15

*P. aphanidermatum*

*P. aphanidermatum*

(Fasihiani, 1991)

)

(Abasi Moghadam *et al.*, 1998; Afzali and Ershad, 2006)

(

(Mahmoudi *et al.*, 2000; Arzanlou *et al.*, 2000)

PCR- RAPD

Irani and Ershad, 1995; )

*P. aphanidermatum*

Davoodee and Afzali, ) (Sheikholeslami *et al.*, 2005

*P. aphanidermatum*

*P. deliense*

(2006

*P.irregular* *P. ultimum* *P. deliense*

*P. paroecandrum*

*P. ultimum* var

.(Afzali and Banihashemi, 2000)

*ultimum*

.(Kashi *et al.*, 2000)

*P. aphanidermatum*

Zamani Noor *et al.* (2004)

*P. ultimum* *Aphanomyces cochlioides*

*Pythium* group *P. aphanidermatum* :

*P. oliganderum* *P. deliense* *Pythium* group "F" "G"

*P. okanoganense* *P. tracheiphilum* *P. salinum*

Babai-Ahari *et al.* (2004) .

*P. aphanidermatum*

CMA (Corn meal agar)

CMA

CMA

(Peever *et al.*, 2000)

Van der Plaasts-)

(Niterink, 1981; Dick, 1990

Hecker and Ruppel, 1977; Scholten *et al.*, 2001; )

( )

(Büttner *et al.*, 2004

( )

( )

( )

*P. aphanidermatum*

: *P. aphanidermatum*

*P. aphanidermatum*

( ) *P. oligandrum*

Cormarc and )

(Moffat, 1961

°C

( )

Büttner *et al.*, )

( )

.(2004

(Mahmoudi *et al.*, 2004)

(Carling *et al.*, 2002)

Area Under )

(Disease Progress Curve

$$\sum_i^{n-1} [(y_i + y_{i+1}) / 2] [t_{i+1} - t_i]$$

y<sub>i</sub>

n

t<sub>i</sub> i

/

:

( )

( )

PDA (potato dextrose agar)

°C ( ) (DI) (Büttner *et al.*, 2004)

PCR Disease Index:  $DI = \frac{\sum (\text{Disease Index})}{\text{Total}} \times 100$

DNA

*P. aphanidermatum* *P. ultimum* *P. aphanidermatum*  
*P. deliense* *P. oligandrum*  
*Phytophthora drechsleri* *Rhizoctonia solani*  
*Fusarium solani*

SPSS SAS DNA (Dellaporta *et al.*, 1983)

UPGMA DNA

SPSS

: OPB-08 OPA-15

*P. aphanidermatum*

*P. aphanidermatum* OPA-15 (5' TTCCGAACCC-3') *P. aphanidermatum*  
Herreo and OPB-08 (5'GTCCACACGG-3')  
Klemsdal (1998)  
*P. aphanidermatum*

CMA

DNA

/ ng/μl /  
/ / dNTP 10x buffer  
MgCl2  
SmarTaq ( ) / ng/μl

°C

*Pythium aphanidermatum*  
**Table 1.** *Pythium aphanidermatum* isolates used in this study

Geographic location	Isolate No
	5P
	7P
	8P
	10P
	11P
	12P
	13P
	14P
	15P
	16P
	17P
	18P
	19P
	20P
	21P
	22P
	23P
	24P
	46P
	64P
	65P
	66P
	69P
	25P*

***P. aphanidermatum***

24P 8P

*P. aphanidermatum*

(HV) *P. aphanidermatum*

( ) (WV) (V)

24P 8P

46P 5P 10P 17P 16P (HV)

11P 20P 19P 14P 18P 13P (V)

17P (WV) 22P 7P 23P 21P 12P 15P

18P ( )

*P. oligandrum*

8P

*P. aphanidermatum*

*P. aphanidermatum*

(8P, 24P)

(18P) (46P)

*P. oligandrum*

CMA (25P)

*Pythium oligandrum* :\*

\*: The *Pythium oligandrum* isolate was used as a non pathogenic check.

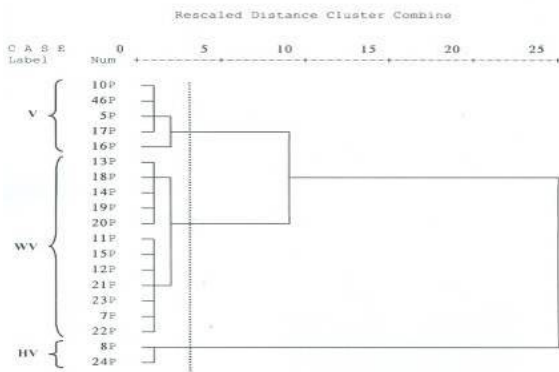
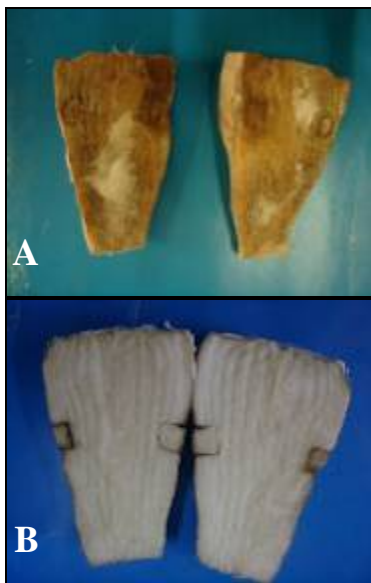


Fig 1. The dendrogram was generated using cluster analysis *P. aphanidermatum* isolates in laboratory tests (HV: Highly virulent, V: Virulent, WV: Weak virulent)



(A) 8P

(B)

Fig 2- Disease severity of 8P isolate (A) in comparison with health check (B) in root inoculation after 10 days

(*P. oligandrum*) 25P

*P. aphanidermatum*

( )

\*

Table 2. Comparison of relative virulence of *P. aphanidermatum* on sugar beet root \* in laboratory with scale (1-9)

Second test 2	First test 1	Isolate No.
---***	5.33 bcd**	5P
---	4.00 cde	7P
8.50 a	8.50 a	8P
3.45 bcd	5.66 bcd	10P
---	3.33 de	11P
---	3.00 de	12P
---	2.00 e	13P
---	2.33 e	14P
---	3.33 de	15P
---	4.66 cde	16P
4.66 bc	6.00 bc	17P
1.91 d	2.00 e	18P
2.33 d	2.33 e	19P
---	2.66 e	20P
---	3.00 de	21P
---	3.66 cde	22P
---	3.16 de	23P
5.33 b	7.66 ab	24P
---	5.66 bcd	46P
2.58 cd		65P
4.58 bc		66P
3.16 bcd		69P
1	1	25P * * * *
1	1	

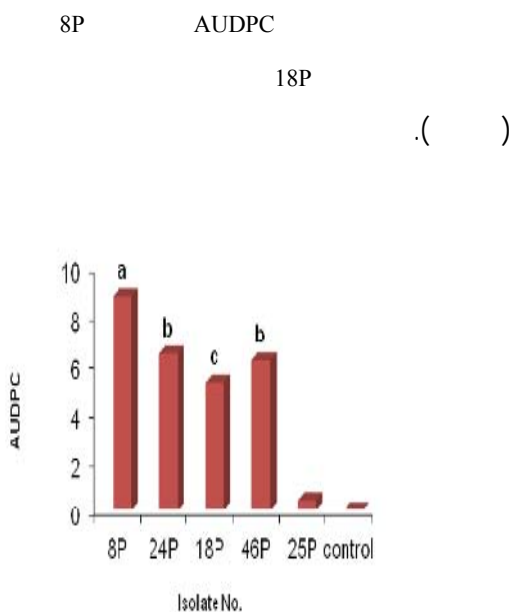
\*\*\*

%

*P. oligandrum*

\* \* \* \*

\*: Approximate weight of used roots was 500-700 gram; \*\*: Values means followed by the same letter in each column are not significantly different at 5%, according to Duncan's multiple-range test; \*\*\*: Not Tested; \*\*\*\*: *P. oligandrum* isolate.



**Fig. 3.** Comparison of AUDPC of representative isolates of *P. aphanidermatum* in *in vitro* tests after two days

*P. aphanidermatum*

*P. aphanidermatum*

*P. aphanidermatum*

8P ( )

18P 46P 24P

**Table 3.** Comparison of relative virulence of *P. aphanidermatum* isolates on germinated seed in direct contact with mycelium in Petri dish with scale (0-4)

Days after inoculation			Isolate No.
3	2	1	
4 a	3.64 a	2.06 a*	8P
4 a	2.55 c	1.06 c	18P
3.97 b	2.92 b	1.39 b	24P
4 a	2.94 b	1.27 bc	46P
0.34	0.31	0.04	25P**
0.02	0	0	Control

%; *P. oligandrum* ;\*\*

\*: Values means followed by the same letter in each column are not significantly different at 5%, according to Duncan's multiple-range test; \*\*: *P. oligandrum* isolate



*P. aphanidermatum*  
 .( )  
 :( )  
*P. aphanidermatum*

*P. aphanidermatum*

**Table 4.** Comparison of relative virulence of *P. aphanidermatum* in seedling stage in greenhouse conditions

Damping - off (%)	Isolate No.
95.99 a*	8P
43.77 b	10P
85.94 a	15P
47.79 b	17P
91.97 a	22P
83.94 a	46P
0	Control

18P

8P

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.( )

.\*

\*: Values means followed by the same letter in each column are not significantly different at 5%, according to Duncan's multiple-range test.

OPA-15 *P. aphanidermatum*  
 OPB-08 (bp)  
 (bp)  
 .( )

*Phytophthora drechsleri* OPB-08  
 (bp)

*P. ultimum*  
*P. oligandrum* (bp)

*Fusarium solani P. deliense*

OPA-15

*Phytophthora drechsleri*

*P. deliense* (bp)

(bp)

*F. solani P. ultimum P. oligandrum*

.( )

*P. aphanidermatum*

8P

/ 18P

.( )

:( )

20P 64P 14P 46P 22P 8P

/ / /

15P

/

- Base pair

*P. aphanidermatum*

( )

**Table 5.** Comparison of relative virulence of *P. aphanidermatum* in mature plants in greenhouse conditions with scale (1-9)

5 Months plants	3 months plants	2 months plants	Isolate No.
---	8 abc	---	5P
7.8 a	9 a	8.33 a	8P
6 b	5 efg	5.25 bc	10P
---	7.66 abcd	---	11P
---	7.33 abcdef	---	13P
---	8.83 a	---	14P
---	3.5 g	---	15P
---	8 abc	6 b	16P
---	5.83 cdefg	5.5 bc	17P
4.75 c	7.5 abcde	3.5 c	18P
---	6.5 abcdef	---	19P
---	8.5 ab	---	20P
---	5.16 defg	---	21P
---	9 a	---	22P
---	6 bcdefg	---	23P
6.7 b	8 abc	7.5 ab	24P
6.16 b	9 a	5.25 bc	46P
---	8.66 a	---	64P
---	4.83 fg	---	65P
---	2	---	25P**
1	1	1	Control

.\*

%

(Cormarc and Moffat, 1961)

*P. oligandrum* .\*\*

\*: Values means followed by the same letter in each column are not significantly different at 5%, according to Duncan's multiple-range test; \*\*: *P. oligandrum* isolate

(Zamani noor *et al.*, 2004; Babai-Ahary *et al.*, 2004)

*P. aphanidermatum*

(Dick, 1990)

(Van der Plaasts-Niterink, 1981)

*P. deliense*

*P. deliense*

*P. aphanidermatum*

*P. aphanidermatum*

*P. aphanidermatum*

*R. solani*

OPA-15

(bp)

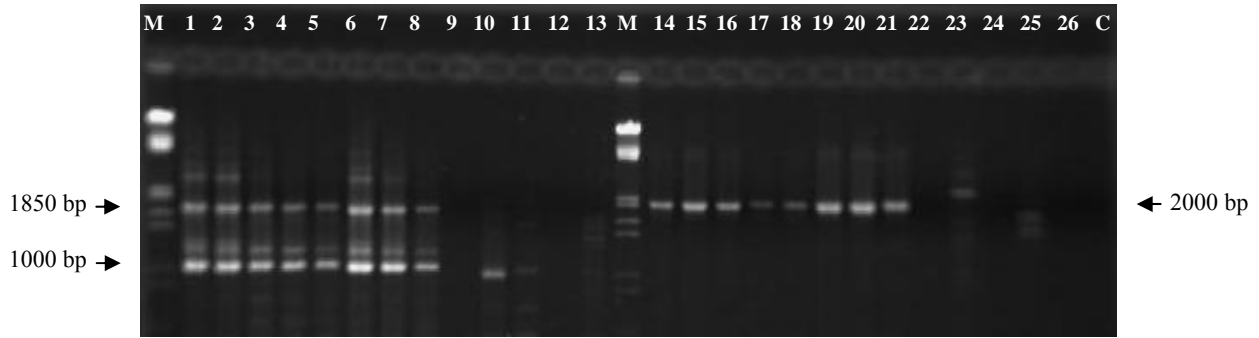
OPB-08

) (bp)

.(

*P. aphanidermatum*

*P. aphanidermatum*



Pythium oligandrum (PO) Pythium deliense (PD) Pythium ultimum(PU) Pythium aphanidermatum (PA)

PA3 PA2 PA1 .OPB-08 OPA- 15 F. solani (FS) Phytophthora drechsleri (PHD)

PA1 OPB-08 PO PD PU PHD FS PA8 PA7 PA6 PA5 PA4

C OPA- 15 PO PD PU PHD FS PA8 PA7 PA6 PA5 PA4 PA3 PA2

(Lambda DNA /EcoRI+HindIII Marker) DNA M (DNA master mix)

**Fig 4.** Banding patterns of *P. aphanidermatum* (PA), *P. ultimum* (PU), *P. deliense* (PD), *Pythium oligandrum* (PO), *Phytophthora drechsleri* (PHD) and *F. solani* (FS) using primers OPA-15 and OPB-08; lanes 1-13, isolates, PA1, PA2, PA3, PA4, PA5,PA6 , PA7, PA8, FS, PHD, PU, PD and PO using OPA-08; lanes 14-26, PA1, PA2, PA3, PA4, PA5,PA6, PA7, PA8, FS, PHD, PU, PD and PO using OPA-15; C: control reaction (Master mix without template DNA). Lanes M: ladder molecular size marker (Lambda DNA /EcoRI+HindIII Marker)

Zang and Yang (2000)

*Pythium*

*P. ultimum*

*P. ultimum*

8P (Zentmayer and Erwin, 1970)

Mahmoudi *et al.* (2004) .

*R. solani*

*P. aphanidermatum*

*P. aphanidermatum*

Al- sa'di *et al.*, 2003; )

Herreo *et al.*, 2003; Zang and Yang, 2000; Al- sa'di *et al.*, (2007

(Ristaino and Duniway, 1989)

(bp)

Erwin and Robeiro (1996)

*P. aphanidermatum*

PCR-RAPD

OPB-08 OPA-15

(Herreo and Klemsdal, 1998)

OPA-15

*P. aphanidermatum*

OPB-08

)

*P. aphanidermatum*

Mc Carter and Littrell .

(1967)

*P. aphanidermatum*

*P. aphanidermatum*

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OPA-15

*P. aphanidermatum*

OPB-08

OPA-15

*P. aphanidermatum*

OPB-08

(bp)

(bp)

*P. aphanidermatum*

Herreo and Klemsdal (1998)

*P. aphanidermatum*

OPB-08 OPA-15

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OPA-15

OPB-08

(bp)

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