J. Crop Prot. 2012, 1 (3): 229-238 _____

Evaluation of genetic diversity of Iranian *Lecanicillium fungicola* isolates using URP marker

Mostafa Mehrparvar, Ebrahim Mohammadi Goltapeh and Naser Safaie*

Department of Plant Pathology, Agricultural Faculty, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Iran.

Abstract: The dry bubble disease, caused by *Lecanicillium fungicola,* is an important fungal disease of white button mushroom in Iranian mushroom production farms. Twenty-three isolates of the pathogen collected in Iran and identified as *L. fungicola* var. *fungicola*, were compared for genetic polymorphism, diversity in growth rate and virulence. Ten Universal Rice Primers (URP) were used to evaluate the genetic diversity of *L. fungicola* var. *fungicola.* URP analysis showed that the genetic diversity of Iranian isolates was low (average 10 % over the 10 primers used) and that they were almost clonal. Relative correlations between geographical origins of isolates and molecular grouping were observed but there was no correlation between mycelial growth rate, virulence assays and URP patterns. Significant differences were observed between isolates based on mycelial growth rate and virulence assays. The high level of genetic homogeneity is attributed to the effect of fungicides used for control of the mushroom diseases which might have imposed a significant selection pressure on the fungal populations.

Keywords: Mycopathogen, *Agaricus bisporus,* universal rice primer, genetic diversity, *Lecanicillium fungicola,* clonal population

Introduction

Dry bubble disease is considered as the most important fungal disease of white button mushroom, *Agaricus bisporus* (Lange.) Imbach causing great losses to mushroom production yearly (Largeteau *et al.*, 2006).

Genetic variability within *L. fungicola* was recently investigated by several authors. Result of RAPD analysis (Bonnen and Hopkins, 1997) subdivided *Verticillium fungicola* (= *Lecanicillium fungicola*) isolates collected during 45 years into four groups. The authors did not find any correlation between RAPD grouping, colony morphology and virulence but they observed a high level of homogeneity between the isolates collected during 1993–1995 for fungicide response, virulence, colony morphology, geographic origin and RAPD grouping. Genetic variability within *V. fungicola* was recently investigated by Collopy *et al.* (2001) in 40 isolates of *V. fungicola* collected from various Pennsylvania mushroom farms in 1999, and 28 isolates of *Verticillium* spp. (*V. lamellicola* and *V. tenerum*) obtained during the last 50 years from various geographic locations. The authors reported the presence of a clonal population of *V. fungicola* var. *aleophilum* among the Pennsylvanian isolates and postulated that the lack of diversity observed may be due to a change in the practices regarding the casing of mushroom crops. Results of six RAPD primers also, demonstrated the existence of clonal population between Pennsylvanian and European isolates. Juarez del Carmen *et al.* (2002) observed notable diversity in pathogenicity, mycelial growth rate,

Handling Editor: Dr. Vahe Minassian

* **Corresponding author**, e-mail: nsafaie@modares.ac.ir
Received: 23 June 2012; Accepted: 11 August 2012

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

extracellular enzyme production and variation in RAPD pattern among the investigated isolates. In this investigation *L. f.* var. *aleophilum* isolates were more homogenous in comparison to *L. f.* var. *fungicola*. Differences in RAPD patterns in their investigation showed that French isolates were not genetically homogenous as much as was expected in Collopy *et al.* (2001) investigation with different markers. Largeteau *et al*. (2006) observed significant differences in physiological and pathogenic feature of *L. f.* var. *fungicola* and showed that based on RAPD and AFLP patterns, European isolates are genetically homogenous but their homogeneity was less than that for *L. f.* var. *aleophilum*. In their observations, a little polymorphism was observed in the French population of the pathogen such that three French isolates, showed more polymorphism than other 15 isolates which were collected during 27 years from the Netherlands, Frence and the United Kingdom. Largeteau *et al*. (2008) when comparing Mexican and French *L. fungicola* isolates, observed no differences in RAPD patterns, sensitivity to chlorotalonil, mycelial growth rate and antibiosis of mushroom. only some primers seperated few isolates from others by only one band in RAPD patterns and showed that populations of the pathogen in both countries were genetically clonal.

Despite the absence of genetic diversity in the pathogen population which was observed using different tools, there were significant differences in antibiosis, sensitivity to fungicides, and hydrogen peroxide, enzyme production, optimum growth temperature and pathogencity between isolates (Bonnen and Hopkins, 1997; Collopy *et al.*, 2001; Juarez del Carmen *et al.*, 2002; Largeteau *et al.*, 2006; Largeteau *et al.*, 2008).

Virulence assays based on spore inoculation of mushroom pilei have also been used to show variation between *L. fungicola* isolates (Bonnen and Hopkins, 1997; Collopy *et al.*, 2001; Juarez del Carmen *et al.*, 2002; Mehrparvar *et al*., In Press).

The URP-PCR technique is a useful tool for grouping of various organisms including plants, animals and micro-organisms at interspecific and intraspecific levels and is designed based on repetitive sequence of rice genome by Kang *et al*. (2002). Though URPs are basically random primers but their greater length and higher annealing temperature in PCR result in better reproducibility of this technique over that of RAPD.

Genetic studies of mushroom pathogens is necessary. Having good knowledge of genetic structures of pathogen populations can assist in breeding for appropriate strains of *Agaricus bisporus* that are resistant to the pathogen. Due to pathogens developing resistance to fungicides, breeding programs would be considered a promising way to control the disease (Juarez del Carmen *et al.*, 2002). This study was carried out to characterize genetic variability using universal rice primers (URP) and to compare mycelial growth rate and virulence of *L. fungicola* var. *fungicala* isolates collected from Iranian mushroom industries.

Materials and methods

Pathogen collection

Samples of diseased mushrooms were collected from Tehran mushroom farms. Isolation was performed by cutting small pieces of diseased mushroom fruit bodies, sterilizing them by immersing in 1 % sodium hypochlorite for one minute and then placing them on potato dextrose agar (PDA, Merck) (Potočnik *et al.*, 2008). Single-spore isolates were kept on potato carrot agar (PCA) at 4 °C. Isolates were identified based on taxonomic criteria presented by Zare & Gams (2008).

Mycelium growth rate

Inoculum plugs (5-mm diam.) from the growing edge of 5-day-old parent cultures were transferred to the centre of 9-cm PDA Petri dishes and cultures were grown in the dark at 24 ± 1 °C and 30 ± 1 °C for 21 days. Mycelial growth of each colony was then recorded across two perpendicular diameters. Two experiments were performed, each with three Petri dishes per isolate and temperature of incubation**.**

Virulence assays

Virulence assay was performed as previously

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

described by Bonnen and Hopkins (1997) with some modification. For each *L. fungicola* isolate, six freshly harvested sporophores of *Agaricus bisporus* were placed into a plastic box used as a moist chamber. Prior to inoculation, stipes were removed from all mushrooms to retard cap opening and basidiospore release. Fifteen microlitres of a conidial suspension of *L. fungicol*a containing 2 × 10^6 conidia/ml were placed on the cap surface. The diameter of the necrotic lesions were recorded after 5 days of incubation at 20 ± 1 °C in the dark. Sterile distilled deionized water was used as negative control.

Statistical analyses

Data were analyzed using SAS software (SAS Institute Inc., Cary, NC) and analyses of variance (ANOVA) were performed and the means were compared when necessary by the Duncan test.

DNA extraction and purification

Potato dextrose broth (2 %) medium was inoculated with mycelium of each isolate and incubated in shaker-incubator (LabTech, LSI-3016R) at 110 rpm for 14 days at 22 °C. Mycelial biomass was harvested by vacuum filtration and washed. The mycelia were lyophilized and ground to a fine powder in liquid nitrogen.

The genomic DNA was isolated using cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modification. Ground mycelia were transferred to polypropylene tubes containing 600 µl DNA extraction buffer (100 mM Tris HCl, 1.4M NaCl, 20 mM EDTA, 2 % CTAB and 1 % β-mercaptoethanol). The tubes were incubated at 65 °C for 30 min with occasional gentle swirling. Two third volume of chloroform: isoamyl alcohol (24:1, v/v) was added to this sample and mixed. The samples were kept at -20 °C for 15–25 min and then centrifuged (Beckman, 64R) at 13000 rpm for 10 min at 4 °C. DNA pellet was rinsed with 70 % ethanol for 10–15 min. This pellet was dried overnight at room temperature. The dried pellet was dissolved in distilled deonized water. For purification of DNA, RNAse treatment was given as described by Murray and Thompson (1980). The purified DNA was qualified and quantified by Eppendorf biophotometer at 280/260 nm absorption spectrum and final concentration was adjusted to 20 ng/µl for use in PCR analysis.

URP primers and PCR amplification

Twelve universal rice primers (URPs) originally derived from the repeat elements of weedy rice by Kang *et al.* (2002) were obtained from the CinnaGen Inc, Iran. Sequences of primers and their characteristics of the amplification products are presented in Table 1.

Amplification was performed in a 25 µl reaction mixture containing 10 × PCE buffer (0.01 % gelatin, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 10 mM dNTPs, 0.2 U *Taq* DNA polymerase (CinnaGen Inc, Iran), 0.2 µM primers and 40 ng of genomic DNA. Reactions were performed in 0.2-ml thin-walled PCR tubes and the thermocycler (Eppendorf, Mastercycler, gradient) was programmed as follows: one cycle of 4 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 46.1-51.1 °C (annealing temperature for each primer was optimized as indicated in Table 1) and 1 min at 72 °C, with a final extension period of 7 min at 72 °C. Products were visualized on 1.4 % agarose gels containing 0.05 µg ml^{-1} ethidium bromide. Numeric images of agarose gels were recorded and the presence or absence of URP products was scored. Each URP was performed twice. Based on similarity matrix, cluster analysis was performed using jaccard's coefficient with the method of UPGMA using NTSYSpc software (version 2.1a) and their dendrograms were drawn.

Results

Identification of the pathogen

Octahedral crystals were produced on the medium. Conidiophores were differentiated from subtending hyphae, at least partly erect and becoming procumbent with age. Conidiophores were without secondary branches. Conidia were produced in globose heads. Hence, all the isolates were identified as

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

Lecanicillium fungicola. All the isolates grew at 24 °C while no growth was observed at 30 °C , therefore according to the key of *Lecanicillium* presented by Zare and Gams (2008)the isolates in this study belonged to *L. f.* var. *fungicola* (figure 1).

There were significant differences at 1 % probability level between isolates, as for mycelial growth rate on PDA (Tables 2 and 4). R1 with 79.3 mm in 21 days had the most rapid mycelial growth and F1 with 54.0 mm had the slowest mycelial growth.

Mycelial growth rate

Table 1 Characteristic of URP primers and amplified loci for *Lecanicillium fungicola* var. *fungicola* genome.

Primer	Sequence (5′-3′)	GC % Content	Annealing temperature	No. of polymorphic loci	No. of monomorphic loci	Total no. of loci	% Polymorphism
1F	ATCCAAGGTCCGAGACAACC	50	50	0	7	7	0.0
2F	GTGTGCGATCAGTTGCTGGG	50	48	0	8	8	0.0
2R	CCCAGCAACTGATCGCACAC	50	50	4	5	9	44.4
6R	GGCAAGCTGGTGGGAGGTAC	50	50	0	5	5	0.0
4R	AGGACTCGATAACAGGCTCC	50	46.1	5	8	13	38.5
9F	ATGTGTGCGATCAGTTGCTG	50	48	1	10	11	9.1
13R	TACATCGCAAGTGACACAGG	50	47.7	0	10	10	0.0
17R	AATGTGGGCAAGCTGGTGGT	55	51.1	0	10	10	0.0
25F	GATGTGTTCTTGGAGCCTGT	50	48.8	0	10	10	0.0
38F	AAGAGGCATTCTACCACCAC	50	50	0	11	11	0.0
30F	GGACAAGAAGAGGATGTGGA	50	-	-	-	-	-
32F	TACACGTCTCGATCTACAGG	50	-	-	-	-	-
total				10	84	94	10.6

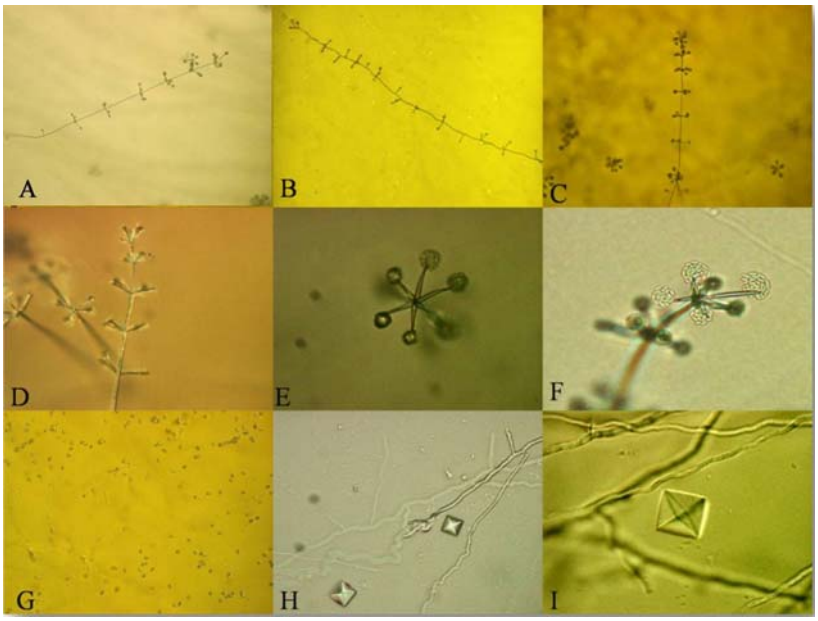

Figure 1 *Lecanicillium fungicola* var. *fungicola*. (A-E) Conidiophores and phialides, (E, F) globous heads containing conidia, (G-I) octahedral crystals.

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

Table 2 Analysis of variance for mycelial growth of 23 isolates of *Lecanicillium fungicola* var. *fungicola*.

Sources	Df	Sum of squares	Mean of squares	F
Treatment	22	3287.30	149.42	115.84**
Error	46	59.33	1.29	
All	68	3346.64		

CV: 1.66
** significant at P < 0.01

Table 3 Analysis of variance for necrosis induction in virulence assays measured for 23 isolates of *Lecanicillium fungicola* var. *fungicola*.

Sources	Df	Sum of square	Mean of square	F
Treatment	23	4208.98	182.99	113.00**
Error	120	194.33	1.62	
All	143	4403.31		

CV: 14.66
** significant at P < 0.01

Virulence assays

There were significant differences between isolates based on the diameter of necrotic lesion (Table 3).

In this investigation, isolates F3 and R3 had the most virulence potency as for necrotic diameter (Table 4). Isolates F6, G6 and P18 did not cause any necrotic lesions on mushroom caps.

Table 4 Year and site of collection, comparison of growth on potato dextrose agar and necrotic diameter for each of 23 isolates of *Lecanicillium fungicola* var. *fungicola*.

Isolate	Year of collection	Site collected	Colony diameter at 24 °C (mm)	Necrotic diameter (mm)†
B1	2010	Shahriar	70.0 d*	5 jk*
B2	2009	Shahriar	70.3 d	14.7 bc
B3	2009	Shahriar	69.3 d	13.2 dc
F1	2009	Shahriar	54.0 h	14.8 abc
F2	2009	Shahriar	78.3 a	9.7 gh
F3	2009	Shahriar	74.3 b	16.8 a
F6	2009	Shahriar	67.0 ef	0 l
F7	2010	Shahriar	64.3 fg	5.3 j
G2	2009	Shahriar	71.0 cd	9.8 fgh
G5	2009	Shahriar	77.7 a	11.8 def
G6	2010	Shahriar	63.0 g	0 l
G8	2010	Shahriar	64.7 fg	6.7 ij
R1	2010	Hashtgerd	79.3 a	15 abc
R3	2009	Hashtgerd	73.7 bc	16.3 ab
R4	2010	Hashtgerd	55.3 h	5.8 ij
R5	2009	Hashtgerd	65.3 fg	14.5 bc
R6	2009	Hashtgerd	74.0 b	7.8 hi
P1	2009	Mohammadshahr	72.0 bcd	5.5 j
P3	2010	Mohammadshahr	65.7 fg	12.3 de
P4	2009	Mohammadshahr	70.0 d	9.7 gh
P16	2009	Mohammadshahr	73.3 bc	3.2 k
P18	2010	Mohammadshahr	65.7 fg	0 l
P21	2010	Mohammadshahr	55.0 h	10.3 efg

*Values within a column followed by the same letters do not differ significantly by Duncan test (P = 0.01).
† Diameter of the necrosis produced by the deposition of a conidial suspension on sporophore caps. Distilled deionized water was used as control where no lesion was observed.

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

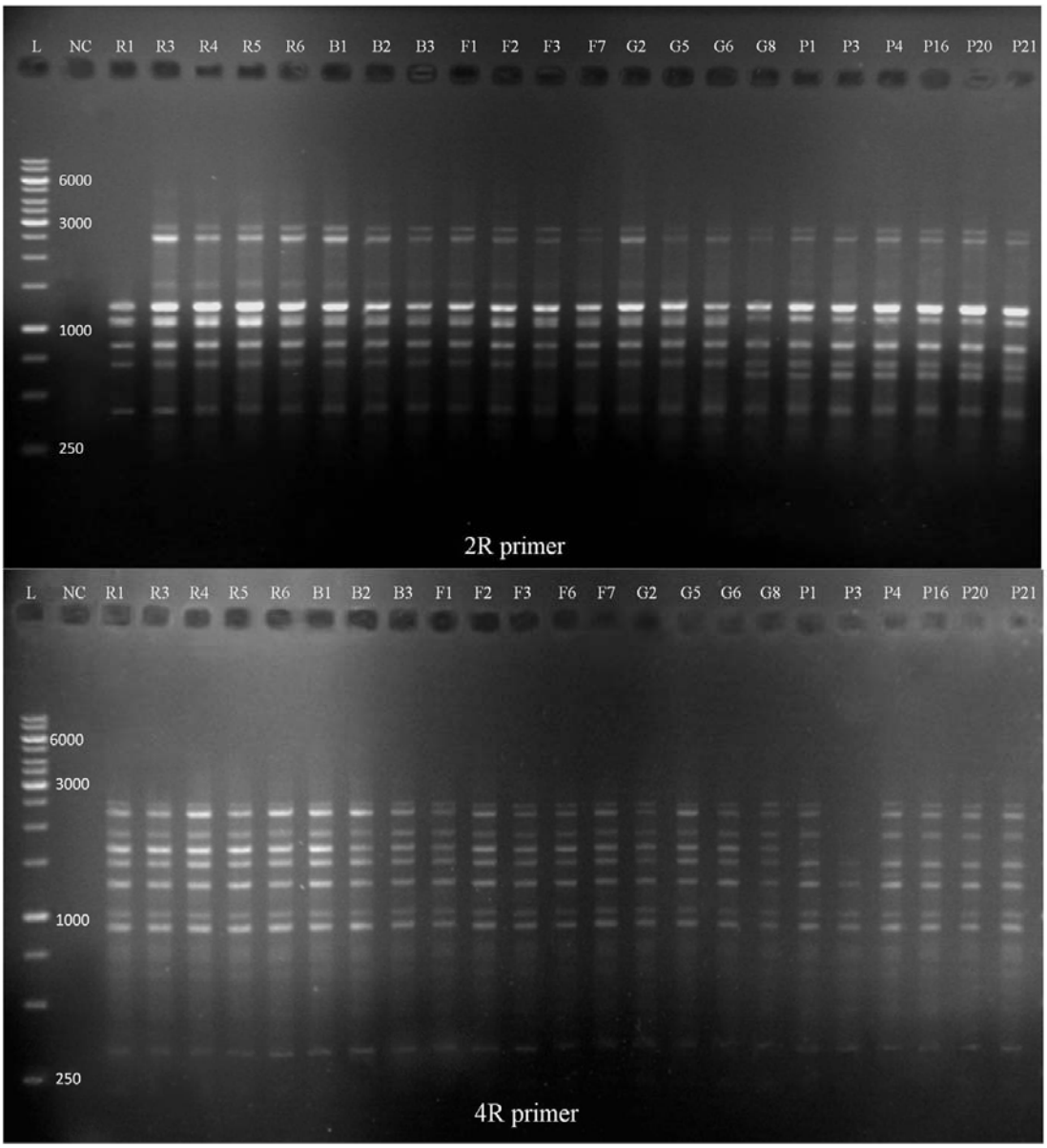

Figure 2 Universal rice primer patterns in *Lecanicillium fungicola* isolates obtained with 2R and 4R primers, NC = negative control, L = ladder. Each set of isolates is flanked on the left with a 1 kbp ladder.

DNA polymorphisms

The DNA polymorphism detected by URP patterns was consistent in replicate experiments. In our study of the genetic diversity of *L. fungicola* var. *fungicola* isolates, primers, 30F and 32F were not able to amplify DNA sequence of the fungal isolates whereas the other 10 primers (2R, 4R, 6R, 13R, 17R, 1F, 2F, 9F, 25F and 38F) amplified the target DNA sequences on the fungal genome (Figure 2).

As a result, 94 amplified loci were obtained of which 10 were polymorphic. Among the primers, polymorphism was only observed in 2R, 4R and 9F primers. Most polymorphism was expressed by 2R with 44.4

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

% and the least by 9F with 9.1 % polymorphism. Primers 6R, 13R, 17R, 1F, 2F, 25F and 38F did not show any polymorphism. The polymorphism scale observed for total of 10 primers was 10.6 % and of the total 94 amplified loci, 10 loci were polymorphic. The characteristics of the amplification products (Total number of loci amplified, number of polymorphic and monomorphic loci of URP primers) for each primer are given in Table 1.

Results of cluster analysis of 2R, 4R and 9F showed that at 86 % similarity level, the 23 isolates of *L. fungicola* were divided into two groups. All isolates of Pedam mushroom farm (P) were placed in one group and separated from isolates of Royan, Bita, Farshad and Golbarg mushroom farms that were placed in another group. In Pedam group (P), P3 was separated from the other Pedam isolates at 89.5 % similarity level and at 96.5 % similarity level, P21 was separated from P1, P4, P16 and P20. In the second group, R1 was separated from other isolates at 89 % similarity level. Isolates of Golbarg, Bita, Farshad and Royan except R1 were placed in a subgroup under this group. At 93.7 % similarity level G8 was placed in one group while G5 and G6 and all isolates of Farshad (F), Bita (B), R3 and R6 were placed in one group. G5 and G6 separated from these isolates at 96.2 percent similarity level (Figure 3).

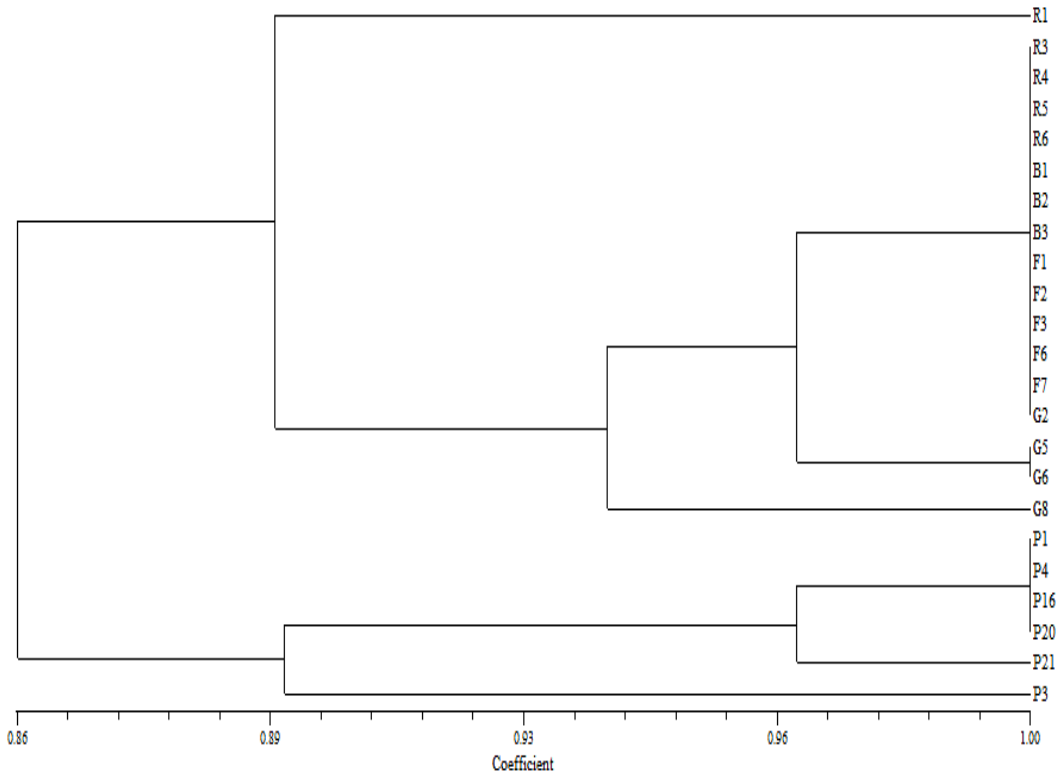

Figure 3 Dendrogram showing the relationship between 23 isolates of *Lecanicillium fungicola* var. *fungicola* in the cluster analysis based on similarity indices calculated from the URP patterns using UPGMA method and Jaccard's coefficient.

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

Discussion

In this study, of the 10 primers, only three were able to show polymorphism between *Lecanicillium fungicola* var. *fungicola* isolates. The rate of polymorphism for 2R, 4R and 9F primers was 44.4, 38.8 and 9.1 %, respectively. Primers 30F and 32F did not amplify any loci on the fungal genome. Results of molecular analysis show that primers 2R and 4R were able to separate the isolates from each other and can be used in genetic studies of this mycopathogen.

Albeit genetic homogeny among *L. fungicola* var. *fungicola* isolates was at high level (86 % similarity), molecular analysis separated Pedam farm isolates from the others. These results show that genetic variation between isolates is very low and isolates are genetically very clonal. This finding corresponds with those of (Bonnen and Hopkins, 1997; Collopy *et al.*, 2001; Largeteau *et al.*, 2006; Largeteau *et al.*, 2008). Adaptation of the pathogen to chemical compounds, intensive management (including fungicide use) of the host crop, high level of genetic homogeneity of the commercial mushroom host and changing in casing soil preparation toward the use of pasteurized peat at casing soil may be the reasons for homogeneity observed in *L. fungicola* isolates in this investigation (Bonnen and Hopkins, 1997; Castle *et al.*, 1987; Collopy *et al.*, 2001; Mehrparvar *et al.,* in press).

In a study by Largeteau *et al.* (2006) low level of polymorphism in French pathogen populations was observed. The differentiation of Pedam isolates from other *L. fungicola* can, in general, be attributed to lower mushroom production standards, different compost formulations and casing soil and finally improper application of chemical mixtures to control mushroom diseases in Pedam mushroom farm.

Efficiency of URP marker in denotation and grouping of various organisms including plants, animals and micro-organisms at interspecific and intraspecific levels has been proved (Jana *et al.*, 2005; Kang *et al.*, 2002; Sharma *et al.*, 2005). Obtained results from URP markers in this investigation show that this marker can reflect genetic relationship *in Lecanicillium fungicola* var. *fungicola* populations but comparative works with other markers (such as ITS, RAPD, RFLP and etc) should be performed to define the efficiency of this marker in comparison to other molecular markers.

Variation in mycelial growth rate between isolates was observed. Relative correlations between sources of isolates and molecular grouping were observed but there was no correlation between mycelial growth rate, virulence assays and URP patterns.

There are reports about lack of correlation between RAPD polymorphism results and virulence potency in *Lecanicillium lecanii* (Mor *et al.*, 1996) and *Verticillium dahliae* (Ramsay *et al.*, 1996) and among physiological traits. Physiological diversity (such as mycelial growth, response to biochemicals produced by *A. bisporus*, fungicide resistance and virulence) has been observed in *Lecanicillim fungicola* populations but no genetic diversity , using RAPD and AFLP markers (Largeteau *et al.*, 2006; Largeteau *et al.*, 2008). In this investigation no correlation was found between virulence assays and URP grouping which corresponds with results of other investigatiors (Largeteau *et al.*, 2006; Largeteau *et al.*, 2008; Mor *et al.*, 1996; Ramsay *et al.*, 1996).

There are several studies indicating resistance of *L. fungicola* to fungicides (Bonnen and Hopkins, 1997; Nair and Macauley, 1987; Potočnik, 2006; mehrparvar *et al*., In Press). We have observed high level of fungicide resistance in *Lecanicillium fungicola* (Mehrparvar *et al*.,in Press). In the present investigation isolates were genetically homogeneous. This genetically clonal population possibly indicates the effect of fungicides on fungal population shift toward more genetic homogeneity and resistance to fungicides.

These findings indicate that the fungicide has gradual selective pressure on the pathogen population and within some years, the sensitivity rate will shift from low to moderate. In the situation of fungicide use for a given disease control, resistant isolates can compete better with sensitive isolates and the population of fungicide resistant isolates will increase. Thus, the use of fungicides

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

acts as a special selection pressure such that after some years there will not be sensitive populations of the pathogen and the pathogen will form a more genetically clonal population (Largeteau *et al.*, 2006, Largeteau *et al.*, 2008).

References

Bonnen, A. M. and Hopkins, C. 1997. Fungicide resistance and population variation in *Verticillium fungicola*, a pathogen of the button mushroom, *Agaricus bisporus*. Mycological Research, 101: 89-96.

Castle, A. J., Horgen, P. A. and Anderson, J. P. 1987. Restriction fragment length polymorphisms in the mushrooms *Agaricus bisporus* and *Agaricus bitorquis*. Applied and Environmental Microbiology, 53: 816-822.

Collopy, P. D., Largeteau-Mamoun, M. L., Romaine, C. P. and Royse, D. J. 2001. Molecular phylogenetic analyses of *Verticillium fungicola* and related species causing dry bubble disease of the cultivated button mushroom, *Agaricus bisporus*. Phytopathology, 91: 905-912.

Jana, T. K., Singh, N. K., Koundal, K. R. and Sharma, T. R. 2005. Genetic differentiation of charcoal rot pathogen, *Macrophomina phaseolina*, into specific groups using URP-PCR. Canadian Journal of Microbiology, 51: 159-164.

Juarez Del Carmen, S., Largeteau-Mamoun, M. L., Rousseau, T., Regnault-Roger, C. and Savoie, J. M. 2002. Genetic and physiological variation in isolates of *Verticillium fungicola* causing dry bubble disease of the cultivated button mushroom, *Agaricus bisporus*. Mycological Research, 106: 1163-1170.

Kang, H. W., Park, D. S., Go, S. J. and Eun, M. Y. 2002. Fingerprinting of diverse genomes using PCR with universal rice primers generated from repetitive sequence of Korean weedy rice. Molecules and Cells, 13: 281-287.

Largeteau, M. L., Baars, J. P. P., Regnault-Roger, C. and Savoie, J. M. 2006. Molecular and physiological diversity among *Verticillium fungicola* var. *fungicola*. Mycological Research, 110: 431-440.

Largeteau, M. L., Mata, G. and Savoie, J. -M. 2008. *Verticillium fungicola* var. *fungicola*: comparison of some Mexican and French isolates. Revista Mexicana de Micologia, 26: 35-40.

Mehrparvar, M., Goltapeh, E. M. and Safaie, N. Resistance of Iranian *Lecanicillium fungicola* to benzimidazole and ergosterol demethylation inhibiting fungicides. Journal of Agricultural Science and Technology (In Press).

Mor, H., Gindin ,G., Ben-Ze'ev, I., Raccah, B., Geschtovt, N., Ajtkhozhina, N. and Barash, I. 1996. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci*. Phytoparasitica, 24: 111-118.

Murray, M. and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8: 4321-4326.

Nair, N. and Macauley, B. 1987. Dry bubble disease of *Agaricus bisporus* and *A. bitorquis*, and its control by prochloraz-manganese complex. New Zealand Journal of Agricultural Research, 30: 107-116.

Potočnik, I. 2006. Causal agents of bubble diseases of white button mushrom (*Agaricus bisporus* (Lange) Imbach) and their sensitivity to fungicides. M.Sc. thesis. Faculty of Biology. University of Belgrade, Serbia and Montenegro.

Potočnik, I., Vukojević, J., Stajić, M., Tanović, B. and Todorović, B. 2008. Fungicide sensitivity of selected *Verticillium fungicola* isolates from *Agaricus bisporus* farms. Archives of Biological Sciences, 60: 151-157.

Ramsay, J., Multani, D. and Lyon, B. 1996. RAPD-PCR identification of *Verticillium dahliae* isolates with differential pathogenicity on cotton. Australian Journal of Agricultural Research, 47: 681-693.

Sharma, M., Gupta, S. and Sharma, T. 2005. Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. Journal of Phytopathology, 153: 449-456.

Zare, R. and Gams, W. 2008. A revision of the *Verticillium fungicola* species complex and its affinity with the genus *Lecanicillium*. Mycological Research, 112: 811-824.

[DOR: 20.1001.1.22519041.2012.1.3.2.7]

بررسی تنوع ژنتیکی قارچ *Lecanicillium fungicola* با استفاده از نشانگر مولکولی URP در ایران

مصطفی مهرپرور، ابراهیم محمدی گل‌تپه و ناصر صفایی*

گروه بیماری‌شناسی گیاهی، دانشگاه تربیت مدرس، صندوق پستی ۳۳۶-۱۴۱۱۵، تهران، ایران
*پست الکترونیکی نویسنده مسئول مکاتبه: nsafaie@modares.ac.ir
دریافت: ۳ تیر ۱۳۹۱؛ پذیرش: ۲۱ مرداد ۱۳۹۱

چکیده: بیماری حباب خشک قارچ خوراکی دکمه‌ای با عامل *Lecanicillium fungicola*، یکی از بیماری‌های مهم قارچی قارچ خوراکی دکمه‌ای سفید در مراکز پرورش قارچ در ایران محسوب می‌شود. بیست و سه جدایه قارچ بیمارگر از مراکز پرورش قارچ خوراکی جمع‌آوری و واریته آن *L. fungicola* var. *fungicola* تشخیص داده شد و از نظر تنوع ژنتیکی، تنوع در میزان رشد پرگنه و ویرولانس با یکدیگر مقایسه شدند. تجزیه و تحلیل URP نشان داد که تنوع ژنتیکی جدایه‌ها پایین بوده (میانگین ده درصد برای ده پرایمر مورد استفاده) و تقریباً از نظر ژنتیکی یکنواخت هستند. ارتباط نسبی میان محل جمع‌آوری جدایه‌ها و گروه‌بندی ملکولی آنها مشاهده شد اما هیچ رابطه‌ای میان میزان رشد میسلیومی، آزمایش ویرولانس و نقوش حاصل از URP جدایه‌ها دیده نشد. میزان بالای یکدست بودن ژنتیکی جدایه‌ها به تأثیر قارچکش‌های مورد استفاده برای کنترل بیماری‌های قارچی قارچ خوراکی نسبت داده شد.

واژگان کلیدی: بیمارگر قارچی، *Agaricus bisporus*، پرایمر عمومی برنج، تنوع ژنتیکی، *Lecanicillium fungicola*، جمعیت همسان

[DOR: 20.1001.1.22519041.2012.1.3.2.7]