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Population genetic structure of *Fusarium verticillioides* causal agent of rice crown and root rot in Ilam province using SSR Marker

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Abstract

Study on the genetic structure of *Fusarium verticillioides* populations, provides different levels of information in management of root rot disease in rice farms. Simple Sequence repeat (SSR) markers were used to determine genetic structure and genetic diversity in 39 *F. verticillioides* isolates from five different regions in Ilam province, such as: Tangehghir, Lomar, Shirvan, Sarableh and Darehshahr. Average number of alleles in populations were 10.6, the number of alleles in populations varied from 9 allele in Tangehghir and Darehshahr as the lowest to 13 allels in Lomar as the highest. Observed number of alleles (*Na*) and effective number of alleles (*Ne*) were higher in Lomar (*Na* = 1; *Ne* = 1.276) compared to other populations. The genetic diversity (*H*) and Shannon's Information index (*I*) were also higher in Lomar (*H* = 0.167; *I* = 0.254) but lower values were estimated for Darehshahr (*He* = 0.141; *I* = 0.204). The lowest genetic distance was found between Tangehghir and Lowmar (0.00) and then Shirvan, while the highest genetic distance was revealed between Darehshahr and Tangehghir (0.018). Total gene diversity (*Ht*) and gene diversities between subpopulations (*Hs*) were estimated 0.125 and 0.116, respectively. Gene diversity attributable to differentiation among populations (*Gst*) was 0.072, while gene flow (*Nm*) was 6.368. Cluster analysis based on UPGMA showed the lowest genetic distance between Tangehghir and Lomar and then between Shirvan. The dendrogram indicated a high genetic distance between Darehshahr and four remaining populations. Results from this study will be useful in developing necessary control methods for Rice crown and root rot disease.
Key words: *Fusarium verticillioides*, Genetic structure, Rice, SSR.

ساختار ژنتیکی جمعیت‌های *Fusarium verticillioides* عامل پوسیدگی ریشه و طوقه برنج در استان ایلام با استفاده از نشانگرهای ریزماهواره‌ای

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چکیده

مطالعه ساختار ژنتیکی جمعیت‌های *Fusarium verticillioides* اطلاعات مختلفی از مدیریت بیماری پوسیدگی ریشه در مزارع برنج را فراهم می‌کند. نشانگرهای SSR برای تعیین ساختار ژنتیکی و تخمین تنوع ژنتیکی در ۳۹ جدایه *F. verticillioides* از پنج منطقه مختلف از جمله: تنگه قیر، لومار، شیروان، سرابله و دره شهر در استان ایلام استفاده شد. میانگین آلل‌ها در هرمکان ژنی ۵/۲ آلل بود و و تعداد آلل‌ها در جمعیت‌ها از جمله جمعیت لومار با تعداد ۱۳ آلل بیش‌ترین آلل و جمعیت‌های تنگه قیر و دره شهر با نه آلل به‌عنوان کم‌ترین تعداد آلل را دارا بودند. تعداد آلل‌های مشاهده شده(*Na*) و تعداد آلل‌های مؤثر (*Ne*) در لومار (*Ne* =۱/۲۷۶ : *Na* =۱) نسبت به دیگر جمعیت‌ها بیش‌تر بودند. تنوع ژنتیکی (*H*) و شاخص شانون (*I*) در لومار بالا بود (*He* = ۰/۱۶۷ *I* = ۰/۲۵۴) اما مقدار آنها در دره شهر کم‌تر بود (*He* = ۰/۱۴۱ *I* = ۰/۲۰۴). کم‌ترین فاصله ژنتیکی بین تنگه قیر و لومار و بعد شیروان مشاهده شد و بیش‌ترین فاصله ژنتیکی بین دره شهر و تنگ قیر (۰/۰۱۸) وجود داشت. تنوع ژنتیکی کل (*Ht*) و تنوع ژنی بین زیر جمعیت‌ها (*Hs*) ۰/۱۲۵ و ۰/۱۱۶ به‌ترتیب تخمین زده شد. تنوع ژنتیکی مؤثر در تمایز بین جمعیت‌ها (*Gst*) ۰/۰۷۲ بود و میزان جریان ژنی(*Nm*) ۶/۳۶۸ بود. تجزیه کلاستر براساس UPGMA نشان داد که بین تنگه قیر و لومار و سپس شیروان کم‌ترین فاصله ژنتیکی وجود دارد و دندروگرام فاصله ژنتیکی بالایی بین دره شهر و چهار جمعیت دیگر را نشان می‌دهد.
واژه‌های کلیدی: برنج، ساختار ژنتیکی، *Fusarium verticillioides* ، ریزماهواره.

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Introduction

Rice (*Oryza sativa* L.), is the most important food for a large part of the world's human population, especially in Asia (Ebadi *et al*., 2013). *Fusarium verticillioides* (Sacc.) Nirenberg [*Gibberella fujikuroi* (Sawada) Wollenw, is a prevalent pathogen of agricultural important crops (Leslie, 1991, 1995). *F. verticillioides* (*Fusarium moniliforme*) has been associated with human and animal toxicities since it was first described in 1881 (Saccardo, 1881). This species and other anamorphs of *G. fujikuroi* are among the fungi that most commonly associated with maize production in Iran and other temperate regions of the world (White, 1999). Members of *F. verticillioides* (Saccardo) Nirenberg (teleomorph: *Gibberella moniliformis*) and *F. proliferatum* (Matsushima) Nirenberg (teleomorph: *G. intermedia*) belong to the Liseola section of Fusarium (Nelson *et al*., 1983), are well-known pathogens of maize, causing stalk and ear rot (Nelson *et al*., 1981; Leslie *et al.,* 1990) and also rice foot rot and stunting of (crown and root rot) (Hsieh *et al*., 1977). Rice Crown and root rot disease caused by *F. verticillioides* (Teleomorph: *G. moniliformis* Wineland) was firstly described in Japan, and widely distributed in Asia (Nirenberg, 1981). In addition, the disease has been reported to be on the rice farms of America and European countries (Desjardins *et al*., 2000; Saremi, 2000). Crown and root rot is primarily a seed borne disease. Soil temperature of 35 ºC is most favorable for infection (Nyvall, 1999). Asexual sporulation is clearly the most successful and impressive reproductive mechanism because of the large number of conidia that can be produced by a single colony (Kendrick, 2003). DNA-based techniques have increasingly become a tool for understanding the genetic diversity and also phylogeny relationships of *Fusarium* spp. Researchers have already worked on molecular variation in *Fusarium* spp. (O'Donnell, 2000; Datta, 2011).

Amoah *et al*. (1995) reported the genetic variability among *F. verticillioides* isolates from different hosts in Ghana by DNA fingerprints detected as the RFLPs of the ribosomal DNA and the RAPDs analysis. They used the RAPD analysis to determine the mating populations of several members of the *Fusarium* in *Liseola* section (Amoah *et al.,* 1996) and they determined differences between A, D, and F mating population members. Genetic variability in the *G. fujikuroi* was reported using RAPD marker (Voigt *et al*., 1995). Knowledge derived from population genetic structures of pathogen populations has direct agricultural applications. For instance, the genetic variation maintained within a population indicates the speed at which a pathogen evolves (McDonald and McDermott, 1993). Several studies have recorded the genetic distinction of the mating populations of the *G. fujikuroi* complex species, including vegetative compatibility (Leslie, 1993), isozyme analysis (Huss, 1996) and electrophoretic karyotyping (Xu and Leslie, 1996). These methods are time consuming and labor intensive, because these species have morphologically nearly similar and it is difficult to distinguish them from each other. Therefore, there is a need for reliable and simple methods for detection of such species using DNA techniques. Molecular techniques have become reliable and highly suitable tools for identifying Fusarium species and for assessing genetic variation within collections and populations (Burgess *et al*., 1996). Accurate and rapid identification of pathogens is necessary for appropriate management of plant diseases (Datta, 2011). Knowledge of genetic structure of *F. verticillioides* populations is useful for development of effective strategies in controlling the disease (McDonald, 2004).

Several molecular techniques are available for investigating genetic variability among populations of fungal plant pathogens. Microsatellites also known as simple sequence repeats (SSRs), which are randomly distributed throughout the genome of fungi and other eukaryotes (Li *et al*., 2002; Wostemeyer and Kreibich, 2002; Sahran and Naef, 2008), provides a powerful tool for taxonomic and population genetic studies (Britz *et al*., 2002). Alleles vary according to the number of repeat units present but other mutations have also been shown to be responsible for allele length variation in SSR analysis (Burgess *et al*., 2001; Slippers *et al*., 2004b).

The objective of present study was to collect isolates of *F. verticillioides* from rice farms in Ilam province and to determine population genetic structure using microsatellites markers.

Materials and Methods

1. Sampling, isolation and identification: Rice plants in panicle stages with symptoms of brown to black lesions on root were randomly sampled from rice fields in five different regions as populations in the west of Iran, Ilam province, in 2012 (Table 1).

Table 1. Location and number of isolates in each *F. verticillioides* populations

populations	No. of Isolates in population.	location	Province
1	8	Tanghehghir	**Ilam**
2	9	Lomar	**Ilam**
3	8	Shirvan	**Ilam**
4	6	Sarableh	**Ilam**
5	8	Darehshahr	**Ilam**

Each sample were cut into 2–5-mm long pieces, Were surface-sterilized with 5% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water and air dried with sterile filter paper. The sterilized samples were placed on a general medium, potato dextrose agar (PDA), and Nash and Snyder medium (Taherkhani *et al.,* 1998; Jo *et al.,* 2008). Samples were incubated 3 days at 25 ^{o}C for production of conidia. Nash and Snyder is a selective medium for Fusarium species and facilitate the formation of large, easily recognizable colonies (Chen *et al.,* 2007). Fusarium colonies were observed microscopically, those colonies identified as *F. verticillioides*, were transferred to carnation-leaf agar (CLA) and potato dextrose agar (PDA). Isolates were identified according to their morphological and microscopic characters as described by (Nelson *et al.,* 1983; Leslie *et al*., 2006).

2. Pathogenicity test and DNA extraction: For inoculation, 100 gr of wheat seeds soaked for 24 hours in distilled water sterilized at 121 °C for 20 minutes, then inoculated with mycelial plugs of 7 days-old cultures on PDA plates. The flasks containing the inoculated seeds were shacked daily to prevent clumping of inoculated seeds. Pathogenicity test was carried out on the susceptible local cultivars such as Anbarbo and Shamshiri under greenhouse conditions. Germinated seeds of rice plants were planted in pots containing sterilized soil. The rice seedling were inoculated by adding propagules to the soils around plant roots, covered with white plastic bags to keep high humidity for 24 hours. Control treatments were some rice plants that were grown in sterilized soil without inoculation. Twenty-one days after inoculation, plants were observed for the development of disease symptoms and *F. verticillioides* were re isolated from inoculated plants to confirm Koch's postulates.

Liquid cultures were initiated by adding 2–4 mm^2 pieces of filter papers containing of *F. verticilioides* to 250-mL Erlenmeyer flasks containing 100 mL PDB medium (potato dextrose broth plus 2 g yeast extract per liter). Flasks were incubated at room temperature approximately 25 °C and shaken on a rotary shaker for 6–8 days. Mycelium was collected by filtration through sterile filter paper with a vacuum funnel. Mycelia were harvested, frozen and stored at −20 °C. DNA was extracted using a modified Cyteltrimethyl-ammonium bromide (CTAB) procedure (Doyle and Doyle, 1990). Mycelia were ground in liquid nitrogen and suspended in 2% CTAB extraction buffer (1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, 0.2% β-mercaptoethanol). Samples were treated with 5 units RNAse at 37 °C for 30 min., and then extracted with chloroform isoamylalcohol 24:1 (v/v). DNA in the supernatant was precipitated with isopropanol, rinsed with ethanol, and adjusted to a final concentration of 20 ng/µl in TE (pH 7.4) and stored at –20 °C (Weising *et al*., 1991; Bayraktar, 2010)

3. SSR amplification and analysis: We selected five SSR primer pairs (Table 2) on the basis of their high PIC as described by Ren *et al*., (2012). Primer aliquots for each marker were prepared by mixing equimolar amounts 20 pmoles of appropriate forward and reverse primer in 1 × TE (1mM EDTA, 10 mM Tris–HCl, pH 8.0) and used for the amplification of individual microsatellite loci. PCR amplification was performed in a 25 µl reaction volume containing 2.5 µl of 10X PCR Buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs mix (100 mM of each dNTPs), 1 µl of each forward and reversed primer, 0.6 U of *Taq* polymerase with 25 ng of template DNA. Amplification was performed using Mnotach 60 thermal cycler, PCR conditions for SSR were as follows; the PCR program had one initial denaturation step at 95 °C for 3 min followed by 30 cycles of 94 °C for 60 s, annealing for 60 s (appropriate annealing temperature were used for each primers set, between 51-59 ^{o}C) and 72 °C for 60s. The thermal cycles were terminated by a final extension of 5 min at 72 °C. Amplified products were resolved in 1.5% agarose gel at 60 V using Tris Boric Acid EDTA (1X TBE) buffer and stained with DNA Safe Stain at 0.5mg/ ml- and photographed under UV Trans laminator with Gel Doc. Intas. A 1kbp ladder was used as a molecular size standard.

Table 2. SSR primers of *Fusarium verticilioides* used in this study (REN *et al*., 2000)

Primer locus	Repeat of cloned allele	Primer sequence (5′→3′)	Estimate size (bp)
4H18	(TTTC)6	F:TGATGCGGTCAAAGAATGG R:ACTGGAGCAGATGAAGAGC	152
5H07	(GAAA)16	F:GTAGCGGTTATGGTTCCCTC R:CGTGATGCGATTCTGGTTG	363
5H08	(CTTT)6	F:ACCAACTAACATCCCGAATC R:CGTAAACTCAAACGCAAGG	410
9H09	(GT)18	F:ATCGGTGGTTTCTTGCTGC R:GCTCCCAACTGCCTACCTACA	263
5H12	(GAAA)7	F:GGCACCAACATTCCTGACG R:AACCGCCTACAAGCACCA	404

4. Statistical analysis: Populations were defined as different geographic regions. The clear and intense amplified DNA bands were scored as binary digit code of "0" (for absence) and "1" (for presence), respectively. The pair-wise distance among the isolates was calculated from the binary matrix using the simple mismatch coefficient (Sneath and Sokal, 1973) that is recommended for haploid fungi (Kosman and Leonard, 2005). Genetic similarity between pairs was estimated using Jaccard`s similarity coefficient. Similarity coefficients were used for the construction of UPGMA (Unweighted Pair Group Method with Arithmetic Average) dendrogram (Rohlf, 1990). For each primer pair, polymorphic information content (PIC), marker index (MI) was calculated. The polymorphic information content (PIC) was calculated using $PIC_i = 2f_i (1-f_i)$, where *i* is the information of marker I, f_i is the frequency of the amplified allele (presence of fragments) and $(1 – f_i)$ is the frequency of the null alleles (Roldan-Ruiz *et al*., 2000). The genetic variation was measured in terms of genetic diversity and was computed by averaging PIC estimates over all loci (Nei and Li, 1979). The marker index (*MI*) was calculated by *MI = PIC x EMR,* where *EMR* is the "effective multiplex relationship" given by the product of the total number of fragments per primer and the fraction of polymorphic fragments (Varshney *et al*., 2007).

Genotypic diversity (*Ht*) among isolates was estimated from allelic frequencies using the equation $H = 1 - \Sigma\, xi^2$, where, *xi* is the frequency of the i^{th} allele of a particular locus (Nei, 1973). The coefficient of population subdivision (*GST*) was computed as *(Ht – Hs)/Ht*, where, *Ht* is the total genetic diversity and *Hs* is the average gene diversity over all subgroups (Nei, 1973). The allele frequencies at polymorphic loci, the *Nm* values (effective migration rate), and the genetic identity among populations for characterize genetic variation, observed number of alleles (*Na*), effective number of alleles (*Ne*), Nei's gene diversity (*He*) and Shannon's information index (*I)* were calculated for each locus and population. Mean values of gene diversity in total populations (*Ht*), gene diversity between populations (*Hs)*, proportion of gene diversity attributable to differentiation among populations (*Gst*) and estimate of gene flow from *Gst* (*Nm*) were estimated across loci (McDermot and McDonald, 1993). Genetic distance of the populations were estimated from the SSR data using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean), clustering method on the basis of Nei's (1978) unbiased genetic distance, all of the above calculations were performed using POPGENE ver. 1.31 (Yeh *et al*., 1999) and Gen Alex ver. 6.501 (Peakall and Smouse, 2012).

Results and Discussion

Fifty six isolates of Fusarium in the section Liseola were recovered from rice plant with symptom of crown and root rot disease. Among them 39 isolates were *F. verticillioides*. Previous studies also showed that *F. verticillioides* predominant in rice fields (Darvishnia *et al.,* 2007). Results proved that all *F. verticillioides* isolates were pathogenic on rice. In the pathogenicity test, inoculated root showed discoloration from brown to black lesions and all isolates caused discoloration of the rice roots while no changes were observed in control treatments.

1. Distribution of alleles at polymorphic SSR loci: All five microsatellite loci were polymorphic with a total of 26 alleles detected between them. 4H18, 5H07, 5H08, 5H09, and 5H12 showed a total of 6, 6, 4, 4, and 6 alleles per locus, respectively (Table 4). Moreover, the average number of alleles per locus in populations was the highest (3 alleles) in Lomar population and the lowest (2 alleles) in Tangehghir population (Table 3).

Table 3. Number of alleles at each locus across the five populations of *F. verticillioides* in Ilam province

SSR primers	Number of alleles				
	Tangehghir	Lomar	Shirvan	Sarableh	Darehshahr
4H18	1	3	2	2	1
5H07	1	4	2	1	3
5H08	3	3	3	4	3
5H09	3	3	3	2	3
5H12	2	2	3	3	3
Average	2	3	2.6	2.4	2.6

2. Primers characteristics: A summary of characteristics of five microsatellite loci is given in Table 4.

Number of alleles, percentage of polymorphism and Polymorphic Information Content (PIC) of SSR primers pairs evaluated, the PIC value varied from 0.371 (primer 5H07) to 0.147 (primers 4H18), with an average of 0.250, which reflects the informative content of the primers used. The total number of alleles, percentage of polymorphic alleles of all SSR markers was 26 and 100% respectively.

A total of 26 amplified alleles were polymorphic, an average numbers of polymorphic alleles per primer was 5.2. Percentages of polymorphic alleles were 100%. Total allelic number in populations varied from 11 (Tangehghir, Darehshahr) to 13 (Lomar). Allele diversity of populations ranged from 0.167 in Lomar to 0.141 in Darehshahr (Table 5).

3. Genetic variability of populations: For each population, the number of observed alleles (*Na*), the effective number of alleles (*Ne*), the gene diversity (*He*) and the Shannon's information index (*I*) we calculated. Various population genetic parameters in five microsatellite loci for all populations are given in Table 6. Observed allele number (*Na*) and effective numbers (*Ne*) of alleles were higher in Lomar and Shirvan compared to other populations. The value of gene diversity (*He*) and (*I*) were also higher in Lomar (H = 0.167; I = 0.254) but lower in Darehshahr population (*He* = 0.141; I = 0.204).

Based on the SSR data, average genetic distance was calculated among five populations. The lowest genetic distance was found between Shirvan and Tangehghir (0.014), while the highest was revealed between Sarableh and Shirvan (0.048) and also Darehshahr and Sarableh (0.048) (Table 7).

Table 4. Number of alleles, percentage of polymorphism and polymorphic information content obtained from SSR primers among *F. verticillioides* populations

primer	Number of alleles	Number of polymorphic alleles	Percentage of polymorphism	Polymorphic information Content (PIC)
4H18	6	6	100	0.147
5H07	6	6	100	0.371
5H08	4	4	100	0.257
5H09	4	4	100	0.310
5H12	6	6	100	0.166
Average	5.2	5.2	100	0.250

Table 5. Number of alleles and mean number of allelic diversity in *F. verticillioides* populations

Population	Number of alleles	Mean number of Allelic diversity
Tangehghir	9	0.142
Lomar	13	0.167
Shirvan	11	0.163
Sarbleh	11	0.152
Darehshahr	9	0.141
Average	10.6	-

Table 6. Genetic diversity estimates for *F. verticillioides* populations based on microsatellite loci

population	*N*	*R%*	*Na*	*Ne*	*H*	*I*
Tangehghir	8	34.62	0.692	1.257	0.142	0.206
Lomar	9	50.00	1.000	1.276	0.167	0.254
Shirvan	9	42.31	0.846	1.287	0.163	0.241
Sarableh	6	42.31	0.846	1.250	0.152	0.229
Darehshahr	8	34.62	0.692	1.255	0.141	0.204
Average	8	40.77	0.815	1.265	0.153	0.227

N population size, *R* percentage of polymorphic loci, *Na* observed number of alleles,

Ne effective number of alleles, *H* Nei's gene diversity (1973), *I* Shannon's information index

Total gene diversity (*Ht*) and gene diversities within subpopulation (*Hs*) were estimated to be 0.125 and 0.116, respectively. Gene diversity attributable to differentiation among populations (*Gst*) was 0.072, while gene flow (*Nm*) was 6.368.

Table 7. Genetic distance between pairs of *F. verticillioides* populations

population	Tangehghir	Lomar	Shirvan	Sarableh	Darehshahr
Tangehghir	0				
Lomar	0.025	0			
Shirvan	0.014	0.021	0		
Sarableh	0.039	0.044	0.048	0	
Darehshahr	0.042	0.031	0.032	0.048	0

4 Genetic relationships among populations: Nei's genetic distances were estimated between populations. Cluster analysis based on UPGMA was used to produce dendrogram and to show the genetic relationships among populations (Fig. 1). The lowest genetic dissimilarity was found between Tangehghir and Lomar and then between Shirvan, Moreover, the dendrogram showed the highest dissimilarity between Darehshahr population and four remaining populations.

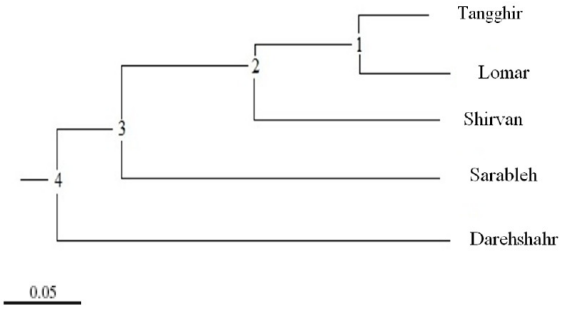

Fig. 1. Dendrogram constructed with UPGMA based on Jaccard`s similarity coefficient among five populations of *F. verticillioides* originated from Ilam province

In this study, genetic diversity within and among five populations of *F. verticillioides* sampled from Ilam was evaluated using SSR markers. The advantage of microsatellite markers over random amplified polymorphic DNA and polymerase chain reaction-restriction fragment length polymorphism markers are their high specificity, high polymorphism, good reproducibility and unambiguous scorability (Tenzer *et al.,* 1999; Sahran and Naef, 2008). The results showed that allele frequencies were different among five SSRs loci. The allele frequencies showed that *F. verticillioides* may spread as asexual stage in all populations. The sample sizes per population and SSRs primers were too small in this experiment for a statistically powerful test of gametic equilibrium. In this study the genetic similarity detected between isolates within the five populations was probably due to exchange of rice seeds between sampled regions and geographical closeness as well.

SSR results indicated that five populations of *F. verticillioides* had low degrees of gene diversity but Lomar and Shirvan maintain higher genetic diversity than the other region (Table 6). Different research has been carried out on international populations of *F. verticillioides,* and a different level of genetic diversity was detected within and between populations (Amoah *et al*., 1995; Mitter *et al*., 2001). High genetic variability among *F. verticillioides* isolates from different hosts has been reported by RFLPs of ribosomal DNA and RAPDs analysis in Ghana (Amoah *et al*., 1995). In a previous study, Mitter *et al*., (2001) in India reported high genetic variation among *F. moniliforme* isolates from different geographical regions and different hosts by RAPD markers.

In Iranian populations, over 93% of the gene diversity was distributed on a local level within populations (Fig. 2). However there was a high degree of genetic similarity among populations separated by low geographical distances like Lomar and Tangehghir. The low level of gene diversity (*Gst*) was detected among all five populations. Low *Gst* value (0.072) indicated little genetic differentiation among the five populations and showed little evidence for geographical subdivision among populations (Bayraktar, 2010). The genetic distances were very small and the geographic distances between populations ranged from 35 to 300 km, this indicates that populations linked by movement of conidia within populations. Gene flow may have occurred among these populations with infected plant debris, infected seeds, agricultural vehicles and fungal

spores. Gene flow has a significant role on the genetic diversity of a population. In the absence of gene flow, genetic drift cause developing different allele frequencies at neutral loci, leading to differentiation in isolate populations (Keller *et al.*, 1997). The high genetic similarity among populations of *F. verticillioides* suggests that gene flow has occurred across long distances. If we assume that the high degree of similarity is due to gene flow and the populations are at equilibrium, we can estimate *Nm*, the average number of migrants that would need to be exchanged among populations in each generation. *Nm* averaged 6.64 in all loci and populations, suggesting a level of gene flow that was 6 times greater than needed to prevent populations from diverging by genetic drift (Keller *et al.*, 1997). Another factor that has an important role on the genetic diversity of *F. verticillioides* populations is the mating system. With this assumption if the sexual reproduction of *F. verticillioides* occurs in nature, the sexual spores (ascospores) may play a major role in population biology. Moreover, infected seed can lead to persistence of genotypes; we consider that infected seed can explain the distribution and diversity of genotypes found at the end of the growing season in natural populations. If the main source of primary inoculums was asexual spores from seeds, we expect to find some clones that were distributed among different locations in a field. Genetic drift and selection would limit the number of genotypes present in field populations, unless sexual reproduction generated new genotypes and wind dispersal distributed ascospores among populations every year (Shah *et al.*, 1995). Depending on environmental factors, genetic diversity of *F. verticillioides* populations may possess potentially a risk of incidence of severs disease and high loss in rice farms in these regions. These data can help breeders for screening resistant cultivars based on genetic diversity of *F. verticillioides* populations for local disease management. Understanding population genetic structure of *F. verticillioides* in the present study may provide insights into the epidemiology and evolutionary potential of the pathogen and could lead to improved strategies for managing the disease

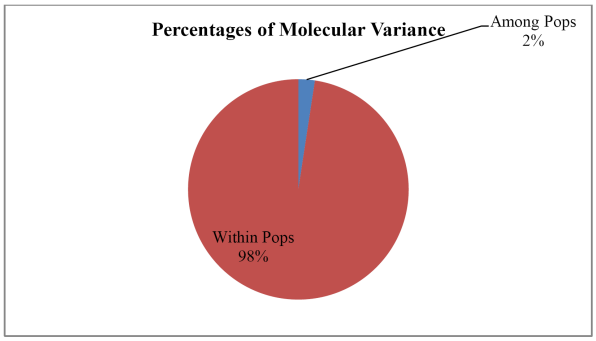

Fig. 2. Results of analysis of molecular variance (AMOVA) in *F. verticillioides* populations in Ilam province

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