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Analysis of genetic diversity of *Trametes versicolor* isolates collected from Northern provinces of Iran using ISSR marker

M.Sheikh Kalukhi

D.Ramezan

A.Rahimian Boogar

Department of Horticulture and Landscape, Faculty of Agriculture, University of Zabol, Zabol, Iran

M.Pirnia✉

Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran

M.Aran

Department of Horticulture and Landscape, Faculty of Agriculture, University of Zabol, Zabol, Iran

Abstract: Due to the variety of vegetation and high humidity, the Northern provinces of Iran are the habitat of many medicinal mushrooms. Genetic variation of medicinal mushrooms in Iran is poorly studied. 30 isolates of *Trametes versicolor* were collected from different localities in Guilan, Golestan, and Mazandaran provinces (North of Iran). The genetic diversity was evaluated by ISSR marker. 86 bands were observed using eight polymorphic primers. Cluster analysis showed that the isolates divided into 18 groups at a level of 0.22 similarity coefficient. SI indices were 0.335, 0.305 and 0.217 in Guilan, Golestan, and Mazandaran populations respectively. 96% genetic variation was observed within populations. The diverse geographic origin of the isolates is an influential factor in the high intra-population genetic diversity of the fungus.

Keywords: DNA fingerprinting, genetics, medicinal mushrooms, molecular variance.

INTRODUCTION

The genus *Trametes* Fr. (Basidiomycota, Polyporaceae), encompasses more than 570 species with poroid hymenophore (https://www.mycobank.org). Basidiomata (fruiting bodies) are semi-spherical to kidney, in different colors with concentric areas, and 2.5-10 cm in diameter. Hyphal system (Kotlaba & Pouzar 1957) and wood-rotting (brown-rot/white-rot) types (Nobles 1958) are recognized for genus delimitation in the Polyporaceae. Welti *et al*. (2012) confirmed close relationship between some genera in the Polyporaceae based on the results inferred from *ITS-rDNA* and *rpb2* regions. Different concepts at the generic level complicated the classification of the genus and allied genera in the Polyporaceae (Olusegun 2014). Molecular data inferred from nucleotide sequencing of different gene loci has changed the genus concept in Polyporaceae (Hibbett & Donoghue 1995; Ko & Jung 1999; Tomšovský *et al*. 2006; Cui *et al*. 2011; Carlson *et al*. 2014; Ueitele *et al*. 2018; Olou *et al*. 2020).

Trametes species are important as the causative of white rot on dead and fallen woods (Kamiyama *et al*. 2013), and medicinal aspects, due to the presence of polysaccharides, proteins, and secondary metabolites in their basidiomata. *T. versicolor* (L.) LIoyd is one of the most common mushrooms in forest ecosystems (Zmitrovich *et al*. 2012).

Genetic diversity is one of the important components of biodiversity, which refers to heritable changes at the molecular level and creates variety in DNA sequence, biochemical and physiological characteristics (Müller-Starck *et al*. 1992). The higher amount of variation reveals that the members will get the alleles that make them survive in the environment (Yang *et al*. 2010). Different mechanisms such as mutation, sexual recombination, migration, gene flow, genetic drift, and selection are involved in

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✉ Corresponding Author: E-mail: pirnia@uoz.ac.ir

http://mij.areeo.ac.ir

genetic variation of fungi (Burdon & Laine 2019). Molecular markers with high polymorphic information content will be very useful for distinguishing closely related isolates. The ISSR (inter simple sequence repeat) is a marker that can show the differences between individuals in a population with high speed and accuracy. The inheritance of inter-simple sequence repeat is dominant-dominant (Yang *et al*. 2010). Due to the longer length of primers and higher binding temperature, the reliability of ISSR was reported higher than other markers (Malekzadeh *et al*. 2011). ISSR has been widely applied to the analysis of the population structure of many fungi (Kauserud & Schumacher 2003; Du *et al*. 2011).

Extensive forest ecosystems in the northern provinces of Iran are the habitat of various species of mushrooms. Ershad (2009) listed 11 species of the genus *Trametes* in his book, but this is the first research on genetic diversity of the genus *Trametes* in Iran.

MATERIALS AND METHODS

Sample collection

Isolates of *T. versicolor* were collected from different localities of Guilan, Golestan, and Mazandaran provinces (North of Iran) (Fig. 1). These isolates were identified as *T. versicolor* according to morphological characteristics (Welti *et al*. 2012; Olou *et al*. 2020) (Fig. 2).

Fig. 1. Northern provinces of Iran around the Caspian Sea. The direction of sample collection is marked with a blue line (approximately 800 km long).

DNA extraction

A small piece of tissue of *T. versicolor* isolates transferred into potato dextrose agar (PDA) and incubated in the dark at 25°C for 7 days. Genomic DNA extracted from mycelia powder using a plant DNA extraction kit (ZandBio Co., Iran). Mycelia powdered separately using liquid nitrogen. The quantity and quality of the extracted DNA evaluated using Nanodrop at 260 nm and electrophoresis on agarose gel (Khanuja *et al*. 1999).

ISSR PCR amplification

Eight ISSR primers (Table 1) were used for evaluating genetic variation among *T. versicolor* isolates. For polymerase chain reaction (PCR), 10ng of template DNA added to 25µl reaction mixture and placed into thermocycler (Eppendorf, Germany). PCR program was pre-denaturation 5min at 94°C, followed by 35 cycles of the PCR reaction (denaturation 1min at 94°C, annealing 1min at TM of each primer, extension 2min at 72°C) and final extension 10min at 72°C (Yang *et al*. 2010). The positions of bands were observed through the Uvitech UV-2100 Gel dock device (Cambridge, France).

Statistical analysis

After electrophoresis, all ISSR bands were scored as present (1) or absent (0). Jaccard's similarity coefficient was calculated through NTSYSpc v.2.02 software. Cluster analysis was performed using the UPGMA algorithm. Molecular variance analysis of the data and genetic variation were performed in Gen Alex v.6.501 software. The contribution of each variance component to the total variance was evaluated. The genetic distance was calculated using the Nie (H) coefficient. Polymorphism rate, number of alleles, SI index, number of effective alleles in each gene locus and genetic diversity performed using Gen Alex v.6.501 software.

Fig. 2. Basidiomata (Fruiting bodies) in *Trametes versicolor*

RESULTS

ISSR analysis

Eight ISSR primers produced 86 countable bands from 30 isolates (Table 1). The ISSR bands showed considerable polymorphism among isolates from different origins. The number of bands detected by the primers was equal to the number of polymorphic bands. Therefore, the polymorphism ratio for all primers was equal to one. The mean number of bands created by each primer was 10.75. $Primer_{11}$ (P_{11}) showed the highest number of polymorphic bands (16 bands), and P_{13} showed the least polymorphic bands (8 bands). The mean percentage of polymorphism of primers was 81.01%, for all 3 populations. Using allelic escape, polymorphic information content (PIC), effective multiplex ratio (EMR), and marker index (MI) were calculated for each primer separately. P_{13} (0.79) and P_1 (0.38) showed the highest and lowest polymorphic information, respectively. The mean ratio of effective polymorphism for all primers was 10.75.

The marker index (MI) of the primers used in this research was 4.51. SI indices were 0.335, 0.305 and 0.217 in Guilan, Golestan, and Mazandaran populations, respectively. The mean numbers of effective alleles (Nie) were 1.236 in the whole population and 1.620 for the number of different alleles in the population.

Cluster analysis

Three similarity coefficients including Dice, Jaccard, and simple matching were calculated for the appropriate clustering of isolates. By comparing the cophenetic coefficient of all 3 matrices, Jaccard's similarity coefficient and UPGMA served as the most appropriate methods for cluster analysis.

Similarity coefficients ranging from 0.03 to 0.42 and the isolates divided into 18 groups at a level of 0.22 similarity coefficient (Fig. 3). Group I consisted of 7 isolates (Guilan1, Golestan9, Guilan9, Golestan6, Guilan3, Golestan1, Guilan6) was collected from two different provinces with relative high geographic distance (approximately 500 km). Groups II, XI, XII with 2 isolates from two different provinces (Guilan5, Golestan5), (Guilan2, Golestan8), and (Guilan10, Golestan3) respectively. Groups III and VIII comprised 2 (Golestan2, Golestan7) and 3 (Mazandaran1, Mazandaran4, Mazandaran8) isolates respectively, from the same provinces. Groups IV, V, VI, VII, IX, X, XIII, XIV, XV, XVI, XVII, XVIII consisted of 1 isolate from different localities of three provinces.

Table 1. Data obtained from eight ISSR primers used for genetic diversity of *T. versicolor* isolates

ISSR Primers	Sequence 5`-3`	TL	PL	FP	PIC	ISIP	Rp	Mean Rp
P_1	$TG(CA)_6C$	12	12	1	0.38	4.08	6.28	0.523
P_2	$GT(CA)_6C$	9	9	1	0.43	3.77	5.92	0.657
P_3	GTGACGA(CT)6	12	12	1	0.40	4.71	6.78	0.565
P_{10}	$(GA)_8C$	9	9	1	0.44	3.93	6.06	0.673
P_{11}	$(AG)_8C$	16	16	1	0.44	7.27	10.68	0.667
P_{12}	$(GA)_8C$	10	10	1	0.45	2.22	7.08	0.708
P_{13}	$(CA)_8C$	8	8	1	0.79	3.91	7.26	0.907
P_{14}	$(TC)_8C$	10	10	1	0.48	4.68	8.06	0.806
Mean		86	86	1	0.42	4.32	7.26	0.688
MI=EMR×Mean PIC=4.51			EMR=MR×Mean FP=10.75			MR=TB/TP=10.75		

TL: Total Loci, PL: Polymorphic Loci, FP: Fraction of Polymorphism, PIC: Polymorphic Information Content, ISIP: ISIR Primer Index, Rp: Resolving Power, Mean Rp; MR: Multiplex Ratio, TP: Total Primer, EMR: Effective Multiplex Ratio, MI: Marker Index.

Analysis of Molecular Variance (AMOVA)

Intra/inter-population genetic diversity was evaluated using analysis of molecular variance (AMOVA), and a significant difference was observed at the level of $P<0.02$ (Table 2). Analysis based on the information for all primers revealed 96% and 4% genetic variation within and among populations respectively (Fig. 4).

Table 2. Analysis of molecular variance of *T. versicolor* isolates

Source of variation	df	Sum of square	Mean of Square	Variance
Inter-population	2	34.733	17.367	4%
Intra-population	27	331.900	12.293	96%
total	29	366.633	4.71	100%
Stat	value	P(rand >= data)	3.93	
PhiPT	40%	2%	7.27	

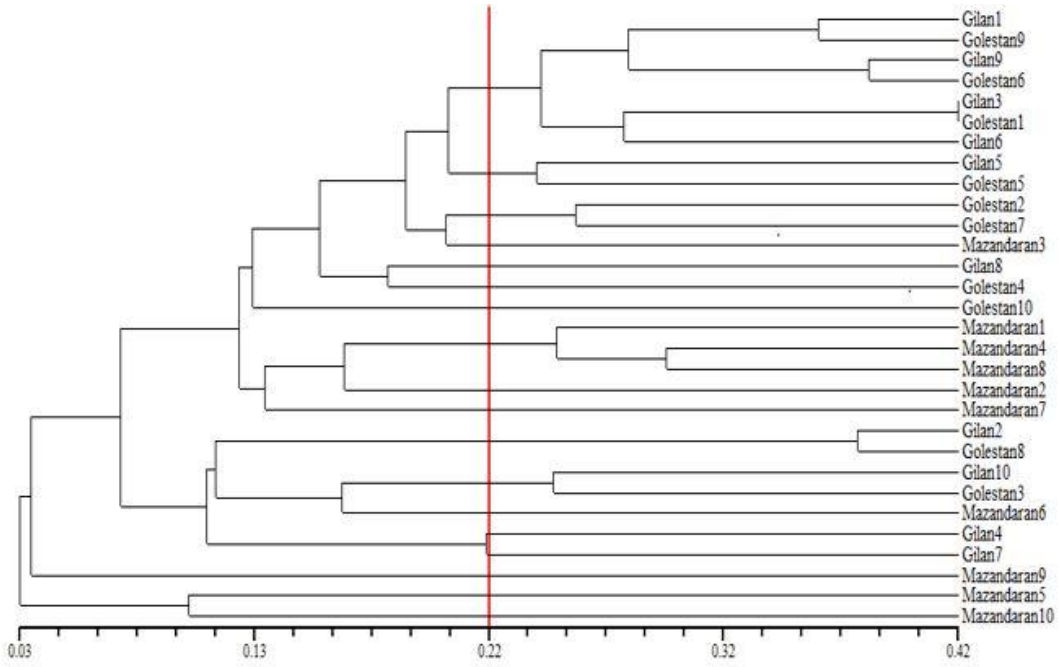

Fig. 3. UPGMA cluster analysis constructed from ISSR patterns obtained from 30 *T. versicolor* isolates.

Principal Component Analysis (PCA)

The placement of each isolate in one area of the diagram shows the genetic similarity or difference. The isolates with more similarity will place close to each other. The two and three-dimensional diagrams are shown in Figures 5 and 6, respectively. The isolates are placed in completely separate groups. A positive correlation was observed between the spatial distances of the points on the diagram with the genetic distances of the isolates, which classified them into separate groups. The results of different methods confirmed each other.

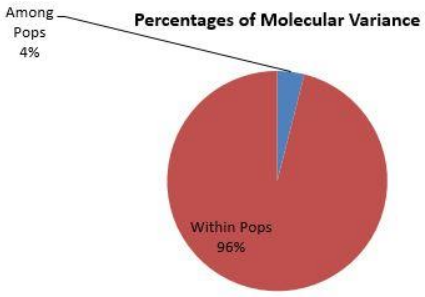

Fig. 4. Intra and inter-population genetic variation based on AMOVA analysis.

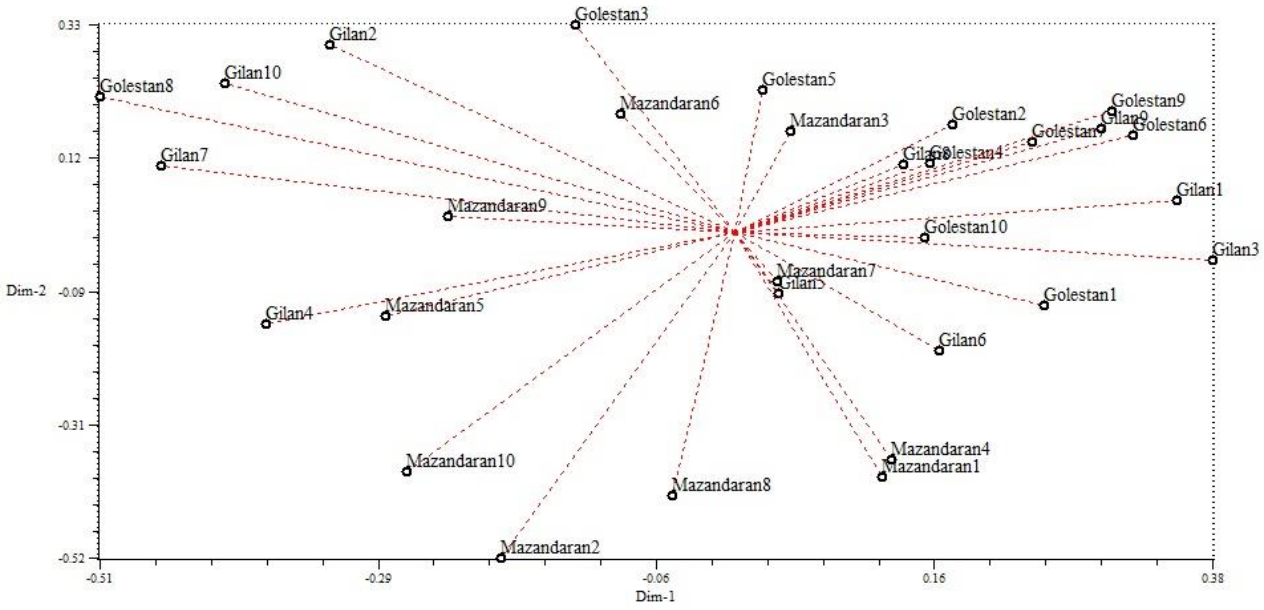

Fig. 5. Position of each isolate in two-dimensional plot.

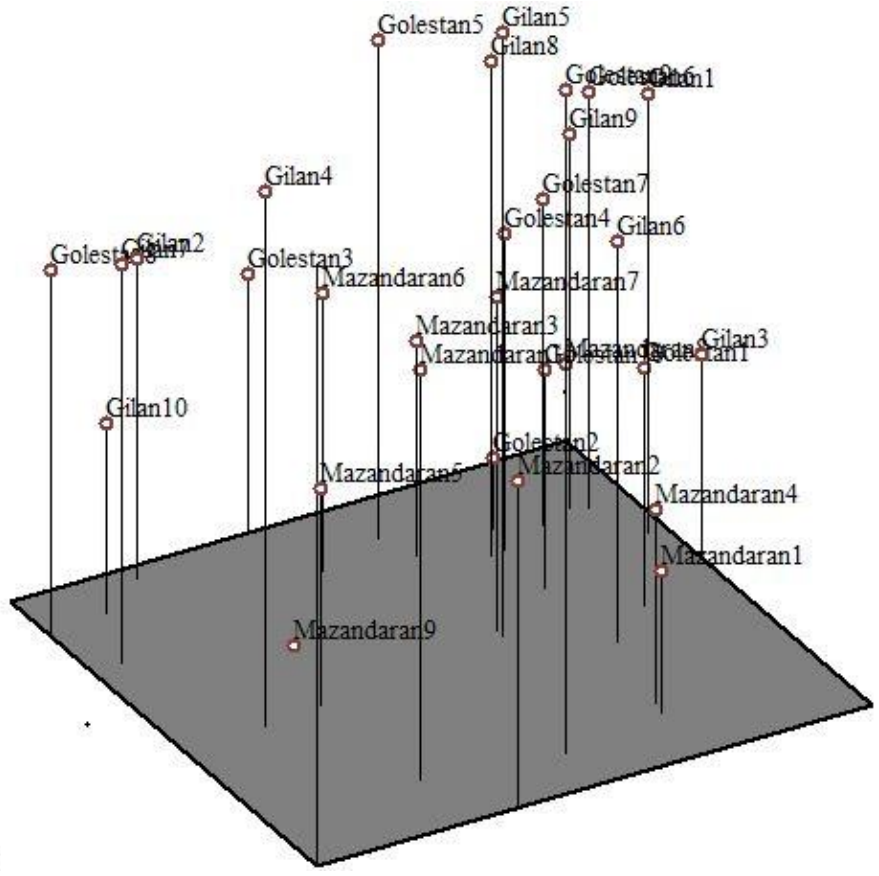

Fig. 6. T Position of each isolate in three-dimensional plot.

DISCUSSION

DNA markers allow direct identification of genomic sequence diversity that can be used to supplement genealogical information. Molecular markers show the process of population genetic changes due to their high accuracy and speed. Our results indicated that ISSR primers used in this research have a suitable distribution throughout the genome of the isolates and are suitable for determining the genetic diversity of *T. versicolor* populations. The number of effective alleles and the number of different alleles in each marker show the suitability of gene location for estimating genetic diversity. Primers with a large number of effective and different alleles are recognized as suitable for investigating genetic diversity. The results of the present research were consistent with the results of Malekzadeh *et al*. (2011), who investigated the efficiency of the ISSR marker to identify the genotypes of button mushroom and recognized 18 genotypes.

In the present research, *T. versicolor* isolates were classified into 18 groups based on the eight ISSR primers and AMOVA analysis revealed 96% intra-population genetic variation. The presence of high diversity within the population can be attributed to the high efficiency of ISSR, the geographical distances of the area under study, and the large number of isolates. According to the results, there is a low similarity between isolates from different localities with long distances. This level of similarity is logical and acceptable in fragmented populations. The primers used in this study showed high content of polymorphic information (0.42), revealing the correct selection and high efficiency of these primers. According to genome and fungal isolates, the effective polymorphism ratio may be different, and different results can be obtained by changing the number and type of primers. Native isolates of each province were not placed in the same group and were distributed in different clusters. Furthermore, a great distinction was observed between the main and sub-groups in the cluster analysis, which was consistent with the findings of Bogacki *et al*. (2010), who reported 91% and 9% intra/inter-population genetic diversity among *Pyrenophora teres* populations, respectively. Furthermore, isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. from each geographic origin were not grouped in one cluster and PIC calculated 0.39 (Wang *et al*. 2005, Yang *et al*. 2010).

Conclusion

This study was the first research on the genetic diversity of *T. versicolor* using ISSR markers in Iran. The results indicated that the ISSR primers used in this study showed suitable distribution throughout the genome, appropriate for determining the genetic and revealed high polymorphism between the isolates. Furthermore, a great differentiation was observed between the main and secondary groups in the clustering. The main reason for high genetic diversity can be attributed to the diverse habitats of the isolates. Some factors including the origin of sample, weather conditions and human activities, can influence the genetic diversity.

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تجزیه و تحلیل تنوع ژنتیکی جدایه های *Trametes versicolor* جمع آوری شده از استانهای شمالی ایران با استفاده از نشانگر ISSR

مرضیه شیخ کلوخی[1]، داریوش رمضان[1]، عبدالرحمن رحیمیان بوگر[1]، مهدی پیرنیا[2]*، مهدی آران[1]

۱-گروه علوم باغبانی و فضای سبز، دانشکده کشاورزی، دانشگاه زابل، زابل، ایران

۲-گروه گیاه پزشکی، دانشکده کشاورزی، دانشگاه زابل، زابل، ایران

چکیده : استان های شمالی ایران به دلیل تنوع پوشش گیاهی و رطوبت زیاد، زیستگاه بسیاری از قارچ های دارویی هستند. تنوع ژنتیکی قارچهای دارویی در ایران خیلی کم مورد مطالعه قرار گرفته است. سی جدایه *Trametes versicolor* از مناطق مختلف استان‌های گیلان، گلستان و مازندران (شمال ایران) جمع‌آوری شدند. تنوع ژنتیکی با کمک نشانگر ISSR مورد ارزیابی قرار گرفت. هشتاد و شش باند توسط هشت آغازگر مورد استفاده مشاهده شد. تجزیه و تحلیل خوشه ای نشان داد در ضریب تشابه ۰/۲۲، جدایه ها به ۱۸ گروه تقسیم شدند. شاخص اطلاعات شانون (SI) در جمعیت‌های گیلان، گلستان و مازندران به ترتیب ۰/۳۳۵، ۰/۳۰۵ و ۰/۲۱۷ بود. درون جمعیت ۹۶٪ تنوع ژنتیکی مشاهده شد. منشاء جغرافیایی متنوع جدایه ها عاملی تأثیرگذار در تنوع ژنتیکی بالای درون جمعیتی قارچ می باشد

کلمات کلیدی: انگشت نگاری DNA، ژنتیک، قارچهای دارویی، تنوع مولکولی

مکاتبه کننده: مهدی پیرنیا Email: pirnia@uoz.ac.ir

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