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GENETIC AND MOLECULAR CHARACTERIZATION OF POPULATIONS OF *Pyricularia grisea* FROM RICE

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Abstract. *Pyricularia grisea*, the causal agent blast disease in rice, is considered as one of the most important fungus in paddy fields. Isolates of *Pyricularia grisea* were analyzed by SSR to determine the amount of genetic variability in populations. Fourteen primers were applied and DNA bands of 95-640 bp were produced. Cluster analysis using UPGMA method gave three groups. Group 1 was the major group and most of isolates which collected from west of Guilan belonged to this group. Groups 2 and 3 belonged to the center of Guilan isolates and the east of Guilan isolates, respectively. The results revealed although genetic distance was high between isolates of west and east of Guilan and the genetic similarity among these isolates of these two populations was low, but there was the maximum of genetic distance or the minimum of genetic similarity between the population of center of Guilan isolates and population of east of Guilan isolates. The minimum of genetic distance or the maximum of genetic similarity there was between the population of center of Guilan isolates and population of west of Guilan isolates. Overall, results confirmed that microsatellite primers are good and suitable markers for analyzing the population structure of *Pyricularia grisea*.

Key words: genetic variation, PCR, *Pyricularia grisea*, rice

INTRODUCTION

Pyricularia grisea, the causal agent of rice blast disease is one of the most important fungi which attack the rice throughout its life cycle from the nursery to the farm [Ou 1985]. Molecular biology techniques have been used to explore genetic variability in fungi [Caligiorne et al. 1999]. Simple sequence repeat (SSR), is among the markers

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based on the PCR [Rabiee and Sabuori 2008]. Levy et al. [1993] collected 151 isolates of *P. grisea* from fields in Columbia and fingerprinted with DNA-repetitive sequence MGR586 and determined 155 haplotypes into 6 genetic groups individually. George et al. [1997] examined *P. grisea* populations by the Rep-PCR method. They demonstrated that this method is highly efficient to understand interactions between host genotypes and pathogen evolution in large populations.

Mitochondrial genome and areas of single copy of genome and nuclease as CUT1, MPG1 and IV1 are used in the study of the different populations of P. grisea. Non-pathogenic genes markers and repetitive elements in the genome of *P. grisea* like MGR and grasshopper used for the determining the diversity of the populations among different host strains and showed the genetic separations among them [Babujee and Gnanamanickam 2000]. Of nine categories of specified repetitive elements in the genome of *P. grisea*, one family of the repetitive sequences DNA is called as MGR. This sequence clearly is kept in the genome of *P. grisea* [Babujee and Gnanamanickam 2000]. In other study, the population structure of Magnaporthe grisea was analyzed in Iran using rep-PCR and differentiated six distinct fingerprint lineages [Javan Nikkhah 2001].

The isolates of *P. grisea* analyzed with SSR markers for determining the genetic diversity and these isolates were divided into 6 groups by cluster analysis [Ying et al. 2004]. The genetic diversity of *P. grisea* was analyzed in Mazandaran province by Hemmati et al. [2005] in RAPD-PCR. They showed that these isolates are divided into three groups. Piotti et al. [2005] identified three Italian major genetic groups with 80% similarity in genetic structures of isolates of *P. grisea* in Italy.

Liu et al. [2008] assessed 46 isolates of rice blast fungus collected from the different geological areas to determine its DNA configuration by the use of SRR markers and revealed every locus has a range of different alleles of 2 to 10 and this result showed SSR genes have polymorphism. The isolates of *P. grisea* analyzed for the configuring DNA using SRR markers. According, these isolates were classified in 7 genetic groups and there was not much difference among the isolates, though the difference among some of the isolates within the group was significant [Jian-Hui et al. 2008]. The genetic diversity of *P. grisea* from different hosts such as barley, rice, weed, wild rice in Thailand was evaluated by using RAPD primers and observed high variations among the separated isolates from northern parts, eastern north and center of Thailand while the genetic differences among southern and eastern strains were very low [Sirihunaya et al. 2008]. Consolo et al. [2008] collected isolates of *P. grisea* from rice fields to DNA-fingerprint population in Argentina. They determined five genetic groups and concluded that *P. grisea* populations were genetically simple in Argentina and the dominant groups still possess the highest genetic diversity.

The phenotypic and genetic diversity of 77 isolates of *P. grisea* collected from two upland rice cultivars was studied and Rep-PCR analysis of isolates using two primers designed from sequences of Pot2 showed that isolates could be clustered broadly into two groups [Prabhu et al. 2007].

Mottalebi et al. [2009] evaluated *P. grisea* populations isolated from rice and weeds and analyzed the genetic diversity of complex species of this fungus by Rep-PCR and concluded that genetic diversity in these isolates is low.

Genetic and phenotypic structure of Magnaporthe oryzae populations of two upland rice cultivars was determined and the ANOVA of virulence data showed high variability within population of each cultivar. There was no significant difference in virulence pattern of isolates from leaves and panicles, independent of collection site and cultivar [Silva et al. 2009].

Genetic diversity and population structure of 72 M. grisea isolates collected from finger millet, foxtail millet, pearl millet and rice from major crop growing areas in India was studied using 24 SSR markers and a model-based population structure analysis of the genomic data identified two distinct populations with varying levels of ancestral admixtures among the 65 M. grisea isolates [Kiran Babu et al. 2013].

Fingerprinting of 11 isolates of P. oryzae by retrotransposon – microsatellite amplified polymorphism (REMAP) showed a high level of variability and polymorphism among them. Phylogenetic analysis using REMAP markers grouped out one rice isolate from others [Vanaraj et al. 2013].

Random amplified polymorphic DNA markers were used to find out genetic diversity in *P. grisea* isolates collected from farmer's fields in Northeast India. 28S rRNA gene sequences of the isolates were compared with other related taxa retrieved from Gene Bank database. The results obtained confirmed the genetic diversity of rice blast fungus in Northeast India [Gad et al. 2013].

In this research, genetic and molecular characterization of *Pyricularia grisea* was investigated using SSR based on the PCR in Guilan province of Iran and the similarities and genetic differences among the populations were determined.

MATERIALS AND METHODS

Collection and culture of fungal isolates

Diseased leaves of rice cultivars were collected from paddy fields in Guilan province of Iran. Leaves were transferred to the laboratory and then isolated the fungi from disease samples. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in Petri dishes at 25ºC for 2–3 days. Potato dextrose agar and water agar media were used for sporulation. Then Petri dishes containing media were incubated at 25ºC in the dark or artificial light supplied by fluorescent light on a 12 h light/dark photoperiod for 15–25 days [Safari Motlagh and Javadzadeh 2010]. Monoconidial isolates of the recovered fungi were maintained on half- strength potato dextrose agar slants in test tubes as stock cultures [Safari Motlagh and Javadzadeh 2010].

Study and identification of fungi

Morphological studies were carried out on PDA and WA media. Cuts of colonies were placed onto PDA medium for 2–3 days. Then, section of colonies was transferred to WA medium for 7–10 days in incubator at 25ºC and 12 h photoperiod. Afterward, morphological observations were taken based on colony, conidium and conidiophore morphology and other characters morphological [Javan Nikkhah 2001].

Molecular analysis

DNA extraction. The dried mycelium of the isolates are used for DNA extraction. DNA from the mycelium samples were extract by Murray and Thompson [1980] method known as CTAB method by a little change; as follows.

The mycelia in liquid nitrogen were grounded to powder with a mortar and pestle. The powders of mycelia were transferred to 1.5 ml tubes. 534 µl extraction buffer (including Tris 0.05 M, EDTA 0.025 M and NaCl 0.06 M) added to mycelia powders and gently were mixed manually. 28 µl of 20% SDS solution was added to the tubes including extraction buffer and mycelia powders to lubricate and to digest the cell wall. Then the tubes were incubated on the ice under stirring at 150 rpm. The samples were put in a water bath for 10 min at 65°C. 92 µl NaCl 5 M was added to each sample and the tubes were shaken gently. 72 µl CTAB solution was added to the tubes and the tubes were shaken gently. The samples were put in a water bath for 10 min at 65°C. 723 µl chloroform and isoamyl alcohol proportional 1:24 was added and the tubes were shaken manually. The samples were centrifuged at 10,000 rpm for 5 min. The supernatant which contains the DNA solution was transferred to another 1.5 ml tubes. 600 µl cold isopropanol was added and the tubes were centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant was thrown away so the DNA sediment remains in the bottom of the tube. The DNA template was washed by the 70% ethanol and it was dried at room temperature under the hood. 70 µl TE was added to the dried DNA in the tube and was shaken gently so the DNA template was solved completely. The tubes containing DNA and TE buffer were incubated in the water bath at 75°C and for 20 min. Then the tubes were spun for 10 min at 10,000 rpm and the supernatant carefully were transferred to the 0.5 ml tubes for keeping at -20°C. The DNA samples are kept at -20°C for the next tests. The extracted DNA was electrophoresed in 0.8% agarose gel for determining DNA quantity and quality.

The extracted genomic DNA dilution. After all the DNA samples on the agarose gel and the related bands under the UV was observed, it was understood some of the DNA samples were not of good quality and the DNA extracting were done again. The extracted DNA samples had different destinies. Finally for the replication of genomic DNA is done well in the thermocycler system; firstly all the samples were diluted with the same destiny. For this, the bands related to the extracted DNA from the lines at first are compared with the bands coming from the lambda phage DNA with 50, 100 and 150 ng/µl and their optical density is calculated. Then all of them are distillation-sterilized with distilled water so they were diluted to the 20 ng/µl and directly were used in PCR reaction.

PCR amplification of alleles at SSR loci. 14 pair primers were used [Liu et al. 2008, Ying et al. 2004, Ma et al. 2008]. The polymerase chain reaction was done by using the Biometra T-Gradient thermocycler system in the final volume of 10 µl, containing 10 ng of DNA, 0.3 µM of each primer, 0.25 mM of each dNTP, 1.5 mM MgCl2, 10X PCR buffer (10 mM Tris-HCl pH 8.5, 50 mM KCl and 0.001% gelatin) and 1 U of Taq DNA polymerase. The reactions were performed on a thermal cycler programmed for one initial cycle with 4 min at 94°C, then 36 cycles with 30 sec at 94°C, 45 sec at 55°C (it ranges from 53 to 56°C in the different primers depending on the length and C

+ G/A + T) 45 sec at 72°C followed by a final extension for 5 min at 72°C. The amplified samples were kept at -20°C. PCR products were electrophoresed in 10% polyacryliamid gel and detected by staining with ethidium bromide.

Data Analysis

The three softwares of NTSYS-pc ver 2.02 [Rohlf 2000], Popgene 1.32 [Yeh and Boyle 1997] and Genealex 6.2 [Peakall and Smouse 2006] were used. The genetic diversity within populations was assessed through calculation of gene diversity (Heterozygosity) and polymorphism within the populations [Takahata et al. 1984]. The number of effective alleles (Ne) and the observed number of alleles (Na) calculated by using Popgene software [Kimura and Crow 1964]. The polymorphism sites in any population calculated as an index of genetic diversity of population by using Popgene software [Nei et al. 1995]. The genotype diversity based on the Shannon information index [Lewontin 1972] calculated by using Popgene software for each of the populations. The genetic similarity and distance between the populations calculated by considering biased and unbiased. All the calculations were done by Popgene software [Nei 1972, 1978]. The analysis of molecular variance of data and calculating the percent of molecular variance between and within populations was done by Genealex 6.2 [Peakall and Smouse 2006].

RESULTS

14 microsatellite primers were used in this research that totally generated 64 polymorphic fragments, ranging from 95–640 bp. The largest fragment was generated by SSR-G5 microsatellite primers and the smallest by the SSR47,48 microsatellite primers. The most generated bands were amplified at the time of application of primers SSR43,44 and the least generated bands were amplified with primers SSR-KMS20. The average of generated bands for each of the primers was 4.75 (tab. 1). All the generated bands were polymorphic.

To study the primers efficiency and to determine the surface of polymorphism, the polymorphism information content (PIC), Nei's genetic diversity, observed number of alleles (na), effective number of alleles (ne) and Shannon's index (I) (tab. 2), were calculated (tab. 2). The observed polymorphism information range in all of the used primers in this research was variable between 0.661–0.852 (tab. 2). The more the number (close to 1) suggests the more number of alleles, polymorphism abundance in supposed genetic location in that population and more uniform distribution of polymorphism bands among the isolates. The results showed among the 14 used microsatellite primers in this research, primers SSR43,44 (PIC = 0.852) have the most PIC and it could show the genetic distances between isolates better than the other primers. The primers KMS20 (PIC = 0.661) showed the less of PIC. The average of PIC was 0.734 for all the microsatellite primers.

According to the results, the most Nei's genetic diversity in all isolates was observed with primers SSR43,44 and the least amount was observed in primers KMS20 (tab. 2). The average of genetic diversity in all the primers for all isolates was 0.734.

Table 1. Number of bands and amplified bands size by primers

Name of Initiator	Number of alleles in every primer	Number of strains possessing each of alleles									Bands size (bp)
		non-bands	1	2	3	4	5	6	7	8	
SSR41,42	5	1	2	5	5	6	11				128–172
SSR43,44	8	1	3	3	4	6	4	5	2	3	252–300
SSR47,48	4	1	9	9	5	6					95–130
SSR77,78	4	–	2	7	10	11					136–200
SSR83,84	6	–	7	7	2	3	7	4			168–210
SSR99,100	5	4	3	6	8	4	5				190–235
SSR101,102	4	–	7	11	4	6					170–200
SSR-D4	4	2	3	12	10	3					350–450
SSR-D5	4	–	5	6	12	7					450–540
SSR-G5	5	3	5	5	6	10					550–640
SSR-KMS20	3	2	9	11	8						180–250
SSR-SMS1	4	–	5	9	11	5					500–550
SSR-SMS10	4	–	6	10	11	3					460–500
SSR-SMS17	4	1	7	4	11	7					370–420

Table 2. Polymorphism information content (PIC), observed number of alleles (na), number of effective alleles (ne), Nei's index (Nei) and Shannon's index (I) in microsatellite sites in all the studied isolates

Name of genetic site	PIC	na	ne	Nei	I
SSR41,42	0.749	5	2.66	0.749	1.38
SSR43,44	0.852	8	3.76	0.861	1.38
SSR47,48	0.735	4	3.06.	0.735	1.18
SSR77,78	0.696	4	2.15	0.696	0.89
SSR83,84	0.804	6	2.68	0.804	1.10
SSR99,100	0.778	5	3.50	0.778	1.24
SSR101,102	0.733	4	2.34	0.733	0.88
D4	0.666	4	2.64	0.666	1.05
D5	0.718	4	1.91	0.718	0.81
G5	0.743	5	2.10	0.743	0.91
KMS20	0.661	3	2.15	0.661	0.82
SMS1	0.720	4	2.87	0.720	1.10
SMS10	0.704	4	3.03	0.704	1.15
SMS17	0.721	4	2.72	0.721	1.12
An average	0.734	4.57	2.68	0.734	1.05

Table 3. Observed number of alleles (na),, number of effective alleles (ne), Nei's index (Nei) and Shannon's index in microsatellite sites in all the studied isolates

Name of genetic site	East of Guilan population				Center of Guilan population				West of Guilan population			
	na	ne	Nei	I	na	ne	Nei	I	na	ne	Nei	I
SSR41-42	3	2.97	0.663	1.09	3	1.81	4.49	0.80	5	3.20	0.688	1.39
SSR43-44	5	4.45	0.776	1.54	4	3.27	0.694	1.27	4	3.56	0.719	1.32
SSR47-48	4	3.77	0.735	1.35	4	3.27	0.694	1.27	3	2.13	0.531	0.90
SSR77-78	3	2.42	0.587	0.97	3	2.33	0.571	0.96	3	1.68	0.406	0.74
SSR83-84	6	3.46	0.711	1.49	3	2.88	0.653	1.08	3	1.68	0.406	0.74
SSR99-100	5	4.17	0.760	1.52	5	4.45	0.775	1.55	2	1.88	0.469	0.66
SSR101-102	3	2.18	0.541	0.88	3	2.88	0.653	1.08	2	1.96	0.490	0.68
D4	3	2.00	0.500	0.83	3	3.00	0.667	1.10	4	2.91	0.656	1.21
D5	3	1.72	0.418	0.73	3	2.33	0.571	0.96	3	1.68	0.406	0.74
G5	5	2.38	0.580	1.18	2	1.80	0.44	0.64	3	2.13	0.531	0.90
KMS20	3	2.28	0.561	0.90	3	2.57	0.611	1.01	2	1.60	0.375	0.56
SMS1	4	3.36	0.702	1.26	3	2.33	0.571	0.96	3	2.91	0.656	1.08
SMS10	3	2.92	0.658	1.08	4	3.27	0.694	1.28	3	2.91	0656	1.08
SMS17	4	3.26	0.693	1.27	3	2	0.500	0.87	4	2.91	0.656	1.21
An average	3.58	2.95	0.635	1.15	3.29	2.73	0.611	1.06	3.14	2.37	0.546	0.94

Also, based on the Nei's genetic diversity index in each of the populations separately, the most genetic diversity content was observed in the isolates of two populations of west and east of Guilan province with primers SSR43,44, and in the population of center of Guilan, the most genetic diversity content was observed with primers SSR99,100 (tab. 3). The least genetic diversity amount in each of studied populations was observed in different primers, so in the population of the west of Guilan, the least genetic diversity with primer D4, in the population of center of Guilan, with primer SMS17 and in the population of east of Guilan with primer KMS20 was observed (tab. 3). These results showed in each of the studied populations, the primers which had the most and the least effects in depicting the diversity among its isolates were different. In other words, the effects of different primers in revealing the genetic differences among the different isolates in the studied populations were different.

The population of isolates of west of Guilan and the population of isolates of east of Guilan had the most and the least of average genetic diversity in all the used primers, respectively.

All the used primers in this research were polymorphic and generated a variety number of alleles with different sizes.

The maximum observed number of alleles (8 alleles), maximum effective number of alleles (3.76) and maximum of Shannon's index (1.38) in all the isolates like the maximum of the PIC and maximum of the genetic diversity were observed in primers SSR43,44 (tab. 2). This suggests that this primer in addition to producing maximum of the number of different alleles in studied isolates; it could have a uniform distribution among the review isolates and this primer can be introduced as one of the perfect microsatellite primers for studying the genetic diversity of *Pyricularia grisea*. The minimum observed number of alleles (3) in all the isolates like the minimum of PIC and Nei's genetic diversity were observed in primers KMS20, but the minimum of effective number of alleles (1.91) and minimum of Shannon's index (0.81) derived from primers D5 (tab. 2).

In isolates of west of Guilan, maximum of observed alleles was observed in primers SSR83,84, but maximum effective number of alleles (4.45) and Shannon's index (1.54) like the maximum of Nei's genetic diversity derived from primers SSR43,44 (tab. 3), that this primer in all the studied isolates showed the maximum of PIC, Nei's genetic diversity, observed number of alleles, effective number of alleles and Shannon's index. The minimum number of observed alleles (3 alleles) in primers SSR41-42, SSR77-78, SSR101-102, D4, D5, KMS20 and SMS10, the minimum effective number of alleles in primer D5 and the minimum of Shannon's index were observed in primer D4 (tab. 3).

The primers SSR99-100 in west of Guilan population isolates had the maximum of observed alleles, effective number of alleles and Shannon's index and also they had the maximum of Nei's genetic diversity. Minimum of observed alleles and effective number of alleles were observed in primers G5. Whereas the minimum of Shannon's index was observed in primers SSR41-42 (tab. 3).

In east of Guilan population isolates, the maximum of observed alleles (alleles) and Shannon's index were observed in primers SSR41-42, but the maximum effective number of alleles like the maximum of Nei's genetic diversity derived in SSR43-42 (tab. 3). This primer also had the maximum amounts related to this statistics in west of Guilan

population isolates and all the studied isolates. The minimum of observed alleles were observed in "SSR99-100, SSR101-102 and KMS20" primers, but the least amount of effective number of alleles and Shannon's index like the Nei's genetic diversity were observed in KMS20. Also, this primer had the minimum amounts related to some of these statistics (number of observed alleles, Nei's genetic diversity and PIC) in all the studied isolates (tab. 3).

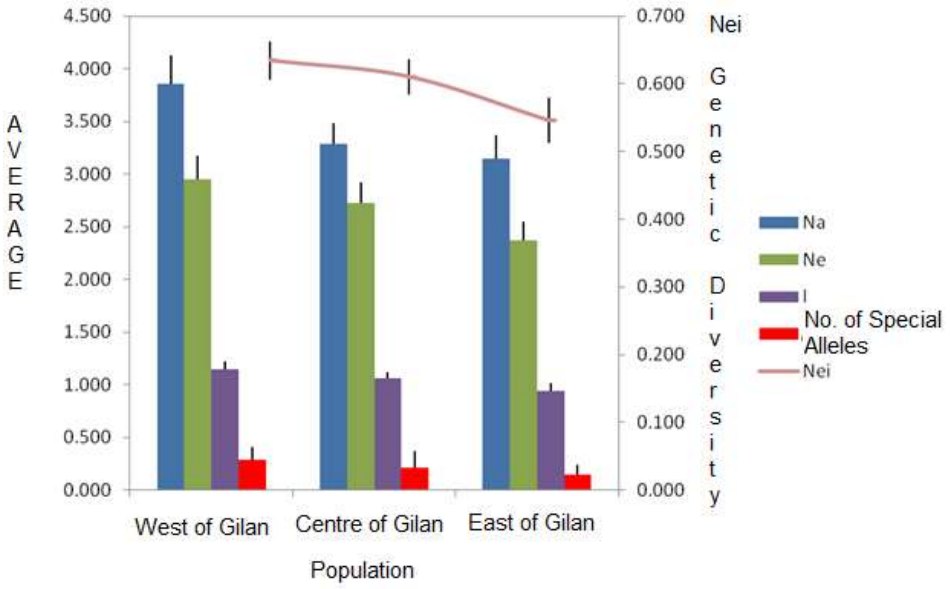

Fig 1. The pattern of allele distribution in the review populations

Table 4. Cophenetic coefficient for the different methods of cluster analysis

Similarity coefficient	Cluster analysis method	Cophenetic coefficient (r)
Simple matching	UPGMA	0.823
	complete linkage	0.732
Jacard	UPGMA	0.798
	complete linkage	0.738
Dice	UPGMA	0.751
	complete linkage	0.684

Number of observed alleles, effective number of alleles, Shannon's index and Nei's genetic diversity in west of Guilan population is more than the other two populations (fig. 1). Also the amounts related to statistics in the center of Guilan is more than east which is a verification for the previous discussions about the high degree of genetic diversity of related isolates in west of Guilan population than the isolates related to the two other populations.

Genotypes grouping was done by NTSYS ver-2.1 and by using similarity coefficient of Dice, Jaccard and simple matching by UPGMA and the complete linkage. Then, cophenetic coefficient was calculated for any of the dendrogram (tab. 4). Finally the cluster analysis was done by the simple matching coefficient and method of UPGMA with maximum of cophenetic coefficient (0.823).

Accordingly, the isolates were grouped in 3 groups. The first group contained 13 isolates that among these isolates, number 16 belonged to the center of Guilan population and 12 other isolates belonged to west of Guilan population which among 15 isolates of west of Guilan population, these 12 isolates were placed in one specific group. This group was divided into 2 subgroups so there were 3 isolates in one subgroup and the other isolates placed in the second subgroup. The second group involved 9 isolates. Among them, numbers 18 and 26 were belonged to the west of Guilan and number 23 was belonged to east of Guilan, but the 6 remaining isolates were belonged to center of Guilan. From 7 isolates related to center of Guilan, 6 isolates placed in this group. By dividing this group into 4 subgroups, numbers 18, 26 and 23 placed in one specific subgroup and were separated from the other isolates of this group. Finally the third group involved 8 isolates that number 12 was belonged to west of Guilan, but the other 7 isolates belonged to east of Guilan population (fig. 2).

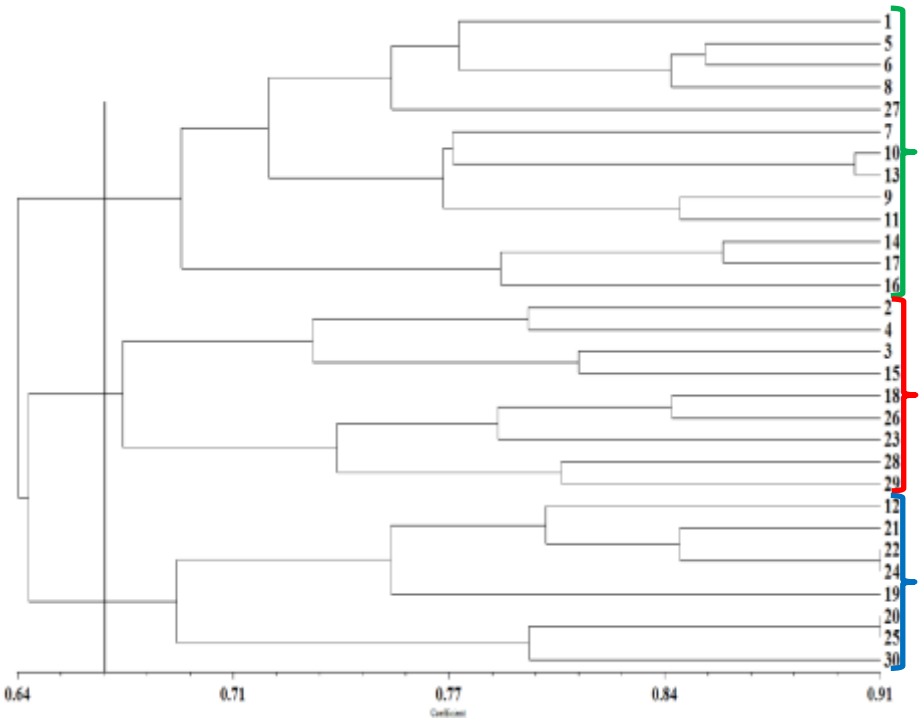

Fig. 2. Dendrogram derived from banding patterns of 30 isolates of *Pyricularia grisea* by the similarity coefficient of simple matching and UPGMA

Table 5. The Genetic distance of populations according to Nei's index

Populations	Population of west of Guilan	Population of center of Guilan	Population of east of Guilan
Population of west of Guilan isolates	0.000	–	–
Population of center of Guilan isolates	0.492	0.000	–
Population of east of Guilan isolates	0.849	0.920	0.000

Table 6. The Genetic similarity of populations according to Nei's index

Populations	Population of west of Guilan	Population of center of Guilan	Population of east of Guilan
Population of west of Guilan isolates	1.000	–	–
Population of center of Guilan isolates	0.611	1.000	–
Population of east of Guilan isolates	0.428	0.399	1.000

Table 7. Molecular analysis of populations of P. grisea

SOV	DF	SS	MS	The estimated variance	The estimated variance percentage (%)
Among populations	2	103.66	**51.83	3.45	15
Within populations	27	527.28	19.53	19.53	85
Total	29	630.93		22.98	–

SOV – sources of variations, DF – degree of freedom, SS – sum of squares, MS – mean of squares,** – significant at p = 1%

The results revealed although genetic distance was high between isolates of west and east of Guilan and the genetic similarity among these isolates of these two populations was low, but there was the maximum of genetic distance or the minimum of genetic similarity between the population of center of Guilan isolates and population of east of Guilan isolates (tabs 5, 6). The minimum of genetic distance or the maximum of genetic similarity there was between the population of center of Guilan isolates and population of west of Guilan isolates (tabs 5, 6).

According to the results, the diversity between the populations was significant and was more than genetic diversity within populations. The estimated variance among individuals within the population justifies 85% of the total diversity whereas the variance between the populations involves 15% of the total diversity (tab. 7).

DISCUSSION

In this research, primers SSR43,44 are the best primers among the used microsatellite primers, because of possessing most of observed alleles and the efficient, maximum of PIC, maximum of Shannon's index and maximum of Nei's index, so they can be recommended for the next studies to evaluating of genetic diversity of *P. grisea*. Then primers SSR83,84, SSR99-100 and G5 are recommended for the next studies. When the circumstantial evidence related to the primers in three populations of east, west and center of Guilan are studied; it is observed that all of these statistics – number of observed alleles, number of efficient alleles, Shannon's index and Nei's index generally in population of west of Guilan is more than the other two (tab. 3). That is because of the abundance of collected samples in west of Guilan than the other two regions and also because of greater diversity of sample-collecting places in this region than the other two.

Generating the different alleles by the microsatellite primers were reported in the previous studies. Liu et al. [2008] by studying of genetic diversity of *P. grisea* by using microsatellite primers reported a range of 2 to 10 alleles. The range of size of generated bands in the different microsatellite primers used in this research were partially the same with size of generated bands in the previous researches. But in some of these primers, sizes of bands were a little bit different with the previous reports. For example, sizes of bands in primers SSR41,42, SSR43,44, SSR47,48, SSR77,78, SSR83,84 , SSR99,100 and SSR101,102 were reported in the previous studies [Ying et al. 2004]. Comparing the size of bands with the observed sizes in this study, it can be said the range of size of observed bands for any primer are almost the same with the range of reported sizes in the previous studies.

Based on PIC amounts in this study, 14 used primers had suitable distribution between isolates; in addition to producing the almost suitable polymorphism bands. A research was done on the genetic diversity of this fungus in the central regions of Brazil by microsatellite primers [Brondani et al. 2000]. Amount of PIC was reported 0.44 to 0.54 for different microsatellite primers. They reported that the use of agarose gel for separating bands coming from microsatellite primers is reason of lower PIC and if polyacrylamide gel to separate the bands is used, in addition to increase in number of alleles, the amount of PIC will be increased [Brondani et al. 2000].

In this research, there was coordination between PIC and Nei's genetic diversity, but if there are lost data in the experiment, this consistency might be lost partially [Farman 2007]. Considering the data on the table 2, this consistency was quite obvious. Most of the observed alleles, the number of efficient alleles, amount of Shannon's index, amount of PIC and amount of Nei's genetic diversity were observed with primers SSR43,44. This suggests that this primer in addition to generate most of the number of alleles in all the isolates; these alleles also had a constant distribution among the studying isolates. This primer can be introduced as one of the suitable microsatellite primers to studying genetic diversity of P. grisea.

The maximum of Nei's genetic diversity, the number of observed alleles, number of efficient alleles and Shannon's index were observed in west of Guilan population. The more collected samples from the west of Guilan and the more diversity among the iso-

lates because of the extension of collecting regions of these isolates were responsible for transcending these indexes in the population of west of Guilan isolates. In a study, was studied the genetic diversity of 46 isolates of *P. grisea* by using 13 microsatellite primers and the Shannon's index for different microsatellite primers was different from the Shannon's index in this research, because isolates and primers were different.

If the similarity or genetic distance was based on geographical distance, there would the most genetic distance between two populations of east and west of Guilan, but, it was not so. In the studies of the other researches such as Séré et al. [2007] which studied genetic diversity of isolates of *P. grisea* in different regions of Burkina Faso and Chadha and Goplakrishna [2005] which evaluated the genetic diversity of isolates of this fungus in India, it has also been pointed out. Therefore, the pattern of SSR bands did not show correlation between polymorphism and geographical diversity.

CONCLUSIONS

This ascertainment demonstrate that SSR markers would be useful tool for study of genetic diversity of *P. grisea* populations and could provide advantageous results for further studies including introduction of tolerant and resistant cultivars of rice.

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GENETYCZNA I MOLEKULARNA CHARAKTERYSTYKA POPULACJI *Pyricularia grisea* Z RYŻU

Streszczenie. *Pyricularia grisea*, przyczyna choroby ryżu, jest uważany za jeden z najważniejszych grzybów na polach ryżowych. Izolaty *Pyricularia grisea* zostały zanalizowane przez SSR w celu określenia zróżnicowania genetycznego w populacjach. Zastosowano czternaście starterów i stworzono pasma DNA 95-640 bp. Analiza skupień przy użyciu metody UPGMA dała trzy grupy. Grupa 1 była główną grupą i większość izolatów zebranych na zachodzie prowicji Guilan należało do tej grupy. Grupy 2 i 3 to izolaty zebrane, odpowiednio, w środkowej i wschodniej części Guilan. Wyniki ukazały, że chociaż odległość genetyczna pomiędzy izolatami z zachodniej i wschodniej części Guilan była wysoka, a podobieństwo genetyczne między izolatami z tych dwóch populacji było niskie, to istniała maksymalna odległość genetyczna lub minimalne podobieństwo genetyczne między izolatami populacji ze środkowej części i wschodniej części Guilan. Najmniejsza odległość genetyczna lub największe podobieństwo genetyczne istniało między populacją ze środkowej części Guilan a populacją z zachodniej części. Podsumowując, wyniki badań potwierdziły, że mikrosatelitarne startery są dobrymi i odpowiednimi markerami do analizy struktury populacji *Pyricularia grisea*.

Słowa kluczowe: zróżnicowanie genetyczne, PCR, *Pyricularia grisea*, ryż

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