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GENETIC DIVERSITY OF *Pyricularia grisea*, THE CAUSAL AGENT OF RICE BLAST BY SRR

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Abstract. *Pyricularia grisea*, the rice blast fungus is the main pathological threats to rice crop in Iran and worldwide. In this research was evaluated the genetic diversity of *P. grisea* collected from different fields of Guilan province by using of 14 microsatellite primers. These primers produced 64 polymorphic bands by an average of 4.57 bands for each marker. An average of polymorphic information content in whole primers was 0.734, an average of effective number of alleles was 2.68, an average of Nei's expected heterozygosity was 0.734 and an average of Shannon's information index was 1.05. Primer SSR43,44 had the most polymorphic information content (PIC = 0.85), observed number of alleles (na = 8), effective number of alleles (ne = 3.76), Nei's expected heterozygosity (Ne = 0.861) and Shannon's information index (I = 1.38). This marker was the best primer between 14 used primers for evaluation the genetic diversity of *P. grisea*. Cluster analysis was done with simple matching similarity matrix and UPGMA method. The results showed that the studied isolates were classified into 3 lineages by cutting off the dendrogram at 0.76 similar linkage level. Number 1 was the major group and represented most of those isolates. Results of principal coordinate analysis also divided the isolates into three groups exactly similar to obtained with cluster analysis. Overall, our results confirmed that microsatellite primers were good and suitable markers for analyzing structure of *P. grisea*.

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

INTRODUCTION

Rice blast disease caused by *Pyricularia grisea* pathogen is one of the most important rice diseases which attack the rice throughout its life cycle from the nursery to the

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farm [Ou 1985]. Blast disease is known as the most important rice disease in prone rice countries including Iran. So, a myriad of researches were done to control it [Khodaparast and Sahragard 2004]. DNA markers showed the changes of DNA. These markers were used because of being unlimited in numbers, not being affected by environmental factors and above all their detection and identification throughout their plant growth processes [Winter and Khal 1995]. These markers had great potential for increasing the efficiency of usually modified methods by choosing according to the molecular markers in concordance with favorite features and not directly related to choosing of the feature but the locus of the used marker should be in high concordance with the related feature. By using the molecular markers techniques, it was possible to map and transfer the genes or the appropriate genes from the wild species to the farm-related ones [Mohan et al. 1997]. DNA markers involved a large group of the markers. It was divided by the two marker categories not based on the polymerase chain reaction (PCR) and markers based on the PCR. Simple sequence repeat (SSR), was among the markers based on the PCR [Rabiei and Sabouri 2008]. The genetic diversity range and instability in *Pyricularia grisea* has been the long-term discussion among the blast researches. The application of the molecular markers in the genetic study of the population found classification of the epidemiologic information which was not possible before. Molecular markers disclosed the genetic contents directly and because of this they could validly refer genetic and phenotypic variations [Babujee and Gnanamanickam 2000]. 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. The primary results revealed every locus had a range of different alleles of 2 to 10 and that result showed SSR genes have polymorphism [Liu et al. 2008]. In 2008, 90 isolates of *P. grisea* were analyzed for the configuring DNA using SRR markers. The conclusion was that there was not much difference among the isolates though the difference among some of the isolates within the group was significant [Jihan-Hui et al. 2008]. Ying et al. [2004] analyzed 105 isolates with SSR markers for determining the genetic diversity and these isolates were divided into 6 groups by cluster analysis. Levy et al. [1993] collected 151 isolates of *P. grisea* from 15 fields in Santa Rosa in Columbia and fingerprinted with DNA--repetitive sequence MGR586 and determined 155 haplotypes into 6 genetic groups individually. Silva et al. [2009] examined the genetic diversity of *P. grisea* on rice in Brazil. The genetic analysis of isolates revealed the highest genetic diversity population belonged to 103 haplotypes in Bonanca and 49 in Primavera. Sirithunya et al. [2008] for analyzing the genetic diversity of *P. grisea* collected 174 isolates from different hosts as barley, rice, weed, wild rice in Thailand and examined them by using RAPD primers. They divided the isolates into 9 groups and high variations were observed among the separated isolates. Consolo et al. [2008] collected 161 isolates of *P. grisea* from 15 rice fields to DNA-fingerprint population in Argentina. They determined five genetic groups of A, B, C, D and E which respectively involved 11, 22, 4, 1, 4 haplotypes. They concluded that in Argentina *P. grisea* populations were genetically simple. Qing-Hua et al. [2004] analyzed 27 isolates which were selected from Guangdong State in China to determine pathotype and genetic diversity by using 10 pair Sequence-related amplified polymorphism (SRAP) primers.

George et al. [1997] examined *P. grisea* population by the Rep-PCR method. The Cluster analysis was consistent with the previous components resistance polymorphisms of MGR586 which were taken from the rice pollutant pathogenic strains. Piotti et al. [2005] to examine the genetic structures of isolates of *P. grisea* in Italy, fingerprinted the DNA of pathogen with using PCR. The cluster analysis of the both showed the existence of three Italian main genetic groups which had 80% similarity. Motallebi et al. [2009] studied *P. grisea* populations isolated from rice and weeds and analyzed the genetic diversity of complex species of this fungus by Rep-PCR. Almost all the *P. grisea* isolates had 42% similarity that genetic diversity in these isolates was low. Hemmati et al. [2005] analyzed the genetic diversity of blast fungus isolates in Mazandaran province by RAPD-PCR. The results revealed those isolates were divided into three groups. Also the population structure of *Magnaporthe grisea* was analyzed by the Rep-PCR in two provinces of Guilan and Mazandaran and based on the cluster analysis, six groups of fingerprinting were identified [Javan Nikkhah 2001].

In this study, the population structure of *Pyricularia grisea* in Guilan province wsa examined by using SSR based on the PCR and also 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 to isolation of the fungus. 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. PDA and WA 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. To avoid of bacterial contamination, sulfate streptomycin antibiotic was used. 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 fungal isolates. 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 were 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 morphological characters [Javan Nikkhah 2001].

Molecular analysis. DNA extraction. The dried mycelium of the isolates were used for DNA extraction. DNA from the mycelium samples were extracted by Murray and Thompson [1980] method known as CTAB method with 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 inclu-

ding 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 gently shaken. 72 µl CTAB solution was added to the tubes and the tubes were gently shaken. 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 with 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 completely solved. 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 was carefully transferred to the 0.5 ml tubes for keeping at -20°C.

The DNA samples were 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 were observed, it was found that some of the DNA samples were not of good quality and so the DNA extracting were done again. The extracted DNA samples had different destinies. Finally for the replication of genomic DNA was done well in the thermocycler system; firstly all the samples were diluted with the same destiny. For that, the bands related to the extracted DNA from the lines at first were compared with the bands coming from the lambda phage DNA with 50, 100 and 150 $ng \cdot \mu l^{-1}$ and their optical density was calculated. Then all of them were distillation-sterilized with distilled water so they were diluted to the 20 $ng \cdot \mu l^{-1}$ and directly were used in PCR reaction.

PCR amplification of alleles at SSR loci. All the SSR primers used in this analysis were bought from Sina-gen Company. 14 pair primers using the microsatellite maps were presented by the Liu et al. [2008], Ying et al. [2004] and 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 $MgCl_2$, 1 × 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 thermocycler 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% poly acrylic amid gel and detected by staining with ethidium bromide.

Data Analysis. The three softwares of NTSYS-pc ver. 2.02 [Rohlf 2000], Popgene ver. 1.32 [Yeh and Boyle 1997] and Genealex ver. 6.2 [Peakall and Smouse 2006] were used.

The percentage of polymorphism sites in any primer was calculated through dividing the number of polymorphism sites by the total number of amplification sites [Nei et al. 1995]. The number of effective alleles (Ne) and the observed number of alleles (Na) were calculated by using Popgene software [Kimura and Crow 1964]. Clustering and UPGMA methods were used for genetic diversity analysis. For confirming the cluster analysis, the Cophenetic correlation coefficient was calculated and to do this NTSYS-pc software was used. Also, the coordinate analysis (PCOA) was calculated [Shlens 2005].

RESULTS AND DISCUSSION

In this research was generated 64 polymorphic fragments. Sizes of bands were floating between 95–640 bp. The largest base pair was generated by SSR-G5 microsatellite primer and the smallest by the SSR47,48 microsatellite primer. The most generated bands were amplified at the time of application of primer SSR43,44 and the least generated bands were amplified with application of primer SSR-KMS20. The average of generation was 4.75 bands for each of the primers (tab. 1). All the generated bands were polymorphic.

Table 1. Number of bands and amplified band size by every primer

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

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.

PIC was the first important index in determination of primers's efficiency [Agrama and Tuinstra 2003]. The amount of PIC was the reflection of allele diversity among the varieties. The high polymorphism in microsatellites showed the high diversity and it suggested the high amount of mutation which occured in microsatellites sequences [Zhou et al. 2003].

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) suggested 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, primer SSR43,44 with PIC = 0.852 had the most PIC and it could show the genetic distances between isolates better than the other primers. The KMS20 primer with PIC = 0.661 showed the less PIC. The average of PIC was 0.734 for all the microsatellite primers which suggested the almost perfect choice for the primers so as to study the genetic diversity of isolates in this research.

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

According to the results, the most Nei's genetic diversity in all isolates was observed in primer SSR43,44 and the least amount was observed in primer KMS20 (tab. 2). The average of genetic diversity in all the primers for all isolates was 0.734 which suggested the perfect allele's diversity used in this research.

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

Genotypes grouping were performed by NTSYS ver-2.1 and by using similarity coefficient of Dice, Jacard and simple matching by UPGMA and the complete linkage. To assess the performance of every method, cophenetic coefficient was calculated for any of the drawn dendrogram (tab. 3). Finally the cluster analysis was chosen by the simple matching coefficient and by the method of UPGMA which had the maximum of cophenetic coefficient (0.823) and the cluster analysis was performed accordingly.

Table 3. Cophenetic coefficient for the different methods of cluster analysis

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

According to merger and similarity coefficients, the cut line was on 0.67 point (fig. 1). Accordingly, the isolates were grouped into three groups. The first group contained 13 isolates. This group was divided into two subgroups so there were three isolates in one subgroup and the other isolates in the second subgroup. The second group involved 9 isolates. By dividing this group into 4 subgroups, the three isolates of 18, 26 and 23 were in one specific subgroup and were separated from the other isolates of this group. Finally the third group involved 8 isolates (fig. 1).

PCOA was performed by NTSYS ver-2.1. In PCOA, the specified components (usually two or three first component) justified a high percentage of changes among molecular data. Amounts related to PCOA involved Eigen value, percentage of variance and cumulative of variance which justified for the 20 first components more than 98% of changes and it was depicted in table 4. The first component justified just 67.87 percent of changes among the genotypes according to molecular data and then the second and the third components were respectively 4.93 and 3.83 variances. Because the specified components were separated from each other and the main initial components justified an

almost small amount of phenotypic variance, it suggested that the primers used in this research were not related with each other and had a uniform distribution on the fungus isolates genome. The lowness of initial components variance in PCOA in the molecular data showed a smaller relation for amplified sites with each other or in other words remoteness of these sites on genome or their perfect distribution on the genome.

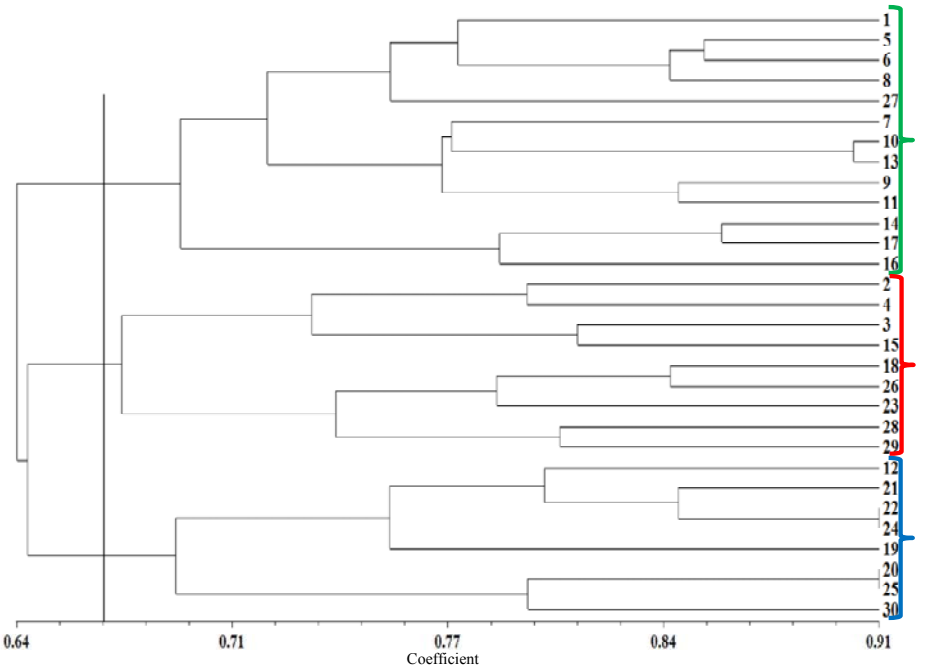

Fig. 1. Dendrogram derived from banding patterns of 30 isolates of *Pyricularia grisea* by the similarity coefficient of simple matching and UPGMA

The two-dimensional transmittance of the reviewed fungus isolates which was made by the principal coordinate according to the first two components by NTSYS and it was depicted in fig. 2. The isolates according to these two components could be divided into three groups. The isolates in these three groups were exactly coincident with the three groups resulting from the cluster analysis, except the isolate number 23 which was in the second group but it was not in this group at this diagram. This result also justified the grouping from the cluster analysis. The results showed that cluster analysis (by using similarity coefficient of simple matching and UPGMA method) and PCOA according to molecular data acted similarly. In other words the results were reliable.

The two dimensional transmittance of reviewed fungus isolates which was made by PCOA according to the first two components by Genealex and it was depicted in fig. 3. The isolates related to any population could be separated in different groups and like the cluster analysis diagram; the isolates can be put into three different groups so the isolates of any population placed in an almost separated group.

Table 4. The Eigen value, percentage of variance and cumulative of variance for the components resulting from PCOA

Component	Eigen value	Percentage of variance	Cumulative of variance
1	20.36	5.68	67.68
2	1.48	4.93	72.80
3	1.15	3.83	76.63
4	1.07	3.57	80.20
5	0.86	2.88	83.08
6	0.58	1.92	84.0
7	0.50	1.66	86.66
8	0.48	1.61	88.24
9	0.41	1.38	89.65
10	0.40	1.32	90.97
11	0.37	1.24	92.21
12	0.35	1.16	93.37
13	0.31	1.04	94.41
14	0.27	0.89	95.30
15	0.21	0.71	96.01
16	0.20	0.67	96.68
17	0.16	0.55	97.23
18	0.15	0.51	97.73
19	0.13	0.45	98.19
20	0.11	0.38	98.57

As it was depicted in tables 1, 2 and 3, primer SSR43,44 was the best primer among the used microsatellite primers in this research 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 it could be recommended for the next studies to evaluate genetic diversity of *P. grisea*. Then SSR83,84, SSR99,100 and G5 primers were recommended for the next studies.

Cluster analysis of isolates divided the isolates into three totally different groups by using similarity coefficient of simple matching and UPGMA method in which in the first group. The isolates grouping also separated the isolates partially similar to the cluster analysis in three different groups according to separating region by PCOA.

The used microsatellite primers in this research totally made 64 bands with the average of 4.57 bands for every primer (tab. 1). Also all the made bands had polymorphism. Making the different alleles by the microsatellite primers were reported in the previous studies. Liu et al. [2008] studied genetic diversity of *P. grisea* by using microsatellite primers reported a range of 2 to 10 alleles. The range of size of made bands in the different microsatellite primers used in this research were partially the same with size of made bands in the previous researches [Ying et al. 2004]. But in some of these primers, sizes of bands were a little bit different with the previous reports. For example sizes of bands in the SSR41,42, SSR43,44, SSR47,48, SSR77,78, SSR83,84, SSR99,100 and SSR101,102 primers were reported in the previous studies [Ying et al.

2004]. Comparing the size of bands with the observed sizes in this study, it could be said the range of size of observed bands for any primer were almost the same to the range of reported sizes in the previous studies.

PIC in all the used primers in this study was between 0.661–0.862 with the average of 0.734 for all the used microsatellite primers (tab. 2). The larger this number (close to one) was represented the more the number of alleles, the abundance of polymorphism for the genetic site in that population and more uniform distribution of polymorphism bands in between of isolates and it suggested the almost perfect choice of primers to studying isolates genetic diversity in this research. So, 14 used microsatellite primers in this study had suitable distribution between isolates; in addition to making 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 reported by Brondani et al. [2000] was 0.44 to 0.54 for different microsatellite primers. They found that the use of agarose gel for separating bands coming from microsatellite primers was the reason of lower PIC and if polyacryl amide gel to separate the bands was used, in addition to increase number of alleles, the amount of PIC would be increased [Brondani et al. 2000].

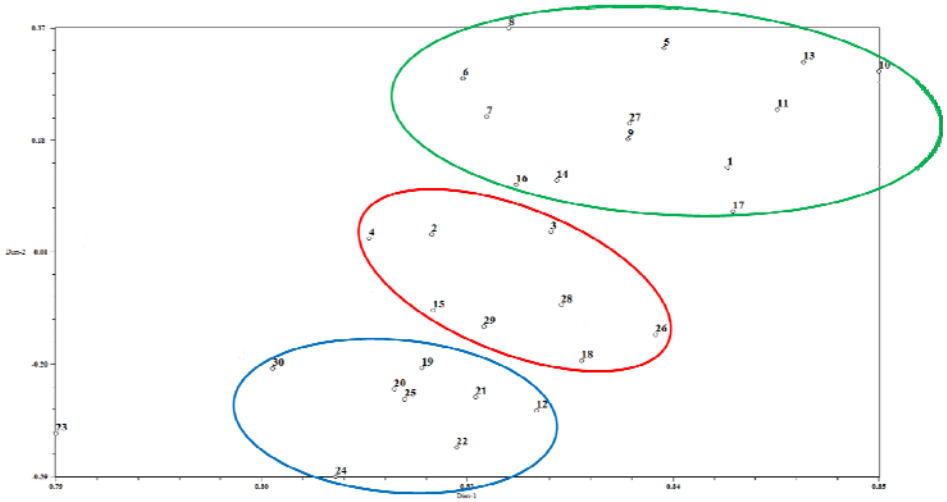

Fig. 2. Two-dimensional transmittance of studied fungus isolates according to the first two components of PCOA by NTSYS

Nei's genetic diversity was ranged from the minimum of 0.661 for primer KMS20 and maximum of 0.861 for primer SSR43,44 (tab. 2). Generally, there was coordination between PIC and Nei's genetic diversity, but if there were lost data in the experiment, this consistency might be lost partially. Considering the data in 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 seen in primer SSR43,44. This suggested that this primer in addition to making most of

the number of alleles in all the isolates; these alleles also had a constant distribution among the studying isolates. This primer could be introduced as one of the suitable microsatellite primers for studying genetic diversity of *P. grisea.*

The cluster analysis of isolates in this research with the molecular data resulting from primers could separate isolates of *P. grisea*. On the other hand, the similarity ranges were from 0.64 to 0.91 which suggested the high similarity of the studying isolates with each other.

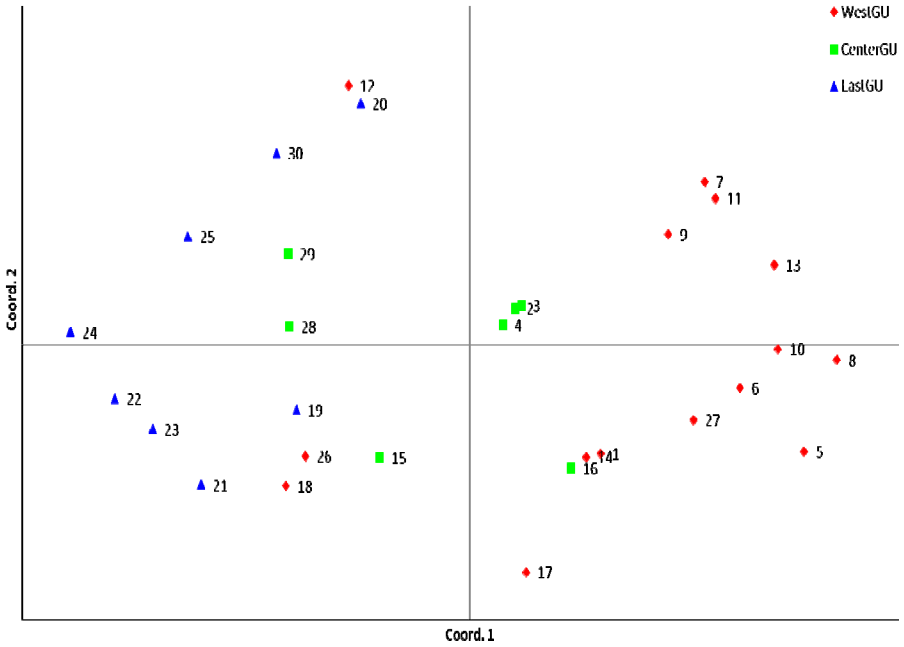

Fig. 3. Two-dimensional transmittance of studied fungus isolates according to the first two components of PCOA by Genealex

Consolo et al. [2008] studied the genetic diversity of 161 isolates of *P. grisea* which were collected from the farms of Argentina and declared these isolates had a high similarity (more than 70%) and were divided into five groups by the cluster analysis. Ying et al. [2004] evaluated the genetic diversity of 105 isolates of *P. grisea* collected from the farms of China by using 7 microsatellite primers and declared that the similarity among the isolates was high and by cutting the cluster analysis diagram from the similarity point of 0.67, they divided isolates into 6 groups. Chadha and Gopalakrishna [2005] investigated genetic diversity of this fungus which were collected from different parts of India and found that the genetic similarity among the isolates was high with ranges from 0.67 to 0.92. They also 20 studied isolates of *P. grisea* divided into two groups and every group into two subgroups [Chadha and Gopalakrishna 2005].

Séré et al. [2007] studied the genetic diversity in 55 isolates of *P. grisea* collected from the different regions of Burkina Faso by using 10 RAPD-PCR primers and declared that the similarity of those isolates was high and the similarity coefficient among the strains was variable from 0.63 to 1. They also divided the isolates into five separated groups by cutting the cluster analysis diagram from the similarity point of 0.65.

Distribution of isolates by using two initial components resulting from PIC divided the isolates into three almost the same groups by the cluster analysis or in the other words the results of these two analyses were the same. In the PIC, the initial main components justified a small amount of phenotypic variance. This suggested that the used primers in this research had a constant distribution on the genome of *P. grisea* isolates. The lower of the initial components variance in the PIC in the molecular data showed the less relation of amplified sites with each other or in other words it was their linkage on the genome and its suitable distribution on the genome.

CONCLUSIONS

This research indicated that SSR markers would be useful tool for analysis of genetic diversity of *P. grisea* and could provide good results for further studies including introduction of resistant cultivars.

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GENETYCZNA RÓŻNORODNOŚĆ *Pyricularia grisea*, CZYNNIKA WYWOŁUJĄCEGO ZARAZĘ RYŻOWĄ PRZEZ SRR

Streszczenie. *Pyricularia grisea*, grzyb zarazy ryżowej, jest zagrożeniem dla plonów ryżu w Iranie i na całym świecie. W niniejszym badaniu przy użyciu 14 markerów mikrosateklitarnych oceniano różnorodność genetyczną *P. grisea* zebranego z różnych pól prowincji Guilan. Markery te tworzyły 64 polimorficznych wiązań przy średnio 4,57 wiązaniach dla każdego markera. Średnia informacji polimorficznych we wszystkich markerach wynosiła 0,734, średnia efektywna liczba alleli – 2,68, średnia heterozygotyczność oczekiwana Nei – 0,734 a średni indeks informacji Shannon – 1,05. Marker SSR43,44 miał największą zawartość informacji polimorficznej (PIC = 0.85), największą liczbę alleli obserwowanych (na = 8), liczbę efektywnych alleli (ne = 3.76), największą heterozygotyczność oczekiwaną Nei (Ne = 0,861) i indeks informacji Shannon (I = 1.38). Był to najlepszy spośród 14 markerów użytych do oceny różnorodności genetycznej *P. grisea.* Analizę skupień przeprowadzono za pomocą prostego współczynnika simple maching oraz metody UPGMA. Na podstawie wyników badań izolaty zaklasyfikowano do 3 linii przez odcięcie dendogramu na podobnym poziomie powiązań 0,76. Numer 1 był główną grupą i reprezentował większość tych izolatów. Wyniki analizy głównych współrzędnych także dzieliły izolaty na trzy grupy podobne do tych, które otrzymano za pomocą analizy skupień. Podsumowując, wyniki badań potwierdziły, że markert mikrosatelitarne to dobre i odpowiednie markery do analizy struktury *P. grisea*.

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

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