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# The Correlation of Genetic Diversity and Geographic Distribution of *Fusarium graminearum* in North Part of Iran

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**Abstract:** Fusarium Head Blight (FHB), caused mainly by *Fusarium graminearum*, is one of the most notorious diseases in North part of Iran. Due to lack of sufficient information regarding genetic diversity of FHB, the correlation between genetic diversity and geographic distribution of 52 isolates that collected from infected wheat kernels from four main grown region of North part of Iran were analyzed with 10 simple Sequence Repeat (SSR) primers and 15 Random Amplified Polymorphic DNA (RAPD) primers. The un-weighed pair group method using arithmetic means (UPGMA) was used and a dendrogram was constructed based on Jaccard's similarity coefficient. Principal Component Analysis (PCA) of SSR and RAPD markers revealed that the first two factors accounted for 51 and 41% of the total variance, respectively. SSR and RAPD data classified isolates into four and seven groups, respectively. SSR analysis showed that there was a correlation between genetic clusters of isolates and geographical origin in *F. graminearum*. However, results of cluster analysis using RAPD data didn't show any relation with geographical distribution but the results of this analysis confirmed a genetic variation among all isolates.

**Key words:** Fusarium head blight, SSR, RAPD, genetic diversity, geographic distribution

## INTRODUCTION

Fusarium Head Blight (FHB), mainly caused by *Fusarium graminearum*, is one of the most notorious and worldwide diseases of wheat and other small cereal grains (Bai *et al*., 2003; Yang *et al*., 1998; Parry *et al*., 1995; Windels, 2000). FHB causes substantial losses in grain yield, quality and performance of storage seed (Olivier *et al*., 2008). Furthermore, the most serious threat of FHB is the problem of grain contamination by fungal mycotoxins such as deoxynivalenol (DON), zearalenone (ZEN) and nivalenon (NIV) (Placinta *et al*., 1999; Cleveland *et al*., 2003; Xu and Nicholsen, 2009). As FHB epidemics have become more frequent and severe in Iran, contamination with mycotoxins may become a major problem for animal and human health and may impact both domestic and export food and feed markets.

Several studies have been done on Diversity of *F. graminearum* in other countries (Zeller *et al*., 2004; Ramirez *et al*., 2006; Akinsanmi *et al*., 2006) and also on different aspects of biology and epidemiology of FHB, grain contamination that caused by mycotoxins and also diagnostic of resistant gene to FHB (Fakhfakh *et al*., 2011; Wu *et al*., 2005; Shah *et al*., 2005; Shaner, 2003), however; studies on genetic variation of spices causing FHB disease and genetic variation in *F. graminearum* isolates have been started with delay in Iran, so there are not enough information available about genetic construction, geographical distribution and genetic diversity of FHB so far. Studies on genetic variation of FHB isolates have shown that FHB has high genetic variation and also has direct effect on management and breeding system of cultivars. Genetic variation of FHB in both sexual and asexual stage lead to produce recombinant population of pathogen with ability to produce mycotoxin that caused overcome to resistant of cultivar and indication of severe epidemic.

Realization and recognition of host genetic resistance to disease agents and detection of resistance genes is the most important factor for the establishment of successful breeding programs and producing resistant cultivars. Due to quantitative heredity of FHB and environment condition effects, the breeding for resistance to FHB disease is time consuming and difficult, therefore molecular markers are used as a useful tool in order to gene mapping of control resistant gene which can simplify

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screening for resistance and also can transfer resistance genes from resistant cultivar to sensitive cultivar in short time.

The aim of this study was to analyze the genetic diversity among 52 isolates of the *F. graminearum* complex collected from important regions with a history of FHB epidemics in Iran. Such an investigation would resolve genetic variation and the genetic distinction of the *F. graminearum* complex in relation to geographic distribution in Iran.

## MATERIALS AND METHODS

**Pathogen isolation and purification:** Fifty two Isolates of *Fusarium graminearum* were collected from four growing states of Iran including Gorgan, Sari, Moghan and Kelardasht (Fig. 1) are listed in Table 1 together with data on their geographic origin. Isolation and purification of fungal isolates were done according to Schmale *et al*. (2006) protocol.

FHB samples were collected around 2 to 4 weeks after flowering. Individual wheat heads were Surface-disinfested in 10% sodium hypochlorite for 1 min and rinse in sterile DI water. Heads cut into three equal portions (bottom, middle and top) and placed on Fusarium-selective medium. Plates were incubated at room temperature for 3-5 days. Fungal colonies transferred to ¼-strength potato dextrose agar (PDA). The cultures grew for 7-10 days at room temperature. Macroconidia streaked onto plates of 2% water agar and single-spore transfer onto ¼-strength PDA. The single-spored culture grew out for 7-10 days. A small square agar block that contains an individual macroconidium was transferred to ¼-strength PDA.

**DNA extraction from fungal culture:** For extraction of genomic DNA, fungi were grown in liquid SNA media (Nirenberg, 1981), Mycelial mass were harvested by

Table 1: Fifty two isolates of *Fusarium graminearum* were selected as agent of places of collection

| Isolate | Location | Isolate | Location | Isolate | Location | Isolate | Location |
|---|---|---|---|---|---|---|---|
| FG4 | Gorgan | FG5 | Sari | FG14 | Kelardasht | FG1 | Moghan |
| FG6 | Gorgan | FG8 | Sari | FG25 | Kelardasht | FG2 | Moghan |
| FG7 | Gorgan | FG15 | Sari | FG32 | Kelardasht | FG13 | Moghan |
| FG10 | Gorgan | FG18 | Sari | FG33 | Kelardasht | FG26 | Moghan |
| FG12 | Gorgan | FG19 | Sari | FG34 | Kelardasht | FG27 | Moghan |
| FG16 | Gorgan | FG20 | Sari | FG3 | Kelardasht | FG29 | Moghan |
| FG17 | Gorgan | FG22 | Sari | FG9 | Kelardasht | FG31 | Moghan |
| FG21 | Gorgan | FG23 | Sari | FG11 | Kelardasht | FG37 | Moghan |
| FG24 | Gorgan | FG36 | Sari | | | FG41 | Moghan |
| FG28 | Gorgan | FG38 | Sari | | | FG44 | Moghan |
| FG30 | Gorgan | FG39 | Sari | | | FG49 | Moghan |
| FG35 | Gorgan | FG40 | Sari | | | | |
| FG46 | Gorgan | FG42 | Sari | | | | |
| FG47 | Gorgan | FG43 | Sari | | | | |
| FG50 | Gorgan | FG45 | Sari | | | | |
| FG52 | Gorgan | FG48 | Sari | | | | |
| | | FG51 | Sari | | | | |

Fig. 1: *F. graminearum* isolates collected from four main growing regions in Iran. (No. in parentheses indicates the total isolates collected from particular location)

Table 2: Characterization of 7 SSR primers in *Fusarium graminearum* (Suga *et al*., 2004)

| Repeat motif | Primer sequences | Locus |
|---|---|---|
| (TCGAAGAGCCAGCTG)6 | F: ACAGGCATCCAAGGACATTT R: GTTTGATGGCGCATTCAAAG | HK1043 |
| (GAA)10(GAG)9 | F: GCAGGACCTGGATGATGAA R: ATGTGTGCAGCCATGAGATT | HK913 |
| (CA)16 | F: ATCTCCCAAGCTGGCTAATT R: AGAACCGGCAAAGTTCGATT | HK917 |
| (GGGAGTCAAT/C)16 | F: TCCGAAGGTAGAAGCGTTGT R: TCAAGCCCATCTATGCTGTT | HK957 |
| (CTTATC)8 | F: GAGATGGCAACATTATTTGCA R: ATTGGCAGCAGGGCTTGATT | HK965 |
| (CAGTGA)5 | F: AAGAGGGCGTGTCTCTGTTTT R: CGCTTCCTTCCTTTCAATTC | HK967 |
| (CACAGG/A)6 | F: AAACGTAAACGGATCAACGG R: AGATTCGCAACTTTGTGCTG | HK977 |

filtration onto paper filters, rinsed thoroughly with sterile water, frozen and ground to a fine powder by liquid nitrogen. Total genomic fungal DNA was isolated according to protocol of Liu *et al*. (2000).

**PCR amplification conditions:** Amplification reactions for RAPD were carried out in volumes of 25 μL containing 50 ng template DNA, 2.5 uL Taq Reaction buffer (10 X), 0.2 mM dNTPs, 0.4 mM primer, 2 mM $MgCl_2$, 0.25% formamide, 1.0 unit of *Taq polymerase*. Amplification was performed in a thermal cycler programmed for 35 cycles at a temperature regime of 94°C for 45 sec, 37°C for 1 min, 72°C for 1 min after an initial denaturation at 94°C for 2 min. This was followed by a final 4 min extension at 72°C. Reaction products were resolved by electrophoresis at 50 V for 5 h in 1% (w/v) agarose gel in 1×TBE buffer and stained with ethidium bromide.

The PCR profile for SSR starts with 95°C for 2 min followed by 25 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min extension at 72°C for 1 min. A final extension 72°C for 10 min was included (Suga *et al*., 2004). Reaction products were resolved by electrophoresis at 60 V for 4 h in 3% (w/v) Agarose gel in 1×TBE buffer and stained with ethidium bromide.

**Data analysis:** Out of the 25 RAPD and 15 SSR primers screened for their polymorphism, the best 15 RAPD and 7 SSR primers (Table 2) were chosen and allowed us to investigate relation between genetic diversity and geographical distribution.

Distinct and reproducible bands were scored as present 1 or absent 0 for each of the RAPD and SSR markers. Genetic similarity between pairs was estimated by Jaccard's coefficient.

$$Sij = \frac{Xij}{(Xi + Xj + Xij)}$$

Sij was the Jaccard's coefficients of similarity between samples i and j, xij was the number of shared bands between both samples and xi and xj were the numbers of bands unique to each sample, respectively.

For each type of molecular marker, RAPD and SSR a dendrogram was constructed using the scorable fragments. All statistical analysis was done using the SPSS 16 program (SSPS, Chicago, IL, USA). In addition, Principal Component Analysis (PCA) based clustering was also done using the subroutine EIGEN.

## RESULTS AND DISCUSSION

Similarity measurement using UPGMA followed by cluster analysis resulted in 4 main groups for SSR marker data based on geographical distribution that generally reflected expected trends between the genotypes.

**SSR markers analysis:** The results of cluster analysis showed that all isolates were grouped into four groups (Fig. 2). The first group includes 22 isolates in which 9 of them were collected from Gorgan and 13 isolates from Sari. The 3rd group is completely for Moghan isolate and the 4th for Kelardasht samples. Most of isolates in the 2nd group belong to Gorgan and Sari but 4 isolate from Moghan and 1 isolate from Kelardasht have been placed in this group. Isolate from Kelardasht showed the most distance from other groups. Isolate from Gorgan and Sari were similar together than the two other region. The final dendrogarm which achieved based on SSR markers approved a high genetic variation among all biotypes of *Fusarium graminearum.* Principal component analysis of SSR marker data was done to evaluate genetic variation among all isolate. Table 3 shows the Results of PCA, the first three components could explain 70% of total variation among isolates.

Figure 3 shows two first factor biplot which explained 51% of total variance. Four groups of isolate can be distinguished very clearly in this plot. Isolate from Kelardasht are far from the other isolates but samples which collected from Gorgan and Sari are close together. These results almost confirmed the cluster analysis grouping pattern.

**RAPD data analysis:** While SSR markers showed a good relationship with geographical pattern but results of cluster analysis using RAPD data didn't show any relation with geographical distribution but the results of these analysis confirmed a genetic variation among all isolates.

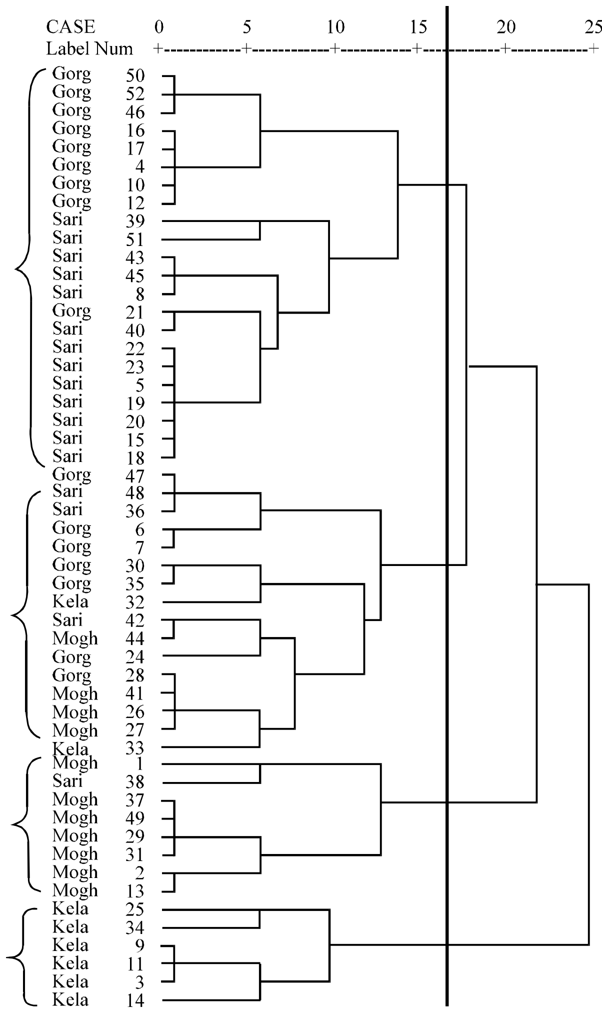


Fig. 2: Dendrogram of 52 isolate of *Fusarium graminerum* based on SSR data

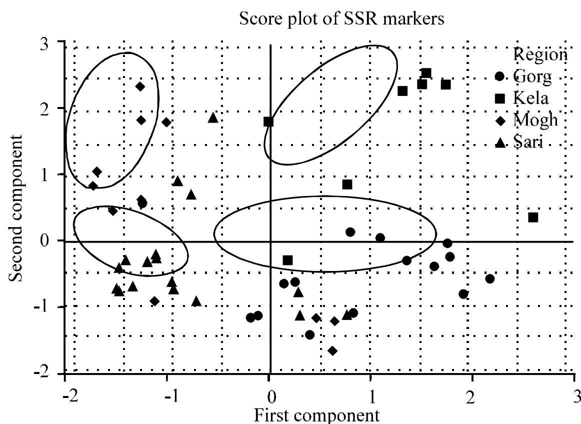


Fig. 3: Two dimensional view of score plot resulted from PCA from SSR data

Using the same cut off point for determining groups, in these experiment isolates can be categorized in to 7 groups. The first groups contain 39 Isolate which are similar to each other. There were also formed 6 groups containing 13 isolate (two groups included just 1 isolate). As it was shown in Fig. 4 in all groups all geographical regions are presented.

Table 3: Principal components (PCS) for 6 SSR markers among isolates

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| SSR1 | 0.205 | -0.268 | 0.646 |
| SSR2 | -0.145 | 0.669 | 0.247 |
| SSR3 | -0.052 | -0.04 | 0.684 |
| SSR4 | 0.628 | 0.224 | -0.186 |
| SSR5 | 0.722 | -0.107 | 0.053 |
| SSR6 | 0.135 | 0.646 | 0.128 |
| Eigenvalue | 1.5459 | 1.5105 | 1.1185 |
| Proportion $\sigma 2$ | 0.258 | 0.252 | 0.186 |
| Commulative $\sigma 2$ | 0.258 | 0.509 | 0.696 |

These results indicate that using RAPD markers in this experiment could not show the relation between genetic variation and geographical pattern.

Although principal component analysis for SSR data showed that the first three component explained 70% of

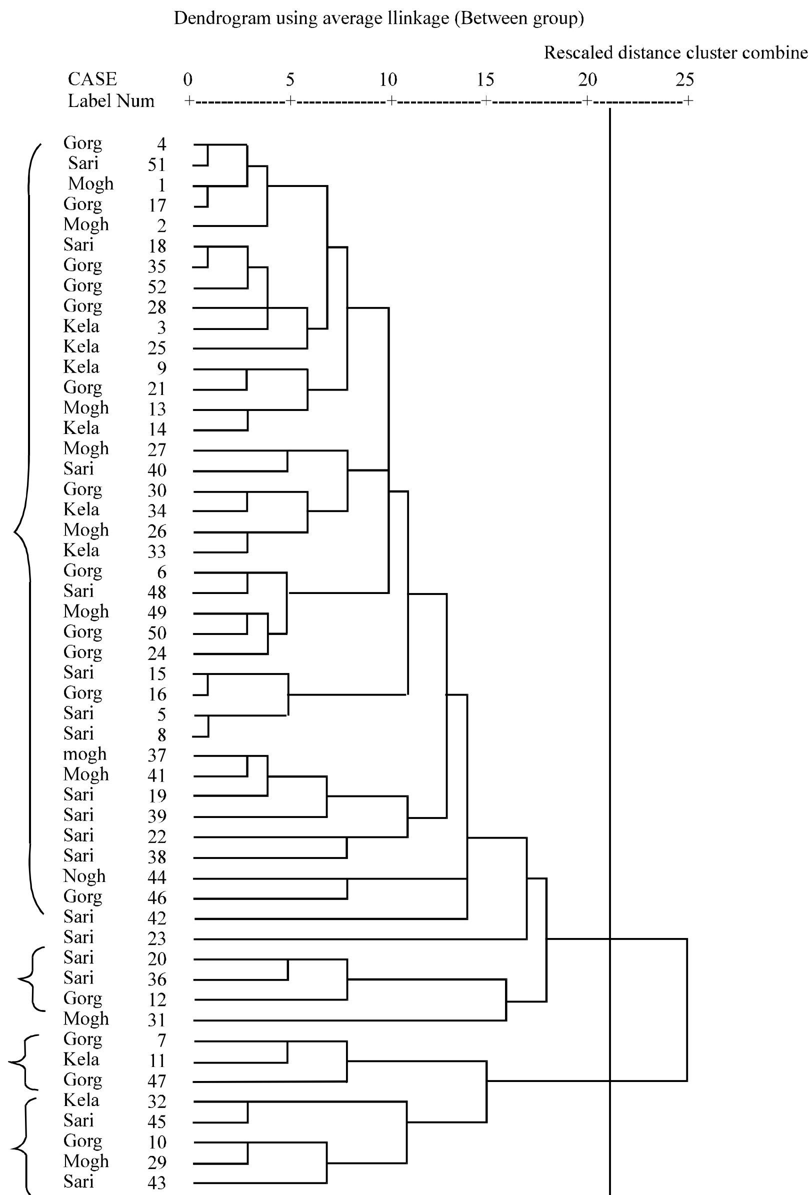


Fig. 4: Dendrogram of 52 isolate of *Fusarium graminerum* based on RAPD data

variability among isolates but for RAPD marker data the first three component only explained 53% of total variance(Table 4), that approved advantages of using SSR marker for diversity studies among *F. graminearum* isolates. Figure 5 shows the biplot of first 2 factors which explained 41% of total variance.

Genetic diversity in *F. graminearum* has been previously studied using DNA molecular primers, AFLP and SCAR (Qu *et al*., 2008), RAPD (Iram and Ahmad, 2007), RFLP (Gale *et al*., 2002) and SSR (Fregene *et al*., 2001) and different correlation between genetic diversity and geographical distribution has been observed. The results obtained from this study showed that using SSR marker not only can explain the variation among isolates of *F. graminearum* better than RAPD markers but also can be applied to a better understanding of relation between geographical distributions of *F. graminearum* isolates. Isolates that collected from each different region were located in nearly same groups which showed the similarity in genetic diversity in each region. Studies have been shown that *F. graminearum* has high genetic variation which is so important in management-control of diseases and cultivars breeding (Goswami and Kistler, 2004). RAPD analysis of isolates of

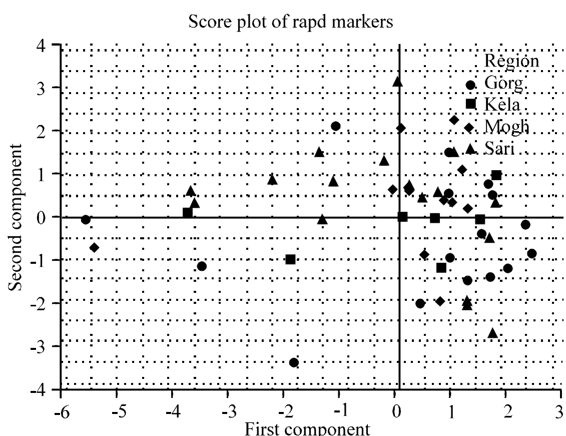


Fig. 5: Two dimensional view of score plot resulted from principle coordinate analysis of RAPD data

Table 4: Principal components (PCS) for RAPD markers among isolates

| Variable | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 |
|---|---|---|---|---|---|---|
| RPD1 | 0.301 | -0.287 | -0.075 | -0.056 | 0.332 | 0.110 |
| RPD2 | 0.143 | -0.458 | 0.15 | -0.189 | 0.362 | 0.064 |
| RPD3 | 0.304 | -0.272 | -0.175 | 0.222 | 0.043 | -0.358 |
| RPD4 | 0.237 | -0.185 | -0.494 | -0.138 | -0.214 | 0.009 |
| RPD5 | 0.332 | -0.19 | -0.382 | 0.094 | 0.031 | 0.148 |
| RPD6 | -0.149 | -0.274 | 0.358 | 0.253 | 0.283 | 0.164 |
| RPD7 | 0.146 | -0.268 | 0.316 | -0.007 | -0.315 | -0.541 |
| RPD8 | 0.36 | 0.133 | 0.286 | -0.025 | -0.248 | 0.146 |
| RPD9 | 0.43 | 0.215 | 0.194 | 0.08 | -0.141 | 0.193 |
| RPD10 | 0.423 | 0.191 | 0.162 | 0.093 | -0.167 | 0.118 |
| RPD11 | 0.038 | -0.252 | 0.301 | -0.686 | -0.141 | 0.188 |
| RPD12 | 0.168 | 0.237 | 0.141 | -0.116 | 0.329 | -0.612 |
| RPD13 | 0.253 | 0.332 | 0.071 | 0.02 | 0.524 | 0.122 |
| RPD14 | 0.013 | 0.294 | -0.248 | -0.566 | 0.136 | -0.129 |
| Eigenvalue | 3.9048 | 1.8723 | 1.6724 | 1.1562 | 1.1104 | 1.0097 |
| Proportion σ2 | 0.279 | 0.134 | 0.119 | 0.083 | 0.079 | 0.072 |
| Commulative σ2 | 0.279 | 0.413 | 0.532 | 0.615 | 0.694 | 0.766 |

*F. graminearum* from different geographical regions has showed that there were high inter species variation among isolates; however, relation between geographical distribution and genetic diversity has not been seen (Miedaner *et al.*, 2001). Considering the large areas and diverse ecoenvironments in Iran where FHB epidemics have occurred, results suggest that geographic isolation and ecological conditions may have had a significant effect on the distribution of *F. graminearum*.

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