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Investigation of genetic diversity of isolate common smut of corn by using RAPD marker in Lorestan Province

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Abstract. *The polymorphism and similarity relationships among 50 mating–type isolates of Ustilago maydis collected from Lorestan Province were determined with random amplified polymorphic DNA(RAPD). These fungal isolates were collected from 4 corn lines. Then DNA was extracted from haploid sporidiums using polymerase chains reaction (PCR) and specific primers, the genome of fungus were examined. Molecular studies of RAPD markers by using 10- primer 10 –nucleotide random primers were used. The highest multiples are established by primer opo1 with 16 bands in isolates and the lowest ones are related to opo 3 primer with 6 bands in isolates. Bands created the primers opo5 a 900 based pair band in 36 isolates and there was a separated model that isolate with other isolates were distinct. The primeres opo9 a 1000 base pairs band into clear 52 isolates as well as a 1000 base pairs band in isolates 52 and 51 can create and cause the rise of large difference between these two isolates with other isolates were. The results of analysis of data obtained by RAPD markers indicate the presence of genetic diversity in different populations of common smut of corn isolates. In this study according of the biggest amplitude slice different isolates of common smut of corn is divided into 8 groups of 52 and 51 each in a distinct cluster. The range of genetic similarity between isolates is from zero to 0.88 percent. The lowest genetic approximate amplitude was 51 with 23, 25, 26 isolates and the highest one was between isolates 33 and 32. Correlation Coefficient value of 0.83 was a good fit, which indicates the data was suitable matching to the dandrogram. Results also showed that the geographical distribution has no effect on genetic diversity.*

Keywords: corn, haploid sporedium, primer marker, population

Introduction

The basiodiomycete *Ustilago maydis* is the causal agent of common smut of maize. Besides its importance as a pathogen, the teliospore-containing tumors (galls) induced by the fungus on young maize ears are edible, an dare gaining appreciation in the North American markets as a delicacy called “huitlacoche” (Pajoohandeh et al., 2000; Khani et al., 2008). The ability of *Ustilago maydis* to cause disease in maize is directly dependent on the capability for compatible mating. This fungus has two mating loci, *a* and *b,* both of which must be heterozygous in mating haploids for compatibility. The *a* locus has two alleles; it encodes components of a pheromone response pathway and controls cell fusion and filamentous growth. The *b* locus, which is represented by up to 25 different compatibility types, encodes a combinatorial regulatory protein and regulates the development of infectious filament ous cells and tumor formation, in other words, pathogenicity. The interdependence of infection and sexual compatibility arises because mating-type loci that control compatibility also control subsequent transition from the saprophytic to the pathogenic phase of the lifecycle (Christense, 1963; Abdou et al., 2006). On the other hand, the fungus is heterothallic and presents an undetermined number of lines or biotypes. In addition, new biotypes arise by hybridization in sexual generations or by mutation. Outbreeding also, contributes to diversity because teliospores are disseminated over long distances by wind (Christensen, 1963). However, *Ustilago maydis* has no naturally occurring morphological or known physiological markers, and there are very few studies of its genetic diversity in natural populations. Recently, a high level of *b* locus diversity withm populations from four Minnesota locations has been found and common *b* mating types were found across broad geographic distance (Peyghami, 2002; Pillay, 2005; Zedchi et al., 2010)

Qualitative and quantitative information on diversity is an essential aspect of many fields in biology, both fundamental and applied (Pillay et al., 2005). Various genetic markers can be used to study fungal phyto pathogen populations, but they are not easy to assay. However, the advent ofgenetic markers based on differences in DNA sequences has made it possible to distinguish among all theindividuals in a fungal population. This has allowed for basic studies on population and evolutionary biology in fungi, thus lack of allelic information is less of a problem in these organisms, where the haploid state prevails (Zaro, 2009). The main application of DNA markers in fungi has been to differentiate among individuals in a population or collection of isolates. For example, PCR technique shave been a powerful tool for identification of the major species in the genus *Phytophthora.* They have also defined phenotypic groups in *Trichoderma* and have been useful for analysing the genetic structure of populations in many other fungi (Zambino et al., 2008). They used b-mating type variety of *U.maydis* fungus in different isolates in Menia, Sohag and Assiut using RAPD method. The results showed high difference of b-mating type among samples. There were b1, b2, b3 allels in all samples ,but not b3 allel in Sohag samples. The variety of GST gene for b-mating type was in subpopulation and the average of genetic variety was 82.79% in population. Also (Martinz et al., 1999) also examined the genetic variety of thirty wild isolates belonging to different strains in Mexico as well as two laboratory strains. The used RAPD method of analysis of loci and polymorphism showed high polymorphism that involved a

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locus with 31 different allels. Based on results, DNA fingerprinting was a more suitable method for discriminating genetic variety in *Ustilago maydis* population and also there is not unity between genetic and geographic distance among isolates. These data proved that there is relatively high genetic variety among population. We studied genetic diversity of common smut of corn by RAPD marker in Lorestan province for the first time.

Material and methods

Fungal isolates

Ustilago maydis were used in this study. Teliospores were collected from smut galls of naturally infected maize from different locations of Lorestan Province during the rainy season in 2008–2009. Within each location, a single ear fully infected with huitlacoche was removed from each of the plants. Spore masses were lyophilized, stored separately by location at 4 °C and were used to obtain the isolates for each group. Sporidia were isolated as previously described (Martinez et al., 1999). Teliospores were germinated on potato-dextrose agar at 28 °C. Individual haploid isolates were obtained as isolated colonies after plating cell dilutions on the same media.

DNA extraction

Sporidial DNA samples were prepared by using cetyl trimethyl – ammonum bromide (CTAB) method as described by Martinez (1999). The purity DNA was quantified by measuring absorbance at 260nm. The purify of DNA was determined by calculating the ratio of absorbance at 260nm to that of 280nm.The DNA samples were then diluted to a concentration of 30 ng/µl for PCR analysis.

RAPD analysis

The PCR reaction mixtre contained 1µl of genomic DNA (about 30ng), 0/4 µl of rTaq DNA polymerase (5µ/µl), 2/5 µl of 10*PCR reaction buffer (with Mg^{2+}), 2µl of 2/5 mM dNTP mixture, 1ul of 5 µM random primers, and ddH2O18/1µl in a total volum of µl. PCR amplification was conducted on a PTC-100tm amplifier. The thermal cycling reaction was started by denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 60s, 38 °C for 45s, 72 °C for 1min, and then a final extension at 72 °C for 8 min. The amplification products were analyzed by electrophroses as described above .The exprement was repeated for at least twice.

Data analysis

Based on the electrophoresis results of amplification products by RAPD-PCR, the samples with DNA band were marked as 1, whereas those without DNA band were marked 0 (only the repeatable bands in DNA electrophoresis analysis were recorded). Jaccard similarity coefficient and UPGMA cluster analyses were conducted by using NTSYS-pc2.10 analytical software (Mcpherson, 2000)

Results

RAPD analysis of Ustilago maydis isolates

We first screened 50 random decamer primers using the genomic DNA of isolate 1 as the template for RAPD analysis. Among them 10 primers were found to produce reproducible bands and thus selected for further analysis of *Ustilago maydis* isolates (Table 1). RAPD analysis of 50 isolates revealed a total of 100 bands with 96 polymorphic bands (96%) (Table1). The number of RAPD bands amplified by each primer ranged from 6 to 17, and the size of bands varied from 100–1700bp. Among these primers, opo5 generated most DNA bands with 17 polymorphic bands, respectively, while opo6, opo4 produced only 6 polymorphic band (Table1).

Genetic diversity analysis

We first conducted UPGMA dandrogram with RAPD data. According to RAPD data, these isolates were grouped into 8 (G1-G2,….G8) with a jaccards similarity coefficient of 0/26 (Figure 1). The highest genetic similarity was found in the isolates 32 and 33, which were more or less expected as they were isolated from different plants of the same corn line (704) at the different geographic location, whereas the lowest similarity was observed between the corn isolates No (51 with 23,25,26), (52 with 11,12, 13, 14,15, 16, 17, 18, 19, 20, 36, 38), while the isolate No.51was collected from corn line 704 in Kouhdasht city, the isolates No 23, 24, 25 were from corn line 404 in Kouhdasht city district of Lorestan Province. Group 1 (G1) includes the *Ustilago maydis* isolates No (1,2,34,5,6,7,8,9,10). The second group consisted of the isolates 35, 36, 37, 38, 39, 40. The third group was included isolates 1, 42, 43, 44, 45, 46, 47, 48, 49, 50. The fourth group consisted of the isolates 11, 12, 13, 14, 15, 16, 17, 18, 19, 20. The fifth group was included single isolate 51. The sixth group was included a single isolate 52. The seventh group was included isolates 1, 22, 23, 24, 25, 26, 27,

Table 1. Characteristics of bands produced by primers.

Primer	Number of multiform bands	All bands	Polymorphism percentage	Band size, bp	Sequence 5'–3'
Opo1	16	16	100%	100 - 1200	5'-GGCACGHAAG-3'
Opo2	13	14	92%	150 - 1500	5'-CTGTTGCHAC-3'
Opo3	6	6	100%	130 - 1300	5'-CCCAGHCACT-3'
Opo4	6	7	85%	750 - 1700	5'-CCACG GGAAG-3'
Opo5	17	17	100%	100 - 3000	5'-CAGCACTGAC-3'
Opo6	6	7	85%	250 - 1550	5'-CCTCCAGTGRT-3'
Opo7	9	9	85%	250 - 1130	5'-TCAGAGCGCC-3'
Opo8	7	8	87%	250 - 1400	5'-CAGTGCTGTG-3'
Opo9	8	8	100%	125 - 1350	5'-CTCGCTATCC-3'
Opo10	8	8	100%	140 - 1450	5' ACAGGAGGT-3'

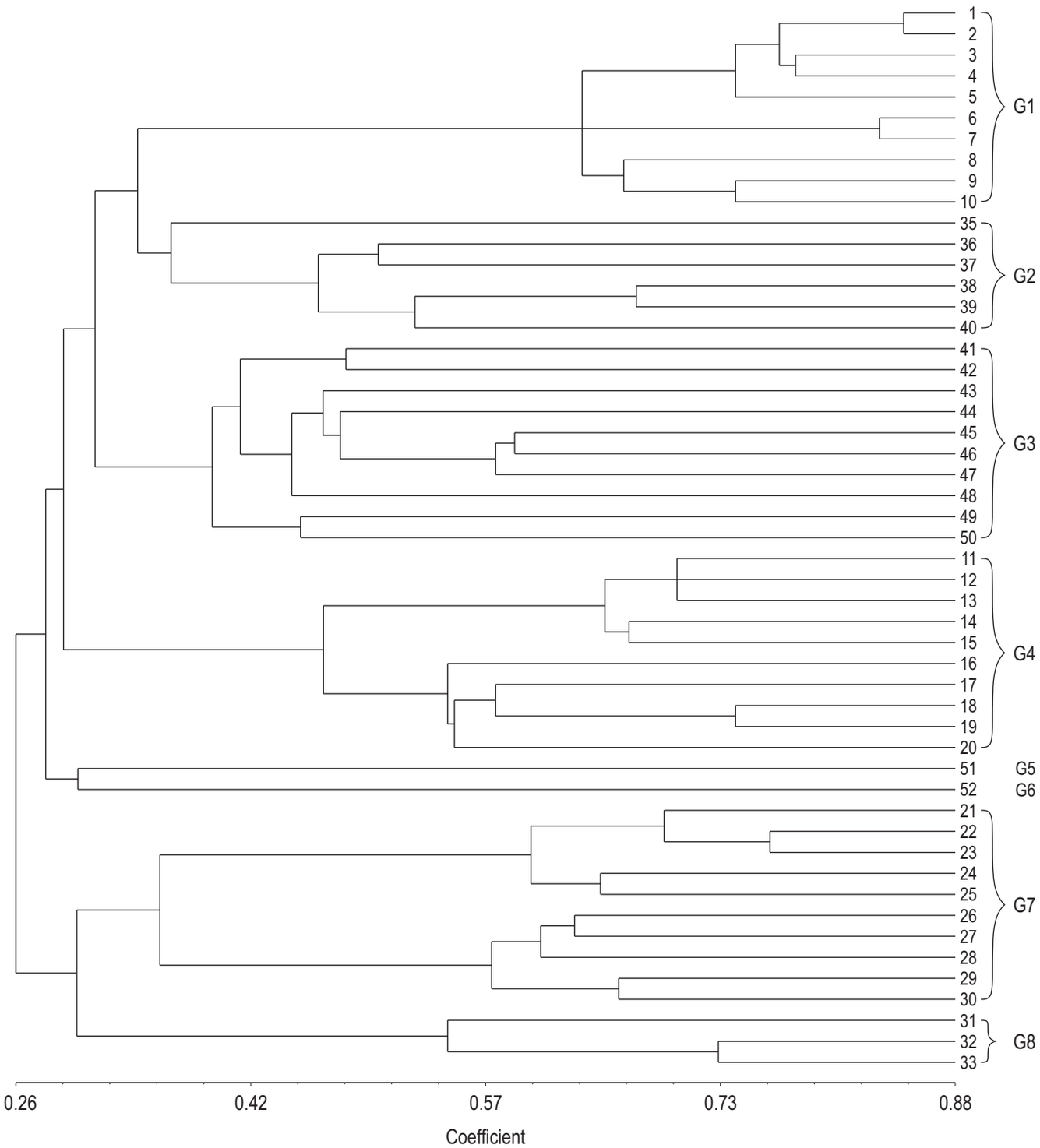

Figure 1. Dandrogram of RAPD results in corn common smut isolates.

28, 29, 30. The eighth group was included isolates 31, 32, 33, 34. The magnitude of genetic similarity among isolates was 0.00-0.88. Co–phonetic coefficient is 0.83 , showing appropriate data adjust to dandrogram.

Discussion

The results of analysis at RAPD method showed high genetic variety among isolates. We collected teliospores, our sampling method assured the heterozygous condition for each individual gall. Obtaining a high value for Nei's (1973) measure of gene diversity reflects the recovery of a large number of isolates in approximately equal frequencies. It is a population level parameter, and high gene diversity values correspond to a low probability that two haploids chosen at random from a population. The values of H that we report are much higher than those reported for other loci in fungal populations. For example, in *Phytophthora infestans*, gene diversity values ranged from 0.126to 0.258 for a DNA fingerprinting locus (Goodwin et al., 1992) and, in *Mycosphaerella graminicola*, values

range from 0.230 to 0.750 (Boeger et al., 1993). The genealogies for many genes under balancing selection demonstrate trans-specific polymorphism. In several plant and fungal systems, bursts of diversification are followed by long time periods when a large number of alleles are maintained, but few new alleles evolve (Joerger et al., 1990). After diversification, nonselective forces could be of increasing importance to the maintenance of variation. In a fungus such as *U. maydis*, which produces a large number of airborne spores and has an extensive geographic distribution, population sizes might be quite large. If this model is correct, we would predict that historic population sizes of *U. maydis* were smaller and selective pressures higher than in the present. To understand the maintenance of genetic variation in *U. maydis*, additional information for pathogen and host are required. We understand little of the life cycle of the fungus in field populations. Whereas in smuts such as *Tilletia* species, the primary sporidia mate immediately after they are produced on the promycelium and may. thus, lead to high levels of inbreeding (Holton et al., 1968) this does not seem to be common in *U. maydis*. However, the likelihood of sibling matings will depend on agricultural practices and the location of teliospore germination, about which we know little. For example, if teliospore germination occurs in the soil before tilling and planting, cultivation could mix populations of fungi (Gordon et al., 1992). Few pathogens for which data exist, information on pathogen migration rates, evolution of virulence, and response to variation in host resistance is invaluable to agricultural practices (Leung et al., 1993).The advantages of using *U. maydis* to address fundamental questions in molecular evolution and in the evolution of plant pathogens are clear. Since smut fungi are major pathogens on many cereal grain crops such as corn, barley, rye and oats, our work with the genetically tractable *U. maydis* may provide a basis for understanding the evolution of many economically important plant-pathogen interactions.

A large amount of genetic diversity distributed over a small spatial scale suggest, The possibility of RAPD adaptation by pathogen to the changing environment (Watson, 2009). The result indicates no relationship between the RAPD profile and the geographic origin sites that isolates were collected from. Such results are in agreement with those of (Zyrochic et al., 2009) who reported that when a genetic fingerprint based on a random sample of genome was compared to a basic suite of geographic variables , the relationship of the two was found to be very poor and not at all predictive.

Conclution

There is high genetic diversity in fungus of common corn smut in the province. The polymorphism analysis conducted in this study strongly suggests that geographical distribution has no effect on genetic diversity because the highest genetic similarity was found in the isolates 32 and 33. Isolate 32 belongs to Azna township and isolate 33 to Aligodarz township and although two township are far from each other, the isolates have high genetic similarity.

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