A Study of the Existence of Various Biotypes of Corn Common Smut Agent, *Ustilago Maydis*, in Lorestan Province of Iran

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**Abstract:**
In order to study the existence of the biotypes of corn common smut in Lorestan province of Iran, the isolates that had the highest percentages of germination were selected from among the isolates collected from each township; then, these isolates were inoculated to selected corn lines after appearance of maize clusters. The results of the present study revealed that the percentage and the severity of infection of isolates were different in various lines. According to these results, it can be argued that K17/2-3 and TV926 lines had the lowest and the highest resistance to used isolates, respectively. The highest and the lowest percentages of pathogenicity belonged to isolate number 1 with %95.3, and isolates number 8 and 43 with 50%, respectively. Isolates were divided in two groups for biotypes identification based on percentage and infection density so isolates 6, 7, 8, 9 were placed in same group.

**Key word:** maize, line, pathogenicity, genetic similarity, gall, severity

**Introduction**
*Ustilago maydis* (syn. *U. Zeae ung.*) is the agent of corn common smut disease. The disease was reported from Europe in 1754 for the first time and then it was seen in United state of America in 1823 (Alizade et al., 1999; Chogan & Zamani, 2000). In Iran, it was reported for the first time from a small field in Semnan City in 1981 and then it was seen in Gorgan and Varamin Cities in 1983 (Nekoyi & Sharif Naby, 2000). High reproduction and relatively long durability of teliospores along with high variability in life manner and special signs that it causes in plants are among distinct features of this fungus (Snetselaar & Mims, 1992; Estakhr & Zamani, 2000). Damage of corn common smut shows itself by reduction of product and formation of galls on each aerial part of plant such as cluster, spike, stem and leaves. Reduction of product caused by this disease ranges from a slightly damage to 100% or more in different areas. In Iran, there is no exact statistics about dispersion and severity of damage caused by the disease, but some researchers have estimated that it is about 10% of product (Banuett & Herskowitz, 1982; Banuett & Herskowitz, 1996; Batra & Sharma, 1968; Martin et al., 1988; Pajohande et al., 2002). Christeien et al. (1963) believed that all corn varieties and lines that are infected by artificial inoculation, become highly sensitive to disease, which is due to the destruction of physical resistance of tissues and stems thickness that prevents disease agent inoculum to reach meristemic tissue. He also believed that this fungus has high genetic diversity and because of heterothallic nature of the fungus, it has many biotypes in nature and constantly produces new biotypes as a result of hybridization and mutation in haploid and diploid stages. Damage caused by this disease in an area is variable due to properties such as race of pathogen, oldness of disease in the area, host sensitivity, agricultural practices and amount of consumed fertilizers (Johnson & Christensen, 1935; Hooker, 1956; Martine et al., 1975; Christensen, 1963; Ullstrup, 1978; Tajbakhsh, 1982; Martin et al., 1988; Thakur, 1989; Valverde et al., 2000). There are different methods to inoculate and infect corn with corn common smut pathogen. Comprehensive studies were carried out by Pope and McCarter titled evaluating different methods for infection of maize, during 1989 to 1991. The purpose of these studies was to achieve a reliable method for formation of galls and recognizing the resistance mechanisms. Methods such as silk cutting, wood cutting and wood injection for infection of corn to common smut are other methods of Pope and McCarter (1992b). In these methods, after the appearance of tassels and before they dry, 105 mm of sporidium suspension in each millimeter was injected to the bottom of maize husk and the same injection was administered to the bottom of the
maize that 1/5 cm of them had been cut. The result was the formation of galls in %97 of inoculation cases (Pope & McCarter, 1992a). Baddueyt et al. (2006) conducted comprehensive studied to survey various biotypes of U. maydis in Anoya and Batazake in Egypt and biotypes A, B, and C were identified among U. maydis samples. According to given results, biotype A was more destructive than biotypes B and C. Studies were done by Sinskey et al. (2006) to recognize the possibility of U. maydis biotypes in India and eventually, 27 biotypes of U. maydis were proved to be existing. According to current evidence, D. S. of various U. maydis biotypes was different. Bruce et al. (2006) carried out studies to determine corn common smut biotypes in Canada; they proved 6 biotypes of the fungus are existing. According to the findings, biotype A, as the most destructive one in Appodemia, caused %98 of infection in line K17. According to Warfied et al. (2006), isolates of fungus of corn common smut with the highest degree of germination (D. S.) have more pathogenic power than isolates with lower degree of germination (D. G.), so producing new fungal biotypes are more possible in these isolates. In this study we examined the possibility of existence of various fungus biotypes of corn common smut in Lorestan province of Iran.

**Material and Method**

*Planting selected corn lines in the field*

Four selected corn lines, k17/2-3, k2817/1, k1259 and TVA926, were used for conducting the experiment (Table 1).

<table>
<thead>
<tr>
<th>line</th>
<th>origin</th>
<th>Ripeness period</th>
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<tbody>
<tr>
<td>17/2-3 k</td>
<td>Karaj</td>
<td>premature</td>
</tr>
<tr>
<td>1/2817 k</td>
<td>Karaj</td>
<td>mature- premature</td>
</tr>
<tr>
<td>1259 k</td>
<td>Karaj</td>
<td>premature</td>
</tr>
<tr>
<td>926 TAV</td>
<td>Former Yugoslavia</td>
<td>too premature</td>
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</table>

The experiment was performed in Randomized Complete Block Design (RCBD) with 4 selected corn lines in an experimental field in Zarghan town (Shiraz, Iran). The field was prepared before experiment and Alachlor and Atrazine herbicides in the amount of 1.2 kg and 5 liter per hectare were used to control weeds. The number of planting lines of each line was two 7-meter lines with 75 cm spacing in each repetition, and there were 30 bushes in each line.

*Preparation of inoculum: Selection of pathogen isolates for the experiment*

From among the isolates of each province, the isolates that had the highest germination percentages were selected to prepare inoculum (Warfied et al., 2006). In order to produce sporidia, 1 mg of each isolate was mixed with 0.323 mg/lit streptomycin sulfate and distilled water for 3 minutes and after disinfection was spread on the medium. Then, it was maintained for 24 hours in 30 ºc under dark conditions. In order to create artificial infection by wounding, after tasseling and pollinating, before the tassels dry, the bottom of each maize was cut by clippers by 2 cm, and 3 ml of suspension containing 106 sporidia was injected to the cut section (10 bushes of each line in each repetition) (Table 2).

*Evaluating corn lines*

After the grains have hardened, the inoculated maize in each line and repetition were separately harvested. Percentage of infection was determined by counting the number of diseased maize, and severity of infection was determined based on progress of disease in each maize through scaling method ranging between 0 (no infection) and 7 (100% infection). Statistical analysis was carried out after converting the data to arcsine square root of the data, and to compare the maens, Duncan’s Multiple Range Test was used (Pope and McCarter, 1991; Pope and McCarter, 1992b; Banuett and Herskowitz, 1996).

**Results and Discussion**

In order to determine the existence of biotypes of Ustilago maydis, the isolates with the highest degree of germination than all other isolates of any province in Iran were selected.

<table>
<thead>
<tr>
<th>Numb er of isolate s</th>
<th>place of collection</th>
<th>Time of collection</th>
<th>sort</th>
<th>pathoge n</th>
<th>severity of germinatio n</th>
<th>percentage of germinatio n after 8 days</th>
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According to the findings, isolate number 1 caused the highest and the lowest percentage of pathogenicity in k17/2-3 and TVA926 lines, respectively. In case of isolate 17, the highest degree was in k2817/1 line and the lowest degree was in k17/2-3 line. For isolate 3, the highest percentage was in TVA926 and the lowest degree was in k1263/1 line. In the cases of isolate 37, 9, 43, 8, 22 and 33 isolates, the highest infection percentages were in k1263/1, k17/2-3, k2817/1, k17/2-3 and TVA926 lines, respectively. Also, the lowest infection percentages were in k2817/1, k1259, TVA926, k1263/1 and k1259 lines, respectively. Zamani and Chogan (2000) studied the resistance of 60 inbred crosses of common smut using injection in cob; and introduced K3165/2*Mo17 as sensitive composition and K1259/3*B73 as resistant composition. Renfro (1983) believes that, in terms of heredity, resistance to common corn smut is polygenic and he suggests that the method of crossing resistant lines with a sensitive line should be used in modification programs, and resistance evaluation in hybrids from F1 and F2 offspring be carried out. Meanwhile, there are so many haploid and diploid biotypes of fungus in nature that are created from hybridization and mutation in haploid and diploid stages; this vast amount results in ongoing assessment of sorghum selective genotypes in regions with high infection. Guly et al. (1980) reported that procedures of wounding the maize cause more infection than the procedures without wounding which go for spraying on the tassel, because the husk prevents the inoculation compound to reach corn seeds in spraying on tassels procedure, thus the infection decreases, so, these methods are not suitable for assessing the genetic resistance, because the low infection may be related to the escape mechanism not to natural resistance of corns. Similarly, Sultan et al. (2001) remarked that increase in disease percentage because of Ustilago maydis after various insects’ attacks indirectly supports the efficiency of wounding methods in maize. Takhar et al. (2007), by comparing the infection percentage and severity percentage of Ustilago
maydis in Yugoslavia, inferred that infection percentage is the variable criterion, since there were different results in two regions with respect to environmental conditions in terms of grouping. But it was not true about severity percentage. It demonstrates that severity percentage is a more stable trait in lines assessment while determining the reaction of various sorghum genotypes, because it specifies host’s genetic resistance based on the involved active resistance. Depper et al. (1990) also believed that when the inoculation method is done through wounding the maize, first, the physiological resistance is assessed, and the next incidence in unwounded seeds depends on apparent resistance. Banuett et al. (1996), in assessing the resistance of 30 lines to common corn smut through two methods of wounding and injection (silk channel), concluded that various sorghum lines showed different reactions to the disease in terms of sensitivity and the disease severity in injection method in silk channel was lower than that of in wounding method. Thakur et al. (1989) believed that when the wounding method for inoculation is used, first, the physiological resistance is evaluated, and next incidence in unwounded seeds depends on apparent resistance; thus both apparent and physiological resistance are involved when selection of resistance to common corn smut after inoculation through wounding the maize is carried out. Therefore, the wounding method is the useful and recommendable method for studying heredity of resistance to disease and screening various genotypes. Delsorb (2005) introduced various biotypes of corn common smut in France. Hanfey et al. (2004) examined various biotypes of corn common smut in 10 regions of Cuba and identified 6 biotypes on 3 line3. After physiological ripening of corn bushes and hardening of inoculated maize grains, each line was harvested separately and percentage and severity of infection of each treatment was determined. There were significant difference (\(p<0.01\)) between isolates in terms of their ability to infect lines and the percentage and severity of infection. The table of variance analysis for percentage and severity of infection of isolates was drawn by SPSS software (Table3). Isolates were divided into two separate groups, based on infection percentage, so as the first group included 6, 7, 9 and 8 isolates and the second group included 4, 5, 2, 1 and 3 isolates (isolate 33=1, isolate1=2, isolate17=3, isolate3=4, isolate 37=5, isolate 9=6, isolate 43=7, isolate 8=8 and isolate 22=9) (Figure 1).

![Figure 1](image1.png)

Figure 1: Resulted dendrogram for isolates based on infection percentages characteristic.

Isolates were divided to two separated groups, based on disease severity, so as the first group included 3, 4, 5, 6, 7, 8 and 9 isolates and the second group included 1 and 2 isolates (Figure 2).

![Figure 2](image2.png)

Figure 2: Resulted dendrogram for isolates based on infection severity trait.
Conclusion
According to the results, percentage and infection severity of various isolates in corn lines were different. The current evidence indicated that new biotypes of U. maydis exist in Lorestan province of Iran. We hope to identify the type of these biotype in the following studies.

References
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