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Determination of Susceptibility of Some Maize Varieties Against Corn Smut Caused by *Ustilago maydis* (DC) Corda

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ABSTRACT

The present study was conducted to determine susceptibility of some maize cultivars against corn smut (*Ustilago maydis* (DC) Corda.) under ecological conditions of Konya, Turkey. For these aims, field trials were established during the growing season in 2005. In the trials, reactions of 10-corn-variety against corn smut were investigated and was not determined any resistant cultivar of all the varieties used in the trials. However, it was established that 35P12, Monton, Bolson and TTM-815 varieties were found as middle resistant to *U. maydis*, whereas, both Falkner and Monzon varieties were very susceptible to the pathogen. In addition, yield losses from *U. maydis* varied from 26.4 % to 51.7 % in varieties used in the trials.

Key wors: Maize, susceptibility, corn smut, *Ustilago maydis*.

INTRODUCTION

Corn (*Zea mays* L.) is an important crop in terms of both human and animal nutrition and has multiple using fields such as using as raw material in starch, glucose, oil, and fodder industry (Kırtok, 1998). Since maize plant has broad adaptation ability and high yield potential, corn can be grown in almost all the regions of Turkey (Gençtan et. al., 1995).

Corn smut caused by *Ustilago maydis* occurs wherever corn is grown. It is more prevalent, however, in warm and moderately dry areas. Corn smut damages plants and reduces yields by forming galls on the aboveground parts of plants, including ears, tassels, stalks, and leaves. The number, size, and location of smut galls on the plant affect the amount of yield loss. Losses from corn smut range from a trace up to 10 % or more in localized areas.

Some individual fields of sweet corn may show losses approaching 100 % from corn smut (Agrios, 2004). Galls on the ears of maize plants may cause severe yield losses and at times disease incidence can rise up to 30-40 % (Sade, 2001). Corn smut occurs wherever maize is grown and can reduce yield considerably (Aktaş, 2001).

In general, in almost every year when following rainfalls dry and warm conditions occurs, particularly, in central part of Turkey, a number of galls from *U. maydis* on maize plants appear as prominent in size in the fields irrigated rather than those of non-irrigated (Tunçdemir and İren, 1980).

Although it has been recommended treatment of maize seeds with some fungicides against corn smut it has been unknown any fungicide that is exactly effective against corn smut. Therefore, it is crucial to be able to control of the disease with cultural practices, one of which is using resistant varieties against corn smut. It was reported by the authors (Agrios, 2004; Aktaş, 2001; Sade, 2001) that using resistant varieties against corn smut is the most effective way in terms of management with the disease. Thus, there has been in need for determining susceptibility of corn cultivars grown commonly in central part of Turkey. For these reasons, the present study was carried out in Konya, central part of Turkey.

MATERIALS AND METHODS

Materials

Galls were obtained from diseased plants in areas of maize production of Bahri Dağdaş International Agricultural Research Institute in Konya in 2004. To get pure culture of *Ustilago maydis* and multiply of sporidia (basidiospores), Potato Dextrose Agar (PDA) and at a rate of 20 % carrot solution were used respectively. In the field trials, 10-dent hybrid maize cultivar (Pol 2001, Goldeclat, Simon, Falkner, Bolson, Monzon, TTM-815, Ranchero, Monton and 35 P12), grown commonly in Konya, were used as host plants.

Methods

Isolation of *U. maydis*

Galls obtained from diseased plants were chopped and chlamydospores (teliospores) were separated from the gall tissues by sieving through a tea strainer. Afterwards, teliospores were surface-sterilized by immersion in a 1 % copper sulfate solution for 20-60 h and filtered through a two layers of sterile cheesecloth not allowing the teliospores to pass through. Then, teliospores on the cheesecloth were washed in three changes of sterile distilled water, dried on sterile filter paper and transferred on potato dextrose agar (PDA) supplemented by antibiotic (streptomycin sulphate) in petri dishes. Dishes were incubated at room temperature or 20-22 ^{0}C for 4-5 days consequently, sporidia (basidiospores) of *U. maydis* emerged. Sporidia about a pinhead in size were taken from cultures and transferred in erlenmayer flasks containing in a 20 % sterile carrot solution and incubated at room temperature or 20-22 ^{0}C for 7 days. In the meantime erlenmayers were shaken vigorously one or twice. In this way, inoculum required for inoculations was obtained by allowing sporidia to multiplication in the carrot solution (Tunçdemir, 1985).

Preparing of the inoculum

Basidiospore suspensions in the erlenmayer flasks were stirred so as to get a homogeneous solution and basidiospores were counted by using a hemocytometer. A small amount of basidiospore suspension was pipetted to both chambers of the hemocytometer and counts were made in the center and four corner squares. The mean was calculated, and the concentration of basidiospores ml^{-1} was estimated. Basidiospore suspensions were diluted to appropriate concentrations by using sterile carrot solution and adjusted to $4x10^6$ sporidia ml^{-1} afterwards, in the same way, teliospore suspensions were adjusted to $1x10^6$ teliospores ml^{-1} and added into the basidiospore suspensions (Tunçdemir, 1985).

Field trials

Field trials were carried out in a randomized complete blocks design with a factorial arrangement with three replications (Düzgüneş et al., 1987). Each plot consisted of three rows, each of which was 3 m long. Row spacing was 70 cm between the rows and 20 cm within the rows. In addition, distance between blocks was planned as 2 m. Varieties were sowed by hand after arranging at randomly in plots within the blocks. Similarly, control plots for each treatment were set up.

Ecological properties of the research area:

Field experiments were carried out in a soil having properties following; pH = 7.8, the amount of total salt = 0.07 %, organic matter = 2.2 %, and nitrogen (N) = 0.2 %.

Table 1. Total rainfall (mm), mean of temperature (° C) and relative humidity (%) in the research area during maize-growing season in 2005.

Month	May	June	July	Aug.	Sept.	Oct.
Rain. (mm)	12.5	3.50	12.2	0.10	20.9	34.7
Rel.hum.(%)	52	49	49	48	61	72
Temp.(ºC)	16.0	20.2	25.3	24.7	17.8	10.6

INOCULATIONS

1. When the plants were 40-60 cm high, 2 ml inoculum ($4x10^6$ sporidia ml^{-1} + $1x10^6$ chlamydospores ml^{-1}) was injected into apical node of the plant by means of a hypodermic syringe (Tunçdemir, 1985).

2. The ear inoculation method was taken from Pataky *et al*. (1995) and used by modifying as follows: for the ear silk of each emerging plant before pollination, 3 ml inoculum ($3x10^6$ sporidia ml^{-1} + $1x10^6$ chlamydospores ml^{-1}) was injected into the ear of each plant through a hypodermic syringe.

Disease assessment

After artificial inoculations, disease severity was calculated according to 0-5 scale of Johnson and Christensen (1935), where 0 = very small galls (<2.5 cm in diameter), 1 = small galls (2.5-5 cm in diameter), 2.5 = medium galls (5-10 cm in diameter), 5 = big galls (>10 cm in diameter).

Disease incidence

Disease incidence was calculated according to Johnson and Christensen (1935) and Walter (1935).

Level of susceptibility of varieties

Susceptibility of the maize cultivars used in the trials were determined by modifying the assessment scale of Dikoneva (1973), where disease incidence (DI) (10 %) = resistant (R), (DI) (11-30 %) = moderately resistant (MR), (DI) = (31-50 %) susceptible (S), and (DI) (>50 %) = very susceptible (VS).

Yield loss

In order to determine yield losses from *U. maydis*, all the ears inoculated and non-inoculated in plots were collected separately at harvest. After all the ears were collected, they were husked and left to dry in open air for 3 days. The percent ratio of kernel / cob was calculated according to Yanıkoğlu *et al*. (1999).

Afterwards, moisture contents of kernels for each treatment were separately determined by keeping the kernels at 72 ^{0}C for 72 h, and yield was adjusted for 15 % moisture content according to the following formula (Poehlman,1987).

$$\textbf{Adjusted Plot Yield} = \text{Plot weight} \times \frac{(100 - \text{moisture \%})}{85} \times \frac{(\text{kernel /cob})}{100}$$

$$\textbf{Yield(kg/da)} = \text{Adjusted plot yield} \times \frac{1000}{\text{Plot area (m}^2\text{)}}$$

In conclusion, yield loss from *U. maydis* was calculated by both using formula above and comparing inoculated plots with controls.

MSTAT-C (Michigan State Univ., East Lansing, MI, USA) statistical software was used for analyses. Differences between factors were determined by F test and mean values determined as different were grouped according to $LSD_{0.05}$ test were applied for multiple comparison of means.

RESULTS

Following artificial inoculations, several weeks later, swellings called as gall on any aboveground parts of the plants in inoculated plots appeared. The galls were observed as semi-fleshy, consisting of the smut fungus intermixed with enlarged cells of the affected organs of the host. Young galls were, at first, firm and light in color and covered with semi-glossy peridium which turned black as the gall matures. When the membrane died, it usually cracked open, exposing the dry powdery spores (teliospores). In general, the galls varied from minute sizes (0.5 cm in diameter) to 20 cm diam. The size, the shape, and the degree of proliferation varied depending to a considerable extent on the location of the gall on the plant and the susceptibility of the host.

The leaf galls differed greatly in size and texture. In general, the galls on the leaves were observed as small in size along the midrib. Many of the very small leaf galls remained firm and frequently contained few spores. The galls occurring on the main stalk usually appeared just above the nodes, however, they were observed on any portion of the main stalk. The galls located on the stalks were rather large, 10-30 cm in diameter, and varied greatly in size and shape. In the ears, the ovaries and glumes were smutted. Sometimes, the entire pistillate inflorescence was converted into a huge smut gall due to severe infection of *U. maydis*. When each floral organs of the tassel were infected by *U. maydis*, generally, a small gall formed each one and the tassel retained its shape. At times, stalk below the tassel was converted into a large mass of smut.

Reactions of the maize varieties used in trials against *U. maydis*

The results of the trials set up to detect reactions of hybrid maize cultivars used were given Table 2 and 3.

Table 2. Severity and incidence of disease caused by *U. maydis* in maize varieties used in the trial

Maize variety	Disease severity *	Disease incidence (%)	Level of Susceptibility
Pol 2001	3.5	34.4	S
Goldeclat	5.3	53.2	VS
Simon	6.4	42.2	S
Falkner	5.6	51.1	VS
Bolson	4.5	28.7	MR
Monzon	6.8	55.6	VS
TTM-815	4.6	28.9	MR
Monton	2.4	23.3	MR
Ranchero	4.1	48.7	S
35P12	1.6	19.4	MR
Mean	4.4	38.5	
Disease severity LSD $_{0.05}$: 4.88			
Disease incidence LSD $_{0.05}$: 27.90			

*: The highest value of disease severity ,accepted as 10.0.
Data are means of three replicates.
S: Susceptible VS: Very susceptible MR: Moderately resistant.

When all the maize cultivars used in the trial were considered; mean disease severity and incidence were determined as a rate of 4.4 and 38.5 % respectively. Any resistant variety was not determined of all the cultivars used in the trial. However, 35P12, Monton, Bolson and TTM-815 varieties were found as moderately resistant to *U. maydis* while, Pol 2001, Simon and Ranchero cultivars were susceptible. As for the others Goldeclat, Falkner and Monzon were determined as very susceptible to the pathogen.

Table 3. Yield losses due to *Ustilago maydis* infection on maize

Maize variety	Yield (inoculated) (kg/da)	Yield (non-inoculated) (kg/da)	Mean Yield (kg/da)	Yield Loss (%)
Pol 2001	656 defgh	1062 abc	859 ab	38.2
Goldeclat	450 h	613 fgh	531 c	26.6
Simon	624 fgh	1226 a	945 ab	50.7
Falkner	557 gh	1155 ab	856 ab	51.7
Bolson	626 fgh	1029 abcde	828 ab	39.1
Monzon	649 efgh	1153 ab	901 ab	43.7
TTM 815	567 gh	965 abcdef	766 bc	41.2
Monton	746 cdefgh	1037 abcd	891 ab	28.0
Ranchero	889 abcdefg	1209 ab	1049 ab	26.4
35P12	880 bcdefg	1245 ab	1063 a	29.3
Mean	664 b	1073 a	869	38.1
Variety LSD $_{0.05}$: 292.7	Disease LSD $_{0.05}$: 130.9			
Variety x Disease LSD $_{0.05}$: 383.2				

Within columns, values followed by the same letter do not differ significantly at P=0.05. Data are means of three replicates.

Differences between values in Table 3 were found as significant statistically ($p < 0.05$). In addition, significant yield differences between varieties were detected**.** Mean yields were determined as 1073 and 664 kg/da in non-inoculated and inoculated ones respectively. Mean yield loss from *U. maydis* was detected as a rate of 38.1 %, the highest and lowest yield losses were obtained from Falkner and Ranchero varieties with rates of 51.7 and 26.4 % respectively (Table 3).

DISCUSSION

It is highly possible that corn smut caused by *Ustilago maydis* can occur wherever corn is grown (Agrios, 2004; Aktaş, 2001; Christensen, 1963). In our field trials, following artificial inoculations, several weeks later, and galls formed on any above-ground part of the plants in inoculated plots. Our results showed that *U. maydis* could cause serious yield losses. Agrios (2004), stated that some individual fields of sweet corn may show losses approaching 100 % from corn smut. In our field trials, it was also detected that the galls on the ears of corn plants not only affected the yield directly but also led to become low quality of the kernels. Similar results were observed by some authors (Johnson and Christensen, 1935; Sade, 2001). Aktaş (2001) reported that big galls, in particular, located on ears could reduce yield up to 40-100%. Agrios (2004), also stated that the galls on ears may cause serious yield losses.

It was not determined any resistant cultivar against *U. maydis* of all the maize varieties used in the research. However, some maize cultivars were found as moderately resistant to *U. maydis* (Table 2). Agrios (2004) stated that any resistant maize cultivar against corn smut has been unknown but some hybrid maize cultivars could be moderately resistant to *U. maydis*. Agrios also reported that *U. maydis* forms constantly new strains, so it is crucial to be selected at least partially resistant maize lines in breedings programs. Hitchcock and Norton (1896) reported that any resistant cultivar against *U. maydis* was not detected among 46-maize-cultivar open-pollinated. A 2-year- study was carried out by Tunçdemir and İren (1980) to determine reactions of 15-maize- cultivar against *U. maydis,* in two different ecological area, Karagöl and Gelemen, located in Samsun, Turkey, under both natural and artificial conditions and consequently no resistant variety against *U. maydis* was determined among the cultivars under artificial inoculation conditions.

Values of disease severity and incidence of the present study were determined as higher than those of values determined by Tunçdemir and Iren (1980) under natural conditions. Potter and Melchers (1925) reported that any maize cultivar, susceptible to *U. maydis* in an area, could be resistant to the pathogen in another area. Konig (1972) stated that host resistance against *U. maydis* might change depending on climate and location in which maize is grown. If climate conditions continue in favor of host in growing season then corn smut caused by *U. maydis* may appear in minimum level (Kyle, 1929). Walter (1935) stated that when maize plants grew fast from seedling stage up to reaching exactly a plant size those plants could be resistant to *U. maydis* or was able to escape from the disease.

It was established that both morphological and physiological structure of maize plant could affect the development of corn smut caused by *U. maydis* (Yanıkoğlu et.al., 1999). Tunçdemir and Iren (1980) and Christensen (1963) also reported similar things. To determine whether pollination of maize hybrid (Early Golden Bantam) ears affects their susceptibility to *U. maydis* infection, ears were treated in one of four ways: pollination only, inoculation with compatible haploid *U. maydis* cells only, pollination followed by inoculation 4 days later, or inoculation followed by pollination 4 days later. Microscopic examination of silks after pollination and inoculation treatments indicated that an abscission zone formed at the bases of pollinated silks and may have prevented fungal infection filaments from growing into the ovaries. These results indicated that pollination rendered ovaries more resistant to infection by *U. maydis* (Snetselaar et.al.,2001). Barnes *et. al*., (2004) conducted a research on the purpose of determine the extent of genetic variation of *U. maydis* in a wide geographical area in which maize is grown (Le Seuer, Minnesota, Tarariras and Uruguay). The samples collected from maize plants infected with *U. maydis* in those areas were examined and consequently genetic variations were determined at the high level between the isolates of the fungus. Genetic variation of *U. maydis* provides the pathogen with infection of different maize varieties.

Tunçdemir and Iren (1980) indicated that rainfall is one of the most important factors affecting development of corn smut,in particular following either slight rainfall or irrigation with the occurrence of dry conditions, which are in favor of development of the disease. The authors also reported that relative humidity can be negligible for occurrence of corn smut but optimum temperature for the development of *U. maydis* ranged from 18 °C to 21 °C. Agrios (2004) stated that corn smut occurs wherever corn is grown. It is more prevalent, however, in warm and moderately dry areas.

As known that any plant disease occurs as depending on three components (host plant, pathogen, environment), called disease triangle. In our research, maize cultivars used in the trials displayed different reactions against *U.maydis*. This does not mean these cultivars may show the same reactions against the pathogen in every region. Because any maize cultivar, which seems susceptible to *U.maydis* in any location or in any growing season, could be resistant against corn smut in another location or in another growing season. Hence, there are some reasons for changes of susceptibilities of maize cultivars against *U.maydis*, which were tried to be elucidated depending on both the results of our trials and the relevant literatures.

ÖZET

BAZI MISIR ÇEŞİTLERİNİN MISIR RASTIĞI HASTALIĞI (*USTILAGO MAYDIS* (DC) CORDA)'NA KARŞI DUYARLILIKLARININ BELİRLENMESİ

Konya ekolojik koşullarında bazı mısır çeşitlerinin mısır rastığı hastalığı (*Ustilago maydis* (DC) Cordo)'na karşı duyarlılıklarını saptamak için bu çalışma yapılmıştır. Bu amaçla 2005 yılı üretim sezonunda tarla denemeleri kurulmuştur. On mısır çeşidinin mısır rastığı hastalığına karşı reaksiyonlarının araştırıldığı tarla denemelerinde hiç bir çeşidin hastalığa dayanıklı olmadığı belirlenmiştir. Bununla beraber Falkner ve Monzon mısır çeşitleri hastalığa karşı çok hassas bulunurken, 35P12, Monton, Bolson ve TTM-815 çeşitleri orta dayanıklı bulunmuşlardır. Denemelerde *U.maydis* den dolayı ortaya çıkan ürün kaybı % 26.4 ile % 51.7 arasında değişmiştir.

Anahtar Kelimeler: Mısır, duyarlılık, mısır rastığı, *Ustilago maydis*

LITERATURE CITED

AGRIOS, G. N.2004. Plant Pathology. (Fifth Edition). Elsevier Academic. Press, Burlington U. S. A.

AKTAŞ, H. 2001. Important diseases of cereals and survey methods. Ministry of Agriculturel. Dept. of Plant Healt. Reseach. Ankara, Turkey.

BARNES, C. W., SZABO, L. J., MAY, G. and GROTH, J. V. 2004. Inbreeding Levels of Two *Ustilago maydis* Populations. **Mycologia**. 96: 1236-1244.

CHRISTENSEN, J. J. 1963. Corn smut caused by *Ustilago maydis.* **Monograph 2**. American Phytopathological Society, St. Paul, MN.

DIKONEVA, L. A. 1973.The resistance of self-pollinated sweet corn lines to blister smut (Abs), **Review of Plant Pathology**. Vol. 54 No: 127.

DÜZGÜNEŞ, O., KESİCİ, T., KAVUNCU, O. and GÜRBÜZ, F. 1987. Research and Trial Methods (Statistics Methods- II). Ankara University, Faculty of Agriculture Publ. No.1021, Lesson Book No: 295. Ankara, Turkey.

GENÇTAN, T., EMEKLİLER, Y., ÇÖLKESEN, M. and BAŞER, I. 1995. Consume projections of cereal crops and production targets. IV. Technical Congress of Turkish Agriculture Engineering. Ankara, Turkey.

HITCHCOCK A. S. and NORTON, J. B. S. 1896. Corn smut. Kansas Agr. Expt. Sta. Bull. 62: 169-212.

JOHNSON, I. J. and CHRISTENSEN, J. J. 1935. Relation between number, size and location of smut infections to reduction in yield of corn. **Phytopathology** 25: 223-233.

KIRTOK, Y. 1998. Production and Using of Maize, Kocaoluk Press, Istanbul, Turkey.

KONING, K. 1972. Investigation on the ecology and control of maize smut (*U.maydis*) (De Corda). (Abstr.) Review of applied mycology. 51:21.

KYLE, C. H. 1929. Relation of husk covering to smut of corn ears. U. S. Dept. **Agr. Tech. Bull.** 120:1-7.

PATAKY, J. K., NANKAM, C. and KERNS, M. R. 1995. Evaluation of a silk-inoculation technique to differentiate reactions of sweet corn hybrids to common smut. **Phytopathology** 85: 1323-1328.

POEHLMAN, J. M. 1987. Breeding Field Crops. Avi Publishing Company, INC. Wesport, Connecticut, USA.

POTTER, A. A. and MELCHERS, L. E. 1925. Study of the life history and ecological relations of the smut of maize. J. Agr. Research 30: 161-173.

SADE, B. 2001. Maize cultivation. Publications of the trade stock of Konya. Publ. No:1 Second press, Konya, Turkey.

SNETSELAAR, K. M., CARFIOLI, M. A. and CORDISCO, K. M. 2001. Pollination can protect maize ovaries from infection by *Ustilago maydis*, the corn smut fungus .**Can. J. Bot.** 79 (12): 1390–1399 (2001). Canada.

TUNÇDEMİR, M. and S,İREN. 1980. The researches on bioecology of corn smut disease (*Ustilago maydis* (DC) Corda.) in the vicinity of Samsun, Turkey. Ankara University, Faculty of Agriculture. The abstracts of master thesis. Ankara Press, 1980.

TUNÇDEMİR, M. 1985. The seminar of the diseases of Wheat and Maize. Central Anatolia Research Inst. 25-29 March, 1985. Ankara, Turkey.

WALTER, J. M. 1935. Factors affecting the development of corn smut *Ustilago zeae* (Beckm.) Unger. Minnesota Agr. Exp. Sta. Tech. Bull. 111.

YANIKOĞLU, S., KÜÇÜK, I., SEZER, M. C. and MERİÇ, H. 1999. The Guide of observation for maize. Ministry of Agriculture and Rural affairs., Tagem Sakarya, Turkey. Agr. researc. Inst. Publ. No.12