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Responses of some maize cultivars to smut disease, *Ustilago maydis* (DC) Corda

Bazı mısır çeşitlerinin rastık hastalığına (*Ustilago maydis* (DC) Corda) tepkileri

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ABSTRACT

Forming galls (tumor) on different aboveground parts of maize plants, *Ustilago maydis* (DC) Corda can cause significant damage to the host. As applying fungicide against *U. maydis* is not feasible yet, using resistant or tolerant maize cultivars is very important for management of the smut. In this regard, a 2-year- field study was conducted to determine responses of some cultivars belonging to different maize variety groups to the smut disease. Inoculations were performed by injecting inoculum into apical node of the plants, 40-60 cm high, and ear silks in plots. Of all the maize cultivars tested, no resistant variety was detected. However, sweet corn varieties (Vega and Merit) were found to be tolerant to the smut disease. It was also detected that Dent corn cultivars (Pioneer-3394, Side), flint corn variety (Karadeniz Yıldızı), and popcorn variety (Antcin-98) all were susceptible, whereas, dent corn (Ada-523) and flint corn varieties (Karaçay) were very susceptible to the disease. Mean disease severity and incidence of the cultivars varied from 0.4 to 8.4 and from 16.6% to 74.1%, respectively.

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ÖZ

Ustilago maydis (DC) Corda mısır bitkilerinin farklı toprak， üstü kısımlarında ur (tümör) oluşturmasıyla konukçuda önemli zarara neden olabilir. *U. maydis*' e karşı fungisit uygulaması henüz mümkün olmadığından, hastalığın mücadelesinde dayanıklı veya tolerant mısır çeşitlerinin kullanılması çok önemlidir. Bu bağlamda, farklı mısır varyete gruplarına ait bazı mısır çeşitlerinin rastık hastalığına tepkilerini belirlemek amacıyla iki yıllık tarla denemesi yürütülmüştür. İnokulasyonlar, parsellerde 40-60 cm boyundaki mısır bitkilerinin en uç boğumuna ve ipeklere inokulumun enjekte edilmesiyle yapılmıştır. Test edilen mısır çeşitleri arasında dayanıklı bir çeşit belirlenmemiştir. Bununla birlikte, şeker mısır çeşitleri (Vega ve Merit) rastık hastalığına karşı tolerant bulunmuştur. Ayrıca, at dişi mısır çeşitleri (Pioneer-3394, Side), sert mısır çeşidi (Karadeniz Yıldızı), ve cinmısır çeşidi (Antcin-98) hassas, at dişi mısır çeşidi (Ada-523) ve sert mısır çeşidinin (Karaçay) ise hastalığa karşı çok hassas olduğu belirlenmiştir. Çeşitlerin ortalama hastalık şiddeti ve oranları sırasıyla 0.4-8.4 ve % 16.6-74.1 arasında değişmiştir.

1. Introduction

Apart from multiple usage fields as a raw material in starch, glucose, oil and fodder industry, maize (*Zea mays* L.) is an important crop for nutrition of human and animal (Kırtok 1998). Having broad adaptation capability and high yield potential, maize can be grown in almost all the regions of Turkey (Gençtan et al. 1995).

Maize smut caused by *Ustilago maydis* occurs wherever maize is grown all over the world. It is more common in warm and moderately dry areas. Maize smut reduces yield of the plant. Unlike other cereal smuts, *U. maydis* gives rise to local infection and remarkably damages stalk and ear of maize by forming colossal galls on them. In case of severe infection at early development stage of the plant, the pathogen also can cause either death or infertility of the plant (Tunçdemir and Iren 1980). However, Christensen (1963) stated that a single gall reduced ear yield about 25% on the average. Aktaş (2001) reported that big galls located on the ears of corn, in particular, could reduce yield up to 40-100%.

In recent years, treatment of maize seeds with some fungicides has been offered by some firms to manage with corn smut. As the pathogen leads to local infection instead of systemic, the seed treatments against *U. maydis* are useless. Likewise, already no effective fungicide against corn smut is known. Consequently, it is crucial to benefit from resistant or tolerant varieties in breeding and management of the pathogen. Several authors (Aktaş 2001; Sade 2001; Agrios 2004) also emphasized that using resistant varieties against maize smut is the most effective way in terms of management of the disease.

The aim of the study, carried out in Antalya Province in 2010 and 2011, was to determine responses of some maize cultivars grown in Turkey.

2. Materials and Methods

Galls (smutty ears) were obtained from smutty plants in maize-producing areas of Batı Akdeniz Agricultural Research Institute, located on Mediterranean region of Turkey in 2009 and 2010. Potato dextrose agar (PDA, Oxoid) and 20% carrot solution were used to get pure culture of *U. maydis* and for propagation of sporidia (basidiospores), respectively. In the field trials, dent corn (*Zea mays* var. *indentata*) cultivars; Ada-523, Pioneer-3394 and Side; flint corn (*Zea mays* var. *indurata*) cultivars; Karaçay and Karadeniz Yıldızı; sweet corn (*Zea mays* var. *saccharata*) cultivars; Merit and Vega; and popcorn (*Zea mays* var. *everta*) variety; Antcin-98 were used as host plants.

2.1. Isolation of U. maydis

The galls 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 to 60 h and filtered through two layers of sterile cheesecloth not allowing the teliospores to pass through. Later, teliospores on the cheesecloth were washed in three changes of sterile distilled water and dried on sterile filter paper, and transferred on PDA supplemented by antibiotic (streptomycin sulphate) in petri dishes. The dishes were incubated at 25 °C for 4 to 5 days until sporidia (basidiospores) of *U. maydis* emerged. When sporidia were about a pinhead in size, they were taken from cultures, and transferred in 500-mL Erlenmeyer flasks containing 20% sterile carrot solution, and incubated at 25 °C for 7 days. At the same time, Erlenmeyer flasks were shaken vigorously for 1 to 2 min once or twice a week. In this way, inoculum required for inoculations was obtained by allowing sporidia to multiply in the carrot solution (Tunçdemir 1985).

2.2. Preparation of inoculum

Basidiospore suspensions in the Erlenmeyer flasks were stirred to get a homogeneous solution and basidiospores were counted by using a hemocytometer (Neubauer, Isolab, Germany). Basidiospore suspensions were diluted to appropriate concentrations using sterile carrot solution and adjusted to 4×10^6 sporidia mL^{-1}, afterwards, in the same way, teliospore suspensions were arranged to 1×10^6 teliospores mL^{-1} and added into the basidiospore suspensions (Tunçdemir 1985).

2.3. Field experiments

Field trials were carried out in a randomized complete blocks design with a factorial arrangement with three replications in Aksu district at maize growing fields of Batı Akdeniz Agricultural Research Institute in Antalya. Each plot consisted of four rows, 5 m long. The row spacings was 70 cm between the rows and 20 cm within the rows. Control plots were established for each treatment.

2.4. Ecological properties of the research area

General soil texture of the research area was clayish and loamy. The area was fertilized with nitrogen, phosphorus and potassium at the rates of 180, 80 and 80 kg ha^{-1} respectively. Field experiments were set up in Antalya province of Turkey. When inoculations of the maize ears were done in August of 2010, monthly rainfall in total was 4.2 mm whereas in the same period of 2011 no measurable rainfall was recorded. However, mean temperature and relative humidity of August in 2010 and 2011 were 30.5 ° C, 59.1% and 29.6 ° C and 50.0%, respectively (Anonim 2013).

2.5. Inoculations

Inoculations were performed in two growth stages of maize plants as follows:

1. When the plants were 40-60 cm high, 2 mL inoculum (4×10^6 sporidia mL^{-1}+1×10^6 chlamydospores mL^{-1}) was injected into apical node of the plant by means of a hypodermic syringe (Tunçdemir 1985). The inoculations of the plants were performed between 6 pm and 8:30 pm on July 15 and July 18 in 2010 and 2011, respectively. Mean daily temperature of the ensuing 3-days was 34.0 °C in 2010 and 29.1 °C in 2011 (Table 1).

Table 1. Daily mean temperature of the research area during inoculations.

Inoculation time (2010)		Daily mean temperature (°C)*					
		2010		Inoculation time (2011)		2011	
July	August	July (°C)	August (°C)	July	August	July (°C)	August (°C)
16	3	34.1	29.8	18	11	30.0	27.9
17	4	35.6	29.5	19	15	29.7	27.2
19	5	32.4	29.7	20	16	29.7	27.5
	10		29.0	21	17	27.9	28.0
	11		29.2		18		28.9
	12		29.4		25		29.4
	20		29.9		26		28.4
	21		32.0		27		27.6
	22		31.8		28		26.8

*Regional Meteorology Station, Antalya.

2. The ear inoculation method, as described by Pataky et al. (1995), was used with some modificatios: For the ear silk of each emerging plant before pollination, 3 mL inoculum (3×10^6 sporidia mL^{-1}+1×10^6 chlamydospores mL^{-1}) was injected into the ear of each plant through a hypodermic syringe. Inoculations of ears were performed on August 3, 10 and 20 in 2010 and August 11, 15, 18 and 25 in 2011, respectively. Mean daily temperatures of the inoculation days and the ensuing 3-day in 2010 and in 2011 were recorded as 30.0 °C and 27.9 °C respectively (Table 1).

2.6. Disease assessments

Disease severity was rated using 0 to 5 scale of Johnson and Christensen (1935), where 0: very small galls (< 2.5 cm in diameter), 1: small galls (2.5 to 5 cm in diameter), 2.5: medium galls (5 to 10 cm in diameter), 5: big galls (> 10 cm in diameter). Disease incidence (%) was calculated by comparing number of the infected ears and non-infected ones.

2.7. Statistical analysis

JMP statistical software (SAS Institute Inc., Cary, North Carolina, USA) was used for variance analysis. Differences between factors were determined by F test and the mean values determined as different were grouped according to $LSD_{0.05}$ test (Düzgüneş et al. 1987).

2.8. Responses of varieties

Responses of the maize cultivars were calculated by modifying the assessment scale of Dikoneva (1973), where disease incidence (DI) (15%) = resistant (R), (DI) (16-35%) = tolerant (T), (DI) **(**36-55%) = susceptible (S), and (DI) (> 55%) = very susceptible (VS).

3. Results

Following injection of the inoculum, about 14 days later, swellings called as gall on any aboveground parts of the plants in inoculated plots appeared. In general, the galls varied from minute sizes (0.3 cm in diameter) to 22 cm in diameter. The size and shape of the galls remarkably varied according to the locations of the galls on the plants and susceptibility of the maize cultivars. The leaf galls varied in their size and textures. However, the galls on leaves were generally in small size along the midrib of the leaves. Most of the tiny leaf galls remained firm and frequently contained few teliospores. The galls occuring on the main stalk usually appeared just above the nodes, however, they were observed on any part of the main stalk. The galls located on the main stalk were highly large, 10 to 20 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*.

Mean disease severity (DS) of the cultivars in 2010 and in 2011 were 0.9 and 4.8, respectively. However, average DS of the varieties was 2.8. DS in 2011 was higher than the one in 2010. The highest DS were established from dent corn varieties (Ada-523, Pioneer-339, Side) but the second, third and lowest DS were found on flint corn varieties (Karaçay, Karadeniz Yıldızı), the popcorn variety (Antcin-98) and sweet corn varieties (Merit, Vega), respectively (Table 2).

Mean disease incidence (DI) of the varieties varied from 16.6 to 74.1%. Average DI in 2011 was higher than the one in 2010 (Table 3).

The highest DS (8.4) and DI (74.1%) were found on dent corn variety (Ada-523) whereas, the lowest DS (0.4) and DI (16.6%) were on sweet corn variety (Vega) (Table 2 and Table 3).

Table 2. Disease severity of the maize cultivars.

Variety	Disease Severity*		
	2010	2011	Mean
Ada-523	2.2**	14.7	8.4
Pioneer-3394	0.1	4.4	2.2
Side	0.5	6.4	3.4
Karaçay	1.1	3.4	2.2
Karadeniz Yıldızı	2.4	5.6	4.0
Merit	0.5	0.6	0.5
Vega	0.3	0.6	0.4
Antcin-98	0.6	3.0	1.8
Mean	0.9	4.8	2.8
Year LSD (0.01)= 0.3 Variety LSD (0.01)= 0.6			
Year x variety LSD (0.01)= 0.8			

*: The highest value of disease severity was accepted as 10.0.
**: Data are means of three replicates.

Table 3. Disease incidence and susceptibility of the maize cultivars against *U. maydis*.

Variety	Disease incidence (%)			Level of susceptibility
	2010	2011	Mean	
Ada-523	55.0	93.3	74.1	VS
Pioneer-3394	10.0	93.3	51.6	S
Side	21.6	71.6	46.6	S
Karaçay	55.0	56.6	55.8	VS
Karadeniz Yıldızı	36.6	71.6	54.1	S
Merit	11.6	28.3	19.9	T
Vega	11.6	21.6	16.6	T
Antcin-98	25.0	63.3	44.1	S
Mean	28.3	62.4	45.3	
Year LSD (0.01)= 3.6 Variety LSD (0.01)= 7.3				
Year x variety LSD (0.01)= 10.3				

S: Susceptible, VS: Very susceptible, T: Tolerant

4. Discussion and Conclusion

In the study, any of the maize cultivars tested in the field conditions was not detected as resistant to corn smut but sweet corn cultivars (Merit, Vega) were tolerant to *U. maydis* (Table 3). In a two-year study, conducted by Tunçdemir and Iren (1980) in two different ecological areas (Karagöl and Gelemen), located in Samsun province of Turkey, no resistant maize variety against *U. maydis* was established among 15 maize cultivars. In addition, Agrios (2004) stated that any maize cultivar, resistant to corn smut disease, had been unknown but some maize cultivars could be tolerant to *U. maydis*. As for, Aydoğdu and Boyraz (2006) reported that none of the 10 dent corn varieties tested under ecological conditions of Konya province of Turkey was resistant to *U. maydis* but 35P12, Monton, Bolson and TTM815 maize cultivars were moderately resistant to smut disease. A-cultivar-trial in Columbia Basin in U.S showed marked differences in susceptibility to corn smut among field corn hybrids (Mohan et al. 2013).

In the present study, dent, flint and popcorn cultivars tested were susceptible to the pathogen (Table 3). However, Aydoğdu and Boyraz (2006) reported that dent corn varieties (Pol 2001, Simon, Ranchero) were susceptible to the fungus. In addition, several authors (Immer and Christensen 1925; Garber and Quisenberry 1925; Christensen and Johnson 1935) emphasized that susceptibility to corn smut could greatly differ among the open-pollinated maize varieties.

In our study, mean DS and DI of the varieties (dent, flint, sweet and popcorn) in Antalya were 2.6 and 45.3%, respectively (Table 2 and Table 3). Aydoğdu and Boyraz (2006) stated that mean DS and DI of dent corn varieties in Konya were 4.4 and 38.5%, respectively. However, Tunçdemir and Iren (1980) reported that mean DI was 9.2% in a survey performed in Samsun province. Thus, it may be suggested that with artificial inoculations, mean DI can be higher than that of natural infections of the pathogen.

Interactions of year, variety, and year x variety were significant (Table 2 and 3). As known that following inoculation, hours and a few days could play an important role in disease development. Tunçdemir and Iren (1980) reported that the most favorable temperature for development of corn smut rests between 18 °C and 21°C. In our study, following inoculation, the mean daily temperature of the ensuing 3-day period was 34.0 °C in 2010 whereas, it was 29.1 °C in 2011 (Table 1). The mean daily temperatures during the inoculation in 2010 were about 5 °C higher than the one in 2011.

Accordingly, this significant difference may have adversely affected germination and penetration of the pathogen in 2010. Therefore, environmental conditions of 2010 were favorable for the host. Kyle (1929) emphasized that when environmental factors continue in favor of the host in maize growing season, smut enfections appear minimum level. In a two-year survey, Görtz et al. (2008) stated that frequency of kernels infected by *Fusarium* spp. ranged from 0.7 to 99.7% in 2006, while the highest incidence of *Fusarium* ear rot was 64% in 2007, and the year-to-year variability in the frequency of *Fusarium* species and in the overall infection rate may be explained by significant differences in temperature and precipitation during the growth periods.

Physiology and morphological structure of the host can also play an important role in disease development. Since the maize cultivars tested have specific physiology and morphological features, different reactions against *U. maydis* were determined in our study. In addition, several authors (Walter 1935; Christensen 1963; Tunçdemir and Iren 1980; Yanıkoğlu et al. 1999) indicated that both morphological and physiological structure of maize could affect development of smut disease. In our study, the maize cultivars showed different reactions against corn smut, which does not mean that these maize cultivars could show the same reactions to the pathogen in every region. Because, any maize cultivar, seems to be susceptible to *U. maydis* in any location or in any growing season, could be resistant to corn smut in another location or in another growing season. Konig (1972) stated that host resistance against *U. maydis* might change depending on climate and location in which maize is grown.

Since no effective fungicide against *U. maydis* is known, testing susceptibility of existing maize cultivars and lines against the pathogen in every location and assessing resistant or tolerant maize varieties or lines in breeding studies are crucial for management of the disease.

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