

## SCREENING OF WHEAT GERM PLASM FOR RESISTANCE TO *MICRODOCHIUM NIVALE* UNDER FIELD CONDITIONS

C. Eken<sup>1,5\*</sup>, S. Bulut<sup>2</sup>, A. Öztürk<sup>3</sup>, E. Dane<sup>4</sup>, Ö. Çağlar<sup>3</sup> and E. Demireci<sup>5</sup>

<sup>1</sup>Graduate School of Natural and Applied Sciences, Ardahan University, Ardahan, Turkey

<sup>2</sup>Department of Agronomy, Seyrani Faculty of Agriculture, Erciyes University, Kayseri, Turkey

<sup>3</sup>Department of Agronomy, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey

<sup>4</sup>Provincial Directorate of Agriculture, Section of Plant Protection, 45010, Manisa, Turkey

<sup>5</sup>Department of Plant Protection, Faculty of Agriculture, Atatürk University, 25240 Erzurum, Turkey

\*Corresponding author e-mail: cafereken@hotmail.com

### ABSTRACT

Pink snow mold, caused by *Microdochium nivale*, is a serious disease of winter wheat (*Triticum aestivum*) in the Northern Hemisphere. A field study with artificial inoculation was conducted using 38 winter wheat cultivars during the 2002-2003 at Erzurum, Turkey. Significant differences were detected among cultivars for reaction and yield components to the *M. nivale*. The most resistant winter wheat cultivars were Harmankaya and Pehlivan, and the most susceptible ones were Aydin-97, Kırgız-95 and Bayraktar. Yield components decreased significantly in inoculated plants. Pink snow mold resulted in decreased number of spikes per m<sup>2</sup>, the grain yield and the plant height of 71.1, 67.3 and 13.2% respectively.

**Key words:** *Triticum aestivum*, pink snow mold, yield components, *Microdochium nivale*

### INTRODUCTION

*Microdochium nivale* (Fr.) Samuels & Hallet, formerly known as *Fusarium nivale* (Fr.) Sorauer causes pink snow mold on grasses and cereals and is the most widespread snow mold species of winter wheat (*Triticum aestivum* L.) in cold to temperate regions of the northern hemisphere (Tronsmo *et al.*, 2001), including Turkey (Demirci and Dane, 2003). Severe damage due to pink snow mold in winter cereals have been reported with a snow cover of two or more months (Ergon *et al.*, 2003). *Microdochium nivale* can also cause other diseases, e.g. head blight, seedling blight and crown rot (Tronsmo *et al.*, 2001). Previous studies have reported that *M. nivale* causes variable but substantial grain yield reductions (15-28%) annually in winter wheat (Humphreys *et al.*, 1995; Parry *et al.*, 1995). Breeding for resistance would provide an important method of reducing the incidence of disease and related yield loss caused by *M. nivale* infection. Evaluation of genetic resources and screening breeding lines for snow mold resistance is routinely carried-out in breeding programs, and testing methods in artificially infested plots or under controlled conditions have been developed (Nakajima and Abe, 1990; Iriki *et al.*, 2002; Ergon *et al.*, 2003).

The objectives of the present study were to determine the resistance to *M. nivale* in winter wheat cultivars in the field and to investigate the effects of *M. nivale* on yield and yield components.

### MATERIALS AND METHODS

**Field experiment:** The snow mold resistance of 38 winter wheat cultivars of different genetic background and origin was tested in a field experiment at Erzurum, Turkey (29°55'N and 41°16'E and 1850 m above sea level) in the 2002-2003. (Table 1). The experimental soil was clay loam with an organic matter content of 1.7-1.9 percent and pH of 7.8. Available P and K contents were 17.3 and 1830.3 kg ha<sup>-1</sup> respectively. The weather data (maximum and minimum snow depth, rainfall and temperature) were recorded at the meteorological station at Atatürk University Agricultural Experiment Station (Figure 1).

Cultivars were seeded (100 seeds per plot) on 15 September. Each plot consisted of a single row of plants with 10 cm spacing between plants in each row and 35 cm between rows. Inoculated blocks were separated from non-inoculated by a 2 m-wide border plot. Inoculated and non-inoculated wheat seeds were planted in a randomized complete block design in a split-plot configuration with three replications. Fertilizers (N – P) were applied before sowing at a rate of 60 – 50 kg ha<sup>-1</sup>. Seedling emergence data were recorded on 28 April 2003.

**Inoculum preparation:** A single-spore isolate of *M. nivale* (ED-46) used in this experiment was obtained from winter wheat and was observed as highly aggressive on wheat seedlings (Demirci and Dane, 2003). This isolate was maintained on potato dextrose agar (PDA) at 10°C and subcultured regularly. Inoculum was prepared by inoculating 50 g of a mixture of sand and maize flour

(98 sand + 2 maize flour) with one mycelial disk (1 cm diameter) and then incubating for 1 month at 15°C. The mixture was wetted to 20% moisture content and autoclaved twice prior to inoculation (Papavizas and Ayers, 1965). Inoculum was spread on the soil surface at a rate of 12 g m<sup>-2</sup> in all experiments (Nakajima and Abe, 1990).

**Agronomic performance:** All inoculated and non-inoculated (control) plants were individually hand-harvested on August 19, 2003. The grain yield, 1000-kernel weight, plant height, number of kernels per spike and number of spikes per m<sup>2</sup> were determined.

**Statistical analyses:** Emergence under field conditions and agronomic performances was subjected to analysis of variance (ANOVA) with the MSTAT-C (1991) software package, and differences among means determined by Duncan's multiple range test.

## RESULTS AND DISCUSSION

During 2002-2003, the winter conditions favoured snow mold development. A durable snow cover promoted development of pink snow mold and orange/pinkish colored spots were observed on the leaves of collapsed plants in the spring after snow melt.

Significant differences in resistance to pink snow mold were found among the 38 genotypes in this experiment (Table 1). Inoculation of seeds with *M. nivale* resulted in decreased seedling emergence of winter wheat ( $p < 0.01$ ) compared with the non-inoculated plants. Emergence of non-inoculated plants varied from 65.7 to 100% and inoculated plants ranged from 4.3 to 50.7%. The most resistant winter wheat cultivars were Harmankaya and Pehlivan and the most susceptible ones were Aytin-97, Kırgız-95 and Bayraktar (Table 1). Decreased seedling emergence was observed for Harmankaya, Pehlivan, Aytin-97, Kırgız-95 and Bayraktar by 47.0, 47.2, 90.2, 90.2, and 95.3% in inoculated plants, respectively.

Cultivars differed significantly for grain yield and all yield components, except number of kernels per spike and 1000-kernel weight, thus demonstrating their genetic differences in the yield potential (Table 2). No difference between inoculated mean number of kernels per spike (33.8 and 37.9 respectively) and 1000-kernel weight, and non-inoculated (31.9 and 38.4 respectively) plants were observed. Compared with non-inoculated plants, grain yield and plant height decreased in the inoculated plants for all genotypes. The number of spikes per m<sup>2</sup> of non-inoculated plants varied from 201.7 to 316.7 and inoculated plants ranged from 28.3 to 140.0. *Microdochium nivale* decreased the number of spikes per m<sup>2</sup> by 71.1%. The grain yield of non-inoculated plants varied from 195.7 to 327.5 kg da<sup>-1</sup> and inoculated plants ranged from 53.6 to 11.3 kg da<sup>-1</sup> for a decrease of 67.3%.

The height of non-inoculated plants varied from 32.2 to 91.9 cm and inoculated plants ranged from 26.8 to 79.7 cm for a decrease of 13.2%.

Winter conditions favoured snow mold development in 2002-2003 (Figure 1). Mean temperatures ranged from -12 to + 20°C. Incubation temperature is a critical factor for resistance testing. Temperatures ranging from 0-1°C (Bruehl *et al.*, 1966) to 15-18°C (Nakajima and Abe, 1990) under controlled conditions in wheat were reported. The importance of temperature in the development of pink snow mold caused by *M. nivale* was emphasized by Miedaner *et al.* (1993). In 2002-2003 snow cover appeared in the middle of December and disappeared in the beginning of April. This could be the reason for the more severe pink snow mold at Erzurum. Snow cover is an important factor in the extent of damage caused by snow mold (Matsumoto and Nissinen, 2001).

The reaction of the 38 winter wheat cultivars to *M. nivale* is presented in Table 1. The inoculation seeds trial indicated that *M. nivale* decreased seedling emergence on winter wheat cultivars ( $p < 0.01$ ), compared with the non-inoculated plants. The disease decreased the seedling emergence of 38 winter wheat cultivars by 73.6% in inoculated plants. Previous study has reported that *M. nivale* is often associated with reduced emergence (Humphreys *et al.*, 1995). In this study, we used sand inoculum (maize flour-sand) and inoculum was spread on the soil surface. Tronsmo *et al.* (2001) reported that for the pink snow mold pathogen, an important means of dissemination and infection is seed-borne inoculum.

Among the 38 winter wheat cultivars, the most resistant winter wheat cultivars were Harmankaya and Pehlivan, and the most susceptible ones were Aytin-97, Kırgız-95 and Bayraktar (Table 1). Susceptible wheat cultivars "Aytin-97", "Kırgız-95" and "Bayraktar" showed higher emergence in non-inoculated plots and lower emergence in inoculated plots, while some cultivars e.g. Gün-91, Hawk (Şahin), Katea-1, Kırac-66, Tir and Yayla-305 showed lower emergence in non-inoculated plots and higher emergence in inoculated plots than the susceptible cultivars. This result would be explained by the level of seed vigor. The high-vigor seeds germinate uniformly and then better field performance and higher yield are expecting. Varietal differences in snow mold resistance exist among winter cereals, and many breeding programs have sought to incorporate snow mold resistance into adapted lines (Iriki *et al.*, 2001b). Artificial inoculation and screening for resistance have revealed significant genetic variation in resistance to *M. nivale* in both winter rye and winter wheat (Miedaner *et al.*, 1993; Hömmö, 1994; Nakajima and Abe, 1990; 1994; Maurin *et al.*, 1996; Iriki *et al.*, 2002; Ergon *et al.*, 2003). Field experiments are most often employed in screening *M. nivale* resistance for winter wheat cultivars.

However, the results from field experiments are often influenced by the prevailing environmental conditions, and many test years and locations are usually needed to reliably assess the *M. nivale* resistance of cultivars (Nakajima and Abe, 1996).

**Table 1. Names and origins of 38 wheat cultivars tested in experiment, their emergence and plant height**

Name of cultivar	Origin	Emergence (%)		Plant height (cm)	
		NI <sup>1</sup>	I	NI <sup>1</sup>	I
Aksel-2000	Ankara, Turkey	91.0	38.0	54.4	58.2
Alparslan	Erzurum, Turkey	100.0	41.7	69.3	56.3
Atay-85	Eskişehir, Turkey	82.0	10.0	55.5	36.0
Aytin-97	Eskişehir, Turkey	92.3	9.0	47.2	54.8
Bayraktar	Ankara, Turkey	92.0	4.3	69.2	65.1
Bezostoja-1	Russia	91.7	27.3	70.9	55.7
Bolal-2973	Eskişehir, Turkey	91.0	30.3	70.4	64.7
Dağdaş-94	Konya, Turkey	77.3	11.7	70.3	68.4
Demir-2000	Ankara, Turkey	75.7	31.7	69.9	50.7
Doğu-88	Erzurum, Turkey	96.7	38.3	60.7	55.1
Gerek-79	Eskişehir, Turkey	80.0	14.3	71.7	56.4
Golia	Italy	75.7	15.7	32.2	26.8
Gün-91	Ankara, Turkey	72.3	22.3	61.3	52.4
Harmankaya	Eskişehir, Turkey	95.7	50.7	58.6	55.3
Hawk (Şahin)	A.B.D.	65.7	20.0	55.0	54.9
Haymana	Ankara, Turkey	84.7	12.7	78.3	54.9
İkizce-96	Ankara, Turkey	89.0	22.7	68.9	63.3
Karasu-90	Erzurum, Turkey	86.3	15.7	72.4	68.2
Katea-1	Bulgaria	72.7	21.0	47.0	50.2
Kınacı-97	Konya, Turkey	93.3	21.7	59.8	54.7
Kıraç-66	Eskişehir, Turkey	71.7	11.0	67.2	51.1
Kırgız-95	Eskişehir, Turkey	92.3	9.0	76.6	63.9
Kirik	Erzurum, Turkey	83.3	35.7	91.9	66.3
Kutluk-94	Eskişehir	85.7	14.0	66.4	53.0
Lancer	A.B.D.	96.7	15.7	87.6	61.3
Mızrak	Ankara, Turkey	90.3	17.7	58.7	53.5
Nenehatun	Erzurum, Turkey	94.0	40.3	66.2	57.7
Palandöken	Erzurum, Turkey	84.7	31.7	62.6	48.6
Pehlivan	Edirne, Turkey	83.3	44.0	51.0	51.0
Prostor	Edirne, Turkey	94.0	36.0	54.6	54.0
Sultan-95	Eskişehir, Turkey	86.3	12.3	60.5	42.9
Süzen-97	Eskişehir, Turkey	94.3	14.3	78.5	71.1
Tir	Van, Turkey	72.0	24.0	76.0	79.7
Türkmen-98	Ankara, Turkey	89.3	17.0	71.2	66.4
Uzunyayla	Ankara, Turkey	96.7	15.7	77.9	61.9
Yakar-99	Ankara, Turkey	85.7	33.0	59.8	59.5
Yayla-305	Eskişehir, Turkey	71.0	20.7	73.8	54.1
Yıldız-98	Eskişehir, Turkey	88.3	12.3	51.6	48.3
Mean		85.9	22.7	65.1	56.5
F- values					
Treatments (T)		1330.47***		100.39**	
Genotypes (G)		3.81***		8.04***	
T x G		2.45***		1.56*	
Lsd (T x G)		21.55		13.64	
Cv (%)		18.62		13.90	

<sup>1</sup>NI: non-inoculated, I: inoculated plots.

Duncan's multiple range test P < 0.05; significant at \*0.05, \*\*0.01 and \*\*\*0.001 levels

Table 2. Means of kernels per spike, spikes per m<sup>2</sup>, 1000 kernel weight and grain yield of 38 wheat cultivars

	Kernels per spike		Spikes per m <sup>2</sup>		1000 kernel weight (g)		Grain yield (kg da <sup>-1</sup> )	
	NI <sup>1</sup>	I	NI <sup>1</sup>	I	NI <sup>1</sup>	I	NI <sup>1</sup>	I
Aksel-2000	21.8	30.6	295.0	91.7	35.1	34.4	271.7	53.6
Alparslan	33.2	39.1	281.7	140.0	34.1	33.6	260.5	79.8
Atay-85	40.7	36.8	201.7	46.7	40.3	38.4	225.8	55.1
Aytin-97	23.0	22.5	235.0	46.7	37.2	36.3	284.2	66.0
Bayraktar	29.3	34.5	270.0	110.0	40.6	41.4	224.5	104.7
Bezostoja-1	32.3	36.6	250.0	63.3	41.4	40.6	245.0	99.4
Bolal-2973	36.4	32.8	216.7	105.0	40.1	37.0	215.5	106.7
Dağdaş-94	36.1	21.0	248.3	33.0	41.1	40.0	233.0	87.0
Demir-2000	32.4	48.9	243.3	78.3	42.5	42.9	256.7	83.2
Doğu-88	25.9	36.2	290.0	118.3	33.3	35.8	277.7	92.7
Gerek-79	31.9	37.2	256.7	66.7	37.2	36.1	240.2	88.8
Golia	36.6	20.1	216.7	35.0	27.7	25.0	241.3	100.3
Gün-91	41.7	39.0	281.7	71.7	38.1	37.2	258.3	71.4
Harmankaya	35.2	39.3	265.0	101.7	41.7	41.3	293.4	114.3
Hawk (Şahin)	35.2	42.5	228.3	86.7	34.4	38.7	235.3	93.1
Haymana	33.3	33.5	260.0	51.7	37.6	44.4	240.2	67.0
İkizce-96	33.7	22.1	280.0	50.0	34.6	36.9	221.5	88.1
Karasu-90	30.1	39.9	236.7	28.3	38.6	36.9	227.6	91.0
Katea-1	31.4	37.5	201.7	58.3	35.6	33.1	222.7	62.7
Kınacı-97	39.1	39.6	266.7	66.7	35.2	38.2	247.6	58.5
Kıraç-66	30.7	22.9	206.7	28.3	37.7	37.9	195.7	78.1
Kırgız-95	37.4	45.9	243.3	46.7	40.4	37.9	266.9	76.3
Kırık	16.4	18.1	280.0	123.3	37.8	39.6	225.0	85.4
Kutluk-94	31.1	30.0	255.0	60.0	40.9	43.2	204.0	48.1
Lancer	30.2	39.3	268.3	61.7	35.3	33.9	327.5	77.7
Mızrak	33.6	33.0	273.3	51.7	35.5	40.3	245.3	57.7
Nenehatun	30.0	46.2	286.7	111.7	37.7	40.8	265.1	86.9
Palandöken	32.4	39.4	300.0	91.7	38.5	37.6	246.2	65.7
Pehlivan	25.7	31.4	233.3	105.0	40.1	41.0	204.6	93.9
Prostor	22.5	24.9	233.3	96.7	41.0	38.0	213.7	64.3
Sultan-95	33.0	28.3	236.7	28.3	41.9	38.0	231.5	57.0
Süzen-97	35.4	40.0	316.7	60.0	36.4	40.8	299.4	94.4
Tir	30.4	28.1	226.7	86.7	48.9	54.6	202.1	72.5
Türkmen-98	36.3	36.6	251.7	108.3	41.4	39.0	257.7	105.7
Uzunyayla	38.0	22.2	265.0	75.0	36.0	38.5	256.9	58.3
Yakar-99	27.4	34.3	251.7	95.0	34.2	34.9	201.9	68.3
Yayla-305	26.6	32.3	246.7	73.3	36.8	39.1	217.7	48.8
Yıldız-98	37.3	41.9	258.3	35.0	34.6	34.1	215.7	105.4
Mean	31.9	33.8	254.2	73.4	37.9	38.4	242.1	79.2
F- values								
Treatments (T)	0.97		149.39**		32.19*		1036.39**	
Genotypes (G)	4.95***		2.00**		12.25***		2.39***	
T x G	2.52***		0.93		1.49		1.88**	
Lsd (T x G)	12.90						62.35	
Cv (%)	18.41		25.16		6.98		18.22	

<sup>1</sup>NI: non-inoculated, I: inoculated plots.

Duncan's multiple range test P &lt; 0.05; significant at \*0.05, \*\*0.01 and \*\*\*0.001 levels

The most practical control for snow molds in winter wheat is through the development of resistant cultivars (Nakajima and Abe, 1996; Iriki *et al.*, 2001b; 2002). In the present study, the most resistant winter wheat cultivars observed were developed in Turkey (Harmankaya and Pehlivan). In addition, high levels of resistance have been reported for some populations of wheat developed in Turkey (Bruehl, 1982; Iriki *et al.*, 2001a).

*Microdochium nivale* inoculation displayed significant effect on agronomic performances (Table 2). Mean number of kernels per spike and 1000-kernel weight, no difference between inoculated (33.8 and 37.9 respectively) and non-inoculated (31.9 and 38.4 respectively) plants were observed. However, compared with non-inoculated plants, grain yield, and plant height

was decreased in the inoculated plants for all genotypes. The *M. nivale* decreased the number of spikes per m<sup>2</sup>, the grain yield and the plant height 71.1, 67.3 and 13.2% respectively. Little has been published on the effect of *M. nivale* on the components of grain yield. In 1 year of field trials, significant correlations occurred between the extent of *M. nivale* seed infection, the number of spikes per m<sup>2</sup> and the grain yield for nine untreated wheat seedlots (Humphreys *et al.*, 1995) and six untreated oat cultivars (Humphreys *et al.*, 1998).

In conclusion, varietal differences in the resistance to *m. nivale* were confirmed. However, this needs further research. the present study is significant in that it is the first demonstration of resistance to pink snow mold caused by *m. nivale* in turkey.

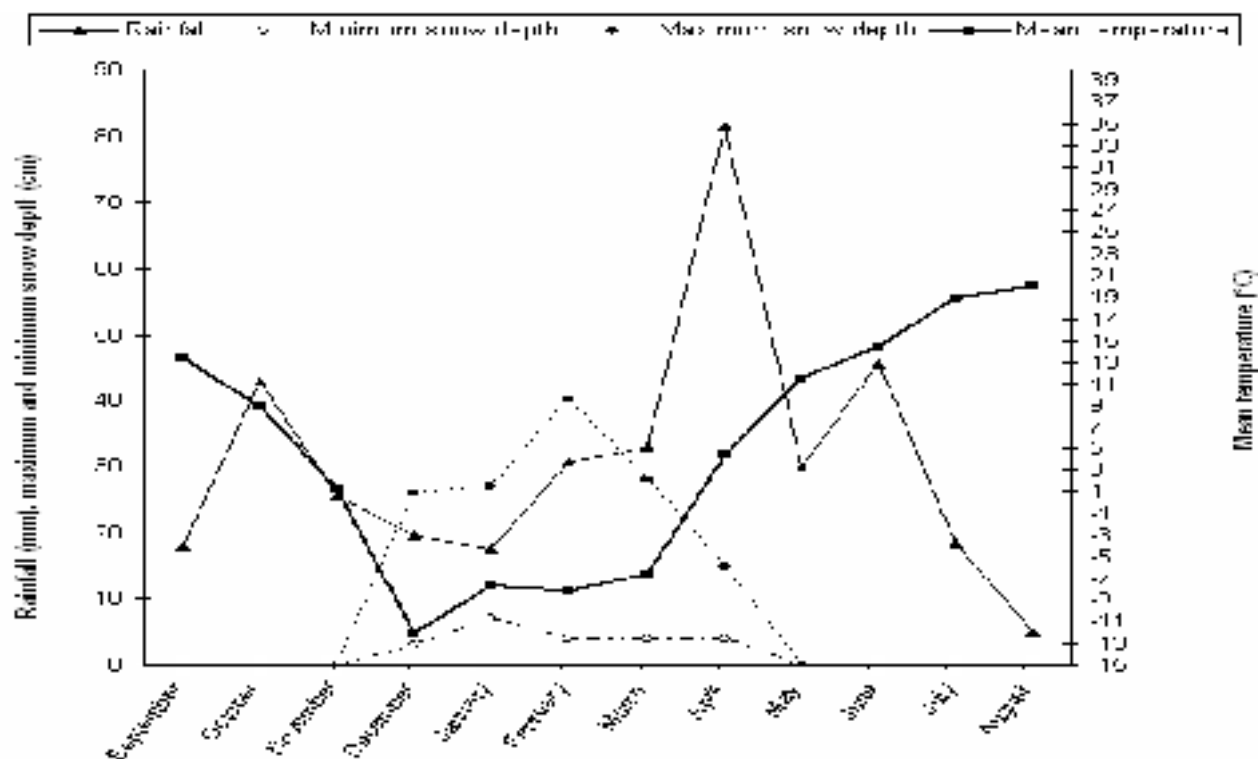


Figure 1. Summary of weather data at Erzurum, Turkey during the field experiment in 2002–2003.

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