

## SHORT COMMUNICATION

# Seedling Resistance to *Stagonospora nodorum* Blotch in Wheat Genotypes

MÓNIKA CSÉPLŐ<sup>1</sup>, MÁRIA CSÓSZ<sup>2</sup>, MARIANN GÁL<sup>1</sup>, OTTÓ VEISZ<sup>1</sup> and GYULA VIDA<sup>1</sup>

<sup>1</sup>Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary;

<sup>2</sup>Cereal Research Non-Profit, Ltd., Szeged, Hungary

### Abstract

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In two independent experiments set up in the greenhouse the seedling resistance to *Stagonospora nodorum* blotch was investigated in 92 varieties, breeding lines and genotypes with a known genetic background. The greatest area under the disease progress curve calculated from lesion type was 37.06, while in the case of the most resistant genotype this value was 0.38. Many of the lines and varieties bred in Martonvásár proved to have excellent resistance in terms of both percentage of infected leaf area and lesion type. Observations indicate that, depending on the aim of the experiment, the efficient selection of breeding lines is possible in the seedling stage either on the basis of the area under the disease progress curve calculated for lesion types, or on the basis of lesion types scored 7, 11 or 14 days after inoculation.

**Keywords:** area under the disease progress curve; disease resistance; *Phaeosphaeria nodorum*; *Triticum aestivum*

Pathogens causing leaf spots in wheat have been known in Hungary for many years (MEZEY 1899; KEPES & TÓTHNÉ 1975; CSÓSZ 2007). Under Hungarian conditions they may cause yield losses of up to 25% (Csósz 2006). The microscopic description of the fungus, considered to be a dangerous pathogen in Hungary, was reported by MESTERHÁZY (1974), together with a description of the disease. Several authors have noted differences in susceptibility between Hungarian varieties in the case of natural infection with *Septoria* spp. (AUGUSZTA *et al.* 1987; FOLLÁRDT & BARKÓ 1994).

Many authors (VAN GINKEL & RAJARAM 1999; FENG *et al.* 2004; LIU *et al.* 2004; SINGH *et al.* 2006,

2007; ALI *et al.* 2008) agreed that under optimum growing conditions the most effective defence against the pathogen was to use sources resistant to several pathogens in breeding. If the resistance of the genotypes is to be reliably tested, a method of artificial inoculation involving a special nursery is required, where satisfactory conditions can be created for the development of the pathogen, allowing resistance to be evaluated even when little or no pathogen attack occurs under natural conditions.

Greenhouse tests on seedlings have the advantage that a large number of genotypes or breeding lines can be tested under controlled conditions in

a short time (FENG *et al.* 2004; PALICOVÁ-ŠÁROVÁ & HANZALOVÁ 2006). A further advantage is that they allow tests to be made on resistance to a single pathogen. In the field, competition may arise between various pathogens (ENGLE *et al.* 2006). This is not a problem if the pathogens induce different symptoms (AL NAIMI *et al.* 2005), but the glume and leaf spot symptoms of *Stagonospora nodorum* blotch are difficult to distinguish from those of *Septoria* leaf blotch and from those of tan spot (BHATHAL *et al.* 2003; ENGLE *et al.* 2006; Csősz 2007).

No data are yet available to breeders on the seedling resistance of Hungarian cultivars to *Stagonospora nodorum*. The primary objective of this study was thus to determine the resistance of Hungarian wheat varieties and breeding lines and of genotypes with known genetic background in the seedling stage after artificial inoculation with *Stagonospora nodorum*. A second objective was to investigate the utilization of the various scoring methods in a wheat resistance breeding programme.

Two independent experiments were set up in the greenhouse of the Agricultural Research Institute of the Hungarian Academy of Sciences. A total of 92 varieties, breeding lines and genotypes with known genetic backgrounds were examined by sowing 20 seeds of each variety in two replications in 15 cm diameter pots filled with a 2:1 ratio of soil and sand. The seedlings were grown at a day/night temperature of 22/18°C with a 16-h day length. The mixture of wheat-derived isolates on a small quantity of infected wheat kernels was kindly provided by Dr. H. Walter, Grünbach, Germany. The inoculum was produced first on SNA (Spezieller Nährstoffarmer Agar) medium (NIRENBERG 1976) at 14–16°C under near-UV light. Sterilized wheat kernels in Erlenmeyer flasks were inoculated with a few drops of a spore suspension and then kept at room temperature for two weeks, before placing them in a vernalization chamber at 4°C for 2–4 weeks for sporulation. The conidia washed off from infected wheat grains were used for artificial inoculation (GÁL & OETTLER 2003). The number of conidia required for inoculation ( $10^6$  conidia/ml) was adjusted under a light microscope by means of Bürker chamber counts. The seedlings were watered and fertilized as needed (Volldünger, 10 g/m<sup>2</sup>). The plants were inoculated at the two-leaf stage with *Stagonospora nodorum* by spraying the spore suspension until runoff using a backpack sprayer. In order to promote infection, the plants were covered with polythene bags for 48 h,

after which the 80–90% relative humidity required for pathogen development was ensured using a humidifier (Netafim, Coolnet Pro; droplet size: 65 micron at 4.0 bar). The genotypes were evaluated at 7, 11 and 14 days after inoculation, scoring the percentage of infected leaf area as described by JAMES (1971 cit. STUBBS *et al.* 1986) and the SNB lesion types using the 0–5 scale described by LIU *et al.* (2004), where 0 = absence of visible lesions, highly resistant (HR); 1 = few penetration points, with lesions consisting of flecking or small dark spots, resistant (R); 2 = lesions consisting of dark spots with little surrounding necrosis or chlorosis, moderately resistant (MR); 3 = dark lesions completely surrounded by necrosis or chlorosis, lesions 2–3 mm, moderately susceptible (MS); 4 = larger necrotic or chlorotic lesions, 4 mm or greater, with little coalescence, susceptible (S); and 5 = large coalescent lesions with very little green tissue remaining; highly susceptible (HS). Plants having equal numbers of two different lesion types were given an intermediate value (e.g. 1.5 in the case of lesion types 1 and 2). The classification of the wheat genotypes into resistance types was based on the mean values of the two experiments, using the following limit values: HR:  $x \leq 0.5$ ; R:  $0.5 < x \leq 1.5$ ; MR:  $1.5 < x \leq 2.5$ ; MS:  $2.5 < x \leq 3.5$ ; S:  $3.5 < x \leq 4.5$ ; HS:  $x > 3.5$  ( $x$  = mean value of lesion type).

The values recorded at different dates were used to calculate the area under the disease progress curve (AUDPC) as described by SHANER and FINNEY (1977). The genotypes were classified into susceptibility groups based on the AUDPC values calculated for lesion types. Varieties and breeding lines with AUDPC values between 0 and 5 were classified as highly resistant, > 5–15 as resistant, > 15–25 as moderately resistant, > 25–35 as moderately susceptible, > 35–45 as susceptible and above 45 as highly susceptible.

The AUDPC values calculated for the lesion types were converted to relative area under the disease progress curve (RAUDPC) values as described by JENKINS and JONES (2003) and modified by GERGELY (2004). According to their RAUDPC values the wheat genotypes were categorised into resistant (R = 0.00–0.20), moderately resistant (MR = 0.21–0.40), moderately susceptible (MS = 0.41–0.60), susceptible (S = 0.61–0.80) and highly susceptible (HS = 0.81–1.00) classes.

The data were evaluated using the MSTAT-C program package (Michigan State University, East Lansing, USA) and with the Analysis Tool Pack

module of the Microsoft Excel software (correlation analysis; Microsoft Corporation, Redmond, USA).

The lack of significant differences between the two independent greenhouse experiments was proved by ANOVA in the case of the AUDPC values calculated for the lesion types and for the percentage of infected leaf area (Table 1). Though significant  $G \times E$  interactions were found for both leaf area and lesion type (Table 1), the order of the most resistant genotypes in the experiments did not change (Table 2). The effect of the experiment was only significant on the 11<sup>th</sup> and 14<sup>th</sup> days. However, the  $G \times E$  interaction did not influence either the lesion type or the infected leaf area values on the 11<sup>th</sup> day. The effect of the genotype was clearly demonstrable for all the traits evaluated.

The AUDPC values calculated from the SNB lesion type showed that of the 92 genotypes three were susceptible, 36 moderately susceptible and 38 moderately resistant. The group of resistant varieties included 7 Martonvásár breeding lines (Mv06-09, Mv15-09, Mv22-09, Mv26-09, Mv19-09, Mv334-09, Mv336-09), three varieties bred in Martonvásár (Mv Béres, Mv Kolompos and Mv Bodri) and the resistant control Atlas 66. Mv Zelma, Mv18-09 and Mv327-09 proved to have excellent resistance, while the penetration of the pathogen into the leaf surface was only detected in traces for line Mv326-09 (Table 2).

Significant differences in the degree of infection were observed between the genotypes. Based on

the AUDPC values for percentage of infected leaf area, 16.3% of the genotypes exhibited significantly greater infection than the mean, 71.7% average infection and 12.0% significantly weaker infection than the mean of the experiment (110.95). The genotypes found to have above-average resistance included Atlas 66, which is held to be a source of resistance to this pathogen (REES & PLATZ 1990), and a number of lines bred in Martonvásár (Mv06-09, Mv15-09, Mv18-09, Mv22-09, Mv26-09, Mv326-09, Mv327-09, Mv334-09, Mv336-09), together with the variety Mv Zelma (Table 2).

While the percentage of infected leaf area was very low for these genotypes even on the 14<sup>th</sup> day after inoculation (the last scoring date) being 6% for Atlas 66, and less than 1% for the breeding lines Mv18-09, Mv326-09 and Mv327-09, the susceptible check variety, ND495 (LIU *et al.* 2004) had a value of 66% (Table 2).

The average disease progress curves calculated on the basis of SNB lesion types for the wheat varieties and lines in various susceptibility groups could be clearly distinguished from each other (Figure 1). On the 7<sup>th</sup> day after inoculation, only traces of necrosis induced by the pathogen could be observed on the highly resistant varieties, while the average level of infection on susceptible varieties had reached 3 on the 0–5 scale. The infection continued to spread more slowly in HR varieties up to the 11<sup>th</sup> day, while the disease progress curves of all the other groups ran parallel to each other.

Table 1. Two-way analysis of variance on the lesion type and percentage of infected leaf area data (mean square values, Martonvásár, 2009)

Source of variability		Experiment (E)	Genotype (G)	E × G	Error
Degrees of freedom		1	91	91	184
Lesion type	AUDPC	110.826 <sup>ns</sup>	276.904 <sup>***</sup>	46.843 <sup>*</sup>	31.639
	7 <sup>th</sup> day	0.011 <sup>ns</sup>	2.732 <sup>***</sup>	0.586 <sup>*</sup>	0.424
	11 <sup>th</sup> day	4.680 <sup>***</sup>	2.548 <sup>***</sup>	0.517 <sup>ns</sup>	0.394
	14 <sup>th</sup> day	6.471 <sup>***</sup>	232.943 <sup>***</sup>	40.239 <sup>**</sup>	49.770
PILA (%)	AUDPC	3234.163 <sup>ns</sup>	37 803.495 <sup>***</sup>	7064.366 <sup>***</sup>	4286.346
	7 <sup>th</sup> day	6.445 <sup>ns</sup>	216.067 <sup>***</sup>	48.569 <sup>**</sup>	30.589
	11 <sup>th</sup> day	27.995 <sup>ns</sup>	489.866 <sup>***</sup>	93.493 <sup>ns</sup>	77.826
	14 <sup>th</sup> day	258.285 <sup>ns</sup>	841.653 <sup>***</sup>	166.646 <sup>*</sup>	113.007

AUDPC – area under the disease progress curve; PILA – percentage of infected leaf area, in the case of mean square values followed by \*, \*\* and \*\*\*; the *F*-test revealed significant differences in the dataset at  $P = 0.05$ ,  $P = 0.01$  and  $P = 0.001$ , respectively; <sup>ns</sup>non-significant

Table 2. Seedling resistance of wheat genotypes to *Stagonospora nodorum* on the basis of lesion types, the percentage of infected leaf area and the resistance types using various methods of evaluation (Martonvásár, 2009)

Genotype	Lesion type					PILA (%)			
	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day	RAUDPC	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day
Mv326-09	0.38 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.25 <sup>a</sup>	0.01 <sup>b</sup>	0.04	0.00	0.00	0.03
Mv18-09	2.81 <sup>a</sup>	0.13 <sup>a</sup>	0.13 <sup>a</sup>	1.13 <sup>b</sup>	0.08 <sup>b</sup>	1.29	0.01	0.01	0.79
Mv Zelma	4.13 <sup>a</sup>	0.00 <sup>a</sup>	0.75 <sup>b</sup>	1.00 <sup>b</sup>	0.11 <sup>b</sup>	6.46	0.00	0.76	2.53
Mv327-09	4.81 <sup>a</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	0.88 <sup>b</sup>	0.13 <sup>b</sup>	0.25	0.00	0.05	0.05
Mv26-09	5.75 <sup>b</sup>	0.00 <sup>a</sup>	1.00 <sup>b</sup>	1.50 <sup>b</sup>	0.16 <sup>b</sup>	4.00	0.00	0.28	2.03
Mv336-09	6.00 <sup>b</sup>	0.13 <sup>a</sup>	0.88	1.50 <sup>b</sup>	0.16 <sup>b</sup>	5.39	0.01	0.29	2.88
Atlas66	7.44 <sup>b</sup>	0.13 <sup>a</sup>	1.13 <sup>b</sup>	1.88 <sup>c</sup>	0.20 <sup>b</sup>	15.78	0.01	1.91	6.01
Mv22-09	8.19 <sup>b</sup>	0.38 <sup>a</sup>	1.00 <sup>b</sup>	1.75 <sup>c</sup>	0.22 <sup>c</sup>	9.36	0.14	0.79	3.90
Mv06-09	9.63 <sup>b</sup>	0.63 <sup>b</sup>	1.13 <sup>b</sup>	1.50 <sup>b</sup>	0.26 <sup>c</sup>	10.66	0.06	1.88	2.50
Mv334-09	10.25 <sup>b</sup>	0.75 <sup>b</sup>	1.00 <sup>b</sup>	1.75 <sup>c</sup>	0.28 <sup>c</sup>	8.04	0.03	1.29	2.26
Mv Béres	11.88 <sup>b</sup>	0.63 <sup>b</sup>	1.50 <sup>b</sup>	2.13 <sup>c</sup>	0.32 <sup>c</sup>	24.31	0.79	3.29	5.65
Mv19-09	12.38 <sup>b</sup>	0.75 <sup>b</sup>	1.50 <sup>b</sup>	2.00 <sup>c</sup>	0.33 <sup>c</sup>	22.51	1.28	2.54	4.41
Mv Kolompos	13.13 <sup>b</sup>	0.75 <sup>b</sup>	1.50 <sup>b</sup>	2.50 <sup>c</sup>	0.35 <sup>c</sup>	25.59	1.01	2.76	6.90
Mv15-09	13.88 <sup>b</sup>	1.13 <sup>b</sup>	1.50 <sup>b</sup>	1.63 <sup>c</sup>	0.37 <sup>c</sup>	11.64	0.16	2.03	2.51
Mv Bodri	14.19 <sup>b</sup>	0.63 <sup>b</sup>	2.00 <sup>c</sup>	2.50 <sup>c</sup>	0.38 <sup>c</sup>	28.02	0.29	3.00	10.63
Mv23-09	15.13 <sup>c</sup>	0.75 <sup>b</sup>	2.13 <sup>c</sup>	2.38 <sup>c</sup>	0.41 <sup>d</sup>	25.17	0.50	3.51	6.75
Mv Lucia	15.88 <sup>c</sup>	0.75 <sup>b</sup>	2.13 <sup>c</sup>	2.88 <sup>d</sup>	0.43 <sup>d</sup>	42.37	1.26	3.43	15.63
Mv Hombár	16.63 <sup>c</sup>	0.75 <sup>b</sup>	2.50 <sup>c</sup>	2.50 <sup>c</sup>	0.45 <sup>d</sup>	41.76	0.53	6.50	10.75
Veranopolis	17.06 <sup>c</sup>	1.13 <sup>b</sup>	2.25 <sup>c</sup>	3.00 <sup>d</sup>	0.46 <sup>d</sup>	78.47	2.26	10.03	20.63
Mv Mambo	17.19 <sup>c</sup>	0.88 <sup>b</sup>	2.25 <sup>c</sup>	3.00 <sup>d</sup>	0.46 <sup>d</sup>	40.28	0.30	4.88	14.38
Mv333-09	17.25 <sup>c</sup>	1.25 <sup>b</sup>	2.00 <sup>c</sup>	2.25 <sup>c</sup>	0.47 <sup>d</sup>	46.59	1.94	4.64	13.14
Mv Ködmön	17.44 <sup>c</sup>	0.88 <sup>b</sup>	2.38 <sup>c</sup>	2.88 <sup>d</sup>	0.47 <sup>d</sup>	39.67	0.79	5.28	11.25
Mv Laura	17.75 <sup>c</sup>	1.13 <sup>b</sup>	2.13 <sup>c</sup>	2.75 <sup>d</sup>	0.48 <sup>d</sup>	52.58	0.54	5.88	19.38
Mv05-09	17.94 <sup>c</sup>	1.13 <sup>b</sup>	2.13 <sup>c</sup>	2.88 <sup>d</sup>	0.48 <sup>d</sup>	34.56	0.31	4.03	12.50
Mv Gyémánt	18.19 <sup>c</sup>	1.25 <sup>b</sup>	2.00 <sup>c</sup>	2.88 <sup>d</sup>	0.49 <sup>d</sup>	25.58	0.65	2.00	10.00
Mv Kolo	18.56 <sup>c</sup>	1.13 <sup>b</sup>	2.25 <sup>c</sup>	3.00 <sup>d</sup>	0.50 <sup>d</sup>	59.04	1.53	7.78	15.63
GK Ati	19.38 <sup>c</sup>	1.38 <sup>b</sup>	2.25 <sup>c</sup>	2.63 <sup>d</sup>	0.52 <sup>d</sup>	44.02	1.04	4.63	14.75
Mv28-09	19.44 <sup>c</sup>	1.25 <sup>b</sup>	2.25 <sup>c</sup>	3.13 <sup>d</sup>	0.52 <sup>d</sup>	47.04	1.04	5.65	14.38
Mv Suba	19.50 <sup>c</sup>	1.38 <sup>b</sup>	2.13 <sup>c</sup>	3.00 <sup>d</sup>	0.53 <sup>d</sup>	37.64	1.04	3.50	13.13
Mv Magdaléna	19.81 <sup>c</sup>	1.38 <sup>b</sup>	2.00 <sup>c</sup>	2.50 <sup>c</sup>	0.53 <sup>d</sup>	36.04	1.04	4.65	9.38
Mv24-09	20.13 <sup>c</sup>	1.25 <sup>b</sup>	2.50 <sup>c</sup>	3.00 <sup>d</sup>	0.54 <sup>d</sup>	54.08	1.15	7.75	13.75
Mv329-09	20.25 <sup>c</sup>	1.25 <sup>b</sup>	2.38 <sup>c</sup>	3.38 <sup>d</sup>	0.55 <sup>d</sup>	52.08	0.54	6.00	18.75
Mv Menüett	20.56 <sup>c</sup>	1.63 <sup>c</sup>	2.25 <sup>c</sup>	2.50 <sup>c</sup>	0.55 <sup>d</sup>	71.13	3.39	8.25	15.75
GK Petur	21.25 <sup>c</sup>	1.50 <sup>b</sup>	2.38 <sup>c</sup>	3.13 <sup>d</sup>	0.57 <sup>d</sup>	60.72	1.76	7.03	17.63
Mv Csárdás	21.94 <sup>c</sup>	1.63 <sup>c</sup>	2.38 <sup>c</sup>	3.13 <sup>d</sup>	0.59 <sup>d</sup>	81.70	2.28	12.00	18.13
Fatima2	22.19 <sup>c</sup>	1.63 <sup>c</sup>	2.50 <sup>c</sup>	3.00 <sup>d</sup>	0.60 <sup>d</sup>	69.06	2.88	8.25	16.25
Mv12-09	22.31 <sup>c</sup>	1.63 <sup>c</sup>	2.38 <sup>c</sup>	3.38 <sup>d</sup>	0.60 <sup>d</sup>	86.63	2.00	10.63	25.63
M-3	22.38 <sup>c</sup>	1.50 <sup>b</sup>	2.75 <sup>d</sup>	3.00 <sup>d</sup>	0.60 <sup>d</sup>	65.13	1.50	8.75	17.50
Mv328-09	22.56 <sup>c</sup>	1.50 <sup>b</sup>	2.75 <sup>d</sup>	3.13 <sup>d</sup>	0.61 <sup>e</sup>	60.33	1.78	9.63	11.25

Table 2 to be continued

Genotype	Lesion type					PILA (%)			
	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day	RAUDPC	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day
Kavkaz	22.74 <sup>c</sup>	1.50 <sup>b</sup>	2.88 <sup>d</sup>	2.95 <sup>d</sup>	0.61 <sup>e</sup>	103.33	1.15	15.13	29.38
Mv09-09	22.75 <sup>c</sup>	1.63 <sup>c</sup>	2.50 <sup>c</sup>	3.38 <sup>d</sup>	0.61 <sup>e</sup>	67.04	2.76	6.50	19.40
Mv Vekni	22.81 <sup>c</sup>	1.50 <sup>b</sup>	2.88 <sup>d</sup>	3.00 <sup>d</sup>	0.62 <sup>e</sup>	65.15	2.05	9.50	13.75
Mv22-01	23.06 <sup>c</sup>	1.63 <sup>c</sup>	2.75 <sup>d</sup>	3.00 <sup>d</sup>	0.62 <sup>e</sup>	86.81	3.00	8.25	27.63
6B365	23.16 <sup>c</sup>	1.63 <sup>c</sup>	2.73 <sup>d</sup>	3.13 <sup>d</sup>	0.62 <sup>e</sup>	100.38	2.00	13.75	27.50
Wattines	23.31 <sup>c</sup>	1.75 <sup>c</sup>	2.63 <sup>d</sup>	3.00 <sup>d</sup>	0.63 <sup>e</sup>	64.07	1.76	9.38	14.38
GK Garaboly	23.38 <sup>c</sup>	1.88 <sup>c</sup>	2.50 <sup>c</sup>	2.88 <sup>d</sup>	0.63 <sup>e</sup>	50.63	1.64	6.00	13.75
GK Csillag	23.44 <sup>c</sup>	1.63 <sup>c</sup>	2.75 <sup>d</sup>	3.25 <sup>d</sup>	0.63 <sup>e</sup>	82.65	2.25	10.65	22.00
Mv21-09	23.63 <sup>c</sup>	1.63 <sup>c</sup>	2.75 <sup>d</sup>	3.38 <sup>d</sup>	0.64 <sup>e</sup>	131.00	5.13	18.13	26.25
Mv20-09	24.31 <sup>c</sup>	1.88 <sup>c</sup>	2.50 <sup>c</sup>	3.50 <sup>d</sup>	0.66 <sup>e</sup>	72.91	3.01	7.53	20.00
Mv335-09	24.50 <sup>c</sup>	2.00 <sup>c</sup>	2.63 <sup>d</sup>	2.88 <sup>d</sup>	0.66 <sup>e</sup>	87.76	3.89	14.40	10.65
Mv29-09	24.63 <sup>c</sup>	1.88 <sup>c</sup>	2.75 <sup>d</sup>	3.13 <sup>d</sup>	0.66 <sup>e</sup>	84.89	2.65	13.13	16.25
Mv07-09	24.75 <sup>c</sup>	1.75 <sup>c</sup>	2.88 <sup>d</sup>	3.38 <sup>d</sup>	0.67 <sup>e</sup>	64.07	2.26	7.25	17.50
Mv Regiment	24.81 <sup>c</sup>	1.75 <sup>c</sup>	3.00 <sup>d</sup>	3.13 <sup>d</sup>	0.67 <sup>e</sup>	118.45	4.53	13.88	30.00
Alcedo	25.38 <sup>d</sup>	1.88 <sup>c</sup>	2.75 <sup>d</sup>	3.63 <sup>e</sup>	0.68 <sup>e</sup>	67.43	0.10	8.13	25.63
Mv330-09	25.81 <sup>d</sup>	2.13 <sup>c</sup>	2.75 <sup>d</sup>	3.00 <sup>d</sup>	0.70 <sup>e</sup>	119.13	5.64	17.63	17.63
Disponent	25.88 <sup>d</sup>	1.88 <sup>c</sup>	3.00 <sup>d</sup>	3.38 <sup>d</sup>	0.70 <sup>e</sup>	104.94	3.26	15.75	21.25
Frontana	26.06 <sup>d</sup>	2.00 <sup>c</sup>	2.75 <sup>d</sup>	3.63 <sup>e</sup>	0.70 <sup>e</sup>	125.01	2.28	18.75	31.25
Mv16-09	26.06 <sup>d</sup>	1.88 <sup>c</sup>	3.00 <sup>d</sup>	3.50 <sup>d</sup>	0.70 <sup>e</sup>	87.48	3.79	9.40	22.50
Mv Mezőföld	26.25 <sup>d</sup>	2.25 <sup>c</sup>	2.63 <sup>d</sup>	3.13 <sup>d</sup>	0.71 <sup>e</sup>	113.88	5.38	13.38	25.00
Mv Makaróni	26.88 <sup>d</sup>	2.00 <sup>c</sup>	2.88 <sup>d</sup>	3.88 <sup>e</sup>	0.73 <sup>e</sup>	131.02	2.04	17.63	38.75
GK Kalász	27.06 <sup>d</sup>	2.13 <sup>c</sup>	3.00 <sup>d</sup>	3.25 <sup>d</sup>	0.73 <sup>e</sup>	155.45	7.53	20.00	29.38
Bánkúti 1201	27.13 <sup>d</sup>	2.00 <sup>c</sup>	3.00 <sup>d</sup>	3.75 <sup>e</sup>	0.73 <sup>e</sup>	178.88	10.14	17.50	41.25
Mv04-09	27.25 <sup>d</sup>	2.25 <sup>c</sup>	2.75 <sup>d</sup>	3.50 <sup>d</sup>	0.74 <sup>e</sup>	100.75	5.88	9.38	23.75
Mv10-09	27.25 <sup>d</sup>	2.25 <sup>c</sup>	2.75 <sup>d</sup>	3.50 <sup>d</sup>	0.74 <sup>e</sup>	182.50	9.13	22.00	36.88
Mv14-00	27.56 <sup>d</sup>	2.25 <sup>c</sup>	3.00 <sup>d</sup>	3.13 <sup>d</sup>	0.74 <sup>e</sup>	108.94	5.38	12.50	23.75
Mv20-01	28.19 <sup>d</sup>	2.38 <sup>c</sup>	2.88 <sup>d</sup>	3.38 <sup>d</sup>	0.76 <sup>e</sup>	161.20	8.78	18.88	31.25
Mv Tamara	28.31 <sup>d</sup>	2.25 <sup>c</sup>	3.00 <sup>d</sup>	3.63 <sup>e</sup>	0.76 <sup>e</sup>	138.88	9.63	14.38	23.75
Mv Palotás	28.50 <sup>d</sup>	2.25 <sup>c</sup>	2.88 <sup>d</sup>	3.13 <sup>d</sup>	0.77 <sup>e</sup>	113.06	3.00	18.75	20.63
Mv19-05	29.06 <sup>d</sup>	2.38 <sup>c</sup>	3.13 <sup>d</sup>	3.38 <sup>d</sup>	0.78 <sup>e</sup>	159.06	7.50	21.88	27.50
Glenlea	29.44 <sup>d</sup>	2.38 <sup>c</sup>	3.13 <sup>d</sup>	3.63 <sup>e</sup>	0.79 <sup>e</sup>	213.63	11.00	28.75	35.00
Mv05-01	29.50 <sup>d</sup>	2.50 <sup>c</sup>	3.00 <sup>d</sup>	3.50 <sup>d</sup>	0.80 <sup>e</sup>	203.81	12.75	22.13	37.50
Coulter	30.13 <sup>d</sup>	2.50 <sup>c</sup>	3.13 <sup>d</sup>	3.63 <sup>e</sup>	0.81 <sup>f</sup>	152.63	6.50	18.13	35.63
Bezostaya-1	30.48 <sup>d</sup>	2.63 <sup>d</sup>	2.98 <sup>d</sup>	3.75 <sup>e</sup>	0.82 <sup>f</sup>	240.38	10.75	32.50	45.00
Mv14-09	30.50 <sup>d</sup>	2.50 <sup>c</sup>	3.13 <sup>d</sup>	3.88 <sup>e</sup>	0.82 <sup>f</sup>	157.50	6.88	20.00	33.13
Martonvásári 4	30.56 <sup>d</sup>	2.50 <sup>c</sup>	3.25 <sup>d</sup>	3.63 <sup>e</sup>	0.82 <sup>f</sup>	216.13	12.25	25.63	39.38
Mv Verbunkos	30.94 <sup>d</sup>	2.75 <sup>d</sup>	3.13 <sup>d</sup>	3.25 <sup>d</sup>	0.83 <sup>f</sup>	155.13	7.13	21.88	26.25
Mv08-09	30.94 <sup>d</sup>	2.38 <sup>c</sup>	3.50 <sup>d</sup>	3.75 <sup>e</sup>	0.83 <sup>f</sup>	139.63	3.00	19.38	36.88
Mv17-09	30.94 <sup>d</sup>	2.50 <sup>c</sup>	3.25 <sup>d</sup>	3.88 <sup>e</sup>	0.83 <sup>f</sup>	237.19	12.50	33.13	35.00
Mv332-09	31.06 <sup>d</sup>	2.63 <sup>d</sup>	3.25 <sup>d</sup>	3.50 <sup>d</sup>	0.84 <sup>f</sup>	164.69	6.25	22.50	34.38

Table 2 to be continued

Genotype	Lesion type					PILA (%)			
	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day	RAUDPC	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day
Mv13-09	31.25 <sup>d</sup>	2.50 <sup>c</sup>	3.50 <sup>d</sup>	3.50 <sup>d</sup>	0.84 <sup>f</sup>	180.88	8.88	23.75	32.63
Salamouni	31.44 <sup>d</sup>	2.88 <sup>d</sup>	3.13 <sup>d</sup>	3.13 <sup>d</sup>	0.85 <sup>f</sup>	189.01	7.15	27.50	33.13
Mv08-06	32.31 <sup>d</sup>	2.63 <sup>d</sup>	3.50 <sup>d</sup>	3.75 <sup>e</sup>	0.87 <sup>f</sup>	173.00	8.75	20.63	35.13
Mv27-09	32.69 <sup>d</sup>	2.75 <sup>d</sup>	3.25 <sup>d</sup>	4.13 <sup>e</sup>	0.88 <sup>f</sup>	288.88	16.50	32.50	56.25
Mv Magvas	32.75 <sup>d</sup>	2.75 <sup>d</sup>	3.38 <sup>d</sup>	3.88 <sup>e</sup>	0.88 <sup>f</sup>	271.88	18.13	29.38	46.25
Mv11-09	32.88 <sup>d</sup>	2.88 <sup>d</sup>	3.38 <sup>d</sup>	3.50 <sup>d</sup>	0.89 <sup>f</sup>	266.56	21.25	25.63	40.00
Mv29-98	33.38 <sup>d</sup>	2.88 <sup>d</sup>	3.25 <sup>d</sup>	4.13 <sup>e</sup>	0.90 <sup>f</sup>	233.19	13.88	26.88	41.88
Mv08-07	33.50 <sup>d</sup>	2.88 <sup>d</sup>	3.50 <sup>d</sup>	3.63 <sup>e</sup>	0.90 <sup>f</sup>	228.75	16.25	23.75	37.50
Mv Pálma	33.94 <sup>d</sup>	3.00 <sup>d</sup>	3.38 <sup>d</sup>	3.75 <sup>e</sup>	0.92 <sup>f</sup>	305.00	25.00	29.38	43.13
Mv331-09	34.13 <sup>d</sup>	3.00 <sup>d</sup>	3.38 <sup>d</sup>	3.88 <sup>e</sup>	0.92 <sup>f</sup>	228.75	14.38	27.50	35.63
Mv25-09	35.94 <sup>e</sup>	3.00 <sup>d</sup>	3.63 <sup>e</sup>	4.50 <sup>e</sup>	0.97 <sup>f</sup>	352.81	25.63	37.50	53.75
ND495	35.94 <sup>e</sup>	2.88 <sup>d</sup>	3.88 <sup>e</sup>	4.38 <sup>e</sup>	0.97 <sup>f</sup>	534.06	43.13	57.50	66.25
Katepwa	37.06 <sup>e</sup>	3.00 <sup>d</sup>	4.00 <sup>e</sup>	4.38 <sup>e</sup>	1.00 <sup>f</sup>	421.94	29.50	46.88	63.75
Mean	22.73	1.70	2.52	3.03	0.61	110.95	5.44	13.45	22.62
CV (%)	43.21	59.92	39.05	31.27	43.21	103.88	165.41	100.78	77.51
LSD <sub>5%</sub>	7.85	0.91	0.88	0.72	0.21	91.34	7.72	12.31	14.83

AUDPC – area under the disease progress curve; PILA – percentage of infected leaf area; RAUDPC – relative area under the disease progress curve; <sup>a</sup>highly resistant; <sup>b</sup>resistant; <sup>c</sup>moderately resistant; <sup>d</sup>moderately susceptible; <sup>e</sup>susceptible; <sup>f</sup>highly susceptible; CV – coefficient of variation; LSD – least significant difference

After the 11<sup>th</sup> day, the slope of the HR curve could no longer be distinguished from that of the other groups. Only slight differences were found between the varieties within each group.

Within the HR group, infection was only detected in traces on the breeding line Mv18-09, while tiny black spots of infection did not appear on line Mv326-09 until the 14<sup>th</sup> day.

The resistance types of the breeding lines were determined on the basis of five criteria: the AUDPC values calculated on the basis of lesion types, the relative AUDPC values, and the lesion types on the 7<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup> days (Table 2). On the basis of evaluation on the 7<sup>th</sup> day, 37 genotypes were classified as resistant to SNB (HR and R types), 18 of which exhibited moderate susceptibility to the pathogen on the basis of lesion type on the 14<sup>th</sup> day (the last evaluation date). A total of 15 resistant and highly resistant genotypes were selected on the basis of the AUDPC value calculated for lesion types, the resistance of which did not change significantly on the 14<sup>th</sup> day after infection compared with the two previous evaluation dates (Table 2). The use

of the relative AUDPC value for the selection of breeding lines proved to be a very strict criterion: only seven of the 92 genotypes could be classified in the R or HR group on this basis.

Correlations between the data obtained with the various evaluation methods were analysed by means of correlation analysis (Table 3), which revealed very close or close positive correlations between all the methods. The weakest correlation ( $r = 0.620^{***}$ ) was found between the infected leaf area on the 7<sup>th</sup> day and the lesion type on the 14<sup>th</sup> day, and the closest between the lesion type AUDPC and the lesion type on the 7<sup>th</sup> day ( $r = 0.982^{***}$ ).

Among the genotypes examined, several Martonvásár breeding lines (Mv06-09, Mv15-09, Mv18-09, Mv22-09, Mv26-09, Mv326-09, Mv327-09, Mv336-09) and the variety Mv Zelma proved to have excellent resistance, in terms of both percentage of infected leaf area and lesion type, while the varieties Mv Kolompos and Mv Béres had good resistance to *Stagonospora nodorum*.

Mv Bodri, found in earlier experiments to have moderate resistance to *Pyrenophora tritici-repentis*

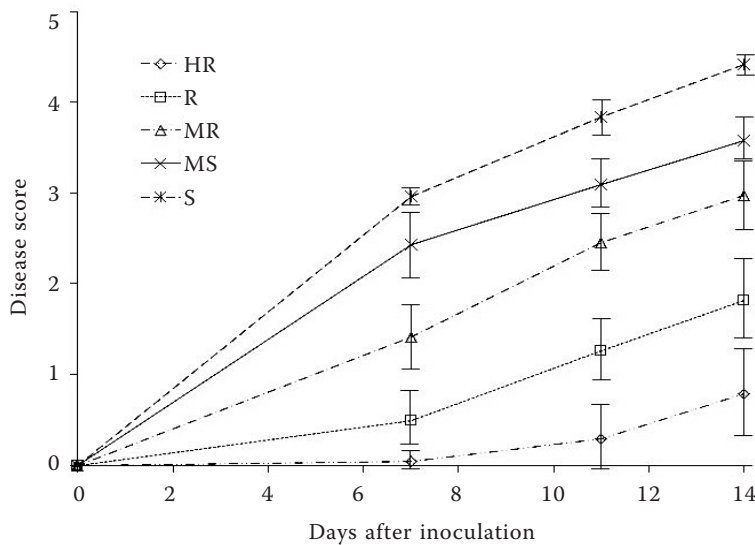


Figure 1. Disease progress curves of wheat genotype groups based on lesion type (Martonvásár, 2009)

HR – highly resistant (AUDPC ≤ 5), R – resistant (5 < AUDPC ≤ 15), MR – moderately resistant (15 < AUDPC ≤ 25), MS – moderately susceptible (25 < AUDPC ≤ 35), S – susceptible (AUDPC > 35); AUDPC – area under the disease progress curve

in the greenhouse (Cséplő *et al.* 2009), also belonged to the group of resistant genotypes in the greenhouse tests.

The results indicated that the best way for breeders to select for SNB resistance was to use AUDPC values calculated on the basis of lesion types, which gave a clear indication of which genotypes had stable resistance throughout the period examined. The coefficient of variation was the 3<sup>rd</sup> smallest for this method, which also allowed changes in the dynamics of disease progress to be monitored. Correlation analysis (Table 3) indicated very close correlations between the AUDPC value calculated from lesion type data and the lesion types determined on the 7<sup>th</sup> ( $r = 0.982^{***}$ ), 11<sup>th</sup> ( $r = 0.972^{***}$ ) and 14<sup>th</sup> ( $r = 0.936^{***}$ ) days. The evaluation of lesion type AUDPC requires experimentation in the green-

house over a 14-day period, with scoring at various dates, which is both costly and time-consuming. As the closest correlation was obtained for the lesion type on the 7<sup>th</sup> day, it appeared that these data might be sufficient for the accurate estimation of resistance to *Stagonospora nodorum* blotch in the seedling stage, thus considerably reducing the time and cost required. This was in agreement with the findings of Liu *et al.* (2004), who determined the seedling resistance of various genotypes to *Stagonospora nodorum* at different dates in order to investigate the genetic background of SNB resistance and concluded from the results that detection could be maximized by disease evaluation 5 to 7 days after inoculation. The present results indicated, however, that evaluation on the 7<sup>th</sup> day was only really efficient for identifying the wheat

Table 3. Correlation coefficients between various scoring methods (Martonvásár, 2009)

		Lesion type				PILA (%)		
		AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day	14 <sup>th</sup> day	AUDPC	7 <sup>th</sup> day	11 <sup>th</sup> day
Lesion type	7 <sup>th</sup> day	0.982						
	11 <sup>th</sup> day	0.972	0.917					
	14 <sup>th</sup> day	0.936	0.875	0.938				
PILA (%)	AUDPC	0.825	0.835	0.777	0.753			
	7 <sup>th</sup> day	0.698	0.719	0.644	0.620	0.961		
	11 <sup>th</sup> day	0.847	0.856	0.801	0.763	0.980	0.900	
	14 <sup>th</sup> day	0.882	0.870	0.848	0.856	0.943	0.839	0.932

AUDPC – area under the disease progress curve; PILA – percentage of infected leaf area; all correlation coefficients are significant at  $P = 0.001$

genotypes with the best seedling SNB resistance (HR group). For wheat varieties and lines in the other resistance groups, the spread of the disease was less easy to predict from 7<sup>th</sup> day data, and some genotypes that appeared to be resistant on the 7<sup>th</sup> day were re-classified in the moderately susceptible group on the basis of evaluation on the 14<sup>th</sup> day (Figure 1). The determination of lesion types on the 11<sup>th</sup> day allowed genotypes in the highly resistant and resistant groups to be clearly distinguished, and from this date onwards there was no further change in the steepness of the average disease progress curves of varieties belonging to the other groups (Figure 1). Observations showed that for the majority of the wheat genotypes tested the lesion type data gradually increased up to the 14<sup>th</sup> day, though the spread of the disease slowed down after the 11<sup>th</sup> day in a few cases. It could be seen from the data that the choice of method for improving seedling SNB resistance should depend on the aim of selection. If the aim is to identify genotypes with outstanding resistance, the lesion type on the 7<sup>th</sup> day is a reliable selection criterion, but if the aim is to score genotype resistance, evaluation should be continued until the 14<sup>th</sup> day. Differences in the dynamics of disease progress, however, can only be detected by scoring at several dates, which allows the seedling SNB resistance of wheat genotypes to be clearly described.

A number of resistance sources providing an excellent level of resistance against several leaf spot diseases have been reported in the literature, but data on the agronomic traits of these genotypes are not available (MA & HUGHES 1995; XU *et al.* 2004; SINGH *et al.* 2006, 2007; ALI *et al.* 2008). The resistance genes to be found in wild or related species may provide protection against the pathogens, but the agronomic and quality traits of these genotypes differ substantially from those of the varieties cultivated in Hungary, so time-consuming backcrossing programmes are needed if these sources are to be modified to satisfy local requirements. The present results indicate that Mv Béres, Mv Bodri and Mv Kolompos combined excellent agronomic traits with resistance to *Stagonospora nodorum*. The identification of new resistance sources and the rapid, efficient selection of breeding lines with good resistance could contribute to the development of wheat varieties with both good agronomic characters and resistance to leaf spot diseases.

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*Corresponding author:*

GYULA VIDA, PhD., Hungarian Academy of Sciences, Centre for Agricultural Research, P.O. Box 19, H-2462 Martonvásár, Hungary  
e-mail: vidagy@mail.mgki.hu

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