P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 48 (no. 2/2) 2003

TMiedaner .[1], H. Wortmann, [2], H. H.Geiger [3]

[1]Univ. of Hohenheim, State Plant Breeding Inst., D–70593 Stuttgart, Germany,
[2]Hybro GmbH & Co. KG, D–76669 Bad Schönborn, Germany,
[3]Univ. of Hohenheim, Inst. of Plant Breeding, Seed Science,
and Population Genetics, D–70593 Stuttgart, Germany;
e–mail: miedaner@uni–hohenheim.de

GENETICS OF DEOXYNIVALENOL (DON) CONTAMINATION CAUSED BY *FUSARIUM* HEAD BLIGHT IN HYBRID RYE

ABSTRACT

Head blight caused by *Fusarium culmorum* or *F. graminearum* affects all cereals including winter rye (*Secale cereale* L.). Besides yield and quality losses, grain is contaminated with the mycotoxin deoxynivalenol (DON) that is harmful to animals and humans. We analysed 76 self–fertile S_2 lines and their corresponding testcrosses for head blight resistance and DON concentration in the grain by inoculating them with an aggressive isolate of *F. culmorum* at two locations in Southwestern Germany in 2000. Disease severity at both locations was low with mean ratings from 2.7 to 3.4 on a 1–9 scale (1=healthy), but still DON occurred in considerable amounts (11.4 – 15.0 mg kg^{-1}). Genotypic variance was significant (P=0.01) in all instances, genotype–location interaction and error variances were more important for DON concentration resulting in a lower heritability of this trait. No association between S_2 lines and their testcrosses was found for head blight rating or DON concentration (r = 0.33 and 0.19, respectively). The coefficient of correlation between head blight rating and DON concentration was low for the S_2 lines and medium for the testcrosses (r=0.24 and 0.60, P=0.05 and P=0.01, resp.). The hybrid rye breeder should select predominantly on testcross performance. Selection for low head blight ratings should result in lower DON concentrations in the grain also. In later generations, DON should be analysed additionally to exploit the maximum selection gain possible.

Key words *Fusarium culmorum* , hybrid breeding, mycotoxins, population parameters, *Secale cereale*

INTRODUCTION

Fusarium culmorum (W.G.Smith) Sacc. and *Fusarium graminearum* Schw. are the major pathogens causing head blight of small–grain cereals including rye (*Secale cereale* L.). Frequently also *Microdochium nivale* (Fr.) Sam. and Hall. (syn. *Fusarium nivale*) can be isolated from infected rye heads. Severe epidemics of *Fusarium* spp. lead to considerable yield loss and reduction of kernel weight. Devastating epidemics of wheat in Europe, USA, China and South America resulted in yield

Communicated by Hartwig H. Geiger

losses up to 30% (McMullen *et al.* 1997). Rye was reported to be less damaged by *Fusarium* head blight than wheat (Arseniuk *et al.* 1999, Miedaner *et al.* 2001). Mycotoxin contamination of the cereal grain is a continuous threat to animal and human consumption. The trichothecenes deoxynivalenol (DON), its acetylated derivatives (e.g. 3–acetyl DON and 15–acetyl DON), and nivalenol (NIV) are the most frequently occurring *Fusarium* toxins world wide (Placinta *et al.* 1999). DON–contaminated grain is less palatable to livestock and may cause emesis, depressed feed intake, and feed refusal in pigs (D'Mello *et al.* 1999). Almost all isolates of *F. culmorum* and *F. graminearum* produce mycotoxins in grains of artificially inoculated winter rye (Gang *et al.* 1998, Miedaner *et al.* 2000). From 68 isolates of both *Fusarium* species investigated, 56 isolates produced DON and 11 NIV. Consequently, natural infections should as a rule lead to the contamination of grain with one or more of these mycotoxins. In a 5–year monitoring, Müller *et al.* (1997) found DON in 69–96% of randomly drawn wheat samples DON with yearly means ranging from 0.15 to 1.69 mg kg^{-1}. DON was also detected in processed human food, though in largely reduced con–centrations (Schollenberger *et al.* 1999). Among rye hybrids and compo–nents of hybrids, three–fold differences in the amount of DON occurred in the grain (Miedaner *et al.* 2001). As a consequence, resistance breed–ing should not only strive for a reduction of yield and technological qual–ity losses, but also diminish the mycotoxin concentration of the harvest.

Resistance to *Fusarium* head blight is quantitatively inherited (for review see Miedaner 1997). Under adequate inoculation conditions dur–ing mid–flowering, moderate to high heritabilities can be achieved. Head blight resistance in rye is mainly controlled by additive gene ac–tion (Miedaner and Geiger 1996). Thus, chances are good for improving resistance by recurrent selection. However, genotype–environment in–teraction was important in all mentioned studies. Little is known about the genetic basis of DON accumulation and about the relationship be–tween resistance traits and mycotoxin concentration in rye.

The objectives of this study were to:

(1) analyse the genetic variance of DON concentration in a represen–tative population of S_2 lines and corresponding testcrosses,
(2) investigate the covariation of S_2 lines and their hybrids, and finally
(3) analyze the relationship between head blight symptoms and DON contamination.

MATERIALS AND METHODS

The genetic materials consisted of 76 S_2 lines of the Carsten (pollinator) gene pool. In 1999, individual S_1 plants were selfed and si–multaneously crossed to a highly susceptible single–cross tester (A x B) of the Petkus (seed parent) gene pool in the greenhouse. In 2000, lines and testcrosses were planted in one–row microplots (0.21 m row dis–

tance, 1.2 m length) in a completely randomized block design with two replications at Hohenheim (HOH) near Stuttgart and Bad Schönborn (BSB) near Heidelberg. Inbred lines and hybrids were planted in separate, but adjacent experiments. A highly aggressive DON–producing isolate of *F. culmorum* (FC 46) was used for inoculation. Spores were produced on autoclaved wheat grain medium incubated for three weeks at 18˚C in glass flasks and spread afterwards under permanent UV at 18˚C for 6–10 days. For inoculation, a suspension of 5×10^5 conidia $\times$ ml^{-1} was applied at a rate of 100 ml m^{-2} with a portable sprayer equipped with a compressor to generate a standardized pressure of 3 bar. Flowering times of lines and their testcrosses were different and, hence, different inoculation dates were necessary. All testcross progenies were inoculated simultaneously at two dates with three days difference. The lines had been splitted into two batches. Each batch was inoculated twice, also with a three–day difference. Head blight rating served as resistance criterion. It was assessed at three consecutive dates on a 1–9 scale on a plot basis: 1 = no symptoms visible, 2 – 9 = =5%, 6–15%, 16–25%, 26–45%, 46–65%, 66–85%, 86–95% and >95%, respectively, diseased spikelets per plot. The arithmetic mean of the three individual ratings was used for further calculations.

DON analysis

The plots were harvested by hand, threshed with a small thresher, and the grain was cleaned several times by sieving under reduced wind. Grain was ground to a particle size of about 1 mm with a laboratory mill, mixed and stored at –20˚C until lab analysis. DON was analysed by a commercially available indirect, competitive enzyme immunoassay (RIDASCREEN®FAST–DON, r–biopharm GmbH, Darmstadt, Germany) with a limit of detection and quantification of 0.2 mg $\times$ kg^{-1}. According to the suppliers' description, the test cannot differentiate between DON and 3–ADON (cross reactivity of 213%), but has no or almost no cross reaction with 15–ADON, triacetyl–DON, nivalenol, triacetyl–nivalenol, and fusarenon X.

Statistical analyses

All analyses were performed on plot basis. Entry means at individual locations followed a normal distribution for both traits, and error variances were homogeneous across locations according to Bartlett's test (Snedecor and Cochran, 1989). Estimates of variance components for σ^2_g (genotypic variance), σ^2_{gl} (genotype–location interaction variance) and σ^2 (error variance) were calculated as described by Snedecor and Cochran (1989). The estimates of variance components were transformed to respective coefficients of variation (CV%, [$\sigma \times$ mean^{-1}] $\times$ 100). This allows direct comparisons between traits of different dimensions. Broad–sense heritabilities (h^2) were estimated on an entry–mean basis by the formula:

$$h^2 = \frac{\sigma_g^2}{\frac{\sigma^2}{r \times l} + \frac{\sigma_{gl}^2}{l + \sigma_g^2}}$$

where:
r is the number of replicates and,
l is the number of locations
All statistical analyses were performed with the computer package PLABSTAT (Utz, 2000). The effects of genotypes, replicates, and locations were assumed to be random variables.

RESULTS

The inoculation resulted in clearly visible disease symptoms of prematurely bleached spikelets in all experiments. Mean disease severities and DON concentrations were rather similar at both locations (Table 1).

Table 1

Means and standard errors for head blight rating and DON concentration of 76 S_2 lines and their testcrosses in Hohenheim and Bad Schönborn 2000

Location	Material	Head blight rating [1–9]	DON concentration [mg × kg^{-1}]
Hohenheim	S_2 lines	2.72 ± 0.47	14.68 ± 3.43
	Testcrosses	2.97 ± 0.28	11.36 ± 2.23
Bad Schönborn	S_2 lines	3.07 ± 0.25	15.03 ± 2.87
	Testcrosses	3.43 ± 0.21	12.29 ± 2.91

Table 2

Coefficients of variation (%) of head blight rating and DON concentration of 76 S_2 lines and their testcrosses evaluated at two locations

Statistics	S_2 lines		Test crosses	
Source of variance	Head blight rating	DON concentration	Head blight rating	DON concentration
Genotype (G)	19.7**	37.2**	18.2**	26.8**
G × location	15.7**	42.3**	9.4**	23.4**
Error	18.3	29.7	10.9	30.9
Heritability	0.65	0.56	0.82	0.58

** Significant at P=0.01 (F–Test)

Head blight resistance and DON concentration were both continuously distributed (Fig. 1). Both frequency distributions were slightly skewed to the left. The S_2 lines were less diseased but more contaminated by DON than the testcrosses and displayed a somewhat larger range for

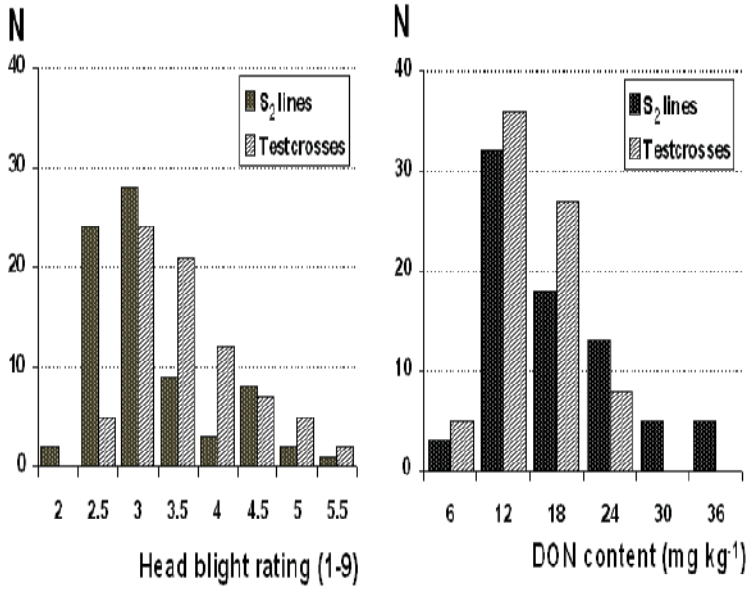

Fig. 1 Frequency distribution of head blight rating and DON concentration of 76 S_2 lines and their testcrosses evaluated at two locations

both traits. Genotypic differences were significant (P=0.01) for head blight rating and DON concentration in both materials with considerably higher coefficients of variation for the latter (Table 2). Genotype–location interaction and error variances were also more important for DON concentration. The higher genotypic variation for head blight rating resulted in higher heritability estimates than for DON concentration. The testcrosses had a somewhat lower genotypic variance than the S_2 lines for both traits, but the relative importance of their genotype–location interaction was also lower resulting in a higher heritability for head blight rating. As a consequence of the highly important genotype–location interaction for both traits, the correlations between locations were, although significant (P=0.01), only medium for head blight rating (r=0.50 for S_2 lines, r=0.70 for testcrosses) and low for DON concentration (r=0.39 for S_2 lines, r=0.43 for testcrosses). Despite significant genotypic variation, no association between S_2 lines and their testcrosses were found for any of the two traits (Table 3). The genotypic coefficients of correlation were low in both cases. The same was true when the correlations were calculated for each location individually. For comparison, the phenotypic and genotypic correlations for plant height are given that were much higher. The coefficient of correlation between head blight rating and DON concentration was low for the S_2 lines and medium for the testcrosses (Fig. 2).

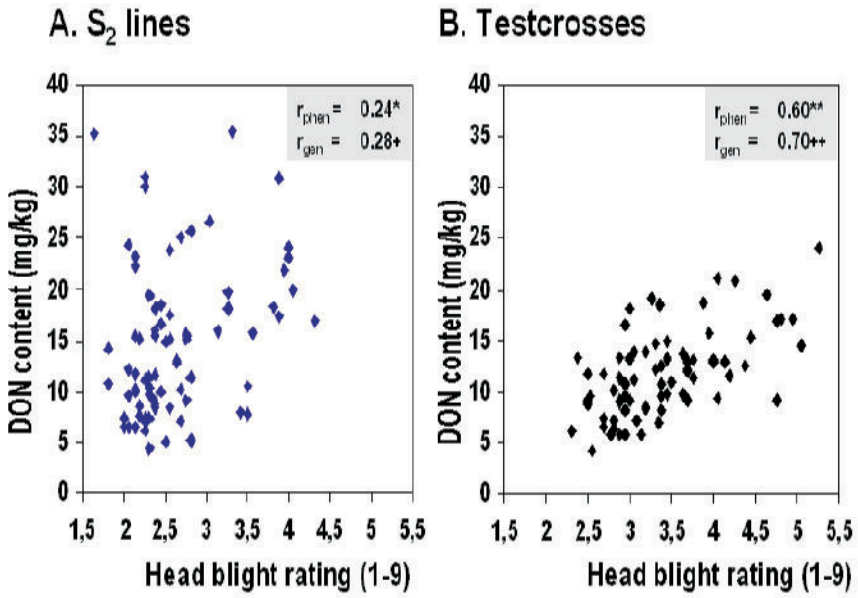

Fig. 2. Association between head blight rating and DON concentration and phenotypic (r_{phen}) and genotypic (r_{gen}) coefficients of correlation for 76 S_2 lines and their testcrosses evaluated at two locations; *,** Significant at P=0,05 and 0.01, resp.; $^{+, ++}$Estimate larger than once and twice its standard error, respectively

Table 3

Coefficients of phenotypic (r_{phen}) and genotypic (r_{gen}) correlation between 76 S_2 lines and their testcrosses for head blight rating, DON concentration and plant height

Trait	r_{phen}	r_{gen}
Head blight rating	0.33**	0.40++
DON concentration	0.19	0.41++
For comparison: Plant height	0.66**	0.95++

** Significant at P=0,01.
++ Estimate larger than twice its standard error.

DISCUSSION

Genotypic variation and covariation of inbred lines and testcrosses are two crucial population parameters for estimating the selection gain and optimizing breeding plans in hybrid rye. In self–fertile and self–incompatible rye populations a large genotypic variance can be used for selection to *Fusarium* head blight resistance (for review see Miedaner 1997). In this study, the testcrosses had only a slightly lower genetic variation than the S_2 lines caused by the high susceptibility of the tester used. This is an important prerequiste to get an optimal differentiation, especially in years that are not so conducive for head blight infections like e.g. the year 2000. According to the large genotypic variance, gain from

selection should be high. It is, however, limited by the low heritability of the DON concentration and the missing association between S_2 lines and testcrosses. This finding confirms an earlier report with a different set of pollinator lines crossed to two testers, where the correlation was totally absent for head blight rating and relative grain weight (Miedaner *et al.* 1995b). Because of the importance of this correlation for hybrid rye breeding we have tested in total 290 S_2 lines for head blight rating at two locations, including those 76 that are shown in this paper. However, coefficients of phenotypic and genotypic correlation were similarly low also for the larger sample (r=0.21, P=0.01, and r=0.32, resp.). No genetic explanation can be given for the missing covariation. Inbred lines displayed no inbreeding depression for resistance. Head blight rating was even slightly lower in the inbreds than in the testcrosses. However, their DON concentration was about 20% higher than that of the testcrosses (Table 1). Both materials were tested at the same locations. Due to their deviating flowering times they were, however, inoculated at different dates. Consequently, temperature and humidity immediately after inoculation were different and this might have influenced the resistance level (Miedaner *et al.* 2001). The stand of the inbred lines at Hohenheim was less dense than that of the testcrosses and that might have contributed to the higher error estimates for head blight rating. Still, the genotypic correlation between lines and testcrosses was only slightly higher than the phenotypic correlation. This finding, however, contradicts to various literature reports in which tight correlations between rye inbred lines and their hybrids were found for other resistances, e.g. to powdery mildew (*Blumeria graminis*), foot rot (*F. culmorum, Pseudocercosporella herpotrichoides*), and leaf rust (*Puccinia recondita,* Miedaner *et al.* 1993, 1995a, 1996). One could speculate that the invasion of the heads by *F. culmorum* and/or the dynamics of resistance reaction might be different in inbred lines and hybrids. A recent molecular genetic study in wheat illustrated that *Fusarium* head blight resistance is not necessarily due to specific alleles of resistance genes but may be caused by a faster expression of genes occurring in both resistant and susceptible genotypes (Li *et al.* 2001). This type of resistance might generally be less effective in inbred lines due to their reduced metabolic activity. By crossing them to a tester all deficiencies caused by inbreeding disappear.

The covariation of head blight rating and DON concentration is crucial for the breeder because of the high costs and the time–consuming procedure of DON analyses. A commercially available immunoassay (r–biopharm GmbH, Germany) proved to be useful for breeding purposes. Its results correlated to those of the internationally used gaschromatography coupled with mass spectrometry (GC MS; r=0.91, *unpubl. data*). A moderate correlation between head blight rating and DON concentration has been found among testcross progenies. A correlated response for low DON concentration can be expected when select–

ing for genotypes with less head blight symptoms. To fully exploit the possible selection gain, DON analyses in later generations would be necessary. The association between disease symptoms and mycotoxin concentration in cereals is still in discussion and was found to be variable in different studies in wheat and rye (Miedaner 1997). Among 27 single–cross hybrids of rye, the phenotypic correlation was absent in the first year and medium in the second (r=0.47, P=0.01; Miedaner and Perkowski 1996). In another study, no association between head blight rating and DON concentration was found among 12 heterozygous rye genotypes across six environments. This was mainly caused by the extreme reaction of one genotype that, nevertheless, was responsible for almost all the genotypic variance (Miedaner *et al.* 2001).

As a consequence of the missing association between inbred lines and their hybrids the plant breeder has to select for *Fusarium* head blight resistance predominantly on testcross level. Lines need a resistance level that secures seed production and seed quality, but no selection for low DON concentration is necessary. To circumvent additional costs, head blight resistance can be selected among testcrosses produced for selection of combining ability in grain yield. Inoculation is done either by spraying a small stripe of the large plots (5 m^2) or in replicated microplots. Because testcross seed is produced on isolated plots, additionally planted microplots should not be a problem.

Rating of head blight symptoms gives a reliable basis for selection, the estimation of yield loss is not necessary (Miedaner 1997). After the first selection for combining ability, a second selection is usually performed in the next year with greater selection intensity (Tomerius and Geiger 2001, this volume). Such a multi–step selection scheme would meet with the requirements for testing in as many environments as possible due to the large genotype–location interaction (Miedaner 1997). Nevertheless, the need for producing testcrosses impairs selection intensity for *Fusarium* head blight resistance compared to selection among early–generation inbred lines. In a recurrent selection programme designed for improving *Fusarium* resistance a two–stage procedure is recommended. Unselected heterozygous plants (S_0) are selfed and crossed to a susceptible tester in the first year. In the second year, the testcrosses and S_1 lines are inoculated at two to three locations. The testcross progenies are intensively selected for their *Fusarium* resistance and mildly for agronomic traits, the S_1 lines, on the other hand, are selected mainly for their agronomic performance (e.g. plant height, lodging resistance, kernel weight, falling number) and only mildly for their *Fusarium* resistance. Index selection should be most useful. Selection gain for *Fusarium* head blight resistance and low DON concentration in rye should be lower than for other resistances, e.g. mildew or leaf rust resistance, caused by the lower heritability estimate of the latter and the missing covariation between lines and testcrosses.

Low DON concentration in cereals might become a more important breeding goal in future, because the European Union and the German Government are discussing to enact a tolerance limit in cereals for humans and animals of 0.1 – 0.5 and 1.0 mg DON kg^{-1}, respectively. Caused by the large genetic variation in rye breeding populations for both traits and their mainly additive gene action, recurrent selection as outlined above should improve resistance and simultaneously reduce DON concentrations.

ACKNOWLEDGMENTS

The technical help of Lucija Feketija and Bianca Schneider is highly acknowledged. This study has been funded by the „Ministerium für Ernährung und Ländlichen Raum (MLR) Baden–Württemberg“, Stuttgart, Germany, and the HYBRO GmbH & Co.KG, Bad Schönborn, Germany.

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