



## Combining ability analysis of resistance to head blight caused by *Fusarium graminearum* in spring wheat

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### Summary

*Fusarium* head blight (FHB) caused by *Fusarium* spp. is one of the most important fungal diseases of wheat (*Triticum aestivum* L.) in regions with wet climatic conditions. Improvement of the FHB resistance by developing new varieties requires sound knowledge on the inheritance of resistance. An 8 × 8 diallel analysis was performed to estimate general (GCA) and specific (SCA) combining ability of resistance to FHB. The F<sub>1</sub>s and parental lines were evaluated under artificial inoculation at the experimental field of IFA-Tulln, Austria during 2001 and 2002. Disease severity was evaluated by repeated scoring of the percentage of infected spikelets and calculating an area under the disease progress curve (AUDPC). The analysis of combining ability across two years showed highly significant GCA and non-significant SCA effects indicating the importance of additive genetic components in controlling FHB resistance. The significant GCA-by-year interaction presented the role of environmental factors in influencing the FHB reaction of wheat lines. The comparison of the crosses with low FHB infection and GCA effects of their parents showed that such crosses involved at least one parent with high or average negative GCA effect. The results revealed that it is feasible to use highly or moderately resistant genotypes and conventional breeding methods to achieve genetic improvement of FHB resistance in spring wheat.

**Abbreviations:** FHB: *Fusarium* head blight; AUDPC: area under the disease progress curve; GCA: general combining ability; SCA: specific combining ability

### Introduction

*Fusarium* head blight (FHB) caused by *Fusarium* spp. is one of the most important fungal diseases of wheat (*Triticum aestivum* L.) in regions with wet climatic conditions (Parry et al., 1995). The hot and humid conditions in Caspian littoral in Northern Iran during flowering and early stages of wheat kernel development favour *Fusarium* epidemics. The disease may cause severe yield reduction, as high as 70%, grain quality losses and germination reduction in years with high epidemics in Iran (Zamanizadeh & Khorsandi, 1995).

Besides the direct losses, high levels of mycotoxin contamination such as deoxynivalenol (DON), causing mycotoxicoses in human and domestic animals, have been reported (A. Alizadeh, personal communication, 2001). Although several species of *Fusarium* are capable of inciting FHB in wheat (Parry et al., 1995), *F. graminearum* and *F. culmorum* are the dominant species in Iran (Zamanizadeh & Khorsandi, 1995).

Several methods such as crop rotation and chemical and biological agents have been used to control FHB. Utilization of wheat cultivars with improved *Fusarium* resistance in combination with appropriate crop

management practices are economic and effective ways to control FHB (Bai & Shaner, 1994; Mesterhazy, 1997; Miedaner, 1997). Variation for FHB resistance has been documented in wheat and its relatives (Bai & Shaner, 1994; Mesterhazy, 1995, 1997; Chen et al., 1997; Rudd, 1997; Buerstmayr et al., 2000). However, the limited information on the genetics and inheritance of the disease resistance and the complex evaluation procedures, have slowed down progress in resistance breeding (Devkota et al., 1999).

Inheritance of FHB resistance was described as being monogenic to polygenic (Bai et al., 1989; Buerstmayr et al., 1999; Liu & Wang, 1991). Results of studies on the combining ability for FHB response indicated that the variation among the crosses was primarily due to the general combining ability (GCA) and thus most of the variation is attributed to additive effects (Snijders, 1990; Miedaner et al., 1993; Jiang, 1998; Buerstmayr et al., 1999; Devkota et al., 1999; Hall et al., 2001). Improvement of FHB resistance by developing new varieties would benefit from knowledge on combining ability effects in potential crossing partners. In this study, eight spring wheat genotypes were analyzed in a half diallel crossing design in order to achieve: (1) a better understanding of FHB resistance in the parental genotypes, (2) estimation of general (GCA) and specific (SCA) combining ability for FHB resistance to design an efficient plan for improvement of the existing materials and (3) identify the most promising combinations for the selection of improved breeding lines.

## Materials and methods

### *Plant materials*

Eight spring wheat genotypes with different levels of resistance to FHB (Table 1), were crossed in a diallel manner without reciprocals. The resulting 28 F<sub>1</sub> progenies along with eight parental genotypes were used in this study.

### *Field experiments*

The wheat lines and crosses were evaluated at the experimental field of IFA-Tulln, 30 km west of Vienna, Austria, located at 180 m above sea level, in 2001 and 2002. The soil type was meadow-czernosem. The preceding crop was corn (*Zea mays* L.). The average temperature and annual precipitation were 9.9 °C and

640 mm for the year 2001, and 10.2 °C and 672 mm for the year 2002. To control seed-borne diseases, the seed was treated with 'Rovral-TS' (Rhone-Poulenc, Lyon, France) seed dressing at a rate of 1.5 g kg<sup>-1</sup> of seed. The trials were carried out in a randomized complete block design with three replicates, each plot consisting of one row (1 m length) with 15 plants sown by hand.

### *Inoculation and disease assessment*

Inoculation was carried out using the pathogenic strain 'IFA-65' of *Fusarium graminearum*. Inoculum preparation was based on the method described by Buerstmayr et al. (2002). The macro-conidia concentration was 50,000 spores ml<sup>-1</sup> in water. At beginning of anthesis and two days later (at full flowering) each plot was inoculated with the conidial suspension by spraying 25 ml of inoculum on each plot using a manual atomizer. Inoculations were carried out every other day after 4 p.m. Inoculated plots were misted using a leaf-wetness controlled mist-irrigation system for 20 h after inoculation. In each plot the percentage of visibly infected spikelets was scored according to a linear scale from 0 (no disease) to 1 (completely infected spikelets) on a whole plot basis 14-days (FHB14), 18-days (FHB18), 22-days (FHB22) and 26-days (FHB26) after inoculation. An area under the disease progress curve (AUDPC) was calculated on a whole plot basis Equation (1).

$$\text{AUDPC} = \sum_{i=1}^n \{[(y_i + y_{i-1})/2](x_i - x_{i-1})\} \quad (1)$$

where  $y_i$  is the percentage of visibly infected spikelets at the  $i$ th observation and  $x_i$  is the day of the  $i$ th observation, and  $n$  indicates the total number of observations (modified from Shaner & Finney, 1977).

### *Statistical analysis*

The mean of each plot was used for statistical analysis. The data recorded on AUDPC were subjected to an analysis of variance for a randomized complete block design for individual years as well as across two years. Least square means based on a general linear model were used to compute general combining ability (GCA) and specific combining ability (SCA) using Griffing's method IV and model 1 (Griffing, 1956) using a SAS program for Griffing's diallel analyses (Zhang & Kang, 1997). Genotype effects were considered as

Table 1. Number, name, origin, pedigree and reaction to Fusarium head blight (FHB) of wheat genotypes used as parents for a diallel crossing scheme inoculated with *F. graminearum* at IFA-Tulln, Austria in 2001 and 2002

No.	Genotype	Origin	Pedigree	FHB-reaction <sup>a</sup>
1	Frontana	Brazil	Fronteira/Mentana	Moderately resistant
2	Falat	CIMMYT, Mexico	KVZ/Buho“s”//Kal/Bb = Seri82	Susceptible
3	Sholeh	Ahvaz, Iran	Landrace	Susceptible
4	Alvand	SPII, Iran	1-27-6275/cf1770	Susceptible
5	Darab	SPII, Iran	RshIrni49(60-61)(271-pk868)	Susceptible
6	Hirmand	SPII, Iran	Byt/4/jar//cfn/sr70/s/jup“s”	Susceptible
7	Sumai 3	China	Funo/Taiwanxiaomai	Resistant
8	Tajan	CIMMYT, Mexico	Bow“S”/Nkt“S”	Moderately resistant

<sup>a</sup>Based on combined data across two years.

fixed, replication and year effects were assumed random. Heritability in the broad sense ( $h^2$ ) was calculated as follow:  $h^2 = V_G/V_P$ ;  $V_G = [(MS_G - MS_{G*Y})/ry]$ ;  $V_P = V_G + MS_E$ , where  $MS_G$ ,  $MS_{G*Y}$  and  $MS_E$  indicate mean square for genotype, genotype-by-year interaction and random error, respectively, and  $r$  and  $y$  are number of replications and years, respectively.

## Results

Based on mean values for all 36 genotypes, highly significant correlation coefficients were observed between AUDPC and FHB26 in 2001 and 2002 ( $r = 0.92$ ,  $P < 0.0001$  and  $r = 0.95$   $P < 0.0001$ , respectively) and medium, but significant correlation coefficients between the individual years for AUDPC and FHB26 means, ( $r = 0.55$ ,  $P < 0.0027$ , and  $r = 0.62$   $P < 0.0005$ , respectively). The diallel analysis demonstrated similar results for AUDPC and FHB26 (data not shown). The combining ability analysis for AUDPC is given in Table 2. The results showed highly significant effects ( $P < 0.01$ ) for the factors genotypes, years, GCA and GCA-by-year interaction. The SCA and SCA-by-year interaction were not significant. The GCA variance was larger than the SCA variance with the ratio of GCA/SCA being 20.55 for AUDPC. Broad sense heritability was 0.46 for AUDPC. The mean AUDPC and general combining ability effects (GCA) of parental lines showed that the genotypes with the lowest AUDPC and highest negative GCA values were ‘Sumai 3’ and ‘Frontana’, the lines with the highest AUDPC and highest positive GCA values were ‘Falat’, ‘Alvand’ and ‘Darab’. ‘Tajan’ with a non significant negative GCA value showed a moderate resistance to

Table 2. Mean squares from combined analysis of variance for area under the disease progress curve (AUDPC) of an 8 × 8 diallel cross of wheat genotypes inoculated with *F. graminearum* at IFA-Tulln, Austria in 2001 and 2002

Source	df	MS	F-value	p-value
Year	1	94.30	29.99	0.0001
Rep. (year)	4	9.21	2.93	0.0231
Genotype	35	22.09	4.27	0.0001
GCA <sup>a</sup>	7	80.56	23.72	0.0001
SCA <sup>b</sup>	20	3.92	1.25	0.2141
G * Y	34	5.17	1.65	0.0243
GCA * Y	7	12.10	3.56	0.0014
SCA * Y	20	4.06	1.20	0.2632
Combined error	135	3.14		

<sup>a</sup>GCA, general combining ability, method 4 model 1 (Griffing, 1956).

<sup>b</sup>SCA, specific combining ability, method 4 model 1 (Griffing, 1956).

FHB (Tables 1 and 3). A list of means of the crosses for AUDPC is displayed in Table 4. A moderate FHB resistance among the hybrids, based on rank-means for AUDPC, was observed for all crosses involving ‘Sumai 3’ and the cross ‘Alvand/Tajan’ (4/8).

## Discussion

### FHB assessment

Uniform infection with FHB depends on a number of factors, apart from resistance, such as time, type and amount of inoculation and environmental variation (Parry et al., 1995). Since FHB resistance is non-specific and horizontal (Van Eeuwijk et al., 1995), the

Table 3. Mean values of the parents and general combining ability (GCA) for area under the disease progress curve (AUDPC) of an 8 × 8 diallel cross of wheat inoculated with *F. graminearum* at IFA-Tulln, Austria in 2001, 2002 and combined data

Parent	2001			2002			Combined data		
	mean	GCA	<i>p</i> -value	mean	GCA	<i>p</i> -value	mean	GCA	<i>p</i> -value
Frontana	3.70	-0.49	0.2444	0.84	-0.78	0.0106	2.27	-0.64	0.0132
Falat	10.33	1.71	0.0001	5.99	0.54	0.0726	8.16	1.13	0.0001
Sholeh	6.51	0.40	0.2369	4.95	-0.21	0.4926	5.73	0.09	0.6787
Alvand	5.79	-0.46	0.2033	7.07	1.49	0.0001	6.43	0.51	0.0295
Darab	9.20	1.50	0.0001	7.92	1.46	0.0001	8.56	1.48	0.0001
Hirmand	6.51	0.23	0.5797	3.90	-0.17	0.5636	5.205	0.02	0.9079
Sumai 3	0.81	-2.30	0.0001	0.29	-2.32	0.0001	0.55	-2.31	0.0001
Tajan	4.31	-0.58	0.1281	2.73	-0.007	0.9825	3.52	-0.29	0.2359

inoculation was carried out using a highly aggressive *Fusarium* isolate at anthesis, which is the most susceptible developmental stage for *Fusarium* ear infection (Pough et al., 1933). In order to account for ear to ear variation in flowering time within each plot, repeated inoculations were applied. Optimal humidity was provided using a mist-irrigation system. However, there was no artificial control of the temperature effect on infection and disease development at the field, replicated trials over two years accounted for the temperature effects. The significant correlation coefficients between the individual years for AUDPC and FHB26 indicated that the inoculation method we used resulted in reproducible disease evaluations of the genotypes under investigation. The percentage of infected spikelets as measured by AUDPC, representing Type I (resistance to initial infection) and Type II (resistance to spread of disease after initial infection) simultaneously (Schroeder & Christensen, 1963; Wilcoxson et al., 1992), could be used as a criterion for selection in a breeding program (Bai et al., 1999; Buerstmayr et al., 2002). None of the genotypes showed an immune reaction to FHB symptoms as reported by earlier studies (Mesterhazy, 1995; Buerstmayr et al., 1999).

#### Combining ability

Combining ability analysis using Griffing's method estimates the average additive and dominance effects of all the genes involved in expression of the trait via GCA and SCA based on progeny performance (Dabholkar, 1992). Due to the highly significant correlation coefficients between AUDPC and FHB26 in two years, the combining ability analysis results were similar for both

traits. In the current study, the value of the AUDPC was demonstrated because it is based on repeated evaluation and measures not only the final percentage of infected spikelets (FHB26) but also the progress rate in early stages of FHB development (Bai et al., 1999; Buerstmayr et al., 2002). However, FHB26 provided similar information on combining ability analysis (Snijders, 1990).

The significant GCA and non-significant SCA effects indicated the importance of additive genetic components in controlling FHB resistance in the studied wheat genotypes. This is in agreement with most of the reports from diallel and factorial crosses indicating GCA is more important than SCA for FHB response (Snijders, 1990; Miedaner et al., 1993; Jiang, 1998; Buerstmayr et al., 1999; Devkota et al., 1999; Hall et al., 2001). Also the ratio between GCA and SCA variances tilted in favour of general combining ability supporting the preponderance of additive gene effects in genetic control of FHB severity. This implies that the parents had a high influence on the performance of the progenies in determining response to the disease. A high level of resistance may be obtained by crossing parents with low AUDPC and high negative GCA effects. On the basis of study, crosses involving 'Sumai 3', 'Frontana' and 'Tajan' are recommended as parents for a crossing program to improve FHB resistance in wheat. The significant genotype-by-year and GCA-by-year interactions for the studied trait indicated the role of environmental factors in influencing the FHB reaction of wheat lines. Despite that, GCA estimates were rather consistent across the two years, at least for the highly resistant lines ('Sumai 3', 'Frontana') and the highly susceptible lines ('Falat', 'Darab').

Table 4. Area under the disease progress curve (AUDPC) of F<sub>1</sub>s in an 8 × 8 diallel cross of wheat inoculated with *F. graminearum* at IFA-Tulln, Austria 2001, 2002 and combined data

Cross <sup>a</sup>	2001	2002	Combined data
1/2	4.63	1.24	2.94
1/3	4.85	2.57	3.71
1/4	4.38	2.86	3.62
1/5	6.83	6.62	6.73
1/6	– <sup>b</sup>	2.81	–
1/7	5.53	1.40	3.47
1/8	2.92	5.45	4.19
2/3	6.66	2.49	4.58
2/4	5.80	5.62	5.71
2/5	7.26	6.73	7.00
2/6	6.19	4.67	5.43
2/7	1.94	0.22	1.08
2/8	7.99	5.08	6.54
3/4	4.23	6.20	5.22
3/5	6.84	3.63	5.24
3/6	6.42	2.63	4.53
3/7	1.92	2.10	2.01
3/8	4.06	4.33	4.20
4/5	4.94	6.37	5.66
4/6	4.38	5.42	4.90
4/7	2.78	2.86	2.82
4/8	2.15	3.83	2.99
5/6	6.20	3.87	5.04
5/7	2.71	0.47	1.59
5/8	5.83	3.62	4.73
6/7	2.30	0.31	1.31
6/8	5.31	3.26	4.29
7/8	2.02	1.04	1.53
Mean	4.70	3.48	4.11
LSD 5%	2.95	2.76	2.82 <sup>c</sup>

<sup>a</sup>Crosses specified by numerical values, see Table 1.

<sup>b</sup>Missing data.

<sup>c</sup>For comparison of means based on combined data.

### Breeding application

The present study showed the possibility of selection of FHB resistant wheat cultivars by evaluating visual disease symptoms under epidemic conditions. Combining ability analysis confirmed that primarily additive gene effects controlled the expression of FHB resistance. Hence, conventional breeding methods can be recommended to achieve genetic improvement of FHB

resistance in wheat. The comparison of the crosses with low FHB infection and GCA effects of their parents indicated that ‘Sumai 3’ and ‘Frontana’ are promising crossing parents. However, their undesirable agronomic traits have negative effects on offspring. Apart from these genotypes, ‘Tajan’ with improved agronomic traits and negative GCA effects on FHB resistance could be utilized in breeding for improvement of FHB resistance in spring wheat.

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