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Genotype-by-environment interaction of barley DH lines infected with *Fusarium culmorum* (W.G.Sm.) Sacc.

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ABSTRACT

Infection of plants by pathogens is a biotic environmental stress. Barley plants are infected, among others, by *Fusarium culmorum*—a pathogen affecting seedling, head, root and stem. The infection can result in reduced yield and grain quality. The aim of the study was to compare the reaction of inoculated and non-inoculated barley doubled haploids (DHs) with *F. culmorum* in various environments. Thirty-four genotypes were inoculated with an isolate of *F. culmorum*. The experiment was carried out over 6 years. Kernel weight per spike, 1000-kernel weight and percentage of plump kernels were observed in control and inoculated plants. Genotype-by-environment (GE) interaction and its structure with reference to the environments and genotypes were analysed. Additional information about the sensitivity of healthy and infected genotypes to environments was determined by the regression analysis. Statistical computation was made using the SERGEN software. Lines were considered as unstable when their GE interaction was significant at $P = 0.05$. Unstable genotypes were classified as intensive or extensive according to the results of the regression analysis. It was found that infection with *Fusarium* decreased the stability of barley lines in different environments. Interaction of unstable infected genotypes with environments, most often, could not be explained by the regression—their response to various environmental conditions appeared to be unpredictable. Selection of lines less susceptible to biotic and abiotic stresses was possible due to comparison of classification of healthy and infected lines, which was made based on their main effects and GE interaction.

1. Introduction

Genotype-by-environment (GE) interaction reflects the various responses of genotypes to environmental conditions. Some genotypes have a stable phenotypic performance in a wide range of environmental conditions, while others display considerable variation across environments. GE interaction can be statistically defined as the difference between the phenotypic value and the value expected from the mathematical model of observations that takes into account the general mean as well as genotypic and environmental main effects. For several phenotypic traits, such as yield and its components, cultivars may be estimated as unstable in multi-environment trials, as they show some variation in performance in different environments. These variations can be partly due to environmental main effects when the mean values of studied traits of all genotypes vary among environments, and also partly can arise from GE interactions, when the differences between genotypes are unequal across environments. Breeders and farmers are interested in stable cultivars or in cultivars only slightly influenced by environment. Evaluation of genotype stability/instability can be performed on the basis of a series of trials. Statistical methods for determination of the mode of response of genotypes to various environmental conditions have been developed by many authors (Neyman, 1932; Yates and Cochran, 1938; Mather and Jones, 1958; Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Wricke and Weber, 1986; Gogel et al., 1995; Denis et al., 1997; Piepho, 1997; Mądry and Kang, 2005; Smith et al., 2005).

Evaluation of genotype-by-environment interaction is often accomplished through phenotypic stability analysis of genotypes. Various stability measures such as regression coefficient, residual variance for genotypes, and determination coefficients were proposed (Kaczmarek, 1986; Becker and Leon, 1988; Kang et al., 1991; Caliński et al., 1997; Piepho, 1998). Methods developed by

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Kaczmarek (1986) and Caliński et al. (1987, 1997, 2005, 2009a,b) for statistical analysis of a series of experiments allow information regarding the behavior of individual genotypes in various environments to be obtained. These methods are based on the multivariate mixed model, in which genotype main effects are fixed, while environmental and genotype–environment interaction effects are treated as random. In these methods, GE effect related to each genotype (measured by the value of the relevant *F*-statistic) is the measure of stability and the regression of the GE interaction effects on the observed environmental means (measured by the value of the relevant *F*-statistic) is the measure of adaptability. The *F*-statistic for GE interaction as the measure of stability can be traced back to the methods originally introduced by Caliński (1960) and independently by Wricke (1962), whereas the *F*-statistic for the regression, considered as the measure of adaptability, is related to the concept of Finlay and Wilkinson (1963) and Eberhart and Russell (1966).

Barley (*Hordeum vulgare* L.) is an important cereal crop and ranks fourth among other cereals (after rice, wheat, and maize). Although barley is known to be adapted to a wide range of environmental conditions (e.g., MacGregor and Bhatty, 1993) many studies have revealed a significant influence of environment and genotype-by-environment interaction on phenotypic performance of agronomically important traits (Eagles et al., 1995; Kaczmarek et al., 1999; Chełkowski et al., 2000). The reaction of genotypes to varying abiotic factors can be evaluated by conducting experiments over several years and/or in different localities, whereas the reaction to biotic factors (e.g., fungal pathogens) is mainly investigated in experiments in which artificial infection is applied. Experiments conducted over several years with control genotypes and genotypes artificially infected allow evaluation of the response of genotypes to both biotic and abiotic environmental factors and help in assessing how the degree of pathogen infection can change the reaction of genotypes to abiotic factors.

Among pathogens infecting barley plants, there are species of the genus *Fusarium* which affect seedling, head, root, and stem (e.g., Bottalico, 1998). *Fusarium* head blight (FHB) disease results in reduced yield and grain quality. The head infection is caused mostly by *F. culmorum* (W.G.Sm.) Sacc. and *Fusarium graminearum* Schwabe (McMullen et al., 1997; Schwarz et al., 1997; Chełkowski, 1998; Buerstmayr et al., 2004; Kulik, 2008). *Fusarium* species produce mycotoxins in kernels of infected heads. Contamination of barley grain with mycotoxins decreases its use for feed and human consumption and significantly affects the malting and brewing quality (Desjardins, 2006; Ma et al., 2009). Barley genotypes vary in their susceptibility to FHB, which is reflected in the various levels of reduction in yield and yield-related traits, e.g., grain weight per ear, 1000-grain weight, and percentage of plump grains (Perkowski et al., 1995; Adamski et al., 1999; Chełkowski et al., 2000; Buerstmayr et al., 2004). Severity of head infection and the level of yield reduction depend not only on plant genotypes, but also on environmental conditions, particularly on temperature and rainfall during several days after infection (Chełkowski et al., 2000; Masterházy, 2002).

The aim of the study was as follows: (1) to evaluate the response to various environments of barley doubled haploids (DHs) infected and non-infected with *F. culmorum*; (2) to assess how the degree of infection with pathogens may change stability and adaptability of genotypes; and (3) to select barley lines stable over a range of environments and more resistant to *Fusarium* head blight.

2. Materials and methods

2.1. Materials

Material for the studies covered 34 spring barley (*H. vulgare* L.) genotypes: 2 parental genotypes (breeding lines 1N86 and R63/1), their F_2 and F_3 populations, and 30 doubled haploid (DH) lines derived from F_1 hybrids. DH lines were developed by the *Hordeum bulbosum* technique. Standard procedures were applied for crossing *H. vulgare* with *H. bulbosum* and for *in vitro* culture of immature embryos (Kasha and Kao, 1970; Devaux, 1986).

2.2. Methods

Field experiments were carried out over 6 years: 1999–2001 at Prusy, South Poland (near Krakow) and 2003–2005 at Cerekwica, West Poland (near Poznań). During each year, the experiment with $k = 34$ genotypes and $t = 68$ treatments was carried out in a group balanced block design according to Gomez and Gomez (1984). The genotypes were divided into two groups: (A) inoculated plants and (B) control plants. The experimental area was divided into three replications, each consisting of $t = 2k = 68$ experimental plots, and each replication was divided into $s = 2$ blocks, each consisting of $k = t/s = 34$ experimental plots. Using one of the randomization schemes, 2 groups of genotypes were assigned at random to the 2 blocks of the first replication and then, independently, for the both remaining replications. Finally, both the groups of genotypes (inoculated and control) were assigned to $k = 34$ plots in the proper block per replication.

In each plot, seeds were sown in six 2 m rows long, 20 cm apart, with each row containing 200 seeds. Each line was artificially inoculated with *F. culmorum*. At full anthesis, 40 spikes of each line in each replication were sprayed (each spike separately) with 2 ml of conidial suspension (5×10^6 in 1 ml) of *F. culmorum* (W.G.Sm) Sacc., isolate KF350 (IPO348-01, ITEM6249) (for details see Warzecha et al., 2010).

Kernel weight per spike, 1000-kernel weight, and plump kernels (kernels of diameter >2.2 mm, in %) were examined in control and inoculated plants.

Mean values of temperature and precipitation in June and July for the years during the experiments are presented in Table 1.

2.3. Statistical analysis

At the first step, one-way analysis of variance (ANOVA) was performed for A and B groups of genotypes examined in a particular experiment, according to Gomez and Gomez (1984). In the next step, statistical analysis of a series of six experiments with 34 genotypes was performed for each group independently using methods described by Caliński et al. (1997) and the computer program SERGEN (Caliński et al., 1998). The methods are based on the multivariate mixed model, which for the observed mean of the trait of genotype i (=1, 2, ..., I) at environment j (=1, 2, ..., J) can be written in the form

$$y_{ij} = \mu + \alpha_i^G + a^E(j) + a_i^{GE}(j) + e_{ij},$$

Table 1
Meteorological data for seasons of experiments.

Year	Temperature (°C)		Precipitation (mm)	
	June	July	June	July
1999[a] (YL1)	17.1	20.3	197	61
2000[a] (YL2)	17.5	16.9	100	177
2001[a] (YL3)	15.3	19.9	79	160
2003[b] (YL4)	19.1	19.5	37	133
2004[b] (YL5)	16.1	17.9	55	49
2005[b] (YL6)	16.7	20.1	19	77
Averages 1976–2005	16.5	18.2	70.5	82.5

Source: Date of the Institute of Meteorology and Water Management, Poland.
[a] Krakow.
[b] Poznan.