Preliminary Evaluation of Locations for Conducting Selection for Resistance to Ergot (*Claviceps purpurea*) in Rye

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Plant breeders conduct selection for resistance in environments that maximize disease development. We studied ergot resistance in rye to assess the suitability of locations as selection environment including one very favourable location for disease development. Sixty-five populations and 245 full-sib families were evaluated in six environments across three agro-climatically diverse locations (Eckartsweier, Kleinhohenheim, Oberer- indenhof [OLI]) and 3 years. Significant genotypic and genotype-environment interaction variations were observed for ergot severity. We further partitioned interaction variation due to linear regression of environments (*b*, genotypic mean at a test location regressed onto average over all environments) and remainder, and also computed simple correlation (*r*) and coefficient of determination (r^2) between these two variables. The *b* and r^2 were used as measures of discrimination ability (DA) and prediction ability (PA) of test locations; respectively. Differences due to heterogeneity among *b*'s were significant in all experiments except one. Ergot severity was distinctly highest at OLI. But OLI had lowest DA and PA whereas Kleinhohenheim had highest values. Our study showed that OLI having higher ergot development, was not the best selection environment. More information encompassing a larger number of environments is needed.

Key Words: ***Claviceps purpurea*, Discrimination ability, Ergot, Prediction ability, Rye, *Secale cereale*, Selection environment**

Introduction

Plant breeders mostly select for quantitative traits. Such traits like grain yield, have complex inheritance as their phenotypic expression is controlled by several genetic factors with small, similar and supplementary effects and is also influenced by the environment and genotype-environment (GE) interactions (Falconer and McKay, 1996). The task of improving these traits is, therefore, a difficult one. In case of quantitatively inherited host-plant resistance, the pathogen is an additional factor that influences the phenotype, and the task of breeding for resistance is complicated further (Geiger and Heun 1989). The genotypes have to be evaluated under specific conditions in a wide range of environments for conducting effective selection and this leads to large expenses. The pathogen is often artificially inoculated so as to promote uniform development of disease and reduce unaccounted variation. Whatever may be the type of selection environment, the endeavor of every plant breeder is to conduct selection in an environment that promotes the identification of genotypes that perform better in the target environment. In addition to mean performance, many criteria have been advocated for the identification of desirable selection environments (Utz, 1972; Becker and Leon, 1988; Dhillon *et al*., 1991). Mostly *r* or r^2 has been used where *r* is the simple correlation coefficient of the genotypic performance in a test environment with that averaged over all environments; and r^2 is referred to as prediction ability (PA) of the test environment. Another criterion that has been used is the linear regression coefficient (*b*) of genotypic performance in the test environment on that averaged across all environments, and is referred to as discrimination ability (DA).

Ergot, caused by *Claviceps purpurea* [Fr.] Tul., is one of the most serious diseases of rye (*Secale cereale* L.). The fungus produces mycelial mass called sclerotium that replaces the kernel, and also produces toxic alkaloids that have harmful effect on the central nervous system in mammals which is known as ergotism (Mainka *et al*., 2007). Thus, breeding for ergot resistance is an important objective in rye breeding. In two previous studies, significant quantitative variation for this trait was reported among self incompatible, fully pollen-shedding rye, among open-pollinated (OP) populations as well as full-sib (FS) families (Mirdita *et al*., 2008; Mirdita and Miedaner, 2009). The objective of the present study was to examine the DA and PA of test environments (location x year combinations), particularly to assess the suitability of one location distinctly more favorable for disease development than others. Many studies have been conducted to evaluate the suitability of different

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locations for conducting selection for quantitative traits, but they have not been performed for quantitative disease resistance in rye, although, they are important since host and pathogen have different ecological requirements and, therefore, contribute differentially to GE interaction. All these factors affect the expression of genetic variability in an experiment.

Materials and Methods

The materials comprised 65 OP populations (indigenous and exotic populations, and cultivars registered in Germany and Poland) and 48 to 50 FS families in each of five OP populations (245 FS families in total), namely the old Polish variety Dankovskije Selekcyjine (Dank. Sel.) that is a selection from a landrace, the modern Ukrainian variety Kharkovskaja (Kharkov.), the Russian strain NEM4 and two older German populations namely Halo and Carokurz, representing the Petkus and Carsten heterotic gene pools, respectively. These gene pools are being commonly used in hybrid breeding. The experiments are referred to as OP populations experiment and FS (with the name of the parent population as a prefix) experiments, respectively.

The materials were evaluated in six environments across three locations and 3 years (see Table 1). The locations were Eckartsweier (EWE, altitude 141 masl, mean annual temperature 9.9°C, mean annual precipitation 726 mm), Kleinhohenheim (KHO, altitude 440 masl, mean annual temperature 8.2°C, mean annual precipitation 700 mm) and Oberer-Lindenhof (OLI, altitude 700 masl, mean annual temperature 6.6°C, mean annual precipitation 952 mm). The OP populations were evaluated during 2002-03 and 2004-05, and FS families during 2003-04 and 2004-05 in three-row plots (0.625 m^2) and six separate lattices, each with three replications. Each test entry in all experiments was separated by four wheat plots planted in a chess-board pattern to avoid plot-by-plot interference (Mirdita *et al*., 2008).

The inoculum, a mixture of *C. purpurea* isolates, was developed and multiplied and artificial inoculations were carried out following Kirchhoff (1929) and Engelke (2002) with some modifications reported in detail by Mirdita *et al*. (2008). Conidium suspension was sprayed with a normal plot sprayer three to four times during full flowering of the entries in the evening with 2 x 10^6 conidia ml^{-1} and about 1,000 l suspension per ha.

The data were recorded as ergot severity (%) on dry weight basis (100 x weight of ergot sclerotia/weight of the mixture of ergot sclerotia and grain). For this, 200-500 g of grains per plot were threshed and analysed, the sclerotia were picked by hand and weighed. The logit-transformed data were subjected to lattice analysis of an experiment in each environment and analysis of variance pooled over environments. In the pooled analysis, the variation due to GE interactions was partitioned into heterogeneity of *b*'s of environments (genotypic means in an environment regressed onto means across environments) and the remainder (Utz, 1972). The *r* between genotypic means in an environment and means across all environments were computed. The *b* and r^2 were used as estimates of DA and PA, respectively. PLABSTAT, a computer package (Utz, 2001) was used for these analyses.

Table 1. Mean (Minimum) expression of ergot severity (%) of open-pollinated (OP) populations and full-sib (FS) families of five OP populations at various locations, namely Eckartsweier (EWE), Kleinhohenheim (KHO) and Oberer-Lindenhof (OLI) [1]

Location (Year)	Mean (Minimum) ergot severity (%)					
	OP Populations (n = 65)[2]	Halo-FS (n = 50)	Carokurz-FS (n = 48)	Dank. Sel.-FS (n = 49)	Kharkov.-FS (n = 49)	NEM4-FS (n = 49)
EWE (2002[3]/2004)	0.66[3] (0.24)[3]	0.32 (0.11)	1.08 (0.24)	0.70 (0.07)	1.07 (0.25)	0.25 (0.05)
KHO (2002)	0.77 (0.22)					
KHO (2004)	0.78 (0.18)	0.21 (0.06)	0.83 (0.11)	0.53 (0.05)	0.25 (0.03)	0.36 (0.09)
OLI (2003)		4.19 (1.53)	7.53 (1.80)	4.34 (3.04)	3.45 (0.92)	4.14 (1.33)
OLI (2004)	6.94 (4.07)	4.42 (1.52)	6.89 (2.53)	4.95 (2.05)	7.13 (3.61)	6.09 (1.36)

[1] Adapted from Mirdita *et al*. (2008) and Mirdita and Miedaner (2009)
[2] *n* is the number OP populations and the number of FS families in five OP populations, namely, Halo, Carokurz, Dankovskije Selekcyjine (Dank. Sel.), Kharkovskaja (Kharkov.) and NEM4.
[3] At EWE OP populations were evaluated during 2002 (marked by superscript) in 2002 and FS families in 2004

Results and Discussion

There was appreciable disease development in various experiments at all locations (Table 1). In the OP populations experiment the lowest ergot severity obtained was 0.18% at KHO (2004). In FS experiments, KHO (2004) had the minimum value (0.03%) for Kharkov. In total there were five FS families (three of Kharkov. and one of Dank. Sel. at KHO, and one of NEM4 at EWE; all during 2004) which had disease severity < 0.05%. All other FS families of five populations had disease severity higher than 0.05%, the threshold limit for the acceptance of grain samples for use as human food in Germany. At OLI, means of ergot severity in individual experiments were 3 to 35 times higher than the other locations in both years.

The mean squares (MS) due to genotypes (OP populations/FS families) in the analyses of variance for each environment and pooled over environments as well as due to environments and GE interactions in the pooled analysis over environments were significant (P<0.01), thereby indicating an environment-dependent response of genotypes to the pathogen (Mirdita *et al*., 2008; Mirdita and Miedaner 2009). Thus, by using our protocol of field inoculation and experimentation, we were able to have appreciable development of the disease and to differentiate the material for their genotypic differences for host-plant resistance in all environments.

Mean ergot severity was distinctly and consistently higher at OLI (3.45-7.53%) than at the other locations (0.21-1.08%). Between EWE and KHO, the severity was higher at KHO in two experiments (OP populations and NEM4-FS) and at EWE in the other four experiments, the average across experiments being higher for EWE. All three locations have natural occurrence of ergot infestation in rye and are highly diverse for climatic conditions. This diversity affects growth and development of the fungus as well as the host and, consequently, the pathogen's pressure and ability of the host to counter that. C. *purpurea* cannot penetrate through the glumes. Hence, its asco- and conidiospores enter the stigma when the flower is open. Cold, rainy weather during flowering delays pollination by restricting the supply of pollen and, thereby, extends the span of flowering phase. Further, wet weather is needed for the germination of ergot spores and rapid development of the fungus (Tudzinksky *et al*., 1995). Thus, cold and rainy weather renders rye more susceptible to ergot. The weather at OLI (the location with higher elevation, lower temperature and higher rainfall) was very favourable for the development of the disease and that led to higher disease severity as compared to other locations.

The MS due to GE interactions was sub-divided into heterogeneity among *b*'s of environments and remainder. The analysis yielded significant MS in all experiments except that due to heterogeneity among *b*'s in Halo-FS experiment (analyses not shown). The results showed that the environments differed significantly for the *b* of genotypic means in an environment onto genotypic means across all environments, that is, the DA, in all experiments except for Halo-FS.

Considering the experiments wherein the differences among *b*'s explained significant portion of GE interaction variation (Table 2), KHO (2004) had always the highest estimate of DA (1.43-1.75). On the other hand OLI had the lowest DA, in four (0.61-0.84) and one (0.62) experiments during 2004 and 2003, respectively. The estimates of PA were also the highest (0.67-0.89) for KHO (2004) in all five experiments. The lowest estimates were obtained for three experiments at OLI (Dank. Sel.-FS and NEM4-FS during 2003, and Carokurz-FS during 2004). In other experiments such estimates were obtained at EWE (Kharkov-FS during 2004) and interestingly at KHO (OP populations experiment during 2003).

Table 2. Discrimination ability (DA = regression coefficient of genotypic means in an environment onto genotypic means across environments) and prediction ability (PA = square of the correlation coefficient between genotypic means in an environment and those across environments) of different environments representing years at three locations, namely Eckartsweier (EWE), Kleinhohenheim (KHO) and Oberer-Lindenhof (OLI) (Results for Halo-FS are not presented as there were no significant difference among environments for their DA)

Location (year)	Discrimination ability (*b*)					Prediction ability (r^2)				
	OP Populations	Carokurz-FS	Dank. Sel.-FS	Kharkov. FS	NEM4 FS	OP Populations	Carokurz FS	Dank. Sel. FS	Kharkov. FS	NEM4 FS
EWE (2002[1]/2004)	0.886[1]	0.880	1.140	0.808	0.870	0.528[1]	0.703	0.676	0.469	0.498
KHO (2002)	0.744					0.407				
KHO(2004)	1.746	1.434	1.576	1.584	1.437	0.670	0.894	0.788	0.799	0.719
OLI (2003)		0.983	0.621	1.002	0.855	0.817	0.508	0.601	0.459	
OLI (2004)	0.624	0.703	0.663	0.606	0.838	0.547	0.662	0.682	0.566	0.529

[1] At EWE, OP populations were evaluated during 2002 (marked by superscript) in 2002 and FS families in 2004

The present study indicated that, though, OLI had distinctly higher disease severity than other locations, but it did not seem to be the best location to conduct selection for ergot resistance. The genotype(s) selected at OLI may not express the same resistance across the target environment as represented by the average across all environments evaluated. This is in spite of the fact that in the FS experiments, OLI accounts for two of the four environments and thus has greater contribution than other locations in defining the target environment. By the same logic, KHO may be expected to have higher estimates of DA and PA in populations experiment. Undoubtedly, it would have been desirable to have the target environment based on a larger number of locations but the results are interesting in view of the high contribution of OLI in defining the target environment. On the whole, this study indicates KHO to be a more appropriate location for deployment as a selection environment. Thus, these results have a significant bearing on breeding for ergot resistance in rye.

Most selection experiments are conducted under conditions favorable for the expression of the traits including disease resistance. Many studies have also been conducted to examine the relationship of mean with DA, PA and related statistics. For example, for maize grain or plant dry matter yield, Weber and Vanselow (1985), Misevic and Dumanovic (1989) and Dhillon *et al*. (1991) reported that more productive locations had better discrimination and prediction, but Pollmer *et al*. (1980) did not observe any such association of DA and PA with environmental means. In conclusion, on the basis of present study OLI did not seem to be an ideal location for conducting selection for ergot resistance. Probably, pathogen pressure was higher than appropriate. Evidently it is difficult to fully elucidate the factors characterizing poor or good DA and PA because of the complex influences of many climatic and edaphic factors on host as well as pathogen. Plant breeders endeavur to have high disease resistance to impart stability of performance to the cultivars across diverse locations which include the ones with high disease pressure. But at the same time selection for resistance under extremely high disease pressure may result in loss of some desirable genes leading to poor performance in the target environment. Thus, it is a dilemma faced by the researcher. Apparently there is a need to collect larger data and undertake more critical analyses of the factors underlying the development of ergot in rye, response of genotypes to the pathogen, and relationship of ergot resistance with other agronomic traits.

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