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ORIGINAL PAPER

Genetic variation for resistance to ergot (*Claviceps purpurea* [Fr.] Tul.) among full-sib families of five populations of winter rye (*Secale cereale* L.)

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Abstract Ergot (*Claviceps purpurea* [Fr.] Tul.) is a serious disease of rye (*Secale cereale* L.) and it adversely affects the quality of grain. The present investigation was undertaken to study genotypic variability among full-sib families (FSF) of five open-pollinated (OP) winter rye populations of highly diverse origin, namely Dankovskie Selekcyine (Poland), Charkovskaja (Ukraine), NEM4 (Russia), Halo and Carokurz, both from Germany. About 50 FSF were developed at random in each population, and the FSF of each population were evaluated in separate but adjacent experiments conducted in four environments under artificial inoculation. A mixture of conidia of *C. purpurea* isolates was sprayed thrice during the flowering period. The materials were manually harvested at yellow-ripe stage. Resistance trait recorded was disease severity, i.e. percent ergot sclerotia in grain by weight. Mean ergot severity ranged from 2.29 to 4.08% for the five populations across environments. Significant genotypic variation ($P < 0.01$) due to FSF and FSF × environment interaction was observed within each population. Genotypic variation within all populations was higher than that among five populations. All populations showed high estimates of heritability (0.72–0.89). The study indicated that the evaluated OP populations are rich reservoirs of genetic variation that should also be used in hybrid breeding. Recurrent selection to further improve ergot resistance should be successful.

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Introduction

Ergot caused by *Claviceps purpurea* [Fr.] Tul. is one of the most serious diseases of rye. It produces dark purplish-black mycelial mass called sclerotium that replaces the kernel in the rye spikelet. The ergot fungus cannot penetrate through the glumes; hence, its infection takes place during the short period of flower opening (Kirchhoff 1929, Tudzynski et al. 1995). The spores invade the floret through the stigma. Rye, being a cross-fertilizing crop, opens the glumes at the time of flowering for cross pollination, facilitating the entry of asco- and conidiospores of *C. purpurea.*

At harvest, many sclerotia fall on the ground, overwinter in the soil, germinate after vernalisation, and produce ascospores, the primary inoculum. The ascospores are spread by wind, splashing rain, and physical contact. The infected floret exudes a white to yellow syrupy substance called 'honey-dew' which contains conidiospores—the secondary inoculum. Honey dew attracts insects and conidia are spread mainly through them and also through physical contact and splashing rain. The dissemination of conidiospores leads to the epidemical spread of the disease.

Claviceps purpurea produces a number of toxic alkaloids, some of which are structurally very similar to lysergic acid (LSD) and cause ergotism (adverse effect on the nervous system) in mammals including human being. In Germany, only grain samples containing ≤0.05 and

0.1% of sclerotia or their pieces are accepted for human food and animal feed, respectively. Similarly, standards are prescribed in the USA and Canada. In Germany, rye is used for bread making and about 60% of the harvest is used for feeding purposes, both as home-grown feed and for industrial mixtures. Therefore, it is crucial to breed for ergot resistance in rye.

To develop an effective breeding program for ergot resistance, it is important to have reliable information on genotypic variability for host-plant resistance, influence of non-genetic factors, and heritability of this trait. Some studies have been conducted but these focussed on parasitic ergot production (Geiger and Bausback 1979; Dotlacil et al. 1997) rather than on the evaluation and improvement of host-plant resistance. In particular, there is no critical information available on the genetic variability within heterozygous open-pollinated (OP) populations. In Germany, about one-third of the rye acreage is devoted to population varieties, and in Poland the percentage is even higher. Moreover, they are important resources for new alleles in hybrid breeding (Geiger and Miedaner 1999).

The objectives of the present study were (1) to evaluate the genotypic variability for host-plant resistance to ergot among FSF of each of five rye populations of diverse origin, and (2) to estimate the variance components (VC) due to genotypic differences and genotype-environment (GE) interaction, heritability (h^2), and the response to selection (R).

Materials and methods

Plant materials

Five OP populations of winter rye used in this study were selected on the basis of their geographical and temporal divergence and their contribution to European rye breeding programs. These were: two German populations namely Halo (registered in 1977) and Carokurz (registered prior to 1953), Dankovskie Selekcyine (Dank. Sel.) a 19th century Polish selection from a landrace, an Ukrainian variety Charkovskaja (Charkov.) registered in 1998, and a Russian breeding strain NEM4 developed in 2000 by the Agricultural Research Institute of the Non-Chernozem Zone at Nemchinovka near Moscow, Russia. Halo and Carokurz represent the 'Petkus-gene pool' and 'Carsten-gene pool', respectively, which were developed, and are still being used in hybrid rye breeding programs in Germany as diverse heterotic groups. All populations are highly self-incompatible, fully pollen shedding, and heterogeneous. In each population, about 50 full-sib families (FSF) were produced in 2002. For this, two randomly chosen plants of a population were cloned into 4–5 pieces and the resulting 8–10 individuals were planted and isolated by polyethylene walls to protect them from alien pollen. Because of the self-incompatibility, each such pair of clones provided seed representing a FSF.

Field testing

The FSF of each population were evaluated in five separate experiments grown adjacent to each other during 2003 and 2004. In each experiment, in addition to the FSF, the respective parental population (each included five times) and a susceptible check was included to result in 56 entries in total. The experimental design was a 8 × 7 rectangular lattice with three replicates. A plot had three-rows each of 1.2 m length and 0.6 m width covering an area of about 0.75 m^2. Each plot was surrounded by wheat plots of the same size on all four sides, by arranging entry and wheat plots in a chess-board pattern. This was done to minimize the spread of disease from a plot to its neighbours. Sowing was done in the last week of September at a density of 300 seeds m^{-2}. Experiments were grown by following cultivation practices of organic farming. No chemical fertilizer or chemical plant protection agent including herbicides was applied. Weeds were managed, when necessary, by mechanical harrowing.

The experiments were conducted at Oberer Lindenhof (OLI: altitude 700 masl, mean annual temperature 6.6°C, mean annual precipitation 952 mm) in 2003 and at OLI, Eckartsweier (EWE: altitude 141 masl, mean annual temperature 9.9°C, mean annual precipitation 726 mm), and Kleinhohenheim (KHO: altitude 440 masl, mean annual temperature 8.2°C, mean annual precipitation 700 mm) in 2004. Hereafter, individual macro environments are designated by an abbreviated location-year combination, e.g. OLI-03 means the experiment conducted at Oberer Lindenhof in 2003. These locations have large differences with respect to their climatic conditions, cover a broad range of agro-ecology of rye cultivation in Germany, and are characterized by natural occurrence of ergot.

The inoculum, an aggressive mixture of *C. purpurea*, was developed and multiplied, and inoculation was done following Kirchhoff (1929) and Engelke (2002) with certain modifications as described in detail by Mirdita and Miedaner (2008).

Resistance trait

The ergot severity defined as percentage of ergot sclerotia in the grain by weight, was used as resistance trait. The spikes in each plot were hand-harvested early at growth stage EC 77 and dried. All spikes per plot were threshed with a single-ear thresher having limited wind supply, so that a majority of the chaff and spindles were separated

from the grain. Thereafter, at first the remnant chaff and spindles were removed from the grain-ergot mixture, and then the grains and sclerotia were manually separated. The cleaned grain-ergot mixture and the hand-picked sclerotia were weighed separately. The ergot severity (%) was calculated as 100 × weight of ergot sclerotia/weight of the mixture of ergot sclerotia and grain.

Statistical analyses

All analyses were based on single plot data. As the residuals were not normally distributed, the data were subjected to logit-transformation for statistically analyses. Lattice analyses were performed separately for each environment and population and combined over environments following Cochran and Cox (1957). Components of variance within each population, due to genotypes (σ^2_G), genotype × environment interaction (σ^2_{GE}), and error (σ^2) were estimated following Searle (1971). Broad-sense heritability (h^2) for each population was calculated as below:

$$h^2 = \sigma^2_G/\left(\sigma^2_G + \sigma^2_{GE}/e + \sigma^2/ek\right)$$

where e and k are the number of environments and replication within an environment, respectively. Expected response to selection per cycle (R) in each population was calculated following Falconer and Mackay (1996):

$$R = ih\sigma_G$$

where i is the selection intensity (assuming a selected fraction of 20%). Phenotypic correlation coefficients among environments were estimated based on the entry mean basis.

The analysis of variance over FSF of all populations was also carried out to estimate components of genotypic variance among populations (σ^2_P), genotypic variance within all five populations ($\sigma^2_{G:P}$), and corresponding interaction involving genotypes and environments (σ^2_{EP}, $\sigma^2_{EG:P}$). All statistical analyses were performed by PLABSTAT (Utz 2005).

Results

An appreciable disease development in all the populations and environments was achieved with an overall average of disease severity of 2.94% (Table 1). The lowest population mean over FSF in an environment was 0.21% in case Halo at KHO-4 (Table 1). The mean disease severity for individual environments ranged from 0.44 (KHO-04) to 5.90% (OLI-04). All individual FSF in all environments showed an ergot severity higher than 0.05% except for five FSF in EWE-04 (data not shown).

Data on disease severity showed a continuous variation across FSF within each population (Fig. 1). The range of resistance reaction in different populations was extended more in the direction of susceptibility (higher severity) rather than that for resistance (lower severity). Halo averaged over its FSF and environments showed the lowest mean and smallest range whereas Carokurz was the most susceptible population with the widest range (Table 1, Fig. 1). Population means across families did not differ from each other except that Carokurz had a significantly higher value than the other four populations. For the range of resistance reaction, population Dank. Sel. had nearly a range as wide as Carokurz, whereas Charkov. had a range comparable to that of Halo. Ergot severity of the populations per se was close to the mean of their FSF as expected.

The ANOVA in each environment showed highly significant ($P \leq 0.01$) genotypic variances for all five populations (data not shown). In the pooled ANOVA over environments, the variance components due to genotypes and genotype × environment interaction were highly significant in each population (Table 2). The estimates of broad-sense heritability were high and ranged from 0.72 (NEM4) to 0.89 (Carokurz). Estimated response to selection was the highest for Carokurz (1.55% points per cycle), closely followed by Dank. Sel. (1.49% points). The lowest value of response to selection was obtained in Halo (0.94% points).

Table 1 Means and standard errors (SE) for ergot severity of full-sib families (FSF) of five populations separately for each environment, means and ranges of FSF and means of the populations per se across four environments

Population	*N*	FSF in individual environments				Across environments		
		OLI-03[b]	EWE-04	KHO-04	OLI-04	FSF		Pop. per se
		Mean ± SE				Mean ± SE	Range	Mean
Halo	50	4.19 ± 0.73	0.32 ± 0.07	0.21 ± 0.07	4.42 ± 0.49	2.29 ± 0.55	1.19–4.82	1.97
Carokurz	48	7.41 ± 1.13	1.08 ± 0.29	0.83 ± 0.22	6.89 ± 0.91	4.08 ± 0.92	1.43–8.07	3.83
Dank. Sel.	49	4.34 ± 0.68	0.70 ± 0.20	0.53 ± 0.13	4.42 ± 0.49	2.63 ± 0.74	1.16–7.57	2.47
Charkov.	49	3.45 ± 0.72	1.07 ± 0.40	0.25 ± 0.09	7.13 ± 1.02	2.97 ± 1.24	1.42–5.40	3.09
NEM4	49	4.14 ± 0.91	0.25 ± 0.09	0.36 ± 0.11	6.09 ± 1.06	2.71 ± 0.20	1.22–6.32	2.65
Susc. Check	1	7.39	1.80	1.74	15.09	-	-	-

N number of FSF, *KHO* Kleinhohenheim, *EWE* Eckartsweier, *OLI* Oberer Lindenhof, 03 = 2003, 04 = 2004

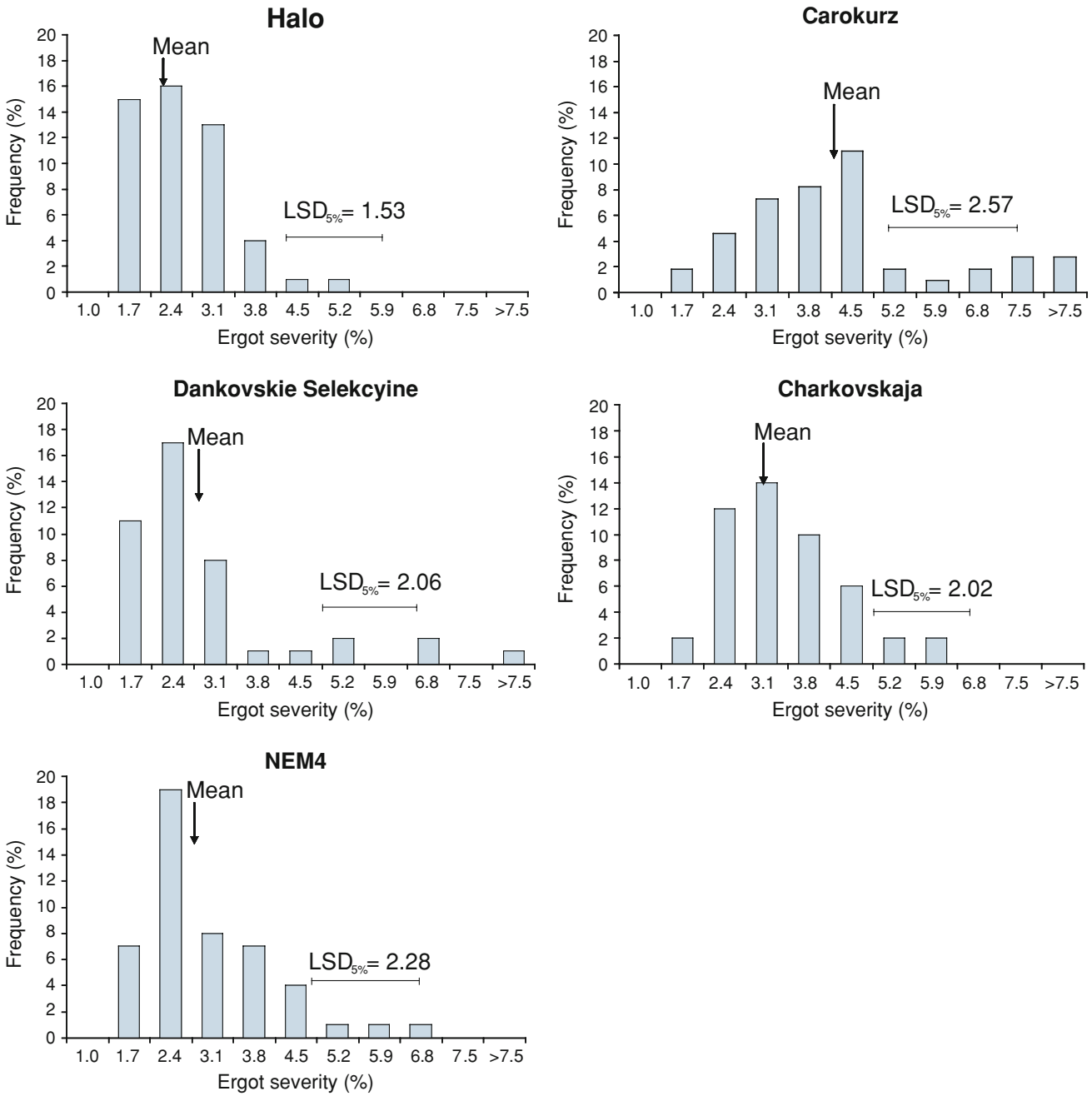

Fig. 1 Distribution of ergot severity of full-sib families of five winter rye populations, averaged across four environments

Phenotypic correlations based on FSF means among four environments were significant in all populations, but the coefficients varied widely over populations (Table 3). Among populations, the lowest and highest correlation coefficients were observed generally for NEM4 (0.28–0.46) and Carokurz (0.55–0.82), respectively.

The pooled ANOVA over populations and environments showed a significant ($P \leq 0.05$) variance among populations and a highly significant ($P \leq 0.01$) variance among FSF within population (Table 4). The interaction variances with environments, both among and within populations, were also significant ($P \leq 0.01$). The genotypic variance component among populations (σ^2_P) was considerably smaller than that within all populations ($\sigma^2_{G:P}$).

Discussion

The data demonstrated that there was appreciable development of the disease in all environments. Disease severity

Table 2 Estimates of components of variance, broad-sense heritability (h^2), and expected response to selection (R) for ergot severity in five populations tested in four environments

Parameter	Halo ($N = 50$)	Carokurz ($N = 48$)	Dank. Sel. ($N = 49$)	Charkov. ($N = 49$)	NEM4 ($N = 49$)
σ^2_G	0.117**	0.270**	0.280**	0.170**	0.142**
σ^2_{GE}	0.068**	0.057**	0.170**	0.117**	0.097**
σ^2	0.240	0.231	0.363	0.285	0.357
h^2	0.76	0.89	0.79	0.76	0.72
R	0.94	1.55	1.49	1.14	1.01

** Significant at $P = 0.01$

σ^2_G genotypic variance among FSF within a population, σ^2_{GE} genotype-environment interaction variance and σ^2 pooled error variance, N = number of full-sib families in each population

Table 3 Range of phenotypic correlation coefficients (r) between each of four environments for ergot severity of full-sib families of five populations

Environment	Range of r over five populations		
	OLI-04	EWE-04	KHO-04
OLI-03	0.35*–0.71**	0.28*–0.67**	0.29*–0.82**
OLI-04		0.42**–0.64**	0.41**–0.68**
EWE-04			0.35*–0.75**

*, ** Significant at $P = 0.05$ and $P = 0.01$, respectively

For abbreviations see Table 1

Table 4 Estimates of components of variance among and within five populations for ergot severity tested in four environments

Source of variation	df	Variance component
Populations (P)	4	$\sigma^2_P = 0.086$*
Families within P	240	$\sigma^2_{G:P} = 0.196$**
P × environment (E)	12	$\sigma^2_{EP} = 0.105$**
Families within P × E	720	$\sigma^2_{EG:P} = 0.102$**
Pooled error	1,820	$\sigma^2 = 0.297$

*, ** Significant at $P = 0.05$ and $P = 0.01$, respectively

was greater than 0.05% with the exception of five FSF in EWE-04. Further, we were able with our protocol of inoculum production, inoculation, and experimental design to differentiate the populations and FSF within populations for their genotypic differences for ergot resistance.

Ergot severity among FSF of five OP populations showed a continuous variation, and the range was wider within Carokurz and Dank. Sel., particularly in the former, than within other populations. Variation for host-plant resistance to ergot in rye has been previously reported by Geiger and Bausback (1979) among 52 pollen-sterile single crosses. This type of variation is thought to be governed by several loci with small supplementary effects and a large impact of environment (Geiger and Heun 1989). Therefore, the study of such a complex trait needs experiments conducted in a broad range of environments and requires larger resources as compared to those dealing with the inheritance controlled by a few genes. Therefore, we conducted the experiment over four location-year environments covering divergent agro-ecological conditions in South Germany.

In each population there was significant genotypic variation among FSF. The FSF responded differentially in the four environments (location-year combinations) to the infection. Significant genotype × environment interaction may be expected for quantitatively inherited traits. In disease resistance experiments, the environmental factors may differentially affect the development of the pathogen as well as the host. In rye, significant genotype × environment interaction have been reported previously for resistance to *C. purpurea* by Mielke (2000) and Engelke (2002) and to other pathogens, e.g. leaf rust caused by *Puccinia recondita* (Wilde et al. 2006) and *Fusarium* spp. (Miedaner et al. 2001).

All five populations showed high heritability (0.72–0.89) for ergot severity. The estimates of response to selection, assuming selected fraction of 20% ranged from 0.94 to 1.55 percentage points per cycle.

Genotypic variance among populations (σ^2_P) was smaller than that among FSF within all populations ($\sigma^2_{G:P}$). This may be expected as rye populations are highly heterogeneous and heterozygous and we had compared five populations versus 245 FSF. Further, these five populations represented a broad range of germplasm in terms of gene pools, ecological origin, and time-frame of development. Also, Hamrick and Godt (1990, 1997) reported larger variation within populations as compared among populations in cross-pollinated plants based on allozymes. Mirdita and Miedaner (2008) evaluated 65 OP populations (52 genetic resources and 13 registered OP cultivars in Germany or Poland) including the five populations of the present study. The FSF of each population in our present study exhibited a larger range than that was found among the 65 populations which was 1.32–4.32% ergot severity (Mirdita and Miedaner 2008). Halo showed the least mean ergot severity in both studies. Therefore, some FSF of Halo was observed having better disease resistance than the parent population per se. Similarly, some FSF of Dank. Sel. and NEM4, the populations which showed disease severity on par with Halo (Mirdita and Miedaner 2008), had a very low ergot severity. Further, the estimates of heritability obtained in the present study were higher than that reported by Mirdita and Miedaner (2008). Thus, the present study shows that the five OP populations evaluated are rich reservoirs of genetic diversity.

Assuming the genotypic variance among FSF to be predominantly additive-genetic variation, high values of response to selection and broad-sense heritability suggest that recurrent selection should be a promising approach to improve resistance to ergot. The choice of the base population(s) for recurrent selection depends on the mean performance for various important traits and variability within the population(s), objective of the program (intra- or inter-population improvement), and type of the variety (population or hybrid variety) to be developed. Despite the lowest response to selection, Halo seems to be a good candidate for recurrent selection within the Petkus gene pool, as it had the lowest mean ergot severity (2.29%), high heritability, and shows good performance for most of the other agronomic traits. For reciprocal recurrent selection the source populations must additionally be diverse and display maximum heterosis. Halo and Carokurz are good candidates and have already been used as opposite gene pools in rye hybrid breeding in Germany (Geiger and Miedaner 1999). It is, however, important to allocate more resources for the improvement of resistance to ergot in Carokurz.

From the present study it may be concluded that severity of ergot in rye has a complex inheritance. Our study reports highly significant differences for host-plant resistance among FSF and a high genotype × environment interaction in the five OP populations. The high importance of GE interaction emphasizes the need to conduct experiments over locations and years under appropriate disease pressure. Molecular studies would be valuable to further analyse the genetic architecture of ergot resistance in rye. Biparental QTL analyses being not feasible in self-incompatible rye, association mapping would be appropriate. For practical breeding the high estimates of heritability and response to selection suggest that recurrent selection should be effective in improving this trait, and extracting inbred lines with higher resistance for hybrid breeding. The ultimate goal should be to have high resistance levels in population and hybrid varieties. To the best of our knowledge, the present study is the first published report on genetic variability for ergot resistance within populations of winter rye.

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