Theor. Appl. Genet. 51, 49-53 (1977)

Genetic Control of Resistance to Crown Rust (*Puccinia coronato* Corda) in *Lolium perenne* L. and its Implications in Breeding

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Summary. A genetic analysis of resistance to crown rust in a series of natural populations of *Lolium perenne* by means of the diallel cross has revealed that heritability is high (58%) and that the genes concerned show complete but ambi-directional dominance for resistance. An environmental correlation was established between the degree of infection and productivity but no genetic correlation was present. The implications of this mode of control for resistance breeding procedures are briefly considered.

Key words: Genetic - Diallel - Rust - Ryegrass - Breeding

Introduction

Recent trends in grass breeding have been towards the production of specialized cultivars for use under closely defined management systems. This had led to the production of pastures with restricted variability with the possibility of increased susceptibility to pathogens (Carr 1975). In order to alleviate this problem it is now necessary for the grass breeder to consider the introduction of genetic resistance to the various diseases likely to be encountered in a sward.

It has been proposed that the successful long term prospect for resistance breeding in the outbreeding crops is dependent upon the use of polygenic as opposed to major gene resistance in order to avoid the breakdown often associated with the latter (Carr 1975).

One of the major fungal pathogens of intensively managed grassland is crown rust of perennial ryegrass which can cause quite substantial losses in dry matter production and its consequent utilization by the grazing animal (Lancashire and Latch 1966). The present report concerns the nature of the gene action governing resistance to this disease in a collection of ryegrass populations and its implications in breeding practice.

Materials and Methods

The material consisted of ten populations of *Lolium perenne* (an outbreeding, diploid species) collected from a range of habitats in North Wales. Within the limits of available knowledge, all derived from pastures with no known history of reseeding and hence may be considered as natural ecotypes.

The genetic control of variation was assessed by application of a diallel mating design. Populations were sampled by taking five individual plants from each and randomly allocating them to a diallel set; thus five complete dialleles including reciprocals were established between all ten populations. Equal quantities of seed of each progeny family of a common population cross were bulked to provide a single population diallel of 90, F_1 progeny families. The ten parental populations were each represented by a bulk sample of seed of a polycross of the five individuals.

The material was established in the field in April 1971 as a spaced plant, randomized block experiment with four replicates, each family being represented by a single row of ten plants per replicate.

The incidence of rust infection was visually scored in September of the establishment year on a 1 to 5 scale, from free to severely infected. The productivi- of the individual plant was also assessed as yield, in grammes of fresh material, one week after they were scored for rust. Family means were used throughout the analysis.

The Analysis

The diallel crossing scheme adopted is a comprehensive mating design which may be analysed according to various procedures (see Mather and Jinks 1971; Griffing 1956; Gardner and Eberhart 1966). For the present experiment the techniques of diallel analysis developed by Hayman (1954) and Jinks (1954) will be utilized. These require certain basic assumptions to be met which are

i) diploid inheritance
ii) homozygous parents
iii) no multiple alleles
iv) independent gene distribution
v) no non-allelic interaction
vi) absence of reciprocal effects

Failure of one or more of the assumptions (ii)-(iv) has been shown to have a characteristic effect upon the analysis and can be recognized as such (Mather and Jinks 1971). For outbreeding species the second assumption does not rigorously hold. However where selection has operated differentially on the parental populations to the extent that clear cut differences exist between them, it is likely that there will be fixation at many of the gene loci concerned. Under these conditions the parent populations will be homozygous for the genes by which they differ there is thus no a priori reason for rejecting any of the above assumptions (see also Breese and Hayward 1972). Nevertheless the 'generalized analysis' of Dickinson and Jinks (1956) considers the effects of heterozygosity and its recognition even in the presence of non-random gene distribution; detection is however complicated if any of the other assumptions fail (e.g., non-allelic interaction). Under these circumstances estimates of the various genetic parameters, particularly levels of dominance, may be distorted and have to be interpreted with caution. The effects of applying the analysis developed for homozygous diallels and the 'generalized analysis' to heterozygous and mixed homo/heterozygous diallels together with further analyses dependent upon the prior recognition of heterozygosity have been examined by Oakes (1967). From theoretical and practical studies he showed that both under and over estimates of the average level of dominance and gene frequencies may be obtained but that no major erroneous conclusion would result from application of these various analyses.

Results

The family means for the incidence of rust are shown in Table 1 from which it can be seen that there are no clear cut qualitative differences between families. Hence the data will be treated as a continuously distributed variable. The analysis of variance of a diallel table of Hayman (1954) has been applied to the data and is shown in Table 2. As the interaction terms of the main effects with blocks are heterogeneous ($P = 0.02-0.01$), each main item of the analysis has been tested against its appropriate error term.

The significant '$\underline{a}$' mean square reveals the presence of additive variation for this character. The overall '$\underline{b}$' term is also significant, but of its components only '$\underline{b}_3$' achieves significance, suggesting the presence of dominance, which, in the absence of '$\underline{b}_1$' would be ambidirectional in nature. The remaining '$\underline{c}$' and '$\underline{d}$' items of the analysis are non-significant, indicating the absence of any variation which may be attributed to reciprocal effects. The significant block item of the analysis should be noted in view of the epidemiology of rust infection, as it was observed that infection arose at several foci before spreading throughout the experimental area.

A more detailed insight into genetic control of rust resistance may be obtained from a consideration of the relationships of the variance (Vr) of progeny fam-

Table 1. Family Means for Rust Score (above diagonal) and Productivity (below diagonal)

	1	2	3	4	5	6	7	8	9	10
1	3.25 886	3.12	3.05	2.80	2.90	3.10	3.00	3.22	2.90	2.26
2	884	3.0 869	3.21	2.63	2.82	2.58	2.65	2.78	2.96	2.60
3	879	887	3.30 1087	2.60	2.70	2.51	3.17	3.21	2.80	2.42
4	723	717	750	2.45 557	2.77	2.76	3.15	2.15	2.70	2.38
5	812	621	906	757	2.75 729	2.82	2.52	2.77	2.83	2.11
6	912	820	1146	843	836	2.4 839	2.53	2.32	2.50	2.45
7	881	788	880	811	801	812	2.67 978	2.35	2.50	2.65
8	833	782	908	772	795	727	930	2.37 874	2.85	2.15
9	800	658	911	581	703	862	613	729	2.65 705	2.04
10	978	926	971	643	1043	908	812	924	858	2.17 910

Table 2. Analysis of Variance of Rust Score and Productivity

Item	d.f.	Rust score M.S.	Productivity M.S.
a	9	2.750***	22754***
b	45	0.349**	2935
b_1	1	0.005	679
b_2	9	0.096	1741
b_3	35	0.424**	3307
c	9	0.301	3843
d	36	0.248	3409
Blocks	3	1.005***	37651***
B × a	27	0.389	3469
B × b	135	0.173	2281
B × b_1	3	0.098	1076
B × b_2	27	0.190	2480
B × b_3	105	0.171	2264
B × c	27	0.150	2239
B × d	108	0.168	1769

** = P 0.01 - 0.001, *** = P 0.001

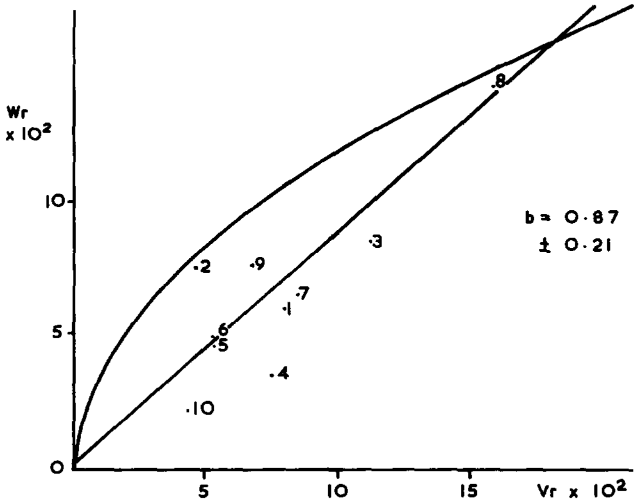

Fig.1. Wr/Vr graph of Rust Infection

Table 3. Analysis of Wr + Vr, Wr - Vr and Joint Regression of Wr on Vr

	d.f.	(Wr + Vr) M.S. × 10^5	(Wr - Vr) M.S. × 10^6
Between arrays	9	1912*	1788
With arrays	30	911	2365
Joint regression	1	8819***	
Heterogeneity	3	579	
Residual	32	341	

Table 4. Components of Variation

$D = 2.1075$	$H_2 = 2.419$
$H_1 = 2.72102$	$F = 0.1143$
$E = 0.189$	$\sqrt{H_1/D} = 1.136$

$h^2n = 0.5844$

ily means within an array with the covariance (Wr) of these progenies with the non-recurrent parent. These are presented graphically in Fig.1. The regression of Wr on Vr departs significantly from 0 but does not deviate from unity, the slope expected for a homozygous diallel situation (Jinks 1954); the triangular distribution of points however would suggest the presence of heterozygosity amongst this set of populations, 2 and 9 being the most heterozygous. The significant regression also indicated the presence of dominance, the distribution of points revealing that population 10 contains a preponderance of dominant genes with population 8 the most recessives. The direction of dominance may be calculated from the correlation of (Wr + Vr) with the corresponding parental value for each array (r = 0.139). It is non-signifcant, reenforcing the evidence of the analysis of variance that dominance is ambi-directional in nature, that is, resistance may be dominant or recessive at the various loci involved. The reliability of this graphical presentation and the regression as a measure of dominance may be determined from an analysis of variance of (Wr + Vr) and (Wr - Vr) over blocks together with a joint regression analysis of Wr on Vr (Table 3).

The value of (Wr + Vr) are significant when tested against their block interaction, indicating that the relative distribution of array points throughout the graph is consistent and confirms the occurrence of dominance, whilst (Wr - Vr) is not significant, showing that the whole of the variation may be accounted for by an additive/dominance model. This is further verified by the significance of the joint regression analysis. The overall regression line cuts the Wr axis close to the origin, suggesting that dominance is virtually complete. This may be corroborated by the relationships of the components of variation presented in Table 4 which have been obtained from the statistics by the methods of Mather and Jinks (1971).

The ratio of $\sqrt{H_1/D} = 1.136$ reveals that dominance is complete, whilst the positive value of F shows that there are more dominant than recessive alleles in these lines. The components allow an estimate of narrow sense heritability to be obtained from the ratio of additive to total phenotypic variation, this value is 58.4% indicating a high potential for selection.

The relationship between the incidence of rust and subsequent plant productivity was investigated using an analysis of covariance, a preliminary analysis of variance having revealed that productivity at this growth period was under simple additive genetic control with no evidence of dominance or other effects (Table 2). Genetic and environmental correlations were estimated from the components of variance and covariance as $\sqrt{\frac{\mathrm{Cov}\,xy}{\mathrm{Var}\,x\ \mathrm{Var}\,y}}$. The genetic correlation (r = 0.1123) was non-significant whilst the environmental was negative and significant (r = 0.4129***).

Discussion

The occurrence of resistance of *Lolium perenne* to crown rust has been well established (Cruickshank 1957; Hanson 1965; Wilkins 1975; McVeigh 1975). Detailed studies of the genetic nature of the resistance have revealed a varying pattern of control. Cruickshank (1957) in New Zealand was able to identify discrete classes of infection whilst McVeigh (1975) in the U.S.A. established that control could be qualitative, conditioned by two recessive genes, or quantitative in nature. The results of these previous studies would suggest that relatively few genes are involved but that the environment obliterates any distinct segregational patterns. In the present experiment as no clear cut qualitative differences in disease reaction were observed amongst the parental and F_1 families of the diallel mating scheme utilized, it was considered more appropriate to analyse the data as a quantitative character although this does not preclude the possibility that the control is by a limited number of loci. A further factor of practical consideration is that the quantitative statistical techniques applied allow an assessment of the mode of gene action operative within this collection of *Lolium perenne* without having to isolate genes and control infection procedures and thus gives breeding information fairly rapidly.

For the present set of populations the important features to emerge from the analysis from the practical grass-breeding angle are that heritability is relatively high; thus phenotypic selection without recourse to progeny testing should be an effective means of raising the resistance of the population, and that dominance is complete but is ambi-directional in nature. This latter fact raises certain problems for its efficient utilization in a breeding programme.

There are two situations where it is desirable to differentiate between dominant and recessive resisters in the construction of a synthetic variety of a forage grass. If the chosen parental plants include a preponderance of one form of resistant parent as opposed to the other, in the event of rust infection occurring during the early multiplication generations of the synthetic variety the overall level of resistance of the ensuring generation will differ if there is a reduced reproductive potential of susceptible individuals. With selection of predominantly recessive resistant parents the population will potentially have a higher mean level of resistance than with selection of dominant resistant parents. On infection, a greater proportion of the population derived from the recessive resistant parents will survive compared with the synthetic based on dominant resistance. With subsequent multiplication however, the latter will give rise to a generation having a higher overall level of resistance as selection will have favoured the homozygous recessive resistant parents leading to fixation at those loci, and also the frequency of the dominant resistant allele will have been raised. In practical terms the choice will undoubtedly be dictated by the number of potential parents within each resistance group and the necessity to consider other agronomic characteristics.

In a breeding programme where it is desired to introduce resistance into an otherwise agronomically acceptable variety by such a scheme as the backcross procedures proposed by Wright and Breese (1966) for Blind Seed Disease Resistance in *L.perenne*, greater efficiency will be achieved by the use of dominant as opposed to recessive resisters. The choice of dominancy will greatly facilitate the identification of resistant genotypes without the need to introduce alternating generations of interpollination and selection with a consequent increase in the length of the breeding programme.

The identification of the two forms of resistant genotypes may be most easily accomplished by the application of the complete diallel analysis as presented here. The graphical representation of the relationship of Wr to Vr allows precise discrimination in that plants with a majority of dominant genes lie closer to the origin of the graph whilst the complete recessive is positioned on the line of slope 1 at the point where Wr and Vr are equal to the variance of the parents. From Fig.1 it can be seen that populations 10 contains most dominants, and is coincidentally the most resistant line, whereas population 8 contains most recessives and is the second most resistant line.

In practive a high level of genetic diversity is undoubtedly desirable in order to minimize the likelihood of a breakdown in resistance (Carr 1975). To achieve this situation an accumulation of both dominant and recessive forms of resistance amongst the parental plants should be considered. This does however necessitate the introduction of a degree of susceptibility. In the grass sward where survival is dependent on continual vegetative reproduction by tillering a proportion of susceptible genotypes may well be tolerated. Indeed the evolution of ambi-directional dominance for resistance could be accounted for by this survival mechanism in contrast to a directional dominance situation where the latter evolves as a means of maintaining a high frequency of desirable genotypes over cycles of sexual reproduction in an outbreeding species (Breese and Hayward 1972).

A further feature to emerge from the present results is the genetic independence of the degree of resistance from the productivity of the plant. The low but significant environmental correlation would suggest that plants which are of minimal productivity due to environmental factors may also be more susceptible to rust infection, or alternatively environmental factors predispose the plant to the pathogen with a consequent decline in productivity. This decline may increase the longer harvesting is delayed after the initial infection, a factor which could have occurred in the present experiment in that foci of infection possibly established at different times in the field blocks. This emphasizes the difficulties which may be encountered in genetic studies of resistance under conditions of field infection.

Acknowledgement

I wish to acknowledge Mr. N.J. McAdam for technical assistance and Mr. P.W. Wilkins, Pathology Department and Dr. E.L. Breese, Herbage Breeding Department, Welsh Plant Breeding Station for much useful discussion during the preparation of this paper.

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Received December 7, 1976
Accepted June 15, 1977
Communicated by R. Riley

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