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The Inheritance of Diastatic Power and Alpha-Amylase Contents in Spring Barley

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Summary. In a diallel cross of 13 spring barley varieties, non-allelic interactions interfered with the analysis of diastatic power. Regression of W_r on V_r was less than unity both in the full experiment and in the sub-set of two-row varieties. The simple additive-dominance model was adequate for the analysis of alpha-amylase. Regression of W_r on V_r indicated directional dominance, increasing alpha-amylase. For both characters non-additive variance was detected and the effects of directional dominance, asymmetry of gene distribution and SCA were evident. Significant GCA effects were also present. No serious obstacles to the improvement of either character in adapted two-row varieties were apparent.

Key words: Spring barley – Diastatic power – Alpha-amylase – Diallel cross

Introduction

The production of sufficient quantities of starch-degrading enzymes, particularly 1 : 4 α-glucosidases, is an essential attribute of malting quality in barley. Diastatic power (DP) is an important factor in cases in which the malt is used, in conjunction with unmalted cereal adjuncts, in the manufacture of grain whisky (Pyke 1965). A high content of alpha-amylase (AA) has been found to be correlated with malting quality where the malt is used for beer production (Atanda and Miflin 1970).

The factors influencing DP have been studied in varietal trials (Streeter and Pfeifer 1966; Hayter and Riggs 1973) which have demonstrated the presence of considerable genetic variation. DP was positively correlated, both environmentally and genetically, with raw grain and malt properties, particularly crude protein (grain N% × 6.25) and alpha-amylase contents. Studies in breeding programmes of selected lines at all stages from F_1 to F_7 (Den Hartog and Lambert 1953; Hsi and Lambert 1954; Rasmusson and Glass 1965, 1967; Rutger, Schaller, Dickson and Williams 1966; Rutger, Schaller and Dickson 1967; Baker, Bendelow and Buchanan 1968) have all shown a positive correlation between DP and grain nitrogen content. The heritability of DP was usually intermediate or high. There have been few genetic studies of DP (Nečas 1960; Rasmusson, Upadhyaya and Glass 1966; Foster, Peterson and Banasik 1967) and little detailed analysis has been published of the underlying genetic mechanism.

There have been few investigations of the mode of inheritance of AA. Gothard (1974) reported a study of sources of genetic variation available to the plant breeder; Hayter and Riggs (1973) showed that AA was not strongly influenced by environmental variation and was largely determined by varietal differences. This paper examines DP and AA in a diallel cross involving nine two-row and four six-row varieties.

Materials and Methods

The 13 spring barley varieties used in the experiments are listed in Table 1. Full experimental details, including the pedigrees and provenances of the varieties, have been given by Riggs and Hayter (1972, 1973, 1975), but briefly, the experiment comprised a 13 × 13 half diallel (13HD) with ten sibs per cross, sown in each of two replicate blocks, in each of two seasons. Data from the 1971 season are presented here and the 1970 season is referred to only where the date provide additional information. Individual sibs were randomly distributed within each block. Analyses could have been made using F_1 hybrid grains but in naturally self-pollinating crops such as barley these grains are atypical as a result of the manipulations involved in their production. The analyses were, therefore, made on samples of F_2 grains from F_1 plants. The enzymes under investigation are synthesised in or have their effects on endosperm tissues, which are triploid and derived from one paternal and two maternal genomes whereas diploid inheritance is assumed for diallel analysis. Greenberg (1977) concluded that this did not affect the analysis of F_2 grains on F_1 plants. However, to test for the absence of maternal effects genotypes 1

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to 9 were arranged in a 9 × 9 full diallel (9FD) with five sibs per cross. By summation of reciprocals in the absence of maternal effects, genotypes 1 to 9 could then be included in the 13HD. Finally, the nine two-row varieties, arranged in a half diallel (9HD), were also analysed to clarify particular aspects of the inheritance.

To estimate enzyme activities samples of ten grains from individual F_1 plants were placed on two Whatman No. 1 filter papers in a petri dish and 4 ml of distilled water was added to each dish. Dishes were incubated at 5°C for 24 hours to allow imbibition to occur, then germinated at 18°C for 72 hours. All ten grains were extracted by maceration in 10 ml of 0.4% w/v calcium chloride hexahydrate. Extracts were centrifuged at 1600 rpm for 5 minutes and the supernatants were assayed for DP and AA activities. The few seed samples with less than 80% germination were repeated.

DP was estimated by the automated method of Scharoun and Saletan (1965) which is based on the recommended manual method of the American Society of Brewing Chemists. Tests showed a close correlation with the Recommended Manual Method of the Institute of Brewing but with one important advantage: whereas in the IB Manual Method the relationship between peak height and enzyme concentration was curvilinear with a plateau at approximately 250° L, in the automated method the relationship was linear over a considerably wider working range. The absolute relationship between peak height and enzyme concentration was subject to change due both to laboratory variations in temperature and to drift in the automated system. Peak readings were expressed as a proportion of the nearest enzyme standard (500 mg Wallerstein β-amylase in 100 ml 0.5% w/v sodium chloride, DP = 250° L). Standards were repeated every 20 samples. The data are termed DP proportions (DPP) to emphasise that caution should be used in equating them with DP determined by manual methods.

The automated method of Trachman and Saletan (1970) was used to determine AA. The recommended manual method of the Institute of Brewing to estimate Dextrinising Units (DU) again showed a linear relationship between the units of measurement and enzyme concentration over a very wide working range (0-50 mg Sigma alpha-amylase, type IV). Peak heights from the automated method were again expressed as a proportion of the nearest enzyme standard (20 mg Sigma type IV alpha-amylase in 100 ml 0.5% w/v sodium chloride, DU approximately 100). The data are termed AA proportions (AAP) and can be directly equated with DU (1.00 AAP = 100 DU). Throughout the experiments batches of starch substrate and enzyme standards were changed between replicate blocks and this has almost certainly increased the magnitude of block effects which are highly significant in many of the analyses.

Details of the methods of genetic analysis have been given by Riggs and Hayter (1972, 1973, 1975) and are based on the procedures described by Mather and Jinks (1971). Briefly, they include analyses of variance of diallel tables, examination of the graphical relationships of variances and covariances and the estimation of genetic components of variance and of genetic correlations between pairs of variates, including those of importance to the plant breeder.

Results and Discussion

The phenotypic means for the 13 parent varieties are shown in Table 1. For DPP, the means for all of the six-row varieties were higher than eight of the nine two-row varieties, the exception being Mosane. Golden Promise and Mosane have been used commercially in Great Britain for the production of diastatic malts. Several two-row varieties had high AAP values, including Boreham Warrior and Maris Baldric, but only the six-row varieties Olli, Pirkka and OAC 21 combined high DPP and high AAP values.

Table 1. Mean diastatic power (DPP) and alpha amylase (AAP) for the parents of the 13 × 13 half diallel

	DPP	AAP
1. Olli*	1.223	0.718
2. Pirkka*	0.986	0.792
3. Cambrinus	0.851	0.532
4. Ymer	0.834	0.605
5. Stock 1	0.713	0.599
6. Scotch Bere*	1.653	0.403
7. OAC 21*	1.038	0.622
8. Golden Promise	0.782	0.569
9. Maris Baldric	0.786	0.750
10. Midas	0.765	0.592
11. Mosane	1.195	0.522
12. Sultan	0.837	0.432
13. Boreham Warrior	0.800	0.829
	± 0.078	± 0.039
Mean		
	0.959	0.613
	± 0.022	± 0.011

* Indicates six-row variety

1. *DPP*

The within-family variances in the 13 HD were heterogeneous and highly correlated with family means. The heterogeneity was reduced by transformation to square roots and almost eliminated by logarithmic transformation. However, the genetic conclusions from the untransformed data and from both sets of transformed data were very similar and for ease of presentation the analyses of untransformed data are given.

For each array V_r, the variance of all offspring of the r^{th} parent and W_r, the covariance between offspring and the non-recurrent parent were calculated. The relationships between V_r and W_r provide tests of the assumptions on which the genetic model is based. The array values of $(W_r - V_r)$ were heterogeneous, indicating failure of the additive dominance model, due to the presence of non-allelic interactions. In the absence of data from F_3 grain on F_2 plants the nature of the interactions could not be determined. The slope of the regression of W_r on V_r (Figure 1) did not differ significantly from unity but the standard error was large. Six-row and two-row varieties

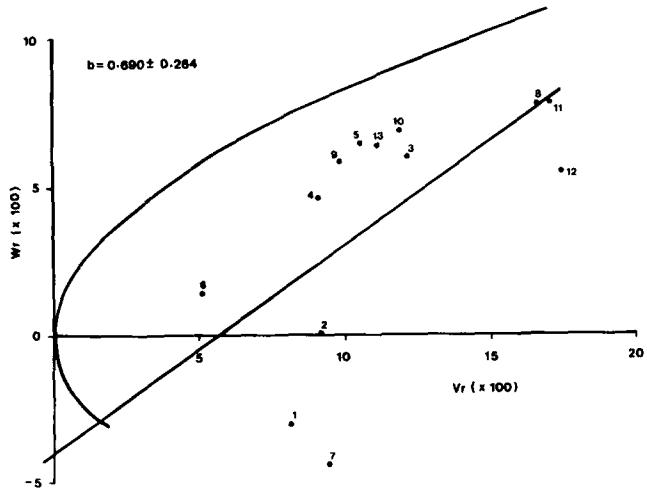

Fig. 1. W_r, V_r regression for diastatic power, 13HD

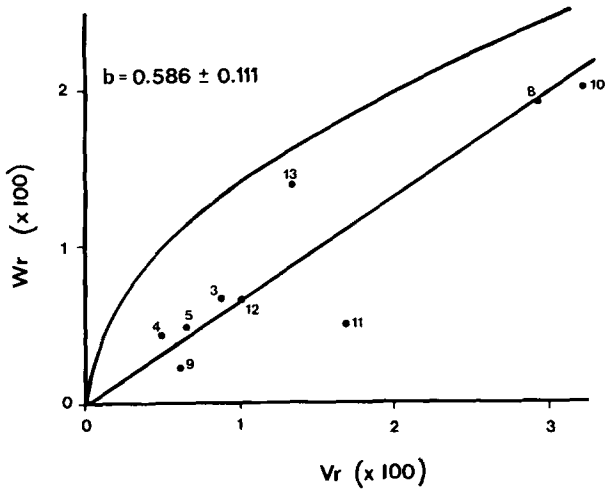

Fig. 2. W_r, V_r regression for diastatic power, 9HD

Table 2. Hayman analyses of variance of diastatic power (DPP) and alpha amylase (AAP) in the 9 X 9 full diallel

Item		DPP	AAP
	d.f.	MS	MS
a (additivity)	8	3.033'''	1.154'''
b (dominance)	36	1.953'''	0.070'''
b_1 (direction)	1	10.526'''	0.784'''
b_2 (distribution)	8	0.540'''	0.053
b_3 (specificity)	27	2.055'''	0.048'
c (maternal)	8	0.139	0.011
d (other reciprocals)	28	0.140	0.043'
Blocks	1	0.643'	0.262''
Bt (pooled block interactions)	80	0.128	0.030
W.f.v. (within-family variance)	625	0.141	0.028

All items tested against the average within family variance of parents and F_1's

', '', ''' indicate significance at $P \leq 0.05$, 0.01 and 0.001 respectively

formed two distinct groups, with six-row varieties occupying positions suggesting that high DPP was determined by an excess of dominant genes. There was a negative correlation between the parent mean, Y_r, and $(W_r + V_r)$ but this failed to reach significance ($0.05 \leq p \leq 0.1$). In the 1970 data this correlation was significant ($r = -0.744$). Apparent over-dominance was indicated by the negative intercept of the regression on the W_r axis. Analysis of the two-row varieties (Figure 2) showed that the non-allelic interactions affecting the slope of the regression were not confined to differences between six-row and two-row groups. However, in these varieties there was no apparent overdominance. Y_r and $(W_r + V_r)$ were not significantly correlated. Genetic components of variance were not estimated as non-allelic interactions were detected by graphical analysis.

Analysis of variance (Hayman 1954) of the full diallel (9FD, Table 2) demonstrated the absence of maternal effects (c) as predicted by Greenberg (1977). Analysis of the 13HD (Jones 1965) detected both additive and non-additive genetic variation (Table 3). The non-additive variation was due to directional dominance (b_1), asymmetry of gene distribution (b_2) and to interactions between specific genotypes (b_3). Heterosis was apparent with the F_1 hybrid mean exceeding that of the parents by approximately 30%. This was presumed to stem, at least in part, from interactions between six-row and two-row parents since overdominance was not detected in the 9HD.

Combining abilities for the two blocks were calculated according to Method 4, Model I of Griffing (1956) and significant effects both for general (GCA) and specific (SCA) combining abilities were detected. Individual GCA effects (Table 4) were consistent over blocks and positively correlated with parental means. All six-row varieties showed large positive effects, while of the two-row varieties all except Mosane showed negative effects. Individual SCA effects were more variable and sometimes differed between blocks. The most interesting positive SCA effects were between six-row and two-row crosses. Six-row by six-row crosses generally produced negative SCA effects, and some two-row varieties, particularly Golden Promise and Sultan, showed a preponderance of negative SCA effects but were strongly positive in combinations with six-row varieties.

2. *AAP*

Within-family variances were homogeneous and were not correlated with family means so that transformation of the data was not necessary. The array values for $(W_r - V_r)$ were homogeneous while those for $(W_r + V_r)$ were heterogeneous, indicating that the simple additive plus dominance model was adequate for the analysis of AAP. Re-

gression of W_r on V_r gave a slope not differing significantly from unity (Figure 3) but this was close to significance. ($0.05 \leqslant p \leqslant 0.1$). (The 1970 data gave a regression slope of less than unity, suggesting that in certain seasons non-allelic interactions may be present). The intercept on the W_r axis was positive, indicating partial dominance. Six-row varieties and Stock 1 occupied positions indicating an excess of dominant genes while Sultan was close to the point expected of a variety with most of the recessive genes. Regression of W_r on V_r for the two-row varieties (9HD) indicated no epistasis in either season (Figure 4 shows the 1971 data). Interactions present in the 13HD in 1970 presumably stemmed therefore from differences between six-row and two-row varieties. Y_r was not significantly correlated with $(W_r + V_r)$ in the 13HD but was in the 9HD (Table 5). The negative correlations in both cases suggest a proponderance of directional dominance effects increasing AAP.

Genetic components appropriate to F_2 grain on F_1 plants were derived by unweighted least squares estimation (Mather and Jinks 1971). Six components (D, H_1, H_2, F, E_0, E_1) were estimated from twelve statistics (V_p, $\bar{V}_r$, $V_{\bar{r}}$, $\bar{W}_r$, E_0 and E_1 from each of two blocks). D, H_1 and H_2 were significant. (Table 5). The ratio $(H_1/D)^{\frac{1}{2}}$ did

Table 3. Hayman analyses of variance of diastatic power (DPP) and alpha amylase (AAP) in the 13 X 13 half diallel

		DPP	AAP
Item	d.f.	MS	MS
a (additivity)	12	9.609'''	1.469'''
b (dominance)	78	1.775'''	0.079'''
b_1 (direction)	1	23.079'''	1.815'''
b_2 (distribution)	12	1.159'''	0.065''
b_3 (specificity)	65	1.561'''	0.056''
Blocks	1	1.028'''	0.480'''
Bt (pooled block interactions)	90	0.143	0.024
W.f.v. (within-family variance)	1602	0.121	0.028

All items tested against the average within family variance of parents and F_1's

', '', ''' indicate significance at $P \leqslant 0.05$, 0.01 and 0.001 respectively

Table 4. General combining abilities for diastatic power (DPP) and alpha amylase (AAP) in the 13 X 13 half diallel

	DPP		AAP	
	I	II	I	II
1. Olli*	0.393	0.350	0.102	0.115
2. Pirkka*	0.299	0.286	0.106	0.097
3. Cambrinus	−0.138	−0.066	−0.044	−0.036
4. Ymer	−0.156	−0.240	0.020	−0.008
5. Stock 1	−0.233	−0.159	−0.036	−0.045
6. Scotch Bere*	0.136	0.265	−0.155	−0.158
7. OAC 21*	0.235	0.198	0.033	0.049
8. Golden Promise	−0.074	−0.102	−0.012	0.004
9. Maris Baldric	−0.152	−0.179	0.067	0.052
10. Midas	−0.183	−0.162	−0.031	−0.040
11. Mosane	0.123	0.068	−0.019	−0.019
12. Sultan	−0.067	−0.083	−0.084	−0.066
13. Boreham Warrior	−0.183	−0.178	0.055	0.054
S.E. $(g_i - g_j)$	± 0.050		± 0.020	
Population mean ± SE	1.255 ± 0.010	1.306	0.718 ± 0.010	0.688

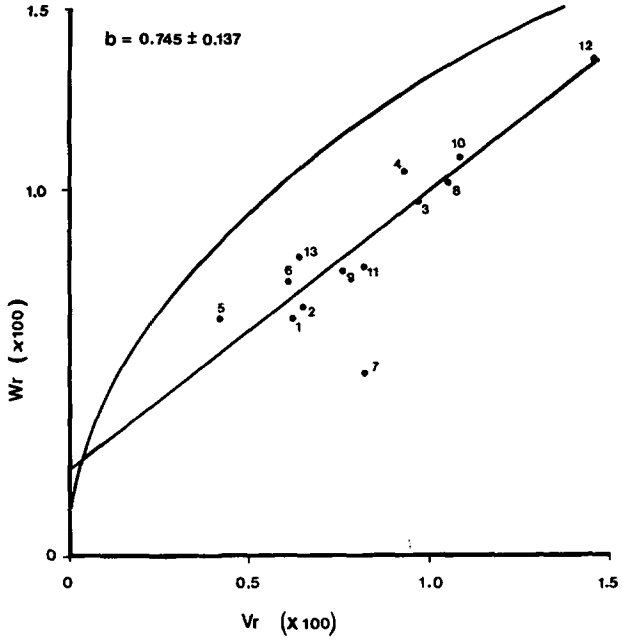

Fig. 3. W_r, V_r regression for alpha amylase, 13HD

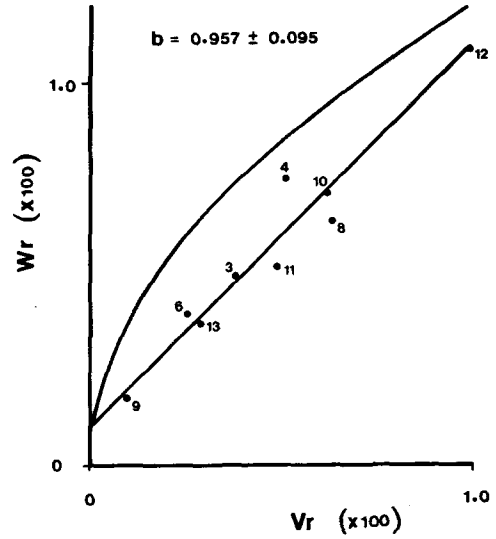

Fig. 4. W_r, V_r regression for alpha amylase, 9HD

Table 5. Genetic components of variance for alpha amylase (AAP) in the 13 X 13 half diallel

	AAP
D	0.016 ± 0.001
H_1	0.034 ± 0.014
H_2	0.027 ± 0.013
F	−0.005 ± 0.005 NS
$(H_1/D)^{\frac{1}{2}}$	1.472
$(H_2/4H_1)$	0.199
h^2_B	0.971
h^2_N	0.653
$r(Y_r, W_r + V_r)$	−0.461 NS
r (two-rows)	−0.749′

′ Indicates $p \leq 0.05$

Table 6. Hayman analysis of variance of alpha amylase (AAP) in the 9 X 9 half diallel of two-row varieties

Item	d.f.	M.S.
a (additivity)	8	0.0573‴
b (dominance)	36	0.0056″
b_1 (direction)	1	0.0683‴
b_2 (distribution)	8	0.0026
b_3 (specificity)	27	0.0042
Blocks	1	0.0417‴
Bt (pooled interactions)	44	0.0023

All items tested against the pooled block interactions

′, ″, ‴, indicate significance at $P \leq 0.05$, 0.01 and 0.001 respectively

not confirm the incomplete dominance detected by graphical analysis but that of $(H_2/4H_1)$ indicated inequality in the frequency of positive and negative alleles. Broad and narrow sense heritabilities were high.

Analysis of variance of the 9FD (Table 2) demonstrated the absence of maternal effects (c) but the item d, due to reciprocal differences not attributable to c, did reach significance. Both additive and non-additive effects were present. The non-additive effects were confined to the b_1 item for directional dominance. In the 13HD (Table 3) the effects of directional dominance (b_1), asymmetry of gene distribution (b_2) and interactions between specific genotypes (b_3) were all significant. There was no significant correlation between parental means and $(W_r + V_r)$ but the negative value suggested that dominance, while being ambidirectional, was still predominantly increasing AAP (Table 5). Only low levels of heterosis, between 5 and 8%, were detected. In the two-row varieties (9HD) the non-additive effects were confined to the b_1 item, for directional dominance (Table 6).

Significant GCA (Table 4) and SCA effects were present. Parental means were positively correlated with GCA effects. In the six-row varieties, only Scotch Bere showed a negative value for GCA. Olli, Pirkka and OAC 21 contained high levels of AAP and DPP and showed large positive GCA effects for both characters. Two-row varieties with high AAP, such as Boreham Warrior and Maris Baldric, showed GCA effects larger than OAC 21 but low values for DPP. SCA effects were sometimes inconsistent over blocks but the pattern detected for DPP was also apparent for AAP although SCA effects were generally smaller. There were only two six-row by two-row combinations exhibiting a large positive SCA, involving Mosane with Pirkka and Scotch Bere.

No serious obstacles were apparent from these analyses to the improvement of DPP or AAP separately in a commercial variety, this would be possible using two-row parents. To improve both DPP and AAP simultaneously it is necessary to use either a six-row parent or three-parent crosses of exclusively two-row varieties. The repeated demonstrations of genetic interactions in crosses between six-row and two-row varieties supports a body of evidence, much of it unpublished, of basic differences between these two crops (see Harlan in Simmonds 1976) and suggests that the use of three-parent crosses might be the more reliable strategy. In practice, the use of Midas and other varieties in crosses with the two-row high DP variety Akka has allowed interim improvements to be made (Hayter and Allison 1975). Further improvements in both DP and AA should result from the use of three-parent crosses of two-row varieties but the use of six-row parents has proved more difficult. Initial selections from two-row by six-row crosses were poorly adapted and susceptible to mildew (*Erysiphe graminis*) and further crossing has been necessary.

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