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A Diallel Cross Analysis of Gum Content in Barley *(Hordeum vulgare)*

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Summary. A diallel cross analysis of gum content in barley *(Hordeum vulgare)* was made using six cultivars of two-rowed spring barley as parents. A Jinks-Hayman analysis of F2 progeny means showed that gum content was controlled by a simple additive-dominance genetic system and that low gum content was strongly dominant. The analysis suggested that gum content was principally controlled by two or three genes showing a high degree of dominance. Some genotype-environment interaction was detected in a comparison between the F2 and F3 generations which were grown in different years and locations. However, the character was found to be highly heritable both within and between generations, suggesting that the selection and breeding of barleys of reduced gum content should not be difficult.

Key words: Barley - Gum Content - Genetics - Genotype - Environment Interaction

Introduction

The water-soluble non-starchy polysaccharides or gums of barley grain have been implicated as a factor influencing malting quality. It has frequently been shown that barley gum is an important determinant of the viscosity of malt extracts (Meredith et al. 1951; Schuster et al. 1967; Bourne and Pierce 1970; Scott 1972). The filtration of the wort from the mash tun may be impeded by an excess of gum due either to enhanced wort viscosity or to a cementing of the mash bed by fine particles and gummy substances (Barrett et al. 1973). The increased use of unmalted barley adjuncts in brewing can be expected to lead to wort filtration problems caused by undegraded gums. There is also some evidence that gums may be involved in determining the feeding quality of barley for chickens. The addition of a highly purified β-glucanase to barley diets fed to chickens has produced considerable improvements in growth rate (Rickes et al. 1962), providing indirect evidence that the gums are detrimental to feeding value. It has also been suggested that high viscosity in the alimentary tract due to the gums is the main cause of this poor feed quality (Burnett 1966). Consequently, breeding barleys with reduced gum content could be of value for both feeding and malting purposes.

Varietal or genotypic differences in the β-glucan and gum content of barley do exist (Schuster et al. 1967; Bourne and Pierce 1970; Reiner and Narziss 1972). However, there appear to have been no studies on the genetic basis of such differences. A rapid method has been devised to screen small samples of barley for variation in gum content, based on measurements of the viscosity of acidic extracts of ground grain (Greenberg and Whitmore 1974; Greenberg 1974). This technique has made possible the genetic investigations of gum content reported here.

Materials and Methods

1. Materials

The Jinks-Hayman analysis of a diallel cross was used (Jinks 1954; Hayman 1954b), as this did not necessitate the estimation of gum content of grain from single plants and provided a large amount of genetic information from a reasonably small number of viscosity determinations. The analyses are normally performed on the F1 grain of a diallel cross. However, grain quality studies generally have to be performed on the F2 because F1 hybrid grain is not typical, being generally naked and shrivelled due to the manipulations involved in making the crosses. Furthermore, sufficient quantities of hybrid grain may not be available for analysis.

A 6 × 6 diallel cross was made using varieties which differed widely in β-glucan content according to Bourne and Pierce (1970). These were all two-rowed spring barleys: 'Golden Promise' and 'Maris Badger' had low β-glucan content, 'Proctor' was

intermediate; and 'Julia' and 'Zephyr' were high in β-glucan. The sixth variety was the selection HB 551/60 from the Plant Breeding Institute, later named 'Maris Mink', which was thought to be intermediate in β-glucan content.

2. Methods

a) Cultural

The diallel set was crossed in the summer of 1972 and the F1 was grown over the winter of 1972/73. The F1 (15 crosses + parents) was grown for the first 5 weeks in a cool growth room under a 10 hour daylength. The light intensity was approximately 70 Watts/m^2 and the day and night temperatures were 11°C and 4°C respectively. The plants were then transplanted into 13 cm diameter pots and moved to a glasshouse where they were grown to maturity under high-pressure sodium vapour lamps providing a supplementary intensity of approximately 20 Watts/m^2. The daylength was 18 hours and temperature was maintained at approximately 14°C. A randomised block layout was used in both growth room and glasshouse with four replications, each plot consisting of five plants in individual pots. The experimental plants were surrounded by two rows of guard plants.

The residual F2 grain after sampling for the estimation of extract viscosity was pooled and drilled in a field trial in 1973. The design used consisted of a randomised block with two replications, each plot measuring 1.5 m × 3.6 m. The F3 grain was combine harvested at the beginning of August, 1973. Severe lodging occurred in this trial as a result of very heavy rain storms in late June and early July. There was considerable growth of secondary tillers, and the immature grain from these could well have altered the results. As much as possible of this grain was removed by running the combine with a high airflow and by carefully sieving the grain and removing green corns after drying.

Three crosses ('Maris Mink' × 'Golden Promise', 'Maris Mink' × 'Maris Badger' and 'Maris Mink' × 'Julia') were made in June, 1971 and the F1's grown in the glasshouse over the winter of 1971/72 under similar environmental conditions to the F1 of the full diallel. The F2's were sown as spaced plants in the field in 1972 and thirty plants were selected from each cross. Each of these provided sufficient seed for the estimation of extract viscosity and also for the inclusion of a sub-sample in a nursery in Palmerston North, New Zealand, during the season of 1972/73. The extract viscosity of the grain from the F2 plants was estimated in Cambridge and a few of the offspring of the extreme phenotypes were returned from New Zealand. These F4 progenies were sown as small plots at Cambridge in 1973.

b) Chemical

Gum content was estimated by measuring the viscosity of acid extracts of barley flour. The grain was milled through a 0.5 mm mesh screen and 2 g of dry flour was extracted in 50 ml of pH 1.5 chloride buffer for four hours at 40°C in a special extraction bath (Greenberg and Whitemore 1974). The extract was cooled to 20°C and its viscosity measured with a rotational viscometer. The logarithm of the extract viscosity was closely correlated with the β-glucan content of the grain (Greenberg 1974).

Results and Discussion

Analyses were performed on $\log_{10}$ extract viscosity values multiplied by 100. The mean extract viscosities for each of the parent varieties in the F2 and F3 trials are given in Table 1, together with an analysis of variance of parental and progeny means in each generation. The Hayman analysis of variance of the diallel cross (Hayman 1954a) is given in Table 2. As the block interaction terms were not found to be significant by a Bartlett test ($\chi^2 = 1.83$, $P \simeq 0.8$ for the F2 data; $\chi^2 = 4.71$, $P \simeq 0.25$ for the F3 data), the pooled block interaction terms (Bt) were used to test the main effects. An analysis of variance (Table 3) was performed to test the heterogeneity of (Wr + Vr) and (Wr - Vr) values between arrays and blocks (Mather and Jinks 1973). The Wr, Vr regression lines for the means over replicates, together with the limiting parabolae $Wr^2 = Vr.Vp$, are shown for the F2 and F3 generations in Figures 1 and 2 respectively. The values of the components of genetic variation estimated in both generations are given in Table 4. The heritability of extract viscosity (Table 5) was determined within the F2 of the diallel and as the correlation coefficient between the extract viscosity of the F3 grain samples and their F5 progenies in the three crosses made in 1971.

Table 1 shows that the viscosity values obtained in the F3 trial in 1973 were considerably higher than those from the F2 trial. There was also some genotype-environment interaction in that the rankings for cv. 'Julia' and cv. 'Zephyr' were reversed, cv. 'Julia' having the highest extract viscosity in the F2 trial and cv. 'Zephyr' having the highest viscosity in the F3. This interaction was also manifested by these two cultivars in other trials grown in 1972 and 1973. There was also a reversal in the rankings of cv. 'Maris Badger' and cv. 'Golden Promise' in the two trials, but the differences in viscosity were smaller and were not significantly different in the F3. The analysis of variance of family means shows that there were highly significant differences between families in both F2 and F3 generations.

Significant additive genetic variance was detected in both generations by the Hayman analysis (Table 2). A significant dominance effect was found in the F2, but none was detectable in the F3, presumably due

Table 1. $100 \times \log_{10}$ extract viscosity values (Centipoises) for the parents in the F2 and F3 trials

Parent	F2	F3
1 'Golden Promise'	52.4 ± 2.6	75.8 ± 1.7
2 'Maris Badger'	42.0 ± 1.7	82.5 ± 8.4
3 'Maris Mink'	46.1 ± 1.4	82.1 ± 5.2
4 'Proctor'	41.9 ± 0.8	75.3 ± 0.4
5 'Julia'	79.4 ± 5.0	88.4 ± 0.6
6 'Zephyr'	60.8 ± 1.3	107.9 ± 1.0

Analysis of variance of parental and F1 family mean $100.\log_{10}$ extract viscosity values

	F2		F3	
Item	df	M.S.	df	M.S.
Replication	3	169.6**	1	75.2*
Families	20	382.4***	20	123.3***
Error	60	33.5	20	16.9

*, **, *** significant at P = 0.05, 0.01 and 0.001 respectively

Table 2. Analysis of variance of diallel table for $100 \times \log_{10}$ extract viscosity

	F2		F3	
Item	df	M.S.	df	M.S.
$\underline{a}$ (additivity)	5	1243.1***	5	435.5***
$\underline{b}$ (dominance)	15	95.5**	15	19.3N.S.
$\underline{b}_1$ (direction)	1	484.8***	1	15.4N.S.
$\underline{b}_2$ (distribution)	5	129.1**	5	31.3N.S.
$\underline{b}_3$ (S.C.A.)	9	33.5N.S.	9	13.1N.S.
Blocks	3	169.5	1	75.4
B$\underline{a}$	15	28.9	5	26.7
B$\underline{b}$	45	35.1	15	13.7
B$\underline{b}_1$	3	72.8	1	1.5
B$\underline{b}_2$	15	25.9	5	28.0
B$\underline{b}_3$	27	36.0	9	7.1
Bt	60	33.6	20	16.9

All effects tested against Bt
, *, significant at P = 0.01 and 0.001 respectively

Table 3. Analysis of variance of (Wr + Vr) and (Wr - Vr) over parental arrays

Item	df	M.S.	F
(Wr + Vr) Array differences in F2	5	42350	2.88*
(Wr + Vr) Block differences in F2	18	14684	
(Wr + Vr) Array differences in F3	5	2038	0.998 N.S.
(Wr + Vr) Block differences in F3	6	2042	
(Wr - Vr) Array differences in F2	5	972.4	1.49 N.S.
(Wr - Vr) Block differences in F2	18	651.5	
(Wr - Vr) Array differences in F3	5	93.8	0.958 N.S.
(Wr - Vr) Block differences in F3	6	98.0	

Wr is the covariance between the individuals in the rth array and their non-recurrent parents
Vr is the variance of the array of the rth parent
* significant at P = 0.05

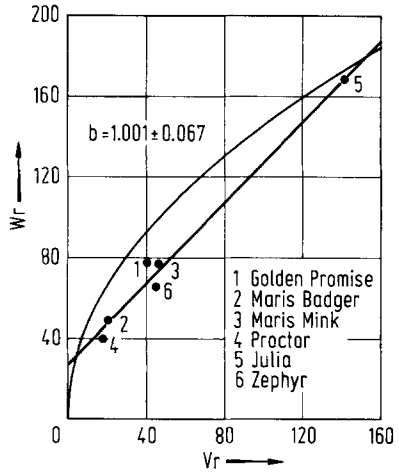

Fig.1. (Wr, Vr) graph for $100 \times \log_{10}$ extract viscosity (Centistokes) in the F2 of the diallel cross

to reduced heterozygosity in this generation. The dominance effect in the F2 was attributable to directional dominance ($\underline{b}_1$) and to differences in the distribution of dominant alleles among the parents ($\underline{b}_2$); there were no specific combining ability effects.

For the estimation of the genetic parameters from the diallel by the Jinks-Hayman analysis, it is necessary that six assumptions about the genetic system are valid (Hayman 1954b):

i) Diploid segregation: although barley is a diploid species, it is likely that the gum content of the grain is an endosperm character since most of the viscous gums are located there (Preece and Mackenzie 1952) and so may be under triploid genetic control. However, the overall means and segregation ratios in the F2 and F3 generations should not in fact be very different from the diploid pattern. If a single gene with alleles X and x is considered, the segregation should

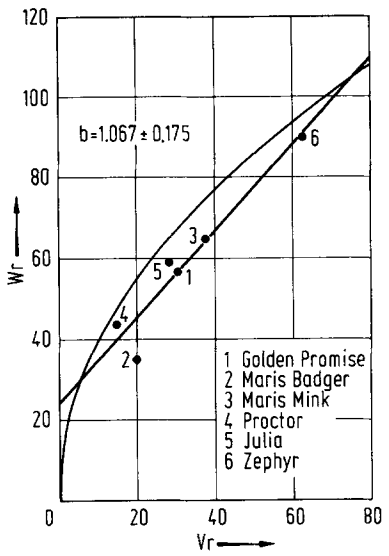

Fig.2. (Wr, Vr) graph for $100 \times \log_{10}$ extract viscosity (Centistokes) in the F3 of the diallel cross

Table 4. Components of variance in $100 \times \log_{10}$ extract viscosity

Component	Generation F2	F3
D	198.8	145.2
F	+ 155.8	+ 237.3
H_1	235.8	77.5
H_2	186.1	- 73.7
E	33.5	16.9
$\sqrt{H_1/D}$	1.09	0.73
$H_2/4H_1$	0.197	- 0.238
$\frac{1}{2}F/\sqrt{D(H_1 - H_2)}$	0.783	0.801
$(\sqrt{4DH_1} + F)/(\sqrt{4DH_1} - F)$	2.12	- 17.9

be as follows:

	♀XX	♂xx	♀xx	♂XX
	Embryo	Endosperm	Embryo	Endosperm
F1	Xx	XXx	Xx	Xxx

The segregation should be identical in reciprocal crosses in the F2 and subsequent generations

	Embryo	Endosperm
F2	XX:2Xx:xx	XXX:XXx:Xxx:xxx
	Embryo	Endosperm
F3	3XX:2Xx:3xx	3XXX:XXx:Xxx:3xxx

It can be seen that an endosperm character showing continuous variation would not be expected to give greatly different results from a diploid character in the F2 and F3 generations. With complete dominance, generation means and variances would be identical to those of a diploid; and with purely additive variation, the generation means would be the same as in the diploid, though their variances might differ. To detect differences between genetic control in the endosperm and embryo, it would be necessary to use F1 grain. The problems resulting from the use of hybrid grain have already been considered.

Table 5. Heritability of $\log_{10}$ extract viscosity
Within generation heritability from F2 diallel data
HBS (broad sense) 0.74
HNS (narrow sense) 0.37
Heritability correlations between F3 and F5 grain

Cross	r(correlation coefficient)
'Maris Mink' × 'Golden Promise' 4 observations (2 low, 2 high)	0.98
'Maris Mink' × 'Maris Badger' 7 observations (3 low, 4 high)	0.87
'Maris Mink' × 'Julia' 6 observations (3 low, 3 high)	0.82

$$HBS = \frac{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{4}H_2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{4}H_2 - \frac{1}{2}F + E}$$

$$HNS = \frac{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{2}H_2 - \frac{1}{2}F}{\frac{1}{2}D + \frac{1}{2}H_1 - \frac{1}{4}H_2 - \frac{1}{2}F + E}$$

ii) No difference between reciprocal crosses: a half diallel was performed due to limitations in space and because reciprocal differences would not be expected in the F2 and F3 generations.

iii) Homozygosity of parents: the parents were all standard varieties and were taken from carefully monitored stocks of this strongly inbreeding species.

iv) Independent action of non-allelic genes.

v) No multiple allelism.

vi) Independent distribution of genes among the parents.

The analysis of variance of (Wr - Vr) (Table 3) showed no significant differences between parental arrays, so these assumptions can be considered to be

valid and a simple additive-dominance genetic system is involved in the control of gum content. However, the parents were not a random sample, but were chosen to give a wide range of gum content, so assumption (vi) is not strictly valid. Similarly, significant differences in the distribution of dominance effects between the parents were found by the Hayman analysis (Table 2). The analysis of variance of (Wr + Vr) showed differences between arrays in the F2, so there was non-additive variation which could be attributed to dominance. No non-additive variation was detected in the F3, as was found by the Hayman analysis.

Table 4 shows that the values of the components of genetic variance estimated from the F3 generation were largely meaningless, with negative values for H_2, $H_2/4H_1$ and $(\sqrt{4DH_1} + F)/(\sqrt{4DH_1} - F)$. This resulted from the very large coefficients by which it is necessary to multiply the statistical components of variance Vp, $W\bar{r}$, $V\bar{r}$ and $\overline{V}r$ due to the low degree of heterozygosity in the F3. The values of the additive genetic variance (D), the estimate of which is of similar accuracy in both generations, are quite similar in both the F2 and F3. The following conclusions can also be made from Table 3: Dominance of low gum content was complete ($H_1/D \simeq 1$). The frequency of increasing and decreasing alleles was not equal over all loci ($H_2/4H_1 < \frac{1}{4}$). The ratio of dominant to additive effects was not constant over all loci, nor was it fully independently distributed ($\frac{1}{2}F/\sqrt{D(H_1 - H_2)}$ was between 0 and 1). There were more dominant than recessive alleles over all the parents in the ratio of approximately 2:1 ($\{\sqrt{4DH_1} + F\}/\{\sqrt{4DH_1} - F\} \simeq 2$).

Figures 1 and 2 both show Wr, Vr regression lines with slopes close to unity and all points are quite close to the regression lines. This indicates that the additive-dominance genetic model was adequate and that there was no interaction. Although there was no significant non-additive genetic variance in the F3 according to the (Wr + Vr) analysis and the Hayman analysis (Table 2), the points for the arrays were still well spread out along the regression line, so some dominance may still have been evident. The correlation between the calculated order of dominance of the parents from (Wr + Vr) and their actual extract viscosity values (yr) was very good in both generations ($r = 0.92$, $p < 0.01$ in the F2; $r = 0.84$, $p < 0.05$ in the F3), showing that dominance was strongly directional and that low extract viscosity was consistently dominant.

The parental varieties fell into three groups on the Wr, Vr regression lines:

i) cv. 'Proctor' and cv. 'Maris Badger' with low extract viscosity and an excess of dominant alleles;

ii) cv. 'Golden Promise' and cv. 'Maris Mink' with intermediate extract viscosity and intermediate dominance;

iii) cv. 'Julia' and cv. 'Zephyr', which were of intermediate or high extract viscosity and exhibiting intermediate dominance or an excess of recessive alleles according to the season.

Despite this genotype-environment interaction in the gum content of these barley cultivars, only a reversal of the rankings of the two cultivars having the highest gum content resulted. The cultivar with the highest extract viscosity was also the one having an excess of recessive alleles, so that dominance was always acting in the direction of low gum content. The arrays with the lowest and highest positions on the regression line fell quite close to the points of intersection of the line and the limiting parabola, so it would not be possible to make selections with extract viscosities much outside the range of the parental varieties.

An estimate of the number of effective factors (K) was made in the F2 using the formula:

$$K = \frac{4(ML_2 - ML_0)^2}{\frac{1}{4}H_2}$$

where ML_2 is the overall F2 progeny mean and ML_0 is the overall parental mean. This is based on the formula given by Jinks (1954) for F1 diallels. This formula estimates only factors with large dominance and estimates have often been disappointingly low (Jinks 1954). The value of K from the F2 of the diallel was 2.46, which may be of a reasonable order as an estimate of the number of genes showing a high degree of dominance. Thus the suggestion may be tentatively made that two or three genes showing dominance were involved in the control of gum content in these genotypes, together with an undetermined number of additive genes.

Apart from the estimate of narrow sense heritability in the F2, the estimates of heritability from Table

5 were all high. The low estimate of narrow sense heritability was due to the high degree of dominance and this value will approach that for broad sense heritability as the degree of dominance becomes lower in subsequent generations. Therefore, selection for low extract viscosity should not be performed until heterozygosity is considerably reduced, i.e. in the F3 or F4 generation at the earliest. These high values for the heritability of gum content tend to reinforce the earlier suggestion that the character is controlled by a comparatively small number of genes of major importance. Although there was some genotype-environment interaction in the gum content of the barley cultivars studied, it was probably not of sufficient magnitude to affect seriously the possibility of selection of barleys of low gum content.

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