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Genetical investigations into β-glucan content in barley

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Summary. Random inbred lines produced by doubled haploidy (DH) and single seed descent (SSD) have been used to investigate the genetics of β-glucan (gum) content in barley (*Hordeum vulgare*). Genetical analyses indicated that gum content is controlled by a simple additive genetic system. Significant negative genetic correlations were observed between β-glucan content, thousand grain weight and height in the DH samples. These correlations were much reduced in the SSD samples and would suggest linkage of the genes controlling these characters. The presence of repulsion linkages could be exploited in a barley breeding programme by producing F1 derived DH to generate recombinants with high thousand grain weight and low β-glucan content. Genetical parameters estimated from DH and F3 samples have successfully been used to predict the number of inbred lines transgressing the parental range for β-glucan content and bivariate combinations involving β-glucan.

Key words: *Hordeum vulgare* – β-glucan – Doubled haploids – Genetics – Correlated characters

Introduction

β-glucan is a collective term for a group of substances containing glucose units connected via β-1,4 and β-1,3 linkages, which together constitute the principle components of barley endosperm cell walls. These water soluble gums are of importance in the breeding of feed and malting quality barley where low β-glucan content is desirable (Greenberg 1977). However, before reduced gum content can be considered in barley improvement programmes it is necessary to devise tests suitable for screening large populations, and therefore the use of infra-red reflectance was proposed (Allison et al. 1978). This screening method has not only allowed breeders to screen large numbers of barley genotypes but also to investigate the genetic control of acid soluble β-glucan content. In this paper the results of such an investigation using random inbred lines are reported. Furthermore, estimates of genetical parameters obtained from early generations are used to predict the number of inbreds falling into defined phenotypic categories.

Materials and methods

The material studied

Twenty doubled haploids (DH) were produced from F1 hybrids of the following three spring barley crosses:

'Golden Promise' × 'Mazurka' (TT1)
'Golden Promise' × 'Ark Royal' (TT3)
BH4/143/2 × 'Ark Royal' (TT4)

In addition, for each cross, 20 F3 families were produced together with a sample of 40 single seed descent lines (SSD). The 4 parents, F3 families, DH lines and SSD lines were grown as part of a larger experiment at the Murrays farm, East Lothian, in 1983. The experimental design was a randomised complete block with two replicates. Within a block each family was represented by a row of up to ten seeds, sown at 5 cm spacings, with a wheat guard at each end of the row. Rows were spaced 22.5 cm apart and the whole experiment was netted to prevent bird damage. Field observations were made on several characters in the manner described by Powell et al. (1984).

β-glucan measurement

Acid-soluble β-glucan was measured on individual 5 gm samples from each row by an infra-red reflectance method (Allison et al. 1978). β-glucan, nitrogen and moisture contents were estimated simultaneously using near infra-red reflectance values at a number of wavelengths; different regression

equations being used for each of the three constituents (Allison et al. 1979). Only β-glucan content will be considered in this paper.

Results

The results from the analyses of variance for β-glucan content in the DH and SSD samples are given in Table 1. There are significant differences between the DH lines for gum content in the case of TT1 and TT3 but not TT4. All three crosses display significant between line variation in the case of the SSD samples. Significant between line variation indicates that there is additive genetic variation and that the parents differ for alleles controlling gum content. When there are genetic differences between the two parents of a cross, their means may be represented as follows:

$$\overline{P1} = m + [d] + [i]$$
$$\overline{P2} = m - [d] + [i]$$

where m is the overall mean, [d] is the contribution of additive genetic effects and [i] is the contribution of additive × additive interactions (Mather and Jinks 1983). The F1DH progeny will segregate 1 : 1 for the alleles at each locus and hence the additive contributions of each allele pair to the DH progeny mean cancel. In the absence of linkage, all possible genotypes will be produced in equal frequency and the interaction between alleles will also cancel. Hence the F1DH progeny is expected to have a mean of m.

The means and standard deviations of the parents, DH and SSD generations are given in Table 2a. The genetical parameters m, [d] and [i] may then be estimated from the means of the parents and F1DH's by weighted least squares model fitting procedures. The results of the model fitting are shown in Table 2b and indicate that a simple additive genetic model is sufficient to explain the differences between means in the case of the TT3 and TT4 cross. However, in the case of the TT1 cross significant [i] type epistasis was detected. It should be noted that an essential component of this test is that DH lines are produced at random. The test for the presence of non-allelic interaction i.e. whether the difference between F_∞ and mid-parent is zero, is

Table 1. Analyses of variance for β-glucan content in the TT1, TT3, and TT4 crosses

	TT1			TT3			TT4		
	d.f.	M.S.	V.R.	d.f.	M.S.	V.R.	d.f.	M.S.	V.R.
DH Families									
Between Reps	1	0.49	24.8***	1	0.84	8.4*	1	0.06	0.6
Between Lines	19	0.22	11.2***	18 (1)[a]	0.26*	2.6*	18 (1)[a]	0.17	1.6
Lines × Reps	13 (6)[a]	0.02		15 (4)[a]	0.10		13 (6)[a]	0.11	
SSD Families									
Between Reps	1	0.27	10.2***	1	0.82	6.6*	1	0.02	0.3
Between Lines	39	0.16	6.1***	37 (2)[a]	0.28	2.2*	35 (4)[a]	0.17	2.9**
Lines × Reps	26 (13)[a]	0.03		27 (12)[a]	0.13		12 (27)[a]	0.06	

[a] Indicates number of missing observations
* $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$

Table 2a. The means and standard deviations for the parents, DH and SSD generations

TT1	$\bar{x}$	SD	TT3	$\bar{x}$	SD	TT4	$\bar{x}$	SD
'Golden Promise'	3.08	0.321	'Golden Promise'	3.08	0.321	BH4/143/2	2.78	0.218
'Mazurka'	2.40	0.119	'Ark Royal'	2.48	0.154	'Ark Royal'	2.48	0.154
F1DH	2.67	0.471	F1DH	2.82	0.511	F1DH	2.61	0.416
SSD	2.77	0.401	SSD	2.66	0.526	SSD	2.60	0.408

Table 2b. Estimates of the genetical parameters obtained by fitting weighted least squares models to the means of the parents and F1DH

m	2.67 ± 0.024	m	2.79 ± 0.012	m	2.63 ± 0.012
[d]	0.34 ± 0.013	[d]	0.30 ± 0.014	[d]	0.15 ± 0.013
[i]	0.07 ± 0.027	[i]	–	[i]	–
		$\chi^2_{[i]} = 1.62$		$\chi^2_{[i]} = 0.68$	

Table 3. Analyses of variance for the random inbred lines for the TT1, TT3 and TT4 crosses

	TT1			TT3			TT4		
	d.f.	M.S.	V.R.	d.f.	M.S.	V.R.	d.f.	M.S.	V.R.
1. Between Lines	1	0.58	23.9***	1	1.46	12.6**	1	0.00	0.02
2. DH×SSD	1 (3+4)[a]	0.22	1.2	1	0.68	2.5	1	0.01	0.08
3. Between DH	19	0.222		18 (1)[b]	0.261		18 (1)[b]	0.173	
4. Between SSD	39	0.161		37 (2)[b]	0.276		35 (4)[b]	0.166	
5. Lines×Reps	39 (19)[b]	0.024		42 (16)[b]	0.117		25 (33)[b]	0.083	

[a] Items used in V.R. test
[b] No. of missing observations

** $0.01 > P > 0.001$; *** $P < 0.001$

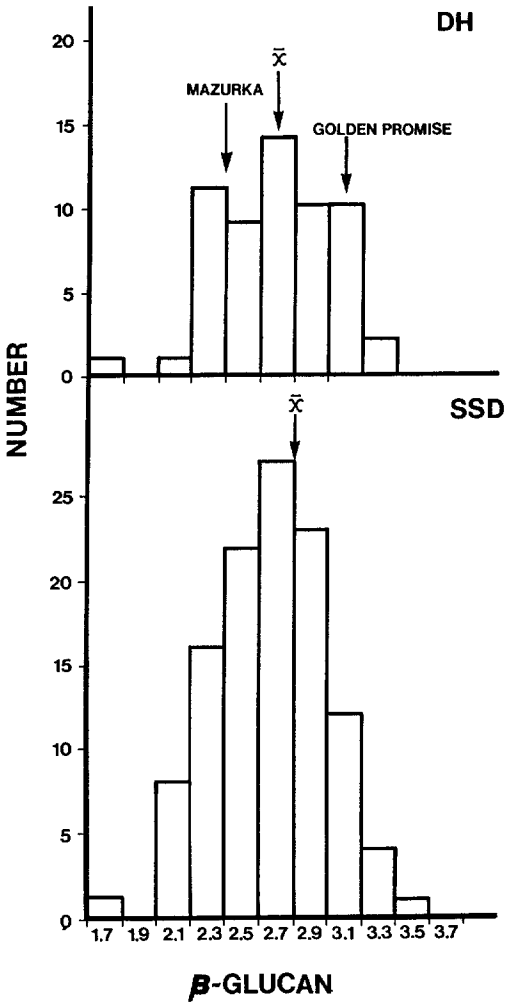

Fig. 1. The frequency distributions for β-glucan content in the doubled haploid (DH), and the single seed descent (SSD) lines

the only test that one can apply for detecting selective elimination during the production of the DH populations (Jinks 1983). The effects of selection and non-allelic interaction are therefore confounded.

Table 4. Estimates of the additive genetic variance (D) for the DH and SSD generations in the 3 crosses

	TT1	TT3	TT4
DH	0.088	0.070	–
SSD	0.067	0.075	0.054
$\chi^2_{[1]}$	0.405	0.009	0.405

In the absence of differential selection and linkage disequilibrium of epistatic genes the means of inbred lines produced by DH and SSD should not be different. The results of the analyses of variance for the three crosses presented in Table 3 clearly indicate that the means of the two samples do not differ significantly and therefore suggest either that the two effects exactly balance or that neither epistasis nor selective elimination are important components in β-glucan content in these crosses. For most characters the plant breeder is concerned with transgressing the parental range. Fig. 1 shows the distribution for β-glucan content in 120 SSD lines and 60 F1 derived DH. In both populations segregants transgressing the parental ranges can be identified.

The component of variance between family means provides an estimate of the additive genetic component of variance, D. Estimates of D obtained from the DH and SSD samples for the three crosses are given in Table 4. The bias, due to linkage, on the additive genetic variation of F_∞ families produced by SSD is less than that of families produced by DH. A comparison of the additive genetic component of β-glucan content from different F_∞ families will therefore provide a test for linkage disequilibrium and its predominant phase. Estimates of D for β-glucan content obtained from DH and SSD samples may be compared by standard maximum likelihood model fitting procedures (Pooni et al. 1980). The method involves fitting a model to the between line mean squares assuming a single value of D but allowing the within line mean squares to

assume their own values (Powell et al. 1985 a). Since three parameters are fitted to 4 statistics there is one degree of freedom remaining to test the goodness of fit of the model. The χ^2 values obtained for the 3 crosses are also given in Table 4. The non-significant values obtained indicate that the estimates of D obtained from the DH and SSD samples are not significantly different. Linkage disequilibrium is not therefore an important component in the genetic control of β-glucan content in barley.

A continuous distribution of phenotypes in a population is frequently interpreted to indicate that a large number of genes, or more strictly effective factors are involved. However, this conclusion is not justified on these grounds alone, since even in diploids two or three loci are sufficient to produce distributions which are in practice indistinguishable from normal distributions (Thoday and Thompson 1976). An estimate of the number of effective factors (k) segregating can be provided by a comparison of the difference between the extreme DH progenies to the genetic variance (Croft and Simchen 1965; Snape et al. 1984) i.e.

$$\hat{k} = \frac{[DH_H - DH_L]^2}{4\sigma_G^2}.$$

Such estimates of k have been calculated and range from 3 to 5. It should be stressed that the estimate of k is very dependent on sample size. The effective factor is, however, a unit in a temporary sense and is a statistical rather than a physical phenomenon (Powell et al. 1985 b). It is nevertheless interesting to note that the estimates of k obtained from this study confirm the findings of Greenberg (1977). This author concluded that gum content was controlled by a simple additive-dominance genetic system and that 2 to 3 genes were involved, but such numbers will undoubtedly be an underestimate.

Estimates of genetical parameters obtained from the early generations of a cross may be used to define the properties of the recombinant inbred lines that can be derived by inbreeding from the F2 of a cross (Jinks and Pooni 1976). All that is required to make these predictions are estimates of m and D. We have already shown that these estimates can readily be obtained from an analysis of variance of F1DH data. Analyses of variance of F3 families will also provide estimates of m and D but in this case one must assume that dominance, i.e. ¼[h] and ⅛H, are negligible. For β-glucan the F3 analyses provide the following estimates:

	TT1	TT3	TT4
m	2.782	2.732	2.637
$\sqrt{D}$	–	0.3291	0.3282

The methods of Jinks and Pooni (1976) were used to calculate the proportion of recombinant inbred lines expected to transgress the parental range. The SSD lines used in this experiment were at the F7 generation and since the material was bulked from a single F4 plant the expected variance of recombinant inbred lines is ⅞D or ⅞(D+I). Therefore when calculating the expected frequency of transgressive segregants ⅞D was used (Caligari et al. 1985).

The expected and observed numbers of inbred lines transgressing the higher and lower scoring parents are given in Table 5. The correspondence between expected and observed numbers is good, indeed, apart from the one expected value from the DH in TT3, the correspondence is very good and it is clear that univariate cross prediction methods may be applied to quality characters such as β-glucan content in barley.

Barley breeders will not however, consider β-glucan content in isolation. For both selection and cross prediction purposes the relationships between β-glucan content and other important agronomic characters need to be considered. The simplest way to examine these relationships is to calculate phenotypic correlation coefficients but such correlations contain both environmental and genetic components. Hence the additive genetic correlations between β-glucan and five agronomic characters have been calculated and are given in Table 6 for the DH and SSD samples. Since the method of calculation of genetic correlations involves components of variances it is not possible to attach significance levels to the correlations. Nevertheless, these correlations do provide a measure of the likely effects of selection for one character upon another, e.g. selection for high thousand grain weight within DH samples in the TT1 and TT3 crosses will probably result in selections with low β-glucan content.

The large negative correlations for β-glucan content with thousand grain weight and with height are very much reduced in the SSD samples when compared to the DH samples. This situation is indicative of linkage disequilibrium between the genes controlling β-glucan content, height and thousand grain weight. Furthermore, the negative correlation in the DH population suggests an excess of repulsion linkages (Jinks and

Table 5. Expected and observed numbers of inbred lines transgressing the parental range

		Obsv	ExDH	ExF3
TT1	>P1	4	3	–
	<P2	5	7	–
TT3	>P1	5	7	6
	<P2	14	4	10
TT4	>P1	10	–	13
	<P2	13	–	13

Table 6. Genetic correlations between β-glucan and five agronomic characters

		Thousand grain weight (TGW)	Height (Ht)	Grain no. (GN)	Awn emergence (AE)	Main stem weight (MSW)
TT1	DH	−0.993	−0.532	–	−0.486	–
	SSD	−0.143	−0.139	0.374	−0.356	0.039
TT3	DH	−0.751	−0.524	–	−0.755	−0.099
	SSD	−0.014	−0.076	–	−0.083	−0.111
TT4	DH	–	–	–	–	–
	SSD	−0.485	−0.401	−0.454	−0.611	−0.599

Table 7. The observed and expected number of inbred lines falling into the 4 phenotypic categories for combinations of characters involving β-glucan

DH-TT1		>P1 >P1,	<P2 <P2,	>P1 <P2,	<P2 >P1
β-/TGW	OBSV	2	0	1	2
	PRED	0	0	0	0
β-/Ht	OBSV	1	0	2	1
	PRED	1	0	0	6
β-/AE	OBSV	3	1	1	4
	PRED	1	0	2	7
DH-TT3					
β-/TGW	OBSV	1	0	1	2
	PRED	0	0	3	3
β-/Ht	OBSV	0	0	2	0
	PRED	0	0	1	2
β-/AE	OBSV	0	1	1	1
	PRED	0	0	2	4
β-/MSW	OBSV	4	6	4	6
	PRED	3	0	1	2
F3-TT3					
β-/Ht	OBSV	0	0	2	0
	PRED	0	0	0	1
β-/MSW	OBSV	4	6	4	6
	PRED	3	1	0	3

The characters referred to in the abbreviations are given in Table 6

Pooni 1981). The three crosses examined in this study each contain an erectoides dwarfing gene. Interestingly alleles at the GP ert locus (Thomas et al. 1984) are not associated with β-glucan content and the linkages identified in this study are independent of the erectoides dwarfing gene.

Pooni and Jinks (1978) have presented a method for predicting the frequency of recombinant inbred lines for two or more characters simultaneously. An essential component of this multivariate prediction system is the additive genetic correlation. The number of inbred lines predicted to fall into 4 of the possible 9 phenotypic categories are given in Table 7. The combinations of characters examined are restricted to those in which there is significant additive genetic variation. Although the relatively small numbers of SSD lines preclude statistical tests, in general the observed and predicted numbers agree reasonably well and indicate that prediction methods can be extended to quality characters in barley.

Conclusions

1. Random inbred lines produced by DH and SSD have shown that gum content in spring barley is controlled by a simple additive genetic system. The number of effective factors detected in the control of this character was in the order of 3 to 5.

2. Linkage disequilibrium does not appear to be an important component of the genetic architecture of β-glucan content in barley. DH or SSD may be used to derive inbred lines which transgress the parental range. However, linkage is important in the relationship between β-glucan content, thousand grain weight and height. Indeed, the production of DH from F1 hybrids provides a means of generating genotypes with high thousand grain weight and low β-glucan content.

3. The procedures of biometrical genetics may also be used to predict what changes might be expected from any given breeding strategy. The number of inbred lines produced by SSD which transgress the parental range for β-glucan content has been successfully predicted from genetical parameters estimated from DH and F3 samples.

4. Breeding programmes are rarely based on single characters and the univariate prediction method has been extended to bivariate predictions involving β-glucan and agronomic characters.

The methods outlined in this study and others (e.g. Caligari et al. 1985) will hopefully provide an incentive for other quality components to be included in cross

prediction strategies in order to meet the objectives of barley improvement programmes.

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