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The effect of selection for protein and isozyme loci on quantitative traits in a doubled haploid population of barley

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Doubled haploid families extracted from the F_1 hybrid of the spring barley cross (Dissa × Sabarlis) by the *Hordeum bulbosum* technique were scored for the segregation of alleles at isozyme loci. Isozyme and protein loci on all seven pairs of barley chromosomes were examined and five out of the seven pairs of barley chromosomes (2I, 3I, 4I, 6I and 7I) were shown to possess polymorphic isozyme and protein loci. The systems tested did not deviate significantly from the expected 1:1 ratio and esterase loci on chromosome 3 were mapped intra-chromosomally. The relationship between marker loci and quantitative trait loci (QTL) was examined. A comparison of the mean scores for each allelic class revealed significant differences in the expression of a range of quantitative traits. Thus, by selecting for isozyme and protein phenotypes one can significantly affect the expression of certain QTL in barley. For example, allelic variation at the *α-Amy*-1 locus makes a large contribution to the genetic variation available for selection in the case of single plant yield. The advantage of using DH families for gene mapping and in dissecting QTL are discussed.

INTRODUCTION

The dissection and manipulation of quantitatively controlled characters are important objectives in both basic and applied genetic research programmes. The identification of specific regions of the genome which enhance the expression of quantitatively controlled characters may allow the development of more efficient plant breeding strategies. The theoretical basis for interpreting the association of marker loci with quantitative trait loci (QTL) has been outlined by Thoday (1961), Mather and Jinks (1982), Geldermann (1975), Tanksley, Medina-Filho and Rick (1982) and Beckman and Soller (1983). Essentially the theory exploits the fact that the marker locus identifies a chromosomal segment and enables that segment to be monitored in subsequent generations of crossing or selfing. Recently, Paterson *et al.* (1988) have outlined a method based on interval mapping which assess the effects of genomic segments located between pairs of marker loci, rather than the effects associated with individual loci. However, reports of the association between marker loci and quantitative traits were first presented by Sax (1923) who found an association between seed size and alleles influencing seed colour in *Phaseolus*. Other earlier reports include those of Rasmusson (1933), Everson and Schaller (1955), Spicket and Thoday (1966) and Breese and Mather (1957, 1960).

The importance of character dissection in plants has been recognised for some time but has been hampered by the lack of suitable marker loci. The development of gel electrophoretic techniques for separating proteins and multiple forms of enzymes, isozymes, has provided considerable impetus for the genetic characterisation of a wide range of plant species (Tanksley and Orton, 1983). Isozymes can be considered as genetic markers, which are well distributed over the genome and interact in a co-dominant manner. Isozyme loci are therefore well suited to the mapping of genes controlling quantitative traits. Isozymes have been used to map QTL in tomato (Tanksley, Medina-Filho and Rick, 1981; Vallejos and Tanksley, 1983; Weller, Soller and Brody, 1988), soybean (Graef, Fehr and Cianzo, 1989), and maize (Stuber, Goodman and Moll, 1982; Stuber, Edwards and Wendel, 1987; Edwards, Stuber and Wendel, 1987). In the case of tomato and soybean interspecific crosses were used to maximise the number

of potential polymorphic markers available in the segregating crosses. Although the level of electrophoretic variability for enzyme coding loci in maize is higher than in tomato and soybean the parents used to investigate the effects of isozyme loci on QTL were again selected to maximise the number of allelic differences in the segregating crosses. These experiments were successful in identifying regions of the genome which can account for substantial fractions of the phenotypic variance in the F_2 generation. However, this approach may not predict which blocks of genes will be of use in intervarietal transfers in plant breeding programmes.

It has been stressed by Brown, Munday and Oram (1988) that there is no *a priori* reason to assume that an isozyme locus will predict the level of expression of a given quantitative trait. A high level of linkage disequilibrium between the marker locus and the quantitative trait is required. The majority of the studies to-date have focussed on the use of F_2 and backcross generations to measure the effects of isozyme loci on QTL. This approach, although valid, assumes that there will be no further breakdown of linkage groups in subsequent selfing and crossing. It is likely that further rounds of gametogenesis will result in the attainment of linkage equilibrium. This indicates that the early segregating generations of a cross may not represent the optimal strategy for the complete evaluation of linkage between an isozyme locus and a quantitative trait locus. Further complications arising from the use of F_2 and backcross generations include the difficulty of obtaining accurate estimates of the environmental and genetical component of variance. The presence of dominance can also introduce a bias to the estimates obtained and the unit of replication is based on single plants (Powell, Caligari and Thomas, 1986). Intergenotypic competition is also an important factor influencing the assessment of quantitative traits (Powell *et al.*, 1985).

Many of the problems associated with the assessment of segregating populations can be overcome by the use of doubled haploid (DH) lines. Haploids are sporophytes which contain the gametic chromosome number (Nitszche and Wenzel, 1977). Haploidisation of diploids and subsequent chromosome doubling to produce DH allows the development of completely homozygous individuals from heterozygous parents in a single generation. Efficient methods of haploid production exist in barley and populations of doubled haploids have been produced by the *H. bulbosum* technique (Kasha and Kao, 1970). Doubled haploids extracted from the F_1 generations are likely to exhibit a higher linkage disequilibrium relative to other generations due to the reduced opportunities for recombination. These families also represent fixed, homozygous genotypes and may therefore be extensively tested in replicated experiments. DH therefore provide a valuable genetical resource which may be used to test for associations between marker loci and QTL. In this paper we present the first report of the use of DH to monitor the effect of selection for isozyme and protein loci on QTL in barley.

MATERIALS AND METHODS

Plant material

Sixty-six doubled haploids (DH) were produced by the *H. bulbosum* technique from the F_1 hybrid of a barley (*Hordeum vulgare*) cross, Dissa × Sabarlis. The parents, DH lines and F_1 hybrids were grown in a randomised complete block field experiment with two replicates in 1986. Within a block each family was represented by a row of up to ten seeds, sown at 5 cm spacing, with a wheat guard at each end of the row. Rows were 22·5 cm apart. After harvest, sheaves were returned to the laboratory and five randomly chosen plants from each row were scored for:

1. Final height, measured from the base of the plant to the collar in cm (Ht).
2. Number of grains per ear on the main stem (GN).
3. Yield of grain on the main stem (MSW).
4. Weight of the main stem straw (WS).
5. Number of fertile tillers per plant (TN).
6. Thousand grain weight, calculated from 2 and 4, above (TGW).
7. Grain yield from the whole plant, single plant yield (SPY).
8. Weight of straw from the whole plant, straw weight (SW).

Electrophoretic enzyme assays

Proteins, including isozymes, were separated by horizontal isoelectric focusing in polyacrylamide gels. Table 1 gives details of the systems used.

RESULTS

Examples of the protein and isozyme polymorphism detected in the parents (Dissa and Sabarlis) are shown in fig. 1. The marker loci

Table 1 List of markers, marker symbols, gene symbols, tissue used, *p*H gradient of IEF gel and assay reference

Marker	Marker symbol	Gene symbol	Tissue used	*p*H gradient of IEF gel	Reference for assay
α-Amylase	α-AMY-1	*α-Amy*-1	Germinated grain	5·0–7·0	Gale *et al.*, 1983
Esterase	EST-3	*Est*-3	Mature endosperm	4·0–6·5	Ainsworth *et al.*, 1984, 1986
Esterase	EST-10	*Est*-10	Mature endosperm	4·0–6·5	Ainsworth *et al.*, 1984
Iodine Binding Factor	IBF-1	*Ibf*-1	Mature endosperm	4·0–6·5	Liu and Gale, 1988
Leaf Esterase	Leaf EST	(*Est*-1, 2 or 4)	First leaf	4·0–6·5	Ainsworth *et al.*, 1984
Protein	WSP-1	*Wsp*-1	Mature endosperm	6·0–11·0	Cawston *et al.*, 1990
Iodine Binding Factor (2)	IBF-2	*Ibf*-2	Mature endosperm	4·0–6·5	Liu and Gale, 1988

examined covered the whole chromosome complement but only seven were polymorphic and were located on five out of the seven barley chromosomes. Table 2 gives the chromosome locations of all the systems studied. Chromosome nomenclature is based on homoeology (Dewey, 1984) thus chromosomes 1, 2, 3, 4, 5, 6 and 7 correspond to barley chromosome numbers 5H, 2H, 3H, 4H, 7H, 6H and 1H respectively. The segregation of alleles at each locus was tested against the expected 1:1 ratio. None of the isozymes tested deviated significantly from the expected ratio, although in the case of IBF-1 an excess of Dissa phenotypes was observed. Esterase loci have been mapped to

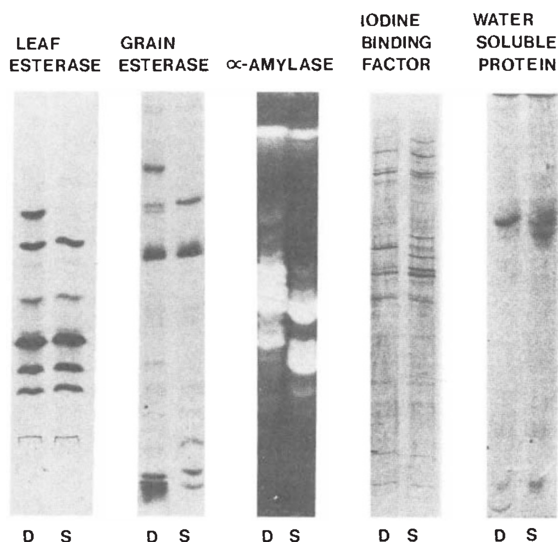

Figure 1 Protein polymorphism detected in the parental genotypes (Dissa and Sabarlis) for: Leaf esterase, grain esterase, α-amylase, iodine-binding factor and water-soluble protein.

the long arm of homoeologous group 3 chromosomes in the *Triticeae* (Ainsworth, Gale and Baird, 1986; Hvid and Nielson, 1977). The joint segregation analysis is given in table 3. The alleles at each individual locus segregate in the expected 1:1 ratio. However, the joint segregation item is significant ($P < 0{\cdot}001$) indicating that the leaf and endosperm loci are linked on 3IL with a recombination value of $0{\cdot}24 \pm 0{\cdot}05$.

A primary objective of this study was to determine if specific marker loci (isozymes) affect any of the quantitative traits measured. The means and standard errors for the parental F_1 hybrid and DH genotypes are given in table 4. For each trait measured the mean scores for each allelic class at the isozyme and protein loci in the DH population was calculated. The difference between the mean values for the alternative alleles at each locus is given in table 5. In the case of *Est*-10 there were significant differences between groups for GN, MSW and WS. For leaf esterase there were significant differences between groups for: Ht, MSW, WS and TGW. Similarly, classification of alleles at the *α-Amy*-1 locus results in significant differences between groups for: GN, MSW and SPY. For IBF-2 there were significant differences between groups for: GN, MSW and TGW. Two variates, GN and TGW exhibited significant differences between classes when selected on the basis of protein profile at the WSP locus. Clearly, selection of alleles at biochemical loci can influence the expression of quantitative trait loci. It is also of interest to note that individual marker loci can influence the expression of more than one quantitative trait locus. For example the *α-Amy*-1 locus significantly affects three out of the eight traits scored.

Comparison of the parental and F_1 hybrid means (table 4) allows us to predict whether the QTL contributed from each parent will have a

Table 2 Chromosomal location and segregation ratios for marker loci monitored in the barley doubled haploid population

Isozyme and protein markers symbols	Chromosomal location* L = long arm S = short arm	Isozyme phenotype		$\chi^2_{(1)}$ 1:1
		Dissa	Sabarlis	
EST-10	3L	34	32	0·06
EST-3	7S	33	33	0·00
α-AMY-1	6L	33	33	0·00
IBF-1	4	40	26	2·97
Leaf EST	3L	36	30	0·55
WSP-1	2	32	34	0·06
IBF-2	Not located	35	31	0·24

* *Triticeae* homoeologous groups.
Other grain isozymes (with chromosomal location) which showed no polymorphism between Dissa and Sabarlis included β-amylase (4), glucose phosphate isomerase (1 and 3), peroxidase (2), phosphoglucomutase (4) and superoxide dismutase (2).

Table 3 Joint segregation analysis for leaf and endosperm esterase loci on chromosome 3

Endosperm *Est*-10	Leaf: Dissa	Leaf: Sabarlis
Dissa	27	7
Sabarlis	9	23
Segregation for endosperm esterase alleles	1	0·06
Segregation for leaf esterase alleles	1	0·55
Joint segregation	1	17·52***
Total	3	18·13

$P = 0·24 \pm 0·05$
*** $P < 0·001$.

positive or negative effect on the character measured. Thus for TGW we would anticipate that the Sabarlis genotype would make a positive contribution to the expression of this character. Reference to table 5 indicates that a number of examples may be identified in which the expression of QTL is in the opposite direction to these predicted by the parental values. Selection for the Dissa leaf esterase phenotype results in a DH population which has a significantly greater TGW value than the Sabarlis genotypes.

Since the chromosomal location of the genes *Est*-10 (Hvid and Nielson, 1977) and leaf esterase, which probably represents one of the closely linked

Table 4 Mean scores for the parents (Dissa and Sabarlis), F_1 hybrid and doubled haploid (DH) population

Genotype	n	Ht	GN	MSW	WS	TN	TGW	SW	SPY
Dissa	50	75·3±0·77	45·9±1·71	1·484±0·067	0·874±0·033	2·5±0·21	32·3±0·78	1·96±0·16	3·3±0·38
Sabarlis	50	80·6±0·97	27·3±0·41	1·208±0·033	0·986±0·033	4·6±0·28	43·9±0·70	3·87±0·26	4·2±0·27
F_1	88	88·6±1·17	26·59±0·65	1·641±0·032	1·306±0·031	4·5±0·24	62·4±0·71	4·54±0·34	5·5±0·35
DH	66	80·1±0·88	34·3±1·64	1·324±0·042	1·022±0·035	3·5±0·17	42·4±1·37	3·91±0·20	4·5±0·20

(Columns Ht to SPY are headed "Quantitative trait".)

Table 5 Difference between mean values for alternative alleles at isozyme loci

Isozyme	Ht	GN	MSW (10^3)	WS (10^3)	TN	TGW	SW	SPY
EST-10	1·28	3·11*	128·20**	−8·17**	0·00	0·64	0·31	0·34
EST-3	1·34	2·25	33·90	45·90	0·31	1·69	0·01	0·10
Leaf EST	−3·13**	1·04	93·20*	−87·10**	0·01	−2·46*	0·27	0·27
α-AMY-1	1·40	−4·64***	−135·10***	48·20	0·30	0·12	0·51	0·72*
IBF-1	0·10	2·70	36·00	−15·00	0·06	1·22	0·00	0·22
IBF-2	−1·98	−6·95***	−131·00**	−10·00	0·01	−3·33**	0·05	0·42
WSP-1	1·08	3·96**	59·00	−16·00	0·17	2·20*	0·12	−0·04

The negative sign (−) indicates examples where differences between groups are not in agreement with the predicted phenotype based on parental and F_1 hybrid performance.

Table 6 Mean values for 4 marker classes produced by two linked isozyme loci *Est*-10 and leaf esterase on chromosome 3

Morphological trait	Genotypic class: Parent 1 DD	Recombinant 1 DS	Recombinant 2 SD	Parent 2 SS
Ht	82·4	74·9	80·1	79·4
GN	35·5	37·2	32·8	32·7
MSW	1·403	1·310	1·259	1·263
WS	1·095	0·934	0·962	0·987
TN	3·6	3·8	3·8	3·6
TGW	43·4	38·4	42·6	41·8
SW	4·20	3·90	3·86	3·82
SPY	4·82	4·58	4·47	4·43

Est-1, *Est*-2 or *Est*-4 genes (Kahler and Allard, 1970) have been established it is feasible to examine the effects of a recombination event occurring between these two loci on the long arm of chromosome 3I. Since the DH were extracted from the F_1 hybrid we can classify the population into four groups representing the parental and recombinant gametes. The mean scores for each genotypic class produced in this way are given in table 6. The analyses of variance for these data, which includes the orthogonal contrasts, are given in table 7. There are significant differences between the parental and recombinant gametes for Ht and WS. The two parental genotypes differ significantly for Ht, MSW, WS. The two recombinant genotypes (classes) differ significantly for Ht and TGW.

The expected mean squares may be used to obtain estimates of *D*, the additive genetic variance for the DH families. This is the heritable portion of the phenotypic differences between homozygotes and is the fixable portion of the heritable variation. For all the characters measured there were significant levels of additive genetic variation and this reflects the genetic variation available for manipulation. The extent of the genetic variation associated with allelic variation at the individual loci is given in table 8. In the case of SPY over 9 per cent of the genetic variation available for manipulation can be accounted for by allelic variation at the *α-Amy*-1 locus and is illustrated graphically in fig. 2. Selection of the Sabarlis *α-Amy*-1 isozyme phenotype results in a higher frequency of greater yielding DH genotypes.

DISCUSSION AND CONCLUSIONS

The mapping of components of polygenic systems depends on the ability to detect the effects of allelic differences which are independent of the variation associated with genetic segregation and environmental variation (Thompson and Thoday, 1979).

Table 7 Analysis of variance for the four marker classes produced by two linked isozyme loci *Est-3* and leaf esterase on chromosome 3

Item	df	Ht	GN	MSW	WS	TN	TGW	SW	SP
Between blocks	1	1040·71	8·67	0·006	0·012	37·38	39·21	150·56	114·30
Between families	65	490·78***	1733·76***	1·14***	0·78***	18·20***	1218·05***	24·55***	26·10**
(P_1+P_2) vs. (R_1+R_2)	1	1245·68*	37·44	0·40	1·11**	3·57	587·62	3·23	2·19
P_1 vs. P_2	1	1156·37*	946·60	2·41**	1·45**	0·25	485·50	23·16	25·63
R_1 vs. R_2	1	1050·71*	789·95	0·10	0·03	0·18	666·91*	0·48	1·55
Between families within groups	62	458·84***	1789·07***	1·14***	0·77	19·02***	1248·92***	25·31***	26·89***
Blocks × families	64 (1)$^+$	186·54	309·94	0·22	0·14	7·42	156·96	10·35	12·35
Replicate error	523 (5)$^+$	30·98	77·32	0·09	0·04	5·05	45·21	5·56	7·22

$^+$ = number of missing values
* $P<0·05$.
** $P<0·01$.
*** $P<0·001$.

Table 8 Estimates of the percentage additive genetic variation associated with allelic differences at isozyme loci in a doubled haploid population

Isozyme locus	Percentage of additive genetic variance associated with a given character
Est-10	MSW (2·2), WS (0·8)
Leaf *Est*	Ht (5·7), MSW (1·19), WS (1·1), TGW (0·0)
α-Amy-1	GN (2·1), MSW (3·3), SPY (9·4),
Ibf-1	GN (6·6), MSW (2·8), TGW (0·9),
Wsp-1	GN (1·0), TGW (0·0)

Isozymes meet these requirements and allow the effects of individual chromosomal regions to be examined. However, the concept of using mapped, monogenic markers to examine the underlying basis of quantitative variation is not new and this approach was pioneered in *Drosophila* genetics (Breese and Mather, 1957, 1960; Thoday, 1961). The availability of codominant markers has allowed an expansion in this area of research, particularly in crop plants (Tanksley and Orton, 1983).

Our results demonstrate that by selecting the isozyme phenotypes one can significantly affect the expression of certain QTL in barley. Of particular relevance is the predictive capacity of the isozyme profile. By examining the parental and F_1 hybrid scores one could predict in which direction a particular phenotype, based on isozyme selection would be biased. Several cases were detected where the selection method produced effects opposite to that predicted by the parental contributions. These cases probably reflect gene dispersion in the parents and may represent genes underlying transgressive segregation (Tanksley *et al.*, 1982). Estimates of the genetic variation associated with individual loci were also calculated. In general, the percentage variation is small and this agrees with information reported for the association of effects of isozyme loci on QTL in maize (Stuber *et al.*, 1987). However, in barley the *α-Amy*-1 locus on chromosome 6 was associated with over 9 per cent of the variation for grain weight. Allelic variation at this locus makes a large contribution to the variation for SPY (fig. 2). This is reflected in the higher number of recombinant DH that exceed a given standard. If we calculate the number of DH that exceed a mean SPY of 5·0 gms, in the case of DH with the Dissa *α-Amy*-1 phenotype we observe 8 such recombinants. However, when we use the Sabarlis *α-Amy*-1 phenotype as an indirect selection tool we observe that 17 exceed the standard.

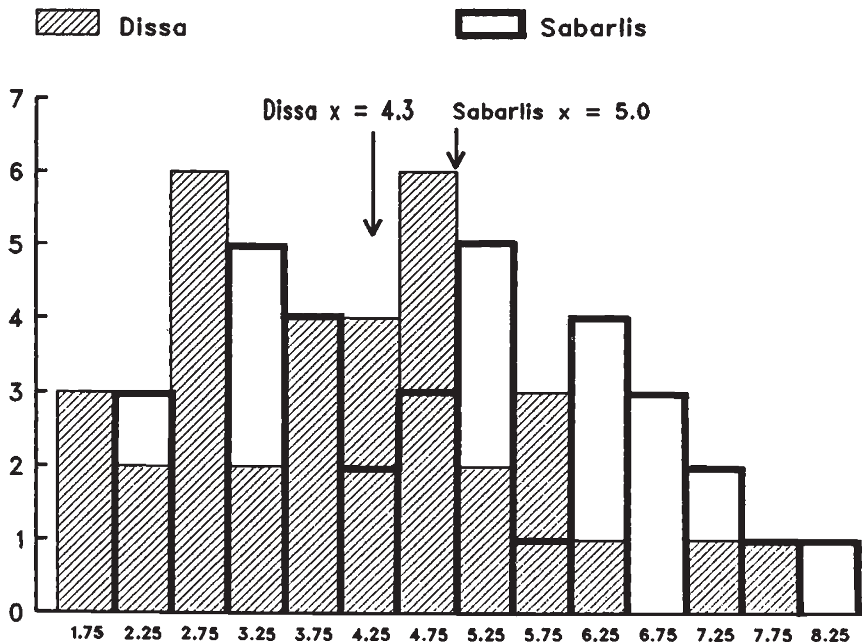

Figure 2 Frequency distribution for the character single plant yield (SPY) based on the Dissa and Sabarlis *α-Amy*-1 isozyme phenotype.

This demonstrates that selection of DH based on *α-Amy*-1 phenotypes can shift the frequency distribution of recombinant inbreds in a positive, favourable direction.

Bailey (1975) and Burr *et al.* (1988) have outlined the advantages of recombinant inbred lines in gene mapping. These include the fact that recombinant inbred lines represent a permanent population that can be used indefinitely for mapping. Thus new loci may be continually added to the linkage map. The intra-chromosomal mapping of loci may be accomplished using DH families. The segregation of alleles at loci in these generations have the same expectations as backcross data and none of the markers deviated significantly from the expected 1 : 1 ratio. Maximum likelihood methods may be used to calculate the linkage intensity (Mather, 1938). Using this approach we were able to map the *Est*-10 and leaf esterase loci intra-chromosomally (table 3). Inbred families can be evaluated in many different environments and more accurate estimates of genetic parameters can be obtained (Mather and Jinks, 1982). These advantages apply with equal force to doubled haploids. Indeed, a comparison of DH and recombinant inbred lines may allow the basis of gene action to be investigated. An association between a marker locus and a quantitative trait locus could be due to pleiotrophy and/or linkage disequilibrium. Since the linkage bias in recombinant inbred lines will be less than that found in samples of DHs derived from F_1 hybrids a comparison of the two samples derived from common parents may allow true estimates of the degree of association between marker loci and QTL to be obtained. In addition, DH offer the possibility of controlling the level of recombination since families may be extracted from any generation (Caligari and Powell, 1986). The potential problem of recombination between a marker locus and a QTL can be circumvented by the use of DH.

Additional marker loci would increase the applicability and power of marker based selection schemes. For example, the availability of genetic markers which are known to map to specific chromosomes will allow us to assess how much recombination occurs during various breeding strategies. Perhaps more importantly one can start to investigate the consequences of such recombinational events. Restriction fragment length polymorphisms (RFLPs) have been used to generate high density linkage maps in tomato (Tanksley, Mutschler and Rick, 1987) and maize (Helentjaris, 1987). The exploitation of such systems in barley will allow the variability available in the gene pool to be exploited to its full potential. The DH approach outlined in this manuscript will play an increasingly important role in relating variation detected at the protein and nucleic acid level to phenotypic variation observed at the whole plant level.

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