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Water-soluble proteins of mature barley endosperm: genetic control, polymorphism, and linkage with β-amylase and spring/winter habit

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Summary. Water-soluble proteins (WSP-2 and WSP-3) and β-amylase (β-AMY-1) were extracted from mature endosperms of 44 spring and 39 winter barley genotypes. The protein and enzyme isoforms were separated in isoelectric focusing gels with a pH gradient of 4–6.5. The *Wsp-3* and *β-Amy-1* loci were located to chromosomes 4H using the wheat/barley chromosome addition lines. Segregation analysis of F_2 and doubled haploid populations showed *Wsp-2* and *β-Amy-1* to be tightly linked, with a map distance of 11 cMorgans. Isoforms of WSP-2 possessed similar pIs to that of WSP-3 and overlapping bands were observed in the gels. These bands segregated independently in F_2 and doubled haploid populations, implying two unlinked genes. All three loci were found to be polymorphic: two alleles were detected at the *Wsp-2* locus, three at *Wsp-3* and two at *β-Amy-1*. The frequency of alleles at all three loci was found to be different in winter and spring genotypes. Spring genotypes possessed a wider range of phenotypes than winter genotypes. Spring and winter genotypes could be distinguished on the basis of WSP-3 and β-AMY-1 phenotypes. The linkage between *Wsp-3* and *β-Amy-1* loci and genes controlling spring/winter habit on chromosome 4H is discussed. It is concluded that *Wsp-3* and *β-Amy-1* can be used as genetic markers for spring/winter habit in barley genetic research and breeding.

Key words: Water-soluble protein (WSP) – Barley – β-Amylase – Linkage – Spring/winter habit

Introduction

Genetic studies in the *Triticeae* have resulted in the construction of detailed genetic maps, which are based primarily on morphological, isozyme and RFLP loci (McIntosh 1988; see also Sogaard and Wettstein-Knowles 1987 for barley). As the density of genetic maps is increased, one objective is to establish linkage between genes of agronomic or scientific importance and genes that can be easily monitored. The latter can then be used as genetic markers for the indirect selection of traits of importance. For instance, *Pch1*, a major gene that conditions resistance to eyespot disease of wheat, can be selected by its linkage with the endopeptidase gene, *Ep-1* (Worland et al. 1988). A prerequisite for the successful use of genetic markers is polymorphism, and various techniques have been exploited to increase the detection of polymorphism between genotypes. Recently, Liu and Gale (1989) have reported a highly polymorphic genetic marker, *Ibf-1* (iodine binding factor, IBF) located on homoeologous group 4 and 5 chromosomes of the *Triticeae*. We provide evidence that IBF in barley corresponds to water-soluble proteins (WSP-2 and WSP-3) as reported by Thompson et al. (1990, 1991). The various synonyms of grain water-soluble proteins are given and the results are discussed with respect to the use of water-soluble proteins and β-amylase as genetic markers for spring/winter habit in barley. The genetic basis of variation at these three loci in barley has been investigated and intrachromosomal mapping studies have been conducted.

Materials and methods

Plant material consisted of *Triticum aestivum* cv 'Chinese Spring' (CS), *Hordeum vulgare* cv 'Betzes' and the wheat/barley disomic chromosome addition lines (Islam et al. 1975). Other barley genotypes included the cultivars and breeding lines mentioned in Table 1.

Water-soluble proteins were extracted from single mature grain, as described by Thompson et al. (1990), and separated

in isoelectric focusing (IEF) gels as described by Thompson et al. (1990) and Liu and Gale (1989). β-Amylase (α-1,4-glucan malthydrolase, E.C. 3.2.1.2) was extracted from single mature grain, separated in IEF gels and visualised using the methods of Thompson et al. (1991).

The linkage relationship between *β-Amy-1* and *Wsp-3*, both located on chromosome 4H, was estimated using an F_2 population from the cross 'Blenheim' × TS264/22 and F_1-derived doubled haploids produced by the *H. bulbosum* method from TS42/3/5 × 'Apex'.

A comparison of iodine and 'Coomassie blue' staining was carried out using 66 *H. bulbosum*-derived doubled haploids from the F_1 of 'Dissa' × 'Sabarlis'. The population represents fixed inbred lines; thus, profiles produced by each stain technique can be compared.

Results

The banding pattern of all barley and wheat grain proteins with pIs between 4 and 6.5 was the same whether stained with the iodine technique (Liu and Gale 1989) or 'Coomassie blue' (Thompson et al. 1990). Iodine stained proteins brown, 'Coomassie blue' stained them blue. 'Coomassie blue' staining proved to be easier, quicker and more reliable than iodine staining and was the preferred technique here. No differences in banding patterns were observed between the two staining techniques when applied to fixed inbred lines (doubled haploids) or to a range of barley cultivars.

Addition line analysis

Water-soluble proteins were distributed throughout the gradient gels used (Fig. 1). Bands corresponding to WSP-H2 and WSP-H3 of *H. vulgare* were located centrally in the pH gradient and at the alkaline side of the major wheat proteins (in the pH range of 4.5 to 5.5; Liu and Gale 1989). WSP-H3 bands corresponded exactly to IBF-H1 reported by Liu and Gale (1989) and mapped to chromosome 4H (Fig. 1). WSP-2 is present as a single band in Betzes and could not be detected in any wheat/barley addition line, as it comigrated with a wheat protein (Fig. 1).

β-Amy-1 has been located to chromosome 4H by Powling et al. (1981) and Ainsworth et al. (1987; see also Fig. 1).

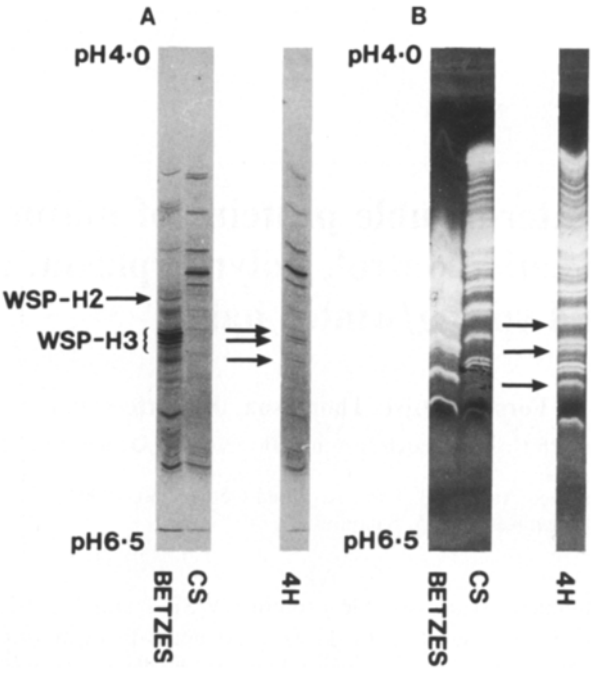

Fig. 1. WSP-2, WSP-3 and β-AMY-1 phenotypes of 'Betzes', CS and the CS/'Betzes' 4H addition line, showing the control of WSP-H3 and β-AMY-H1 by genes on chromosome 4H

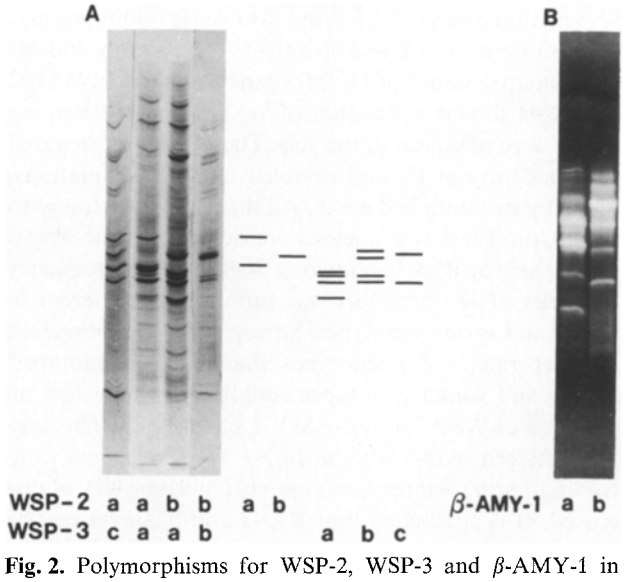

Fig. 2. Polymorphisms for WSP-2, WSP-3 and β-AMY-1 in barley

Genotypic variation in barley

Two alleles were found at the *Wsp-2* locus, three at *Wsp-3* and two at *β-Amy-1*. These have been designated *Wsp-2a*, *Wsp-2b*, *Wsp-3a*, *Wsp-3b*, *Wsp-3c*, *β-Amy-1a* and *β-Amy-1b*. The protein profiles corresponding to these loci are shown in Fig. 2. The results of classifying the winter and spring barley genotypes based on allelic constitution at each of the three loci are given in Table 1.

The distribution of alleles at the *Wsp-3* and the *β-Amy-1* loci does not appear to be randomly distributed between winter and spring genotypes (Table 1). This is particularly evident in the case of the *Wsp-3b* allele, which is very rare in the winter group of barley cultivars. 'Panda' is the only winter genotype tested that possesses this allele. Similarly, the majority of winter cultivars possess *β-Amy-1a*; 'Dido', 'Panache' and 'Panda' are the only winter cultivars to possess the *β-Amy-1b* allele. These results suggest that the frequency of certain alleles

Table 1. Spring and winter barley genotype carrying the various alleles at the *Wsp-2*, *Wsp-3* and *β-Amy-1* loci

	Wsp-2a	*Wsp-2b*	*Wsp-3a*	*Wsp-3b*	*Wsp-3c*	*β-Amy-1a*	*β-Amy-1b*		*Wsp-2a*	*Wsp-2b*	*Wsp-3a*	*Wsp-3b*	*Wsp-3c*	*β-Amy-1a*	*β-Amy-1b*
Winter types															
Bronze		+	+			+		Masto		+	+			+	
Cashmir		+	+			+		Melusine			+			+	
Classic	+		+			+		Mimosa		+	+			+	
Dido	+				+		+	Panache		+			+		+
Eagle		+	+			+		Panda		+		+			+
Fiesta	+		+			+		Paris			+			+	
Fighter		+	+			+		Pastoral		+	+			+	
Finesse		+	+			+		Pipkin	+		+			+	
Firefly		+	+			+		Plaisant		+	+			+	
Frolic		+	+			+		Posaune		+	+			+	
Gerbel		+	+			+		Puffin		+	+			+	
Gypsy	+		+			+		Revue			+			+	
Halcyon	+		+			+		Sarah	+		+			+	
Igri		+	+			+		Shire		+	+			+	
Jennifer			+			+		Target			+			+	
Kaskade		+	+			+		Torrent		+	+			+	
Kira			+			+		Trixi			+			+	
Magie		+	+			+		Waveney	+		+			+	
Marinka		+	+			+		CEB85634		+	+			+	
Maris Otter	+		+			+									
Spring types															
Alexis		+		+			+	Klaxon		+		+			+
Apex		+	+			+		Kym	+				+		+
Atem		+		+			+	Magnum		+		+			+
Betzes	+		+			+		Maris Mink		+	+				+
Blenheim		+		+			+	Midas	+		+				+
Carmargue		+	+			+		Molak		+	+				+
Celt	+				+	+		Murrayfield		+		+			+
Corniche		+	+			+		Natasha		+		+			+
Cytris		+		+			+	Oboe		+		+		+	
Dandy		+		+		+		Prisma		+	+			+	
Delphine		+		+			+	Regatta	+				+	+	
Digger	+				+		+	Sabarlis	+		+				+
Dissa		+	+				+	Sherpa		+	+			+	
Dorrett		+		+			+	Sundance	+		+				+
Doublet		+	+			+		Totem		+		+		+	
Egmont	+				+		+	Triumph		+		+			+
Fergie		+	+			+		Tyne		+	+			+	
Golden Promise	+		+			+		E224/3		+	+				+
Golf		+		+			+	PEG125/6/3		+		+			+
Heriot		+		+			+	TS42/3/5		+		+			+
Heritage		+	+				+	TS264/22	+		+			+	
Imber		+			+		+	TS240/15/2/3		+		+			+

Wsp-2 alleles have not been determined for cultivars 'Jennifer', 'Kira', 'Melusine', 'Paris', 'Revue', 'Target' and 'Trixi'

at the *Wsp-3* and *β-Amy-1* loci is not constant between the spring and winter groups. Furthermore, the classification of spring and winter genotypes based on allelic variation at the *Wsp-3* and *β-Amy-1* loci results in a similar grouping of genotypes and thus provides indirect evidence that *Wsp-3* and *β-Amy-1* may be physically linked.

Linkage analysis between Wsp-2, Wsp-3 and β-Amy-1

To test the hypothesis that *Wsp-3* and *β-Amy-1* are linked, an F_2 and a doubled haploid population segregating for alleles at both these loci were used to test for independent segregation. Table 2 shows the results of the segregation of alleles at *Wsp-2*, *Wsp-3* and *β-Amy-1* loci in the F_2 populations from the cross 'Blenheim' × TS264/22. It was difficult to distinguish between the heterozygote and

Table 2. Segregation analysis of *Wsp-2*, *Wsp-3* and *β-Amy-1* in an F_2 population derived from the cross 'Blenheim' × TS264/22

				df	2 (1)
β-Amy-1					
Wsp-3	B1	TS + Hyb	Segregation for *β-Amy-1*	1	2.74
B1	28	4	Segregation for *Wsp-3*	1	0.35
			Joint segregation	1	79.3***
TS + Hyb	9	76	$r = 0.111 + 0.022$		
β-Amy-1					
Wsp-2	B1	TS + Hyb	Segregation for *β-Amy-1*	1	2.74
B1	12	28	Segregation for *Wsp-2*	1	5.27*
			Joint segregation	1	0.0009
TS + Hyb	25	52			
Wsp-2					
Wsp-3	B1	TS + Hyb	Segregation for *Wsp-2*	1	4.67
B1	12	22	Segregation for *Wsp-3*	1	0.27
			Joint segregation	1	0.17
TS + Hyb	30	62			

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

the parental TS264/22 phenotypes due to overlapping bands and gene dosage in hybrid triploid endosperms; consequently, frequencies for these two genotypes are grouped and the χ^2 test was calculated on the basis of an expected 3:1 ratio. The values obtained indicate uniformity with Mendelian segregation ratios at two out of the three loci tested. Alleles at the *Wsp-2* locus depart significantly from the expected 3:1 ratio ($P = < 0.05$, > 0.02). Significant linkage was detected between *Wsp-3* and *β-Amy-1* ($\chi^2_D = 79.3$, $P = < 0.001$), confirming that *Wsp-3* is located on chromosome 4H. A recombination fraction of 0.111 ± 0.022 was obtained. Joint segregation tests for the other pairs of loci were nonsignificant, having taken into account the disturbed segregation at the *Wsp-2* locus. This indicates that *Wsp-2* is not linked to *Wsp-3* and *β-Amy-1* and therefore is probably not located on the long arm of chromosome 4H.

Discussion

The major storage proteins of mature barley grain are the hordeins which map to chromosome 1H (Shewry et al. 1980). Other proteins of barley grain have gene loci on chromosomes 2H, 3H, 4H, 5H and 7H (Table 3), and these include the water-soluble proteins studied here. The nomenclature of these proteins is confusing since several synonyms have been used (see Table 3). The WSP symbol is a temporary designation, which may change once a specific function is ascribed to these proteins. *Wsp-2* segregates independently of *Wsp-3* (Fig. 3); it could not be located to a specific chromosome, using wheat/barley addition lines, and is not linked to *Wsp-3* on chromosomes 4H. Since these proteins have identity with IBF (Liu and Gale 1989), possible expected locations for *Wsp-2* would be 4H or 5H; wheat *Ibf-1* alleles are located on homoeologous group 5 chromosomes and those of *Aegilops sharonensis* are located on chromosomes $5S^L$ and $4S^L$.

WSP-3 has been shown to have identity with IBF; it is not, however, equivalent to 'protein albumin Z' (PAZ1), a major antigenic protein in beer (Hejgaard 1982, 1984). This is concluded from genetic distance from the *β-Amy-1* locus on chromosome 4H; *Paz1* is 48 cMorgans from *β-Amy-1* (Nielsen et al. 1983), whereas the genetic distance in the present study was estimated to be 11 cMorgans.

All seven alleles (two at *Wsp-2*, three at *Wsp-3* and two at *β-Amy-1*) were found among the 44 spring genotypes, but *Wsp-2c* was absent in the 39 winter genotypes tested. The combination of alleles was also more diverse in the spring types. For instance, of the six possible combinations of *Wsp-3* and *β-Amy-1* alleles, all six were found in the spring types, but only three were found among the winter genotypes. It is possible that selection for winter type, either vernalisation requirement and/or winter hardiness, has influenced the spectrum of variability at the *Wsp-3* and *β-Amy-1* loci.

RFLP analysis using a β-amylase probe has been used to link β-amylase with spring/winter habit (Chojecki et al. 1989). Spring and winter barley cultivars could be distinguished, but again with the exception of 'Panda'. It therefore seems likely that both *β-Amy-1* and *Wsp-3* are closely linked to the spring/winter gene, *Sh/sh*, lo-

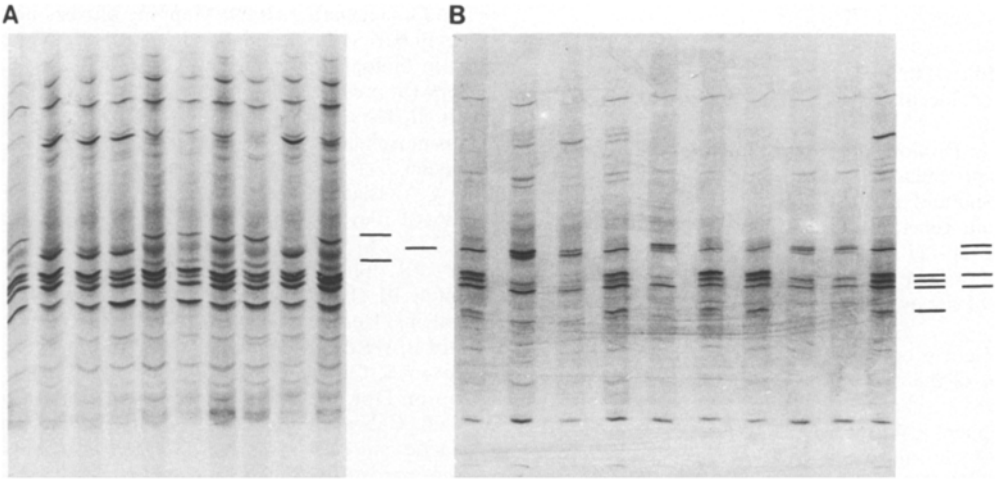

Fig. 3A, B. Segregation of *Wsp-2* and *Wsp-3* genotpyes in ten lines from two doubled haploid populations: **A** 'Dissa' × 'Sabarlis' segregation of *Wsp-2a* versus *Wsp-2b*; **B** TS42/3/5 × 'Apex', segregation of *Wsp-3a* versus *Wsp-3b*

Table 3. Water-soluble proteins of mature barley grain, protein symbols, gene symbols, chromosoma location and references to synonyms

Protein (synonym)	Protein symbol (synonym)	Gene symbol (synonym)	Chromosomal location	Reference
Water-soluble protein-1	WSP-1	*Wsp-1*	7	Liu et al. (1989)
Water-soluble protein-2	WSP-2	*Wsp-2*	Not located	Thompson et al. (1990, 1991)
Water-soluble protein-3 (Iodine binding factor-1)	WSP-3 (*IBF-1*)	*Wsp-3* (*Ibf-1*)	4	Thompson et al. (1990, 1991) Liu and Gale (1989)
Protein Z4 (Protein albumen Z)	PAZ1	*Paz1*	4	Hejgaard (1982, 1984)
Protein Z2	PAZ2	*Paz2*	5	Hejgaard (1984)
Basic protein N	PBN1	*Pbn1*	3	Nielsen and Hejgaard (1990)
Basic protein Q	PBQ1	*Pbq1*	2	Nielsen and Hejgaard (1990)

Pbq1 may correspond to a water-soluble protein gene also located to chromosome 2H (Liu et al. 1989)
WSP-1 reported by Thompson et al. (1991) does not correspond to WSP-1 reported by Liu et al. (1989)

cated on chromosome 4H; *β-Amy-1* has been estimated to be 4 cMorgans from the *Sh* locus (Chojecki et al. 1989). In the present study the majority of winter barley genotypes possessed the *β-Amy-1a* allele, with 'Panda', 'Panache' and 'Dido' carrying *β-Amy-1b*. It is of interest here to note that although 'Panda' ('Katy' × 'Gerbel') is classed as a winter barley, it has low vernalisation requirement, and that 'Panache' has 'Panda' in its parentage ('Panache' = 'Panda' × 'Halcyon'); the low vernalisation requirement may have originated from the spring cultivar 'Kenia', which is in the parentage of both 'Katy' and 'Gerbel'. 'Panda' is also exceptional in that it is the only winter genotype to carry a *Wsp-3b* allele. This therefore reinforces a linkage between *Wsp-3* and *β-Amy-1* and the *Sh* locus.

The products of the *β-Amy-1* and *Wsp-3* genes are easily detected in IEF gels; they can be extracted simultaneously from the endosperm half of the grain, leaving the embryo intact for future regeneration after analysis. Both genes are polymorphic. These characteristics make β-AMY-1 and WSP-3 extremely valuable as biochemical markers for the spring/winter trait in pure and applied aspects of barley genetics and breeding. For instance, genes can be transferred between spring and winter cultivars and the progeny can be selected for recombinants in the desired habit.

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