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Milling energy requirement of the aneuploid stocks of common wheat, including alien addition lines

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Summary. Aneuploid stocks, which included *Triticum aestivum*/alien, disomic, chromosome addition lines, wheat/alien, ditelosomic, chromosome addition lines, and the available aneuploids of 'Chinese Spring' wheat, were used to locate genes that influence milling energy requirement (ME). Genes that affected ME were found on all seven homoeologous chromosome groups. The addition of complete wheat chromosomes *1B*, *1D*, *2A*, *2D*, *5B*, *6B*, *7B* and *7D* increased ME. Positive effects were also found in specific chromosome arms: *1BS*, *2DS*, *5AS*, *5BS* and *6BL*. Wheat chromosome *3B* conditioned low ME and the gene(s) responsible was located on the short arm. Other negative effects were attributed to wheat chromosome arms *4BL*, *4DL*, *5DS* and *6DS*. Alien chromosome additions that conferred high ME included *2H*, *5H*, *6H* and *7H* of barley, *Hordeum vulgare* and *2R*, *2Rα*, *4R*, *4RL*, *6R*, *6RL* and *7RL* of rye, *Secale cereale*. Those that conferred a low ME included $1H^{ch}$ of *H. chilense*, and *6U* and *7U* of *Aegilops umbellulata*, *5R* and *5RS* of *S. cereale* and $5R^{m}$ and $5R^{m}S$ of *S. montanum*. Although the control of ME is polygenic, there is a major effect of genes located on the short arms of homoeologous group 5 chromosomes.

Key words: Milling energy (ME) – Common wheat – Aneuploid analysis – Alien chromosome – Triticeae

Introduction

The aneuploid stocks of wheat (*Triticum aestivum*) and related species have been used routinely for the past 30 years to located genes to specific chromosomes, as can be easily confirmed by consulting the catalogue of gene symbols (McIntosh 1988).

Allison et al. (1976) described the use of milling energy (ME) to assay barley genotypes with good malting potential. This technique has since been developed and used in the barley breeding and research programmes of the Scottish Crop Research Institute (Swanston 1987; Ellis et al. 1989; Swanston and Taylor 1990). Essentially, low milling energy indicates the potential for good malting quality, but only if high enzyme levels are developed during malting. High milling energy can be more certainly associated with poor malting performance.

In this paper, we aim to determine the genetic basis of ME by identifying chromosomes that influence the character. The effects of individual chromosomes of seven distinct genomes were assessed, using the wheat aneuploid stocks that contain or lack specific chromosomes or chromosome arms. Chromosome nomenclature is based on chromosome homoeology.

Materials and methods

The species used included: wheat, *Triticum aestivum* cultivars 'Chinese Spring' (CS) and 'Holdfast' (Hold) (AABBDD, 2n = 6 × = 42); barley, *Hordeum vulgare* cultivars 'Betzes' and 'Natasha' (HH, 2n = 2x = 14) and rye, *Secale cereale* cultivars 'King II' and 'Imperial' (RR, 2n = 2x = 14). The aneuploid stocks included: CS tetrasomics (2n = 44), except for chromosomes *4A* and *4D*; 29 available CS ditelosomics (2n = 40 + 2t); 18 compensating nullisomic/tetrasomic combinations (2n = 42). The available wheat/alien addition lines for both complete and telosomic chromosomes included: CS/*H. vulgare* cv 'Betzes' (AABBDDHH, 2n = 44), *H. chilense* (AABBDDH^{ch} H^{ch}, 2n = 44, or 42 + 2t); CS/*S. cereale* cultivars 'King II' and 'Imperial' (AABBDDRR, 2n = 44, or 2n = 42 + 2t); CS/*S. montanum* (AABBDDR^{m}R^{m}, 2n = 44, or 42 + 2t) and CS/*Aegilops umbellulata* (AADBBDDUU, 2n = 44). The 'Hold/King II' complete and telosomic additions were also included (2n = 44 or 42 + 2t).

Caryopses of the above genotypes were germinated, and cytological checks were made in root-tip squashes to ensure

plants carried the corect chromosome complement. Five plants of each checked line and parents were grown to maturity under glasshouse conditions from March to September, 1989. Mature grains were threshed from harvested, ripe plants and samples were taken for the measurement of milling energy.

Milling energy requirement (ME) was measured by the use of the Comparamill (Allison et al. 1979). The sample size was reduced to 1 g, and variation was assessed relative to the least significant difference calculated after replicated milling of parent cultivars. Mean ME values for parental lines were: 'Chinese Spring', 70 J; 'King II,' 77.5 J; and 'Betzes', 125 J. The pooled standard deviation was 2.2 J and the $LSD_{5\%}$ was 4.5 J.

Results

The ME requirements of the genotypes tested are given in Tables 1 to 3. These are summarised below according to species.

Triticum aestivum

Tetrasomic lines with significantly higher ME values than the control CS include *1B*, *1D*, *2A*, *2D*, *5B*, *6B*, *7B* and *7D*. Interestingly, the positive effects of *1B* are also seen when *1B* is present as a tetrasomic in nulli-*1D*/tetra-*1B* (*1D*/*1B*), and this is converted to a negative effect in nulli-*1B*/tetra-*1D* (*1B*/*1D*). Since *1BL* also has a negative effect, the short arm of *1B*, which is absent in the ditelosomic *1BL* line, must carry genes with a positive effect. Tetrasomy of *6B* also confers a high ME in *6A*/*6B*, but *6D*/*6B* shows no significant effect. The absence of *7A* increased ME in both *7A*/*7B* and *7A*/*7D*. However, *7D*/*7A* and tetra-*7D* showed a significant decrease in ME. The ditelo-*7AL* showed a significant increase in ME, indicating that genes influencing the expression of this character are located predominantly on the short arm. Similarly, chromosome arms *3BS*, *4BL*, *4DL*, *5DS* and *6DS* are also implicated as carrying genes with negative effects. This is verified in the case of *3B*, with positive results when *3B* is absent (both *3B*/*3A* and *3B*/*3D*) and a negative value when *3B* is present in extra dosage (tetra-*3B*). In the case of group 5 wheat aneuploids, the conclusions are supported by the results of the various *5R* addition lines (see below).

Aegilops umbellulata

Addition of *6U* and *7U* significantly lowered ME relative to CS wheat. Effects of other chromosomes were not significant.

Hordeum vulgare

ME values greater than the euploid CS were found in addition lines for chromosomes *2H*, *5H*, *6H* and *7H*.

Hordeum chilense

The addition line with a short $1H^{ch}$ telosome showed low ME, but none of the other *H. chilense* addition lines were significantly different from CS.

Table 1. Milling energy requirement of the aneuploid stocks of 'Chinese Spring' wheat[a]

Homoeologous group	Tetrasomic		Nullitetrasomic (nulli/tetra)		Ditelosomic[b]	
1	*1A*	NS	*1A*/*1B*	NS	1AL	NS
	1B	+7.9	*1A*/*1D*	NS	*1BL*	−11.8
	1D	+5.7	*1B*/*1A*	NS	*1BS*	NS
			1B/*1D*	−5.3		
			1D/*1B*	+4.7		
2	*2A*	+9.3	*2B*/*2D*	NS	*2AS*	NS
	2B	NS	*2D*/*2B*	NS	*2BL*	NS
	2D	+6.4	*2D*/*2A*	NS	*2DL*	−8.0
					2DS	NS
3	*3A*	NS	*3A*/*3D*	+5.1	*3AL*	NS
	3B	−8.3	*3A*/*3B*	NS	*3AS*	NS
	3D	NS	*3B*/*3A*	+7.8	*3BL*	+10.8
			3B/*3D*	+5.8	*3DL*	NS
			3D/*3A*	NS		
			3D/*3B*	NS		
4	*4*Tri*A*[c]	NS			*4AL*	NS
	4B	NS			*4BL*	NS
					4BS	+7.3
					4DL	NS
					4DS	+9.3
5	*5A*	NS	*5B*/*5D*	−5.2	*5AL*	−5.5
	5B	+4.6	*5D*/*5A*	+19.6	*5BL*	−9.9
	5D	NS	*5D*/*5B*	+15.8	*5DL*	+10.0
					5DS	NS
6	*6B*	+8.6	*6A*/*6B*	+13.0	*6BL*	NS
			6A/*6D*	+9.8	*6BS*	−11.6
			6D/*6B*	NS	*6DL*	+15.7
					6DS	NS
7	*7A*	NS	*7A*/*7B*	+7.7	*7AL*	+5.6
	7B	+5.6	*7A*/*7D*	+6.7	*7AS*	NS
	7D	+10.1	*7B*/*7A*	NS	*7BL*	NS
			7B/*7D*	NS	*7BS*	NS
			7D/*7A*	+7.0	*7DS*	NS
			7D/*7B*	NS		

[a] The effect of a particular chromosome is given as the deviation from the wheat parent. The mean value of Chinese Spring was 70.0 J

[b] S and L−Telosomic for short and long arm, respectively

[c] Trisomic for chromosome *4A*

NS−Not significant

Secale cereale

Secale cereale cv 'King II' chromosomes were present as addition lines in two wheat genetic backgrounds: Chinese Spring and Holdfast. In both sets of additions the intact chromosome *5R* had a negative effect. The telosomic addition of *5RS* conferred significant lowering of the ME of the respective wheats. High ME values were produced by addition of *6RS* (CS/KII) and *6R* and *6R* (Hold/KII), with the long arm having the greatest effect. Genes affecting ME on *6RL* are probably in the same homoeallelic series as those on *6DL* of wheat. Addition of *2R* and *2Rα* also conferred significantly high ME values.

Table 2. Milling energy requirement of the alien addition lines of 'Chinese Spring' wheat *Aegilops*, *Secale montanum* and *Hordeum* chromosomes[a]

Homoeologous group	*Aegilops umbellata* (*U* genome)		*Secale montanum* (R^m genome)		*Hordeum chilense* (H^{ch} genome)		*Hordeum vulgare* cv 'Betzes' (*H* genome)	
1			$1R^m$	−12.8	$1H^{ch}S$	−7.5		
2	*2U*	NS	$2R^m$	NS	$2H^{ch}$	NS	*2H*	+10.0
3							*3H*	NS
4			$4R^m$	NS			*4H*	NS
			$4R^mS$	NS				
5	*5U*	NS	$5R^mS$	−7.8	$5H^{ch}$	NS	*5H*	+6.0
6	*6U*	−9.4	$6R^m$	NS	$6H^{ch}$	−4.5	*6H*	+10.0
			$6R^mS$	NS				
7	*7U*	−5.0			$7H^{ch}$	+7.0	*7H*	+7.0

[a] S and L – Addition of the short and long arm, respectively
NS – Not significant

Table 3. Milling energy requirement of the rye (cv 'King II' and 'Imperial') chromosome addition lines to common wheat cultivars 'Chinese Spring' and 'Holdfast'[a]

Homoeologous group	'Hold/King II'				'CS/ King II'		'CS/ Imperial'	
1	*1R*	NS	*1RL*	NS	*1R*	NS		
			1RS	NS				
2	*2R*	+14.2	*2Rα*	+16.9				
3					*3R*	NS		
4	*4R*	+12.4	*4RL*	+8.4				
			4RS	NS				
5	*5R*	NS	*5RL*	NS	*5R*	NS	*5R*	−5.8
			5RS	−6.8				
6	*6R*	+15.7	*6RL*	+15.4			*6R*	+9.1
			6RS	NS				
7	*7R*	NS	*7RL*	+10.2				
			7RS	NS				

[a] S and L – Addition of the short and long arm respectively. α, arm not assigned
NS – Not significant

Secale montanum

The addition of chromosome $1R^m$ produced the greatest reduction in milling energy. Chromosome $5R^mS$ also resulted in a low ME.

Discussion

Genes affecting ME were found on all seven homoeologous groups of chromosomes of the Triticeae. It is possible that grain morphology, anatomy and composition contribute to these differences (Gaines 1986). Grain morphology is affected by tetrasomy or by the addition of certain chromosomes or chromosome arms. For instance, the grain characters – vitreous, narrow, coarse, round and wrinkled – are conditioned by the addition of homoeologous chromosomes or chromosome arms *2*, *2L*, *5L*, *6L* and *7*, respectively (Miller and Reader 1987). Altered grain morphology may thus account for some of the present results, e.g., the positive effects of adding chromosomes or chromosome arms *2A*, *2D*, *2H*, *2R*, *2Rα*, *5B*, *5H*, *6B*, *6H*, *6R*, *7B* and *7D*, and the negative effects of adding *6U* and $6H^{ch}$ chromosomes.

Addition of alien chromosomes of homoeologous group 1 to wheat has not been associated with altered grain morphology, and therefore the high ME values of telosomic lines *1BS*, $1H^{ch}S$ and *1RS* are probably due to some other factor. Grain hardness, measured from estimates of particle size index, has been associated with the presence of particular gliadins (Campbell et al. 1987). Group 1 chromosomes carry genes for both the major endosperm proteins, i.e. gliadins and glutenins (McIntosh 1988). Thus, it is possible to infer that gliadin encoded by *Gli-1* and glutenin encoded by *Glu-2*, located on the short arm of group 1 chromosomes, but probably not the glutenin encoded by *Glu-1* on the long arm (Payne 1987) may have contributed to high ME values. Gliadin protein is also encoded by a gene, *Gli-2* on homoeologous group 6 chromosomes (Payne 1987), and could thus explain the result of the addition of the complete *6B* chromosome; this indicates that the alleles on *6BS* and *6DS* both have an effect of similar magnitude.

Although the addition to wheat of the long arm of group 5 homoeologues affects grain morphology, the short arm has no such effect. Again, some other factor must be involved to produce the major effects of lowered milling energy by *5S* homoeologues. Endosperm proteins associated with group 5 chromosomes include a nonstorage protein (Payne et al. 1985) and iodine binding factor (IBF) (Liu and Gale 1988). Both can be detected by the relatively insensitive stain, Commassie blue, and therefore abundant and may affect ME. However, it has been shown that an increase in grinding time, which can be related to ME (Ellis et al. 1979), resulted from the

substitution of CS *5D* with *5U* from *Aegilops umbellulata* (Morrison et al. 1984, 1989). These effects were ascribed to a gene conditioning grain softness (*Ha*) on the short arm of chromosome *5D* or a closely linked gene. Two genes controlling levels of free lipid were provisionally identified (*Fpl-1*, *Fpl-2*). *Fpl-2* was located on the short arm of chromosome *5D* and may be the same gene as *Ha*, while *Fpl-1* was located on the long arm of *5D* proximal to *Vrn3* conditioning spring habit. Morrison et al. (1989) suggested that *Fpl-1* may also be associated with grain hardness and although our results agree with the effect of *5DS*, no effect of *5DL* on ME was found.

Greenwell and Schofield (1986) suggested that the presence of a relatively small protein (15S) on the surface of starch granules could be the biochemical basis of grain hardness. The location of the gene encoding for this protein on chromosome 5 was inferred by its absence in durum wheats and presence in triticale, and was confirmed by *5D* addition lines in Chinese Spring. The analysis of recombinant lines indicated close linkage to the *Ha* gene (Schofield and Greenwell 1987). Thus, there appear to be a series of genes on chromosome 5 that are important for the technological properties of wheat. While the lack of a sufficiently refined gene map hampers selection and recombination of desirable attributes, it would appear that the use of ME would assist the wheat breeder in selection for quantitative improvements in grain hardness. By the same token, breeding for malting quality would be greatly assisted by the use of milling energy to select for softer endosperm texture.

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