HGCA

PROJECT REPORT No. 63

A RAPID TEST FOR MALTING QUALITY IN BARLEY

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A RAPID TEST FOR MALTING QUALITY IN BARLEY

by

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SUMMARY

Grain milling energy (GME) was devised as a rapid test to identify genotypes with endosperm properties similar to Triumph. The rapidity of the test and the small sample size required make it suitable as a preliminary screen in a breeding programme. A study of the effects of environmental factors indicated that GME is not affected by variation in grain nitrogen as is HWE. While storage of grain samples can cause considerable changes in HWE, GME does not change.

GME can be dissected into a number of components which are related to the milling energy requirement of the major tissues. The husk and aleurone require more energy to mill than the starchy endosperm but, as they together form only some 15-20% of the total weight of the grain, 70% of GME can be attributed to the starchy endosperm. Even so the true nature of a genotype with a soft endosperm can be masked by a particularly tough husk, formed from the fusion of the pericarp/testa and lemma and palea.

The integrity of the starchy endosperm depends on the strength of the cell walls and the nature of the protein matrix. Protein and starch granules are intimately associated in the endosperm, particularly the small granules. It has been possible to associate GME with particular proteins which can be extracted from the surface of starch granules isolated from barley endosperms with centrifugation through CsCl. An examination of the milling energy of malt during the malting process has shown genotypic differences in endosperm degradation. GME can show significant correlations with components of malting quality but the level of correlation improves markedly when malt milling energy (MME) is considered. The milling energy of barley is greater than that of wheat and selection for important wheat properties could be achieved with the milling energy test.

ABBREVIATIONS

ANS	8-anilino-1-naphthalene sulphonic acid
CsCl	Caesium chloride
DH	Doubled haploid
GC	Germinative capacity [100 grain germinated in 4ml distilled water observed at 120 hr]
GE	Germinative energy [as GC observed at 72 hr]
GME	Grain milling energy. Megajoules (J) per 5g.
HWE	Hot water extract
IoB	Institute of Brewing
LMW	Low molecular weight protein markers
LP	Living pedigree demonstration grown 1983
ME	Milling energy, while this is the original definition GME is preferred to distingush grain and malt milling energy
MEA/MEB	ME estimates taken at 2 and 4 seconds from the start of milling
MME	Malt milling energy
PAGE	Polyacrylamide gel electrophoresis
RIL	Field trial of random inbred lines from the cross Blenheim * E224 grown in 1988
SAC	Scottish Agricultural College
SCRI	Scottish Crop Research Institute
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscope/microscopy
SD88	Sowing date trial grown 1988
SSD	Single seed descent
TCW	Thousand corn weight
VNT86	Variety by nitrogen trial grown 1986
VNT87	Variety by nitrogen trial grown 1987
VNT88	Variety by nitrogen trial grown 1988
WS	Germination, as for GE and GC but with 8ml water

INTRODUCTION

The objective of this project was to develop further the milling energy of barley grain (**GME**) as a rapid test for malting quality. Additionally, by analysing properties of grain that contribute to milling energy, it is possible that a more complete understanding of the malting process would be achieved. The definition of further components of malting quality then would permit selection for such traits in breeding programmes and in turn lead to improved malting performance. Several approaches have been used to analyse genetic and environmental effects on GME.

For example GME has been examined in relation to:-

(i) Genetic differences between cultivars
(ii) Environmental effects such as variation of sowing date and the application of fungicides and nitrogen
(iii) The changes in milling energy (ME) as each of the major tissues was sequentially removed by abrasion
(iv) The effects of grain storage on malting performance
(v) The composition of the starchy endosperm, changes during malting and malt milling energy (MME)
(vi) The relationship between GME and MME with standard malt and barley tests
(vii) The characteristics of isolated starch
(viii) A comparison between wheat and barley

Historical Review

Malting quality in barley is a complex trait associated with grain composition, germination rate and the production of enzymes that degrade endosperm constituents such as starch, protein and cell wall components. In commercial malting practice much attention is given to ensure that germination is even and carefully controlled to minimise malting losses. The major contrast between this situation and that faced by breeders, and also by maltsters on intake into maltings, is the need for rapid processing and the inability to apply an optimum regime for each genotype (Ellis *et al.* 1989).

Micromalting was developed by Whitmore and Sparrow (1957) to allow selection for genetic differences in malting quality, in the laboratory. Micromalting, with modifications such as air rests during steeping, can be

supplemented by specific tests such as that for cell wall degradation described by Aastrup *et al.*, (1981), enzyme levels (Hayter and Riggs 1973) and assays of the extent of modification (Ellis *et al.* 1989). Nevertheless, any process that relies on malting cannot be applied when time or seed availability is limiting. These difficulties have lead to the development of small scale tests that can be applied in breeding programmes. However, Gothard (1984) has pointed out a major problem with the calibration of small scale tests because grain samples, in addition to recovery from dormancy, go through a process of maturation or conditioning. It is even possible for feed quality cultivars to produce hot water extract at the same level as good malting quality cultivars.

Malting quality is greatly influenced by the environment during growth and maturation of the grain. This will in turn affect the success of selection within breeding programmes. In fact, with hindsight, it is possible to see that the introduction of intensive agricultural systems led to the selection of cultivars, such as Triumph, which can produce good hot water extracts at higher grain nitrogen levels than Proctor (Gothard, 1983). Germination has a major influence on malting quality, especially when complicated by post-harvest dormancy. In Scotland the grain ripening and harvest period is typically colder and wetter than in much of the rest of Europe. The result is a long period of post-harvest dormancy which accounts for the, often unconscious, selection for short dormancy periods in lines bred in Scotland. It is possible to envisage environmental components that relate to: field conditions, eg seen as variation in grain size and nitrogen content; to storage conditions, e.g. the degree of dormancy; and to malting systems, eg the speed of modification.

Milling energy (ME) was developed at the Scottish Crop Research Institute (SCRI) for the rapid assessment of malting quality in barley (Allison *et al.*, 1976). A well-malted sample is one where the cell walls and protein matrix have been sufficiently degraded enzymatically for the starch granules to be readily hydrolysed during mashing to give a high extract of fermentable sugars. Barley endosperms that can be readily modified enzymatically have properties that also facilitate their mechanical disruption during milling (Allison, 1986; Swanston *et al.*, 1990). However, correlations reported between grain milling energy (GME) and hot water extract (HWE) vary (Allison *et al.*, 1979; Ellis *et al.*,

1979; Thomas *et al.*, 1991), so if GME is to be developed as a test for malting quality, it is important that the factors governing kernel hardness and their relationship to GME and HWE are better understood.

In wheat, much work has been carried out to identify which components of the grain determine its hardness (Anjum & Walker, 1991). It has been suggested that, in hard-milling wheats, the starch granules and surrounding protein matrix are tightly bound, but that in soft wheats there is a low molecular weight protein on the surface of the starch granules (Greenwell & Schofield, 1986). More recent studies have indicated that the tensile strength of tablets made from flour of soft and hard wheat reflected the grain texture. Fractionation and reconstitution of treated flour components indicated that a 15 kDa protein was responsible for endosperm texture (Malouf and Hosney, 1992, Malouf, Lin and Hosney, 1992). It may be however, that friabilin cannot be used as a marker in all crosses as Glenn and Saunders (1990) report a lack of correlation between intensity of friabilin and texture.

Bread wheat is hexaploid so it is possible to examine the effects of the genes on single chromosomes or chromosome arms in genetic stocks that have been especially developed for such studies (Forster & Ellis, 1990). These authors showed a major effect of genes on the short arm of chromosome 5 but also showed genes on other chromosomes which increased or reduced GME. Ellis & Forster (1990) suggested that such effects could explained by genes known to affect grain size and morphology.

There may be parallels between wheat and barley, although barleys have higher GME than hard wheats (Ellis & Forster, 1990). Barley grain differs from wheat grain in having a very fibrous husk, an aleurone layer at least three cells deep (Figure 5) rather than one or two and endosperm cell walls containing much higher levels of ß-D-glucans (Henry & Cowe, 1990).

Ellis *et al.*, (1991) demonstrated that the milling energy of *H. spontaneum* populations from Israel ranged from that similar to malting cultivars while the highest values observed were much greater than found in *H. vulgare*. The highest levels of milling energy in *H. spontaneum* were

related to the toughness of the husk and lemma awn and thus selection for low milling energy would be useful in programmes designed to improve cultivated barley by the use of *H. spontaneum*.

Powell *et al.* (1991) used doubled haploids (Powell *et al.*, 1990) to investigate genetic linkage between a biochemical marker, r-DNA, on barley chromosome 7 and milling energy. In the cross E224 * Blenheim this linkage accounted for some 7% of GME and in the reciprocal cross for some 28%. This is the first report of linkage between GME and a marker gene.

MATERIALS AND METHODS

Field plots were grown on the SCRI farms at Mylnefield or Gourdie and, unless indicated specifically, were the source of all grain samples. Milling energy estimates on grain and malt were made with the Comparamill described by Allison *et al.*, (1979). Control samples, of contrasting cultivars, were included in each run and the results were used to monitor the performance of the mill over time (Figure 1). Grain samples were, experimentally, conserved by kilning at 60°C or by freezing at -20°C, followed by storage in airtight containers, for comparison with samples stored at 10% moisture and ambient temperatures (Figure 2). Samples that were frozen gave GME results very similar to grain stored at laboratory temperatures. Kilned samples, however, showed a reduction of 50-90J in GME. Scanning electron micrographs of the cut surface of kilned grain indicated that the starch granules had become separated from the shrunken protein matrix (Figure 3).

Scanning electron microscopy was used to examine the particles from the Comparamill. Unlike a roller mill, a hammer mill causes greater attrition of particle shape, so that genotypic differences were not striking. However, Klaxon showed greater abrasion of the protein matrix than Tyne, which is indicative of higher milling energy in the latter (Figure 4).

1 PEDIGREE ANALYSIS

A series of cultivars, that formed a living pedigree demonstration (LP) of SCRI bred cultivars, was grown in 1983. After harvest the grain samples were dried at 25 °C and stored at ambient temperatures. In 1987 samples were sieved over a 2.25mm screen and assessed for GME. Inbred lines were derived from crosses between spring and winter barley cultivars and assessed by the same procedure for GME.

2 GENOTYPE X ENVIRONMENT INTERACTION FOR GME

The effect of different nitrogen top dressing rates on malting quality and GME was assessed in three spring barley Variety x Nitrogen Trials (VNT86, VNT87, VNT88) grown in 1986-8. Nine cultivars were sown, by

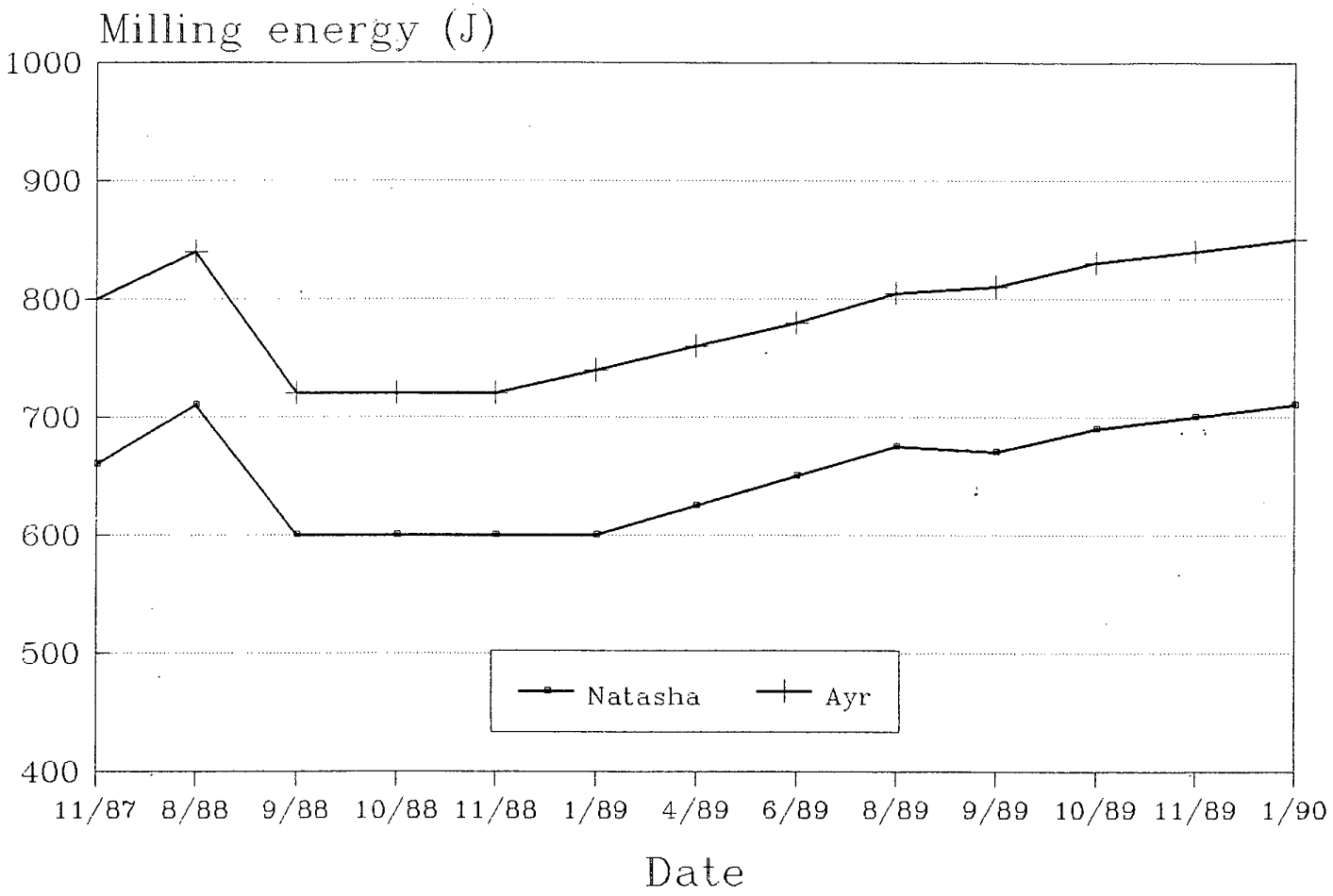


Figure 1. Variation of control samples milled with the comparamill.

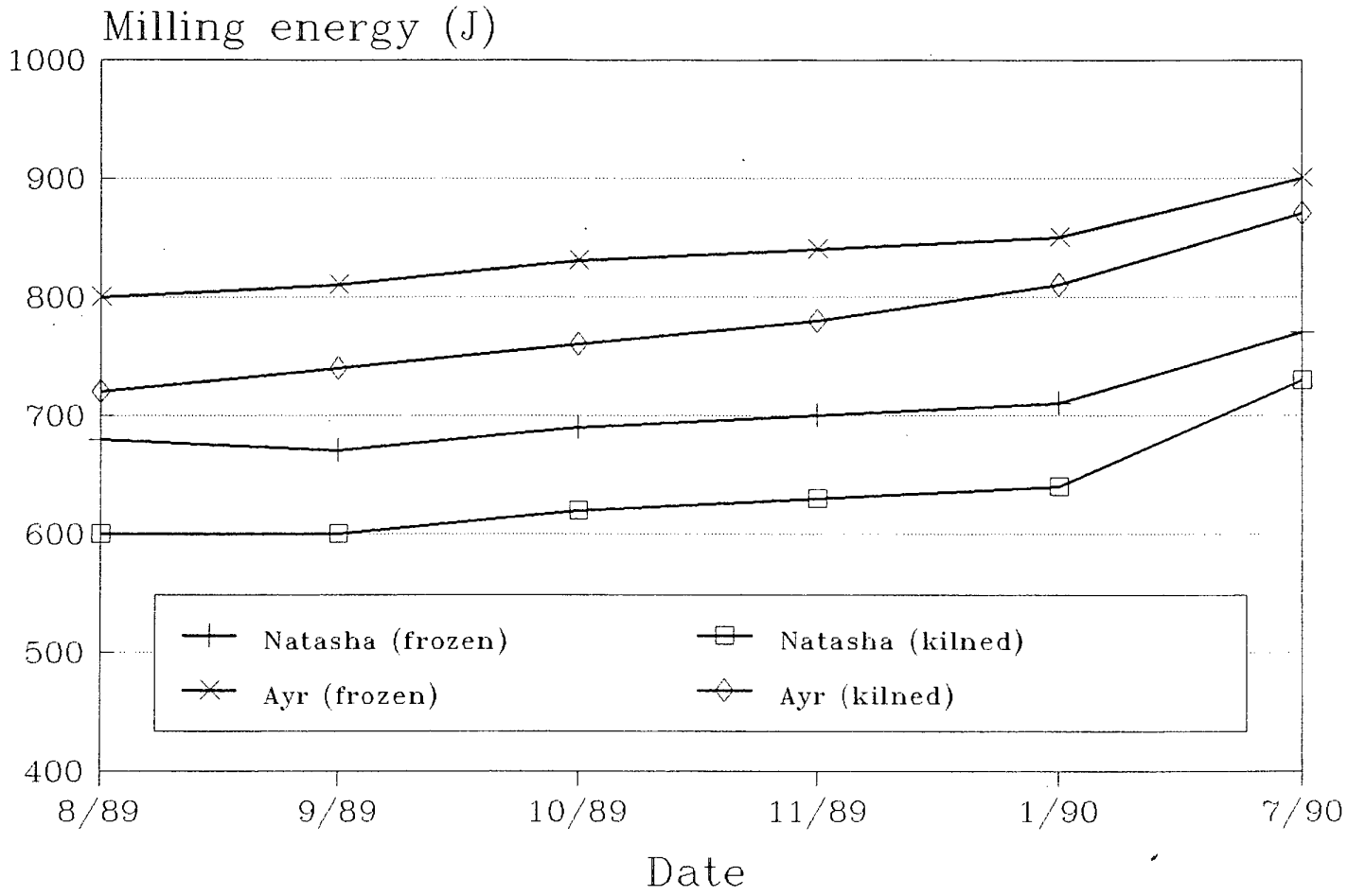


Figure 2. Variation of control samples after kilning and freezing.

Figure 3. Scanning electron micrograph of the cut surface of a grain after kilning. The protein matrix has separated from the starch granules in contrast to the normal structure of the starchy endosperm (see Figure 5). s = large starch granule, c = crack. Scale bar = 10μ.

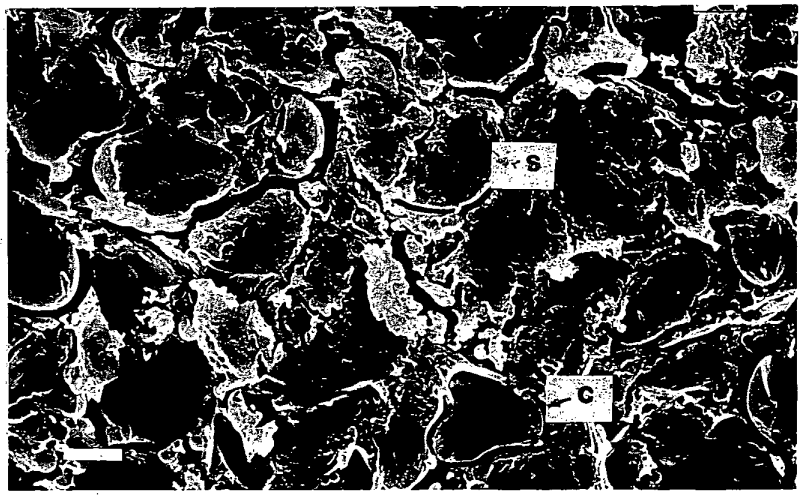
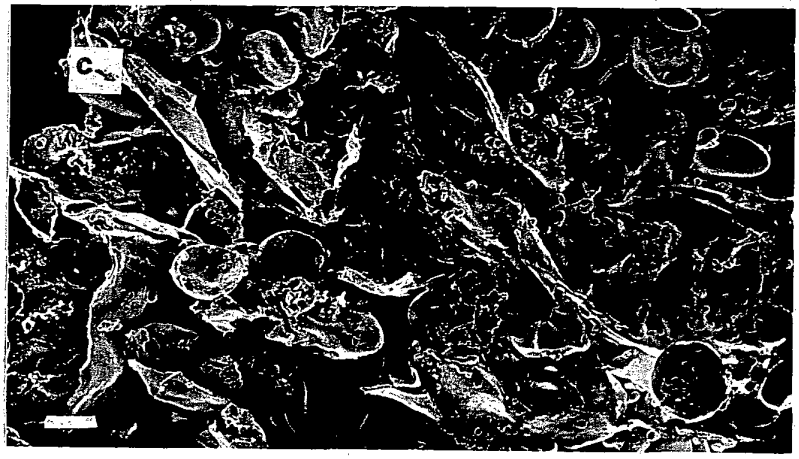


Figure 4. Scanning electron micrographs of flour particles from (a) Klaxon and (b) Tyne. c = cell wall, s = large starch granule. Scale bar = 10μ.

{a}

{b}



Dr. W.T.B. Thomas (SCRI), in a split plot design with four replicates. Nitrogen top-dressing was at the rates of 80, 100 and 120 kg/ha in 1986 and 1987 while 77, 89 and 111 kg/ha were used in 1988.

The combined effects of variation in sowing date and fungicide treatment were examined in a trial (SD88) which contained two spring and seven winter barleys sown on 10th September, 10th October and 10th November 1988.

3 ABRASION

Two experiments were set up to examine the effects of abrading the outer grain tissues. In the first experiment a survey was made of seven cultivars, from which Leith, Tay and Tweed are derived (Figure 8d). In the second four cultivars from VNT87, were used. These were chosen to represent high (Tyne), moderate (Natasha and Klaxon) and low GME (Doublet) in combination with good and poor malting quality.

Grain samples were screened to provide grain samples of >2.5mm and <2.75mm and then successively abraded with a laboratory abrader to produce **dehusked** grain, **pearled** grain (aleurone removed) and **inner** endosperm (subaleurone removed). The abrasion process was controlled by timing and direct observation of the progress of each sample. For example to produce pearled grain the abrasion was stopped when the aleurone layer had been removed from the dorsal surface and cheeks of the grain (Figure 5c). Some husk and aleurone remained in the ventral groove (Figure 6). 5g samples were then taken from each stage and milled with the Comparamill. The Comparamill results were interpreted by the use of the average grain composition (Table 1) derived from the loss of tissues during abrasion.

Table 1. Average percentage of tissues milled at each stage of abrasion. Superscripts $^{a-j}$ indicate values used to calculate the weight of tissue per 5g sample in the equations below.

	Stage of abrasion			
Tissue	Inner	Pearled	Dehusked	Grain
Inner	100^{a}	85^{b}	77^{c}	69^{d}
Subaleurone	0	15^{e}	13^{f}	12^{g}
Aleurone	0	0	10^{h}	10^{i}
Husk	0	0	0	9^{j}

The milling energy per gram of each tissue was derived in the following way:-

[A] **inner** =

$ME/5^{a}$

[B] **subaleurone** =

$(MEpearled - ([A]x4.26^{b}))/0.74^{e}$

[C] **aleurone** =

$(MEdehusk - (([A\ x3.82^{c} + ([B]x0.66^{f}))) / 0.52^{h}$

[D] **husk** =

$(MEgrain-(([A]x3.47^{d})+([B]x0.60^{g})+([C]x0.47^{i})))/0.46^{j}$

See over

Figure 5. Scanning electron micrographs of the cut surfaces of barley grain to illustrate structure and the process of abrasion.

(a) Outer tissues of the grain, h = husk, pt = pericarp/testa, a = aleurone, sa = subaleurone. Scale bar = 10μ.

(b) "Husk" removed by abrasion, a = aleurone, sa = subaleurone. Scale bar = 100μ.

(c) Husk and aleurone removed, the fractured surface shows the shape of the cells in the subaleurone. Scale bar = 10μ.

(d) Inner region of the starchy endosperm. The starch protein/matrix is continuous unlike that of kilned grain (Figure 3). Scale bar = 100μ.

{a b}
{c d}

Figure 5.

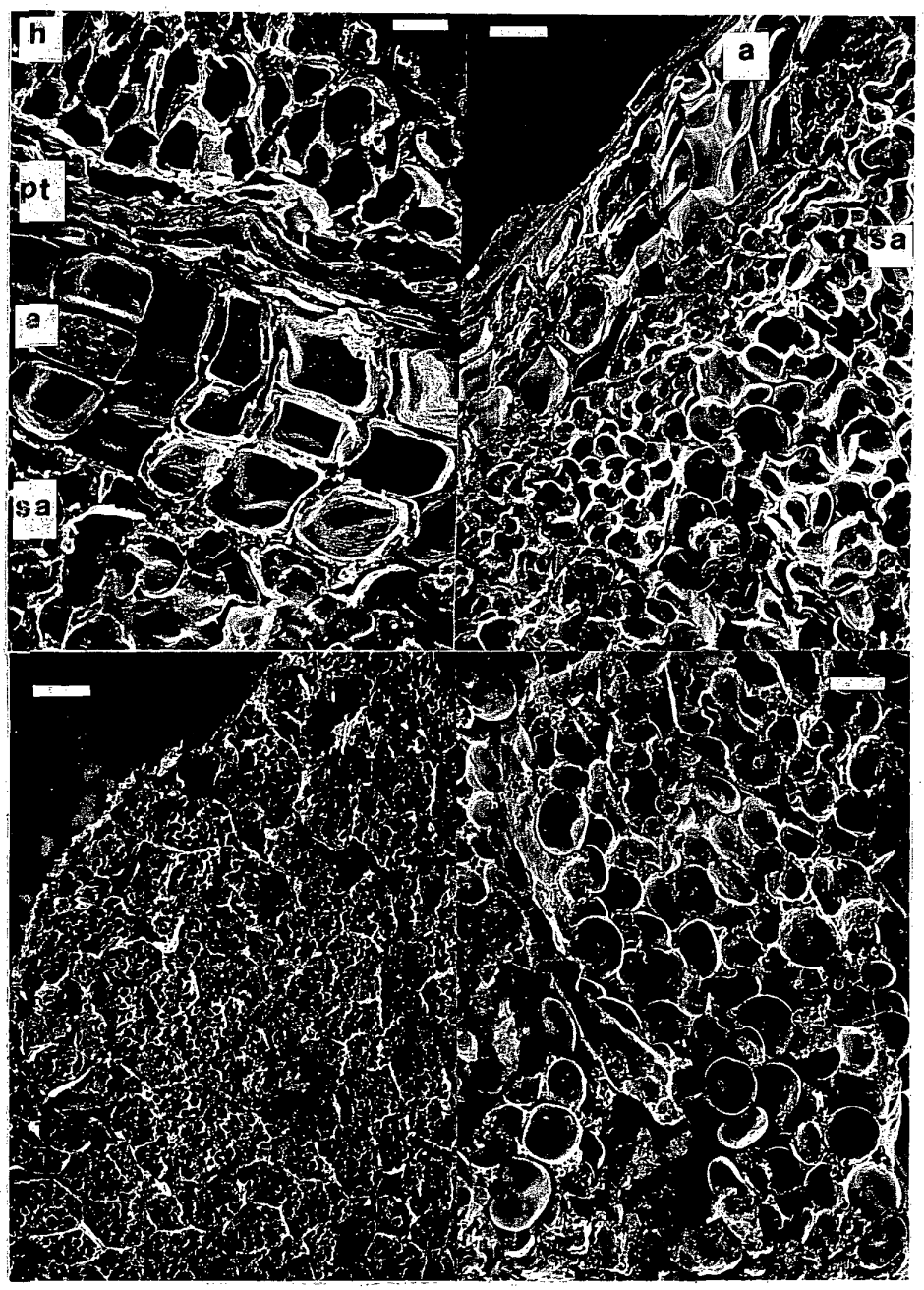
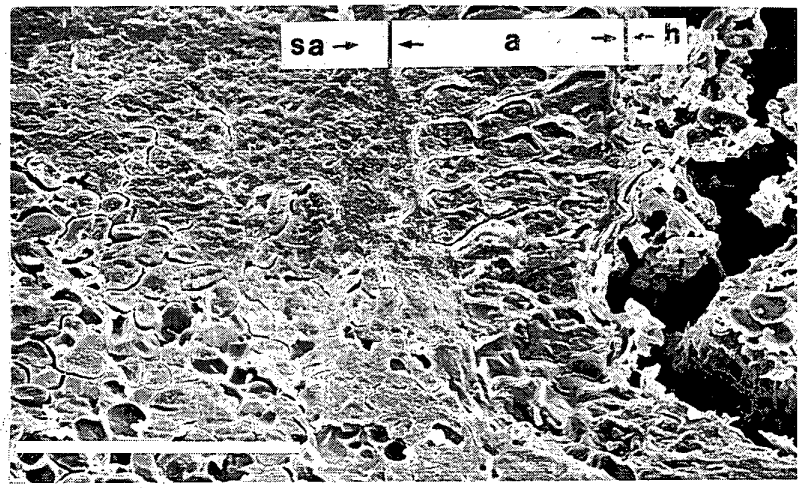


Figure 6. Scanning electron micrograph of the cut surface of a grain to illustrate that abrasion treatments did not remove the aleurone and husk from the ventral groove. a = aleurone, h = husk with pericarp and testa remnants, sa = subaleurone Scale bar = 100µ.



4 EFFECT OF STORAGE

The effect of storage of grain samples on milling energy and malting quality was determined by malting grain from a Variety x Nitrogen Trial grown in 1987 (VNT87). After harvest samples were dried and stored at ambient temperatures in an unheated warehouse from September 1987 and then malted in December 1987, March 1988 and June 1988. Samples were screened over a 2.2mm sieve and 60g micromalted (Taylor and Swanston, 1987). Germinative energy (GE), germinative capacity (WS), water sensitivity (WS) and thousand corn weight were estimated by Institute of Brewing (IoB) methods (1971). Similar procedures were used in 1989 except that malting was carried out in December 1989, March 1990, June 1990 and October 1990.

5 ENDOSPERM COMPOSITION AND CHANGES DURING MALTING

Samples of Triumph, Golden Promise and Koru were obtained from a time-course experiment designed to investigate the use of rapid screening tests to assess modification at different stages of micro-malting (Swanston & Taylor, 1988) Percentage modification of cell walls in malt was assessed with the Malt Modification Analyser (Aastrup *et al.*, 1981).

In preparation for scanning electron microscopy (SEM), barley and malt kernels were fractured along the crease and then transversely midway along the grain, mounted and sputter coated with a thin layer of gold and viewed with a Jeol JSM T200 scanning electron microscope.

Before fluorescence microscopy barley and malted barley kernels were split longitudinally. This was followed by fixation in 3% glutaraldehyde in 0.025M phosphate buffer (pH 6.8-7.0) at 4°C for between 24h and 1 week. After rinsing with buffer, the tissues were dehydrated through ethanol and infiltrated with London Resin for 12h and polymerised at 50°C for 24h. Longitudinal and transverse sections, 1 μm thick, were cut with glass knives on a Reichert Ultracut microtome. Serial sections were attached to slides pre-coated with 2% aminoalkylsilane (Henderson, 1989). Consecutive sections were then treated with either Calcofluor, or 8-anilino-1-naphthalene sulfonic acid (ANS). Sections were viewed with a Olympus BH-2 fluorescence microscope equipped with epi-illumination at a wavelength of 365 nm.

6 GRAIN AND MALT CHARACTERISITICS

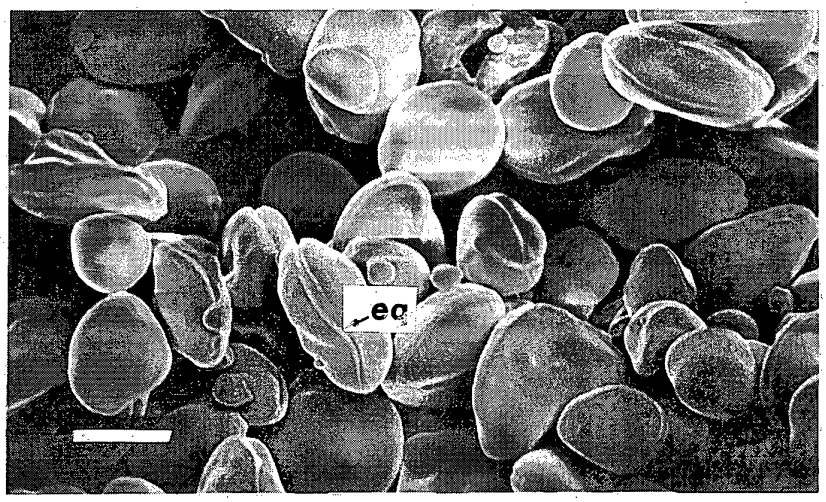
Fifty samples of barley and malt from a Scottish Agricultural College (SAC) cultivar trial, with grain and malt analyses carried out on them by Institute of Brewing Recommended Methods (1971), were obtained from Robert Kilgour Ltd, Kirkcaldy. GME and MME were measured and correlated with these results. Cell wall modification in malt, was assessed by using the fluorochrome Calcofluor, as a specific marker for ß-D-glucans (Fulcher and Wood, 1980) and measuring the loss of fluorescence with a Malt Modification Analyser (Aastrup *et al.*, 1981).

7 STARCH CHARACTERISTICS

Constant Environment

Four cultivars were used to study the relationship between GME, TCW and starch composition. These comprised two widely used malting cultivars: Triumph and Golden Promise and two cultivars which had mutations for altered starch composition, Waxy Oderbrucker (low amylose) and Glacier [Pentlandfield] (high amylose). They were grown under continuous illumination from high pressure sodium lights at constant temperatures of 10, 15 and 20°C. Transfer to these conditions took place at anthesis after an initial period in a glasshouse. At harvest grain was divided into sub-samples for GME determination and starch analysis at the University of Strathclyde (Tester *et al.* 1991)

Figure 7. Starch isolated from barley by homogenisation of macerated grain in water. Adhering protein was removed by centrifugation through CsCl. (Compare with Figure 18c). eq = equatorial groove on large starch granule. Scale bar = 100μ.



Field Trials

A preliminary examination of starch characteristics of material from the field was carried out on plots grown in 1987. The cultivars in this study included six spring and five winter types and represented a range of malting quality and GME.

A field trial (RIL) was grown in 1989 by Dr. W.T.B. Thomas to investigate the performance of inbred lines from the cross Blenheim x E224. Inbred lines had been generated by selfing the F_1 to F_6 generations (SSD) or from doubled haploids (DH) generated by anther culture at the F_1 generation. Grain was harvested from the plots and used to determine thousand corn weight, milling energy, grain nitrogen and hot water extract.

Starch Isolation

Samples of grain, from RIL, were dehusked, steeped overnight, macerated in a mortar and pestle and then repeatedly extracted in a 50ml pot homogeniser. The suspension was filtered through a 30 mm sieve and the starch extracted based on the method of Sulaiman and Morrison (1990) but using 12ml CsCl in 25ml ultracentrifuge tubes and with an additional centrifugation through 6mls 80% CsCl in 10ml centrifuge tubes. This was to ensure the starch was free from protein contamination (Figure 7) as this appears to be much more of a problem in barley than in wheat.

Electrophoresis

The starch surface proteins were preferentially extracted by adding 3ml of 2% SDS to 300mg of purified starch and agitating at 20°C for 30 min on an horizontal shaker. The proteins were then run on SDS polyacrylamide gradient gels (PAGE 10-20%) (Greenwell and Schofield, 1986).

Starch Granule Size

The determination of starch granule sizes was based on the technique of Morrison and Scott (1986) with a Coulter Counter (ZM) and Multisizer equipped with a 70 μm orifice.

RESULTS

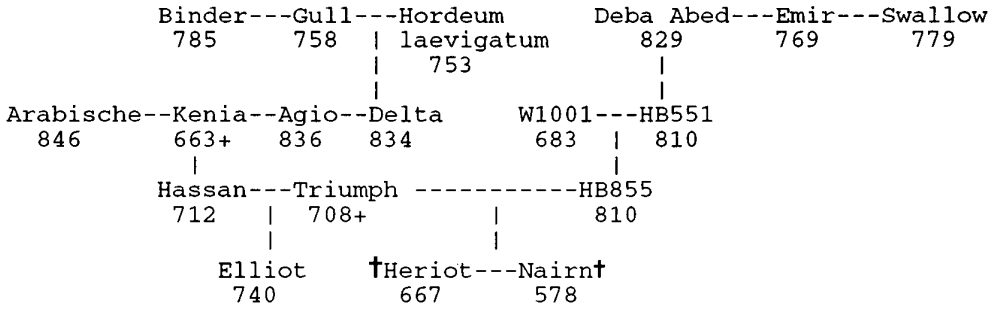
1 PEDIGREE ANALYSIS

In spite of storage for four years, and some loss of vigour of germination, differences for GME were found in the living pedigree (LP) . GME (Figure 8) varied continuously from the very soft (Nairn), to the very hard (Arabische) with no obvious sub-groups. This indicates that GME is not under the control of simple Mendelian genes and is influenced by environmental factors. This conclusion is supported by the distributions observed when the GME of inbred lines from two winter x spring barley crosses was assayed (Figure 9). The spring parent, a selection from the cross Tyne * Scots Bere, was common to both crosses. Thus differences in the distributions reflect the differences in GME of Mallard and Gerbel. Mallard was rated as A (good) malting grade by the National Institute of Agricultural Botany (1989) while Gerbel was rated as D (poor). The distribution of GME seen in the SCRI85 * Mallard inbred lines suggests that transgressive segregation for low GME is possible but dominant alleles condition high GME.

The introduction of mildew resistance has resulted in cultivars with high milling energy eg Arabische (Figure 8a), and *Hordeum laevigatum* (Figure 8c). Successful selection for improved malting quality has, on several occasions, resulted in cultivars with "softer" kernels i.e. with lower GME. Nairn has lower milling energy than either of its parents (Figure 8a) and the mean milling energy for each of the preceding generations (779 J) is greater than that for those which include Triumph (743 J) and Nairn (662 J).

Figure 8. GME of spring barley cultivars related to their pedigrees. Values in parentheses were from other trials. *** = milling energy not determined. †=selection for malting quality which resulted in a reduction in milling energy. + = see Figure 8b for full pedigrees of Triumph and Kenia.

a) Elliot, Heriot and Nairn .



b) Almond and Donan

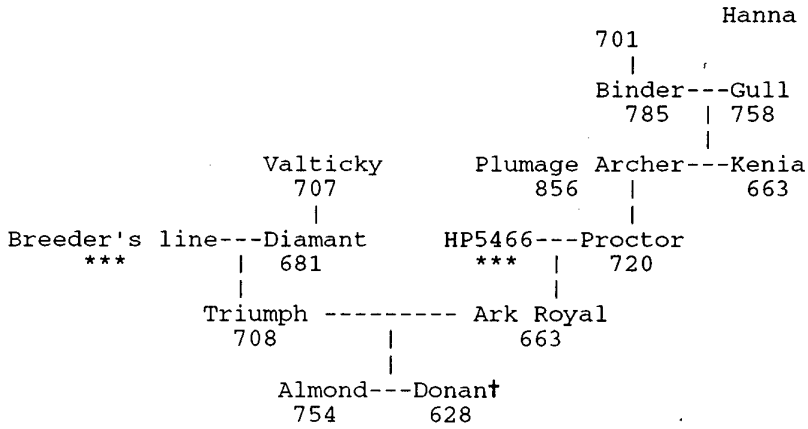


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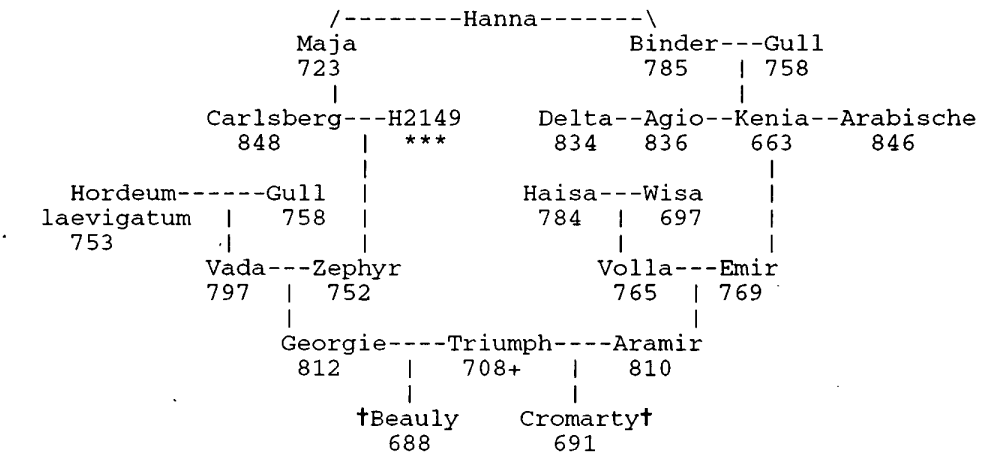
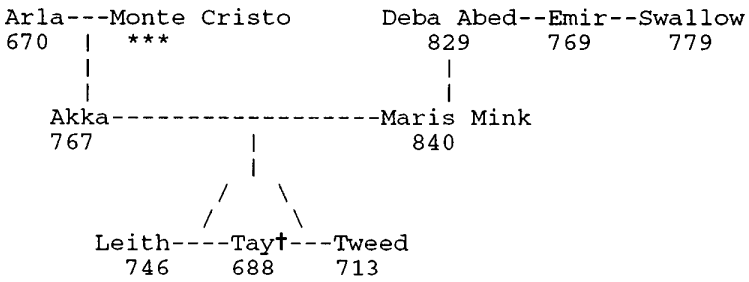
Figure 8 continued.

c) Beauly and Cromarty



d) Leith, Tay and Tweed



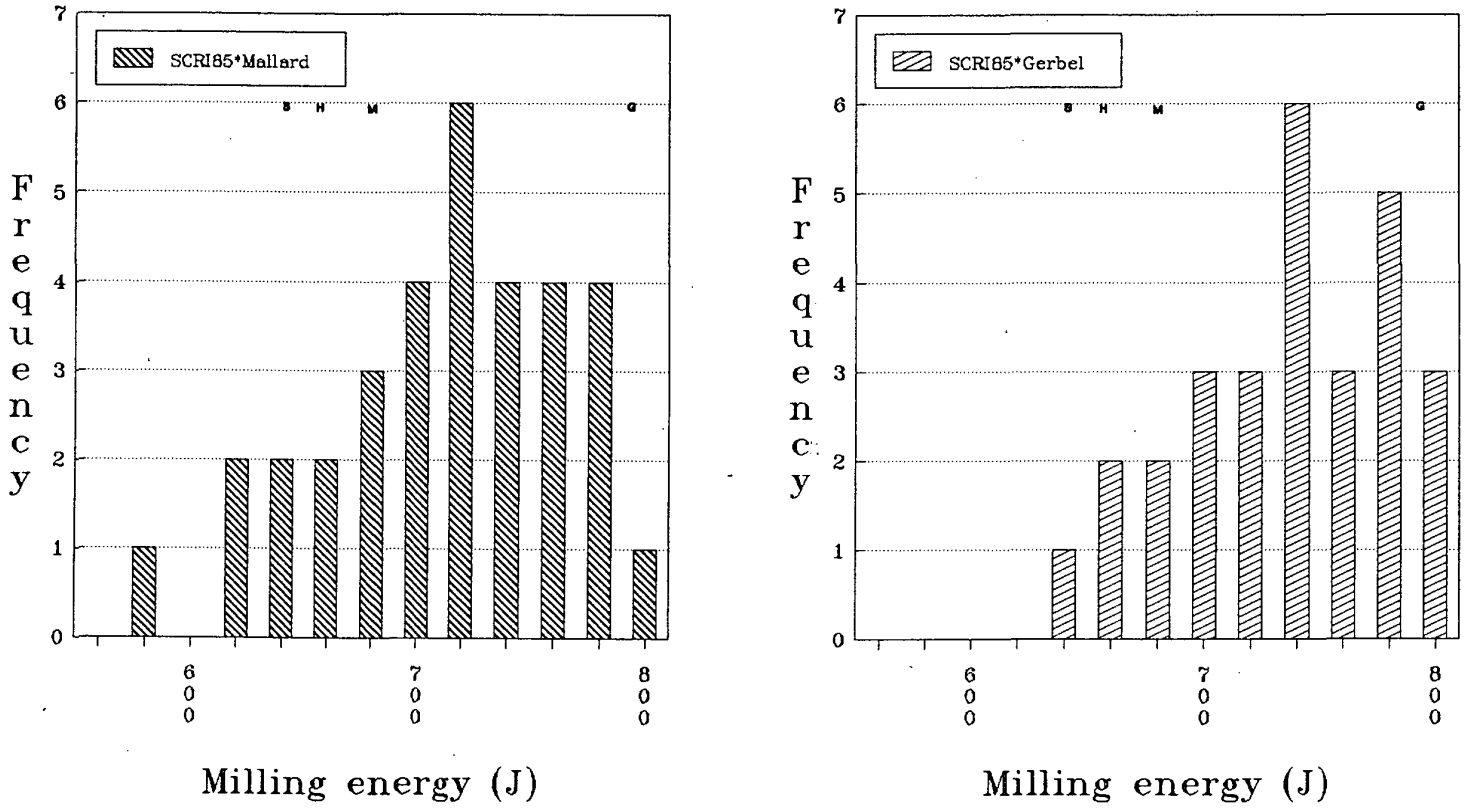


Figure 9. GME of inbred lines from winter*spring barleys.

G = Gerbel, M = Mallard, H = Halcyon, S = Spring parent

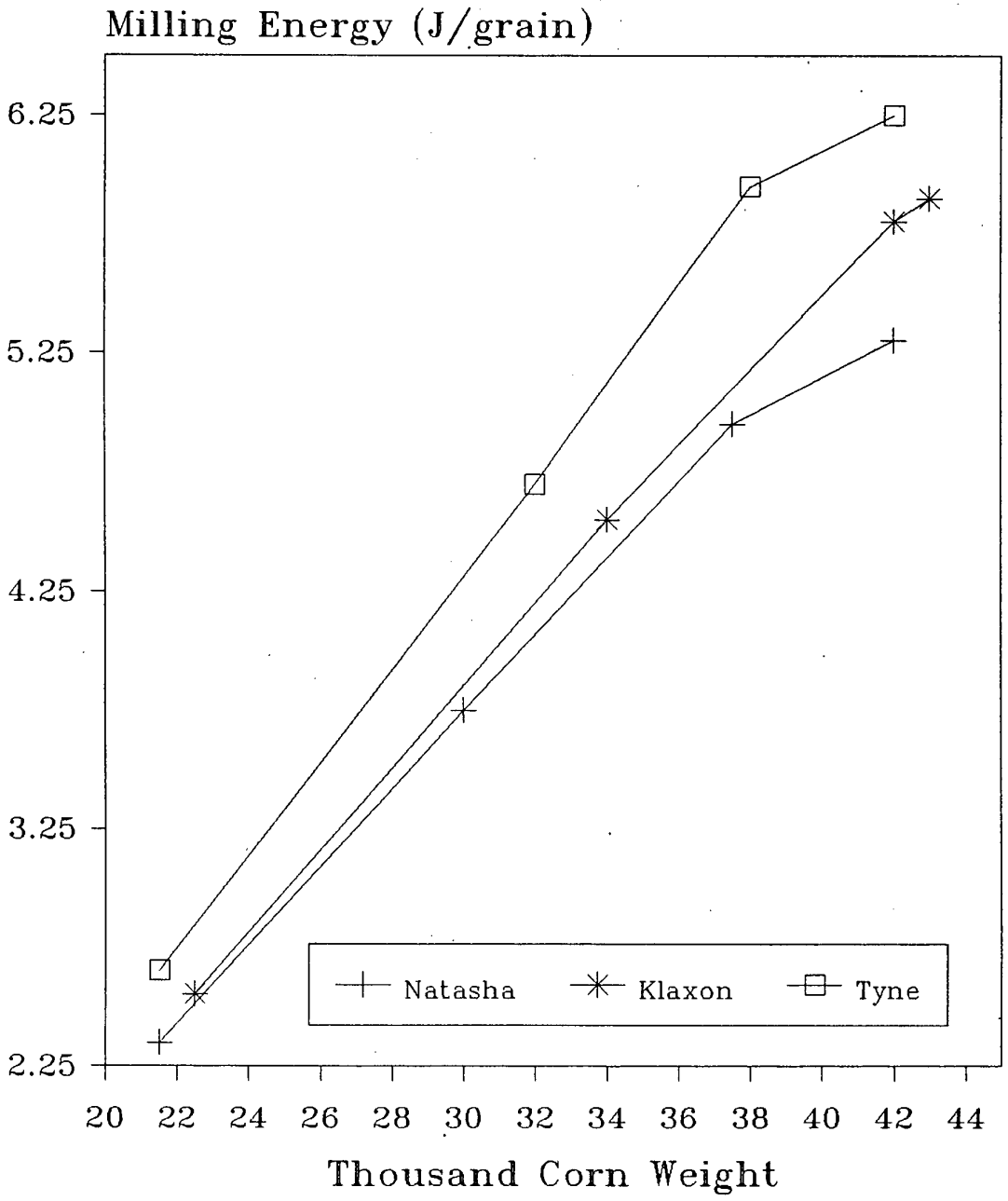


Figure 10. Relationship between milling energy and TCW of grain from VNT87.

2 GENOTYPE X ENVIRONMENT INTERACTION FOR GME

When GME, grain nitrogen and TCW were measured in Klaxon, Natasha and Tyne samples from VNT86 large genotypic differences were found only for GME and TCW (Table 2). Grain nitrogen levels were high, because of late top dressing, and were not significantly different between genotypes. In samples from VNT87 slightly lower grain nitrogen levels were detected but there was still little effect of grain nitrogen on GME. When grain samples were sieved into fractions and the GME per grain of each fraction was plotted against mean TCW a near linear relationship was observed (Figure 10) which was characteristic of each cultivar.

Table 2. GME, grain nitrogen content and TCW for three cultivars from VNT86 and VNT87.

	Nitrogen dressing kg/ha								
	80			100			120		
	GME	N	TCW	GME	N	TCW	GME	N	TCW
VNT86									
Klaxon	681	1.9	42.8	677	2.0	43.2	675	2.1	43.9
Natasha	662	2.1	39.8	662	2.1	38.7	652	2.1	40.2
Tyne	744	2.0	37.6	742	2.0	36.0	738	2.0	37.8
VNT87									
Klaxon	679	1.8	40.0	677	1.9	39.1	682	1.9	39.0
Natasha	647	1.8	35.6	660	1.7	35.1	652	1.8	35.4
Tyne	765	1.7	38.0	769	1.7	38.0	772	1.9	37.3

Analysis of variance revealed highly significant genotypic differences and effects of nitrogen top dressing for GME in VNT88 (Table 3). Higher applications of nitrogen resulted in significant increases in grain nitrogen content ($P<0.001$) (Table 4). The result was a small but significant ($P<0.001$) rise in GME across cultivars of between 20 to 40 J per 0.1%

increase in grain nitrogen content. Hot water extract fell significantly with higher nitrogen content and GME (P<0.001).

Table 3. Analysis of variance for GME in VNT88.

	df	MS
Genotype (G)	8	93307***
Residual(1)	24	1666
Nitrogen (N)	2	5891***
G x N	16	1082
Residual(2)	32	935

*** = $P < 0.001$

Table 4. The mean effects of nitrogen application on GME and HWE for 9 cultivars in VNT88.

	Nitrogen dressing (kg/ha) 80	100	120	se (54 df)
Grain nitrogen %	1.60	1.61	1.65	0.012
GME	654	662	669	4.162
HWE	285	282	278	1.478

The relationships between GME and malting parameters in VNT87 are considered in section 4 "The effects of storage".

In SD88 the effects of genotype, sowing date and fungicide on GME were all highly significant (Table 5). The relationship between good malting cultivars and low GME was also confirmed (Table 6a). Although application of fungicide significantly decreased GME in material sown in September, it had no significant effect in plots sown later (Table 6b). The largest effect of sowing date on GME was in early sown plots unprotected by fungicides.

Table 5. Analysis of variance for grain nitrogen (%N x 10^3), GME and HWE in SD88.

	df	Mean Squares		
		N	GME	HWE
Genotype	8	153***	88505***	2005***
Residual(1)	16	8	680	39
Fungicide	1	2	7245***	302**
Genotype x Fungicide	8	7	2	99**
Residual(2)	18	10	465	26
Sow Date	2	600***	10907***	204***
Genotype x Sowing date	16	22**	769	64***
Fungicide x Sowing date	2	13	3569***	485***
Residual(3)	72	10	692	9

*** = $P < 0.001$
** = P 0.01-0.001

Table 6. The effects of sowing date and fungicide on grain nitrogen (N%DM), GME and HWE in SD88. The genotypes are arranged in descending order of GME.

(a) Cultivar x Fungicide

	N		GME		HWE	
	F-	F+	F-	F+	F-	F+
Heriot	1.73	1.70	635	606	303	297
Maris Otter	1.76	1.72	639	622	300	298
Halcyon	1.69	1.74	639	636	301	297
Golden Promise	1.67	1.70	657	644	289	291
Plaisant	1.73	1.73	691	665	283	286
Torrent	1.80	1.81	750	758	283	285
Gerbel	1.77	1.71	776	751	278	271
Igri	1.87	1.93	766	766	279	268
Vixen	1.94	1.97	807	793	277	276
se (9 df)	0.03		11.24		2.7	

(b) *Sowing dates*

September	1.90	1.89	729	697	285	284
October	1.71	1.71	705	699	287	288
November	1.70	1.72	686	684	293	283
se (27 df)	0.03		6.21		1.42	

(c) *Fungicide*

	1.77	1.78	707	693	288	285
se (81 df)	0.02		3.36		0.80	

(d) Cultivar characteristics

	Malting quality	Habit
Heriot	Good	Spring
Maris Otter	Good	Winter
Halcyon	Good	Winter
Golden Promise	Medium	Spring
Plaisant	Medium	Winter
Torrent	Poor	Winter
Gerbel	Poor	Winter
Igri	Poor	Winter
Vixen	Poor	Winter

F- No fungicide
F+ Fungicide applied

3 ABRASION

ME was reduced significantly by abrasion of all the cultivars (Table 7) from which Leith, Tay and Tweed were derived (Figure 8d).

Table 7. Mean TCW, nitrogen and ME of barley from LP to show the effect of abrasion.

	Grain	Dehusked	Pearled	Inner
TCW	43.09	40.38	36.8	32.46
ME	608.6	568.6	533.3	512.1

In all cultivars the **husk** and **aleurone** tissues were much more resistant to milling than the **subaleurone** or **inner** endosperm tissues (Figure 11). This explains why the contribution of the **husk** and **aleurone** tissues to GME (Figure 12), was greater than would be predicted from their proportion of the grain by weight. Even in this small sample it was possible to observe genetic variation which resulted in differences in the ranking of the cultivars. For example Akka had the husk with the greatest milling resistance combined with average endosperm ME. Tay showed the softest endosperm with a husk of average milling requirement.

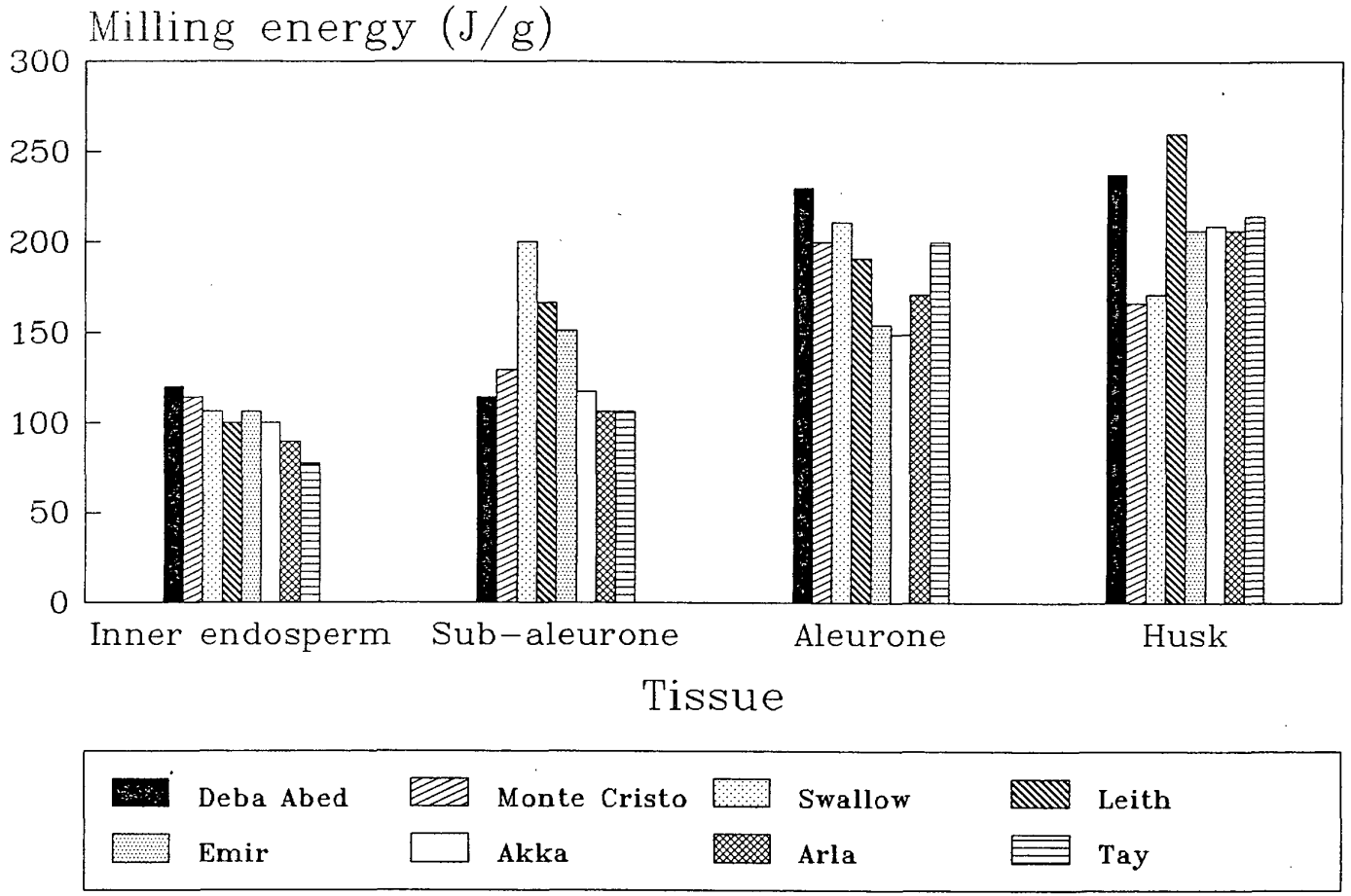


Figure 11. Milling energy of per gram of tissue in cultivars selected from LP.

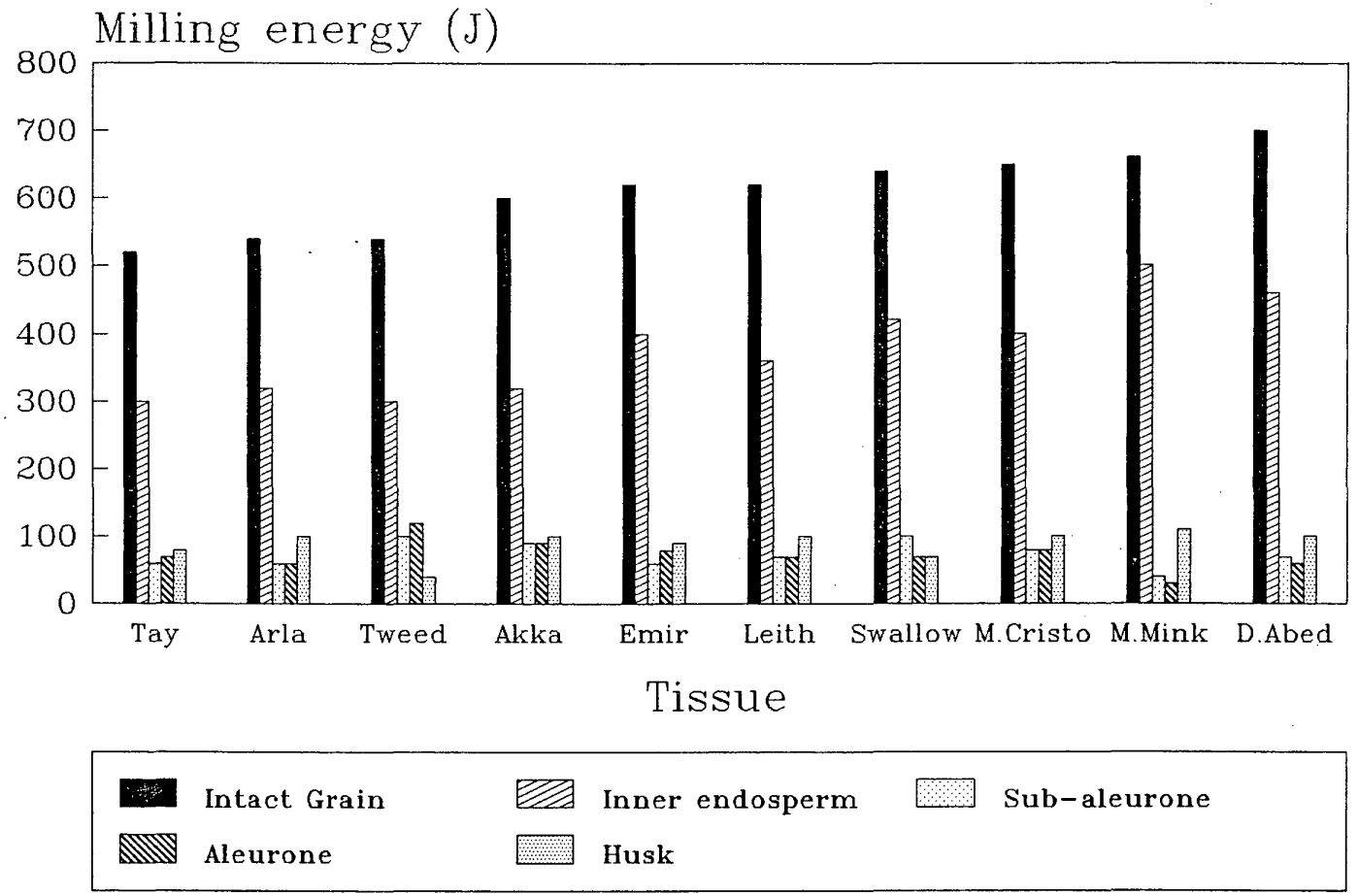


Figure 12. Milling energy per tissue from cultivars selected from LP.

Figure 12 also reveals how the contribution of husk and starchy endosperm to GME can vary between cultivars and that the influence of the external tissues was most pronounced in the softer cultivars (where <70% of ME could be attributed to the starchy endosperm). Consequently whilst the contribution of the starchy endosperm is the largest component of GME, the external tissues are also significant.

In order to confirm these findings and assess how these factors could be influenced by environmental factors, a second abrasion experiment was set up with replicated samples from VNT87.

Table 8. Analysis of variance for ME of grain from VNT87 after abrasion treatments.

Source	df	MS
Genotype	3	92084***
Residual 1	3	607
Nitrogen	1	85
Genotype x Nitrogen	3	727
Residual 2	4	553
Abrasion	3	35173***
Genotype x Abrasion	9	754**
Nitrogen x Abrasion	3	103
Residual 3	9	215

df = Degrees of freedom
MS = Mean Square
*** = P<0.001
** = P 0.01-0.001

There were significant differences in ME due to the effects of genotype and abrasion (Table 8) and there was also significant genotype by abrasion interaction. This is explicable because Tyne had the hardest and smallest grain but Doublet had the grain with the husk with the greatest resistance to milling (Figure 14). There were not, however, as would be expected, any effects arising from the application of different levels of nitrogen dressings.

Because the husk and aleurone tissues are more resistant to milling, loss of these tissues by abrasion caused a significant fall in GME ($P<0.001$) in all four cultivars from VNT87. However, the large proportion of starchy endosperm in the grain (Figure 13) accounted for some 72% of total GME. As a result the different GME between Doublet and Tyne can be attributed to differences in the composition or structural integrity of the starchy endosperm. Only in contrasts, such as that between Natasha and Klaxon, where the ME of the inner endosperm is similar, does the aleurone and husk contribute significantly to genotypic differences in GME.

Figure 14 indicates the milling energy (ME) that is required to mill one gram of each tissue for four contrasting genotypes from VNT87. The findings of the LP survey were confirmed in that that the inner endosperm and subaleurone tissues are of very similar "hardness" despite the higher protein content of the latter and that both have significantly ($P<0.05$) lower ME than the aleurone and husk.

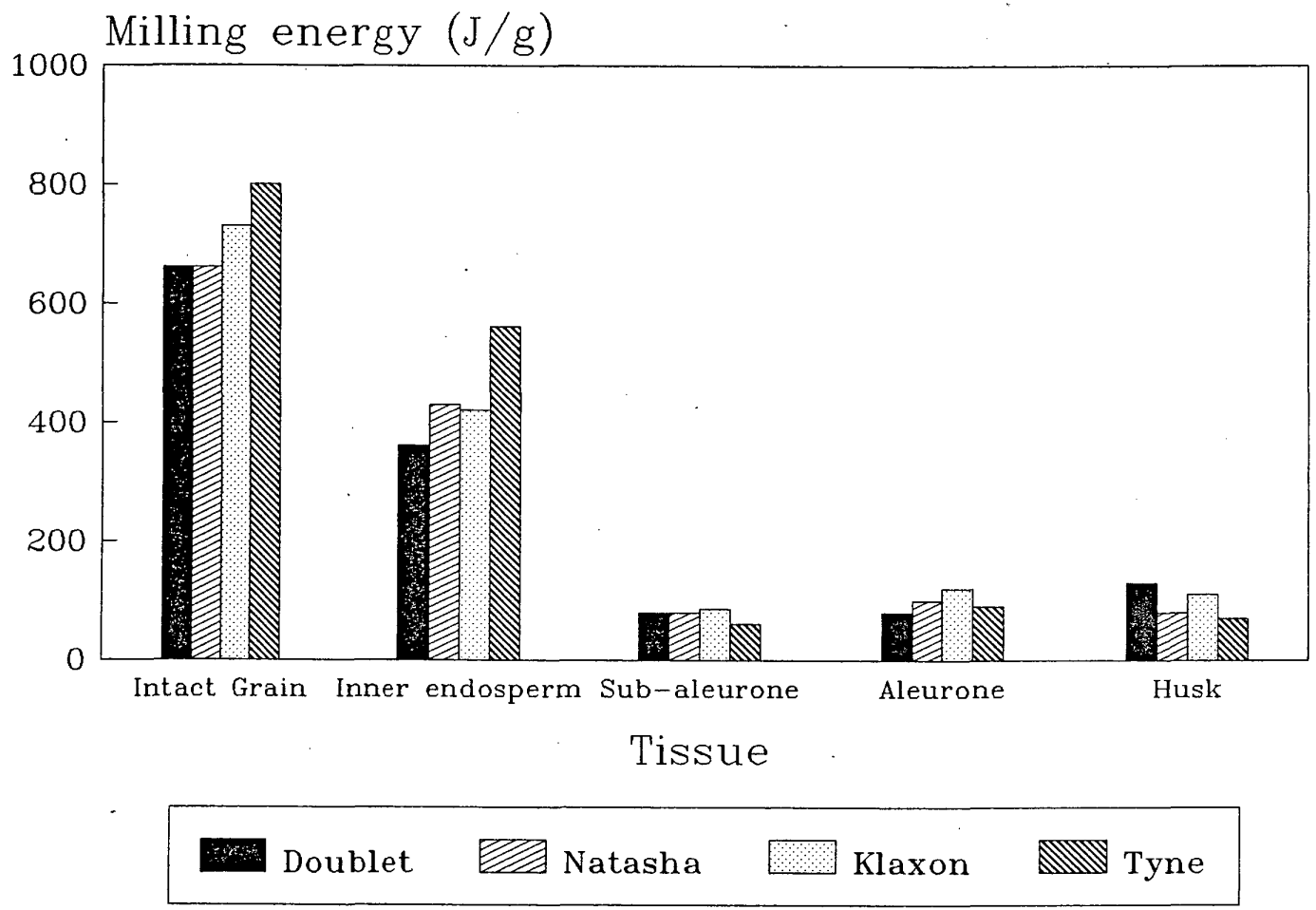


Figure 13. Milling energy per tissue from cultivars grown in VNT87.

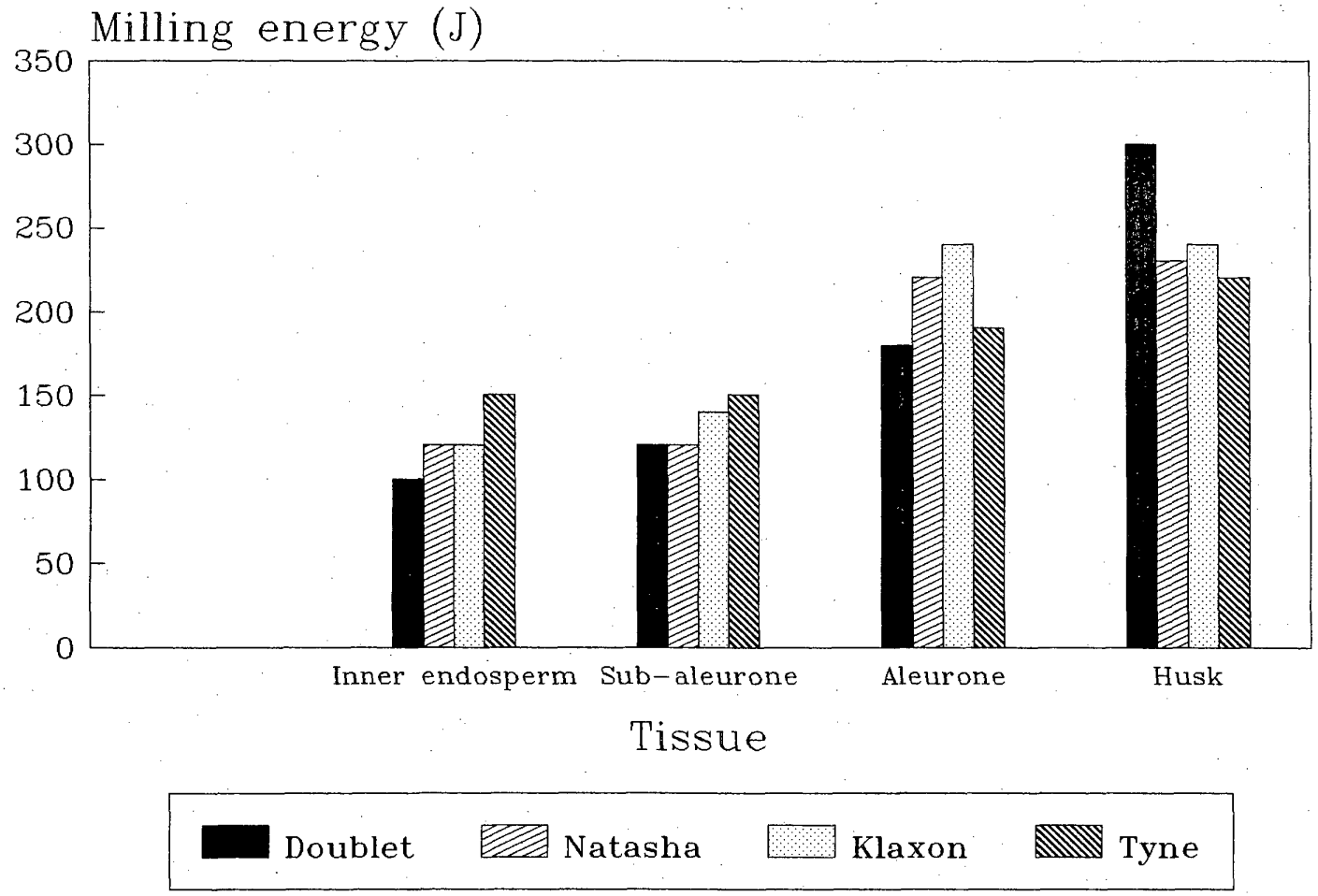


Figure 14. Milling energy per gram of tissue from cultivars grown in VNT87.

Samples from VNT87 showed significant genetical differences for TCW, grain nitrogen and milling energy (Table 9). TCW and grain nitrogen, but not milling energy, showed significant effects of nitrogen top dressing and TCW showed an interaction between genotype and nitrogen dressing. In contrast to those characters only genetical differences were detectable for Germinative Energy (GE), Germinative Capacity (GC) and Water Sensitivity (WS) [see methods section for definitions] as these characters were unaffected by nitrogen top-dressing level (Table 10).

Analysis of variance for water uptake, malting loss and hot water extract (Table 11) over three dates showed significant genotypic and date effects and also genotype by date interaction.

Table 9. Grain characters from VNT87. Analysis of variance for samples before sieving to obtain grain fractions.

Source	df	Mean Squares		
		TCW	Grain nitrogen	Milling energy
Genotype	7	62***	0.026**	36061***
Residual(1)	21	6	0.007	401
Nitrogen	2	5***	0.065***	383
Genotype x Nitrogen	14	0.4**	0.004	274
Residual(2)	48	0.5	0.005	237

Table 10. Germinative energy, germinative capacity and water sensitivity measured in December on VNT87.

Source	df	Mean Squares		
		Germinative energy	Germinative capacity	Water sensitivity
Genotype	7	314***	58***	2610**
Residual(1)	21	37	4	649
Nitrogen	2	21	1	34
Genotype x Nitrogen	14	16	1	184
Residual(2)	48	19	7	340

Table 11. Analysis of variance for water uptake, malting loss and hot water extract from VNT87.

Source	df	Mean Squares		
		Water uptake	Malting loss	Hot water extract
Genotype	7	10.0***	6.3**	522.5***
Residual(1)	21	0.5	1.2	22.9
Nitrogen	2	0.7	1.5	103.9*
Genotype x Nitrogen	14	0.9*	0.7	73.9**
Residual(2)	48	0.4	0.9	31.1
Date	2	5.2***	172.8***	7859.9***
Nitrogen x Date	4	1.8***	0.6	269.6***
Genotype x Date	14	4.6***	5.4***	149.8***
Residual(3)	96	0.2	0.6	23.3

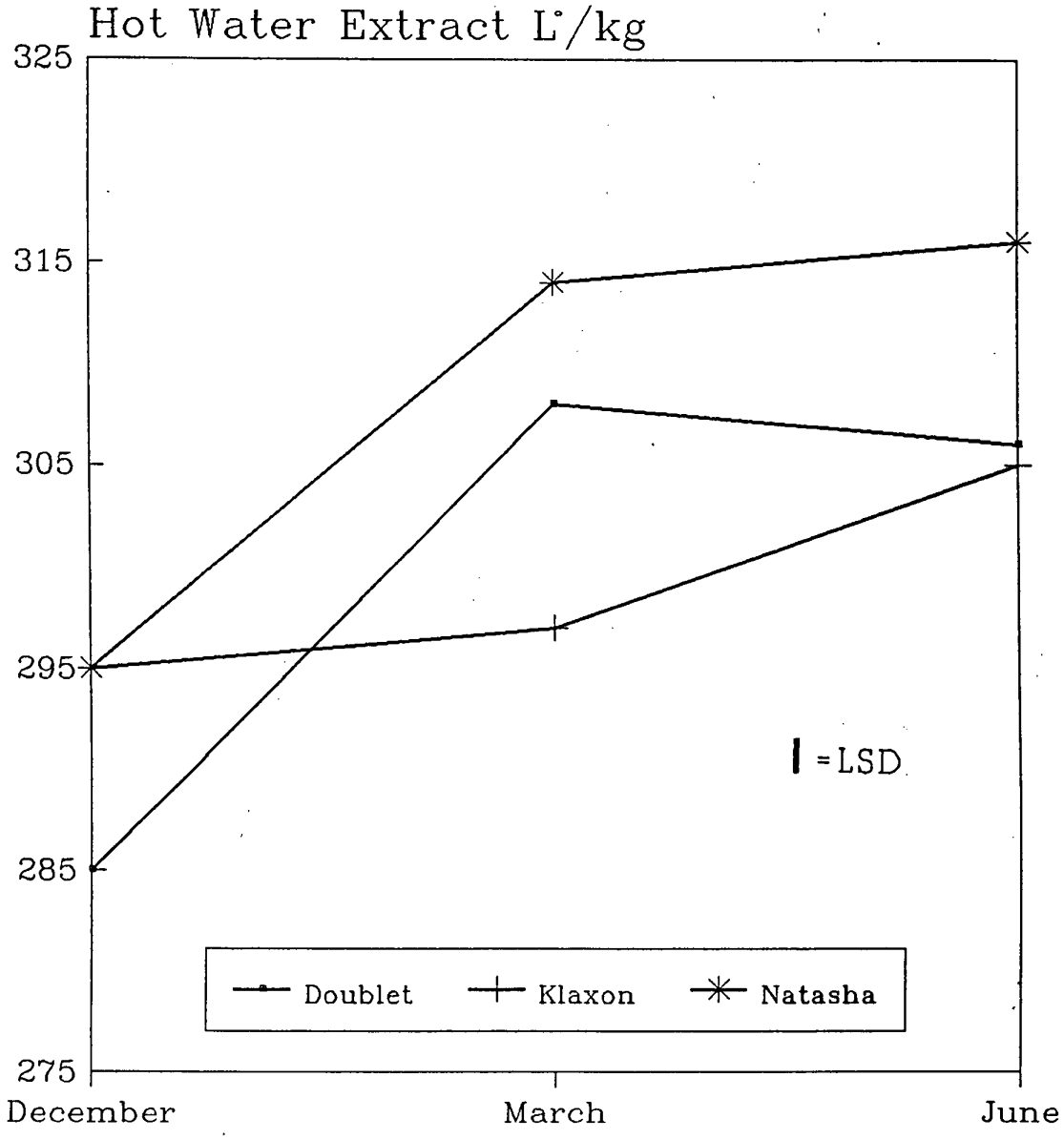


Figure 15. Changes in hot water extract after storage of samples from VNT87.

Malt made from VNT87 grain samples stored between September and June showed a significant increase in HWE (Figure 15), with Doublet exhibiting the greatest change.

The correlations between HWE and other characters (Table 12) indicated a significant relationship between HWE in malt made in December and germinative energy. The relatively poor extract seen in the December malting therefore appears to result from dormancy, particularly in Doublet. However, there was no significant correlation between HWE and germinative energy in the March or June maltings. This indicates that a reduction in dormancy could not be responsible for the increase in HWE between these dates.

The significant negative correlation between HWE in March and TCW of grain from the middle sieving fractions indicates that, after dormancy has broken, grain size can have a limiting effect on HWE, because large grains modify more slowly.

The relationship of grain size to hot water extract (Table 12b) in June was not so close, suggesting that further grain maturation had taken place and facilitated grain modification. This study indicated that barley grown in Scotland produces dormant grain whose extract improved further after recovery from post-harvest dormancy and that selection for malting quality in a breeding programme should be based on at least two assessments of HWE.

Milling energy was monitored throughout the storage process. A catastrophic accident, when the circlip retaining the hammers disintegrated, necessitated an overhaul of the mill. As a result GME values for controls (Figure 1, August 1988-September 1988) were some 100 J lower. GME values were adjusted to allow for this base line change by calculating a regression and correcting for the average change with time. The storage experiment was repeated in a second season with seed from VNT88.

Table 12. Correlations between HWE, determined on malts made from VNT87 samples, in December, March and June and other characters. TCW, grain nitrogen and GME were measured on grain retained by a 2.5mm sieve.

(a) Grain characters

Date of malting	TCW	Grain nitrogen	GME
December	-0.03	0.27	0.22
March	-0.58**	-0.29	-0.57**
June	-0.38*	-0.31	0.48**

(b) TCW of sieve fractions

Date of malting	%<2.25 mm	%2.25-2.50mm	2.50-2.75mm	>2.75 mm
December	-0.0	0.0	-0.2	0.0
March	-0.3	-0.6**	-0.6**	-0.2**
June	-0.2	-0.4*	0.4*	0.0

(c) Germination

	Germinative energy	Germinative capacity	Water sensit.
December	0.49**	0.43*	0.11
March	-0.06	-0.06	0.02
June	0.17	0.13	0.04

* p<0.5, ** p<0.01, *** p<0.001

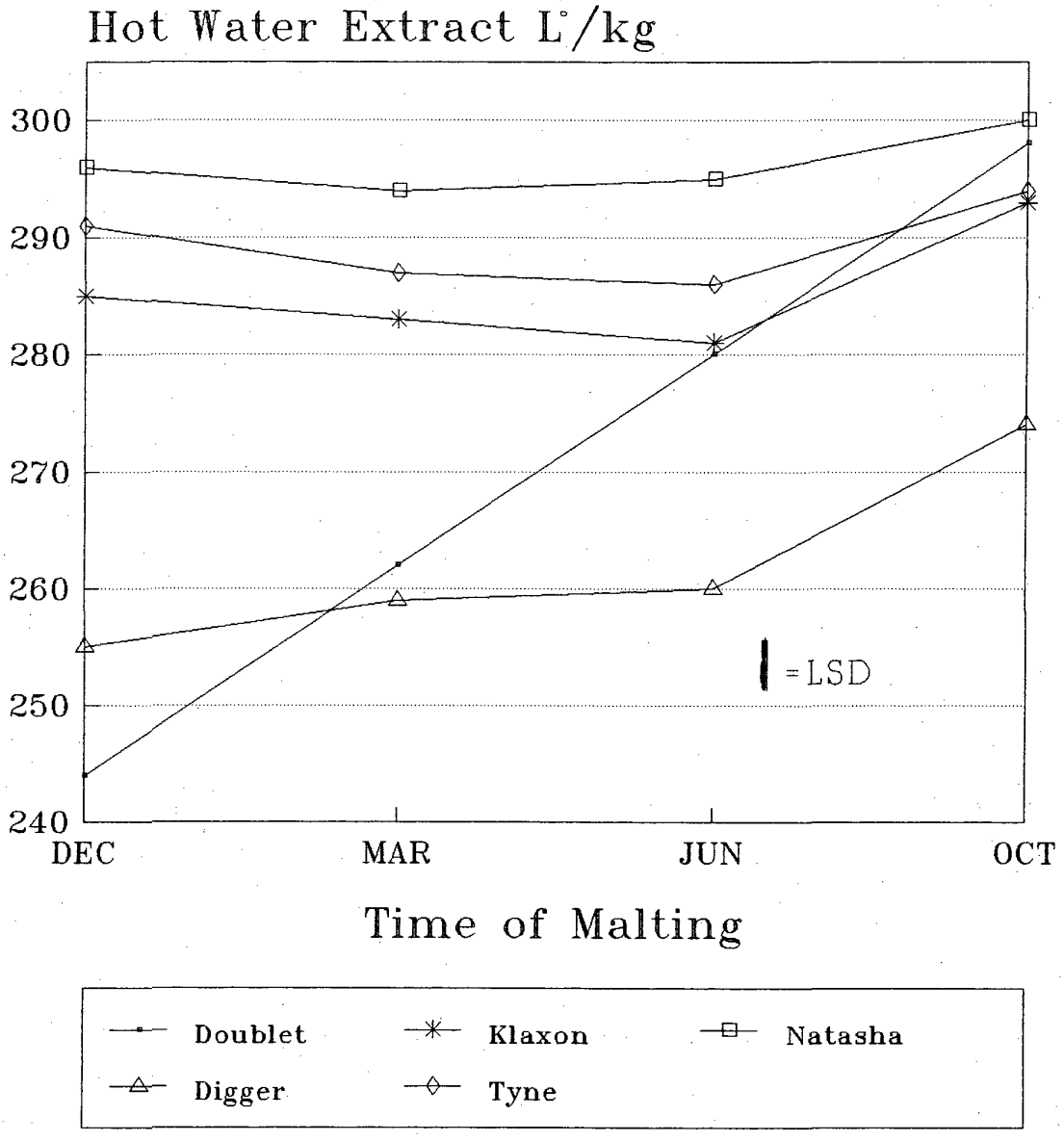


Figure 16. Changes in HWE with storage of cultivars from VNT88

Table 13. Analysis of variance for GME and related characters in VNT88, with correction for mill wear, analysed in December 1988, March 1989, June 1989 and October 1989.

Source	df	Mean squares			
		N($1x10^3$)	GME	HWE	MME
Genotype	7	67**	33425***	7614***	37638***
Residual (1)	21	13	967	104	2519
Nitrogen level	2	161***	7928***	1358***	20993***
Genotype x Nitrogen	14	6	465	24	1760
Residual (2)	48	11	679	93	1953
Date	3	127***	30670***	4197***	29257***
Genotype x Date	21	11**	30572***	654***	10995***
Nitrogen x Date	6	25***	239	16	775
Residual(3)	216	5	559	29	216

Samples from VNT88 (Figure 16) also showed variation in hot water extracts in maltings between December and October. All cultivars showed a tendency to an increase in HWE between December and October, even Digger. However, the most spectacular change was seen in Doublet which, while recovering more slowly than in VNT87, changed from the worst to the second best extract performance.

5 ENDOSPERM COMPOSITION AND CHANGES DURING MALTING

Scanning electron and fluorescence microscopy failed to reveal marked differences in the structure of unmalted grain between cultivars of widely differing malting quality. However, the distribution of protein, revealed by ANS, indicated that not only were protein deposits heaviest in the subaleurone zone, but also that this "band" of high protein was deepest in the ventral cheek areas and distally where the dorsal and ventral subaleurone zones merge. The loss of ANS fluorescence during malting revealed that the degradation of protein in the subaleurone layer was more rapid in the dorsal and lateral regions than ventral. This confirmed SEM observations which showed that the ventral cheeks and the tip of the grain

distal from the embryo contained the largest residues of poorly modified protein.

Triumph, Golden Promise and Koru were micromalted (Taylor & Swanston, 1987) under identical conditions for seven days and samples taken throughout were examined by fluorescence microscopy, SEM and were milled. Fluorescence microscopy of sections stained with Calcofluor indicated that cell wall degradation proceeded from the aleurone to the subaleurone. At the completion of malting very little fluorescence was visible. Similar sections stained with congo red showed greater fluorescence from the starch granules as the surrounding protein matrix was degraded.

Scanning electron microscopy indicated that there was considerable variation between cultivars, and also within grains, for the rate of degradation of starch, protein and cell walls. In Triumph much of the protein matrix was degraded within the first day of malting (Figure 17 a,b) and starch granules showed the initiation of degradation (Figure 17 c). By the third day of malting starch damage was visible in Triumph and Koru adjacent to the dorsal region of the scutellum (Figure 18). The area of modification was restricted and, even in Triumph (Figure 18a), there was still enough protein to bind starch granules together in the endosperm near the ventral part of the scutellum. A similar condition was achieved in Koru at the seventh day of malting in the dorsal region (Figure 19d). Triumph showed extensive protein and starch degradation at day seven but in Golden Promise it was still possible to find starch granules closely associated with protein (Figure 19a). It is interesting to compare the fibrillar protein associated with the large starch granules in Fig 19c with the morphology of starch granules extracted from the intact endosperm (Figure 7). At a point approximately 0.5mm from the tip of the grain there was comparatively little degradation of the protein matrix and apparently none of the starch (Figure 20). When kilned malt was examined the surface of the starch granules appeared to be smooth (Figure 21).

The percentage loss of MME was plotted against the modification of cell walls, revealed by Calcofluor fluorescence (Figure 22). This showed that by day two, cell wall modification had progressed further in Triumph,

Triumph, than in Golden Promise or Koru and this was associated with a greater fall in MME. However after day two, the MME of Triumph fell more slowly than in Golden Promise or Koru, despite maintaining a rapid loss of Calcofluor fluorescence. Consequently when malting was terminated at day seven, the difference in the percentage loss of MME between Triumph and Koru had been reduced.

See following pages for Figures.

Figure 17. Changes, near the scutellum, in grain during the first day of malting revealed by scanning electron microscopy (SEM). Scale bar = 10μ.

Triumph ventral(a) and dorsal (b), surface of starch granules which show the initiation of enzyme attack (c).
Koru dorsal (e).
Golden Promise dorsal (d).

(a) sgs = small starch granules still embedded in protein
(b,c) cw = cell wall material
(d) p = protein surrounding large starch granules
(e) pg = starch granule pitted by enzyme degradation
cw = cell wall material

{a d}
{b }
{c e}

Figure 18. Changes, near the scutellum, by the third day of malting revealed by scanning electron microscopy (SEM).
Triumph (a)ventral and (b)dorsal; Koru dorsal (c). Scale bar = 10μ

{a}
{b}
{c}

Figure 19. Changes, near the scutellum, in grain by the seventh day of malting revealed by scanning electron microscopy (SEM). Scale bar = 10μ

Triumph dorsal (a); Golden Promise dorsal (b) and ventral (c); Koru dorsal (d) and ventral (e).

(c) pf = protein fibrils associated with the equatorial grooves of large starch granules
(d) sg = small starch granules still adhering to large starch granules

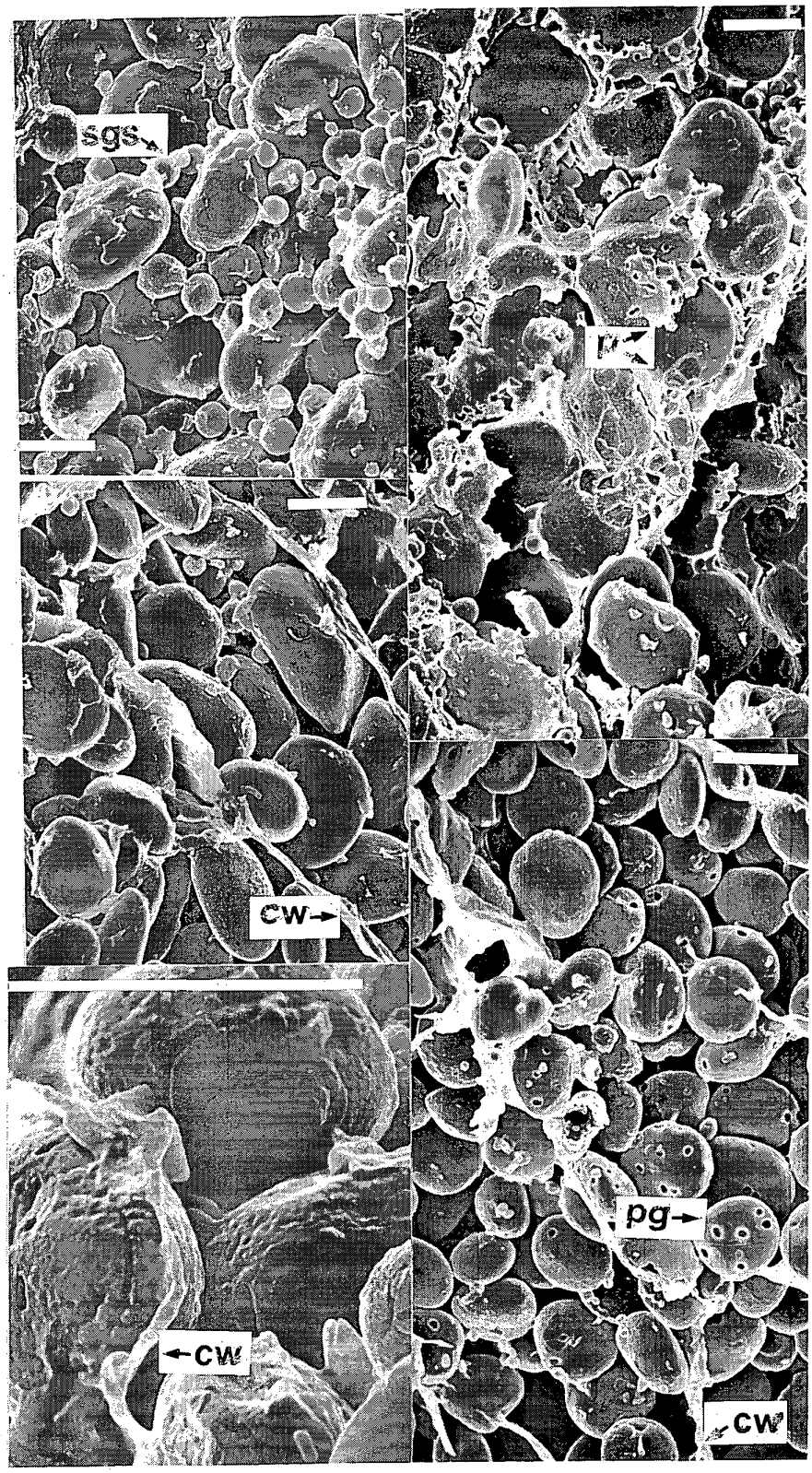
{ a }
{b c}
{d e}

PTO

Figure 20. Changes in the tip of the grain during malting revealed by scanning electron microscopy (SEM).
Triumph (a) day 1 Scale bar = 100μ
Triumph (b) day 7 Scale bar = 10μ
After one day of malting the tip of the grain shows little degradation of protein but at the end of malting a reduction of the matrix is visible.

{a}
{b}

Figure 21. Changes in the grain during malting revealed by scanning electron microscopy (SEM). Scale bar = 10μ.
Triumph malt with angular starch granules, formed by contact during grain development.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cw
cw
pg
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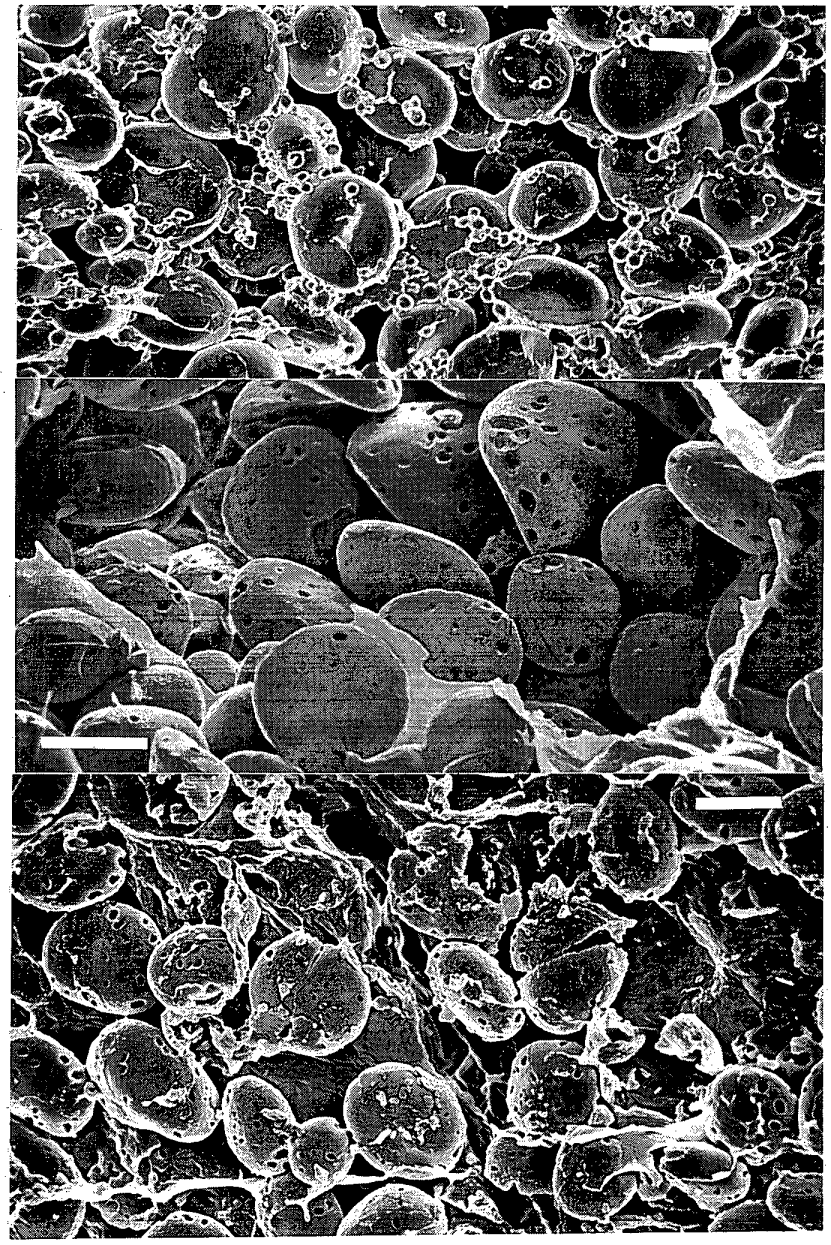

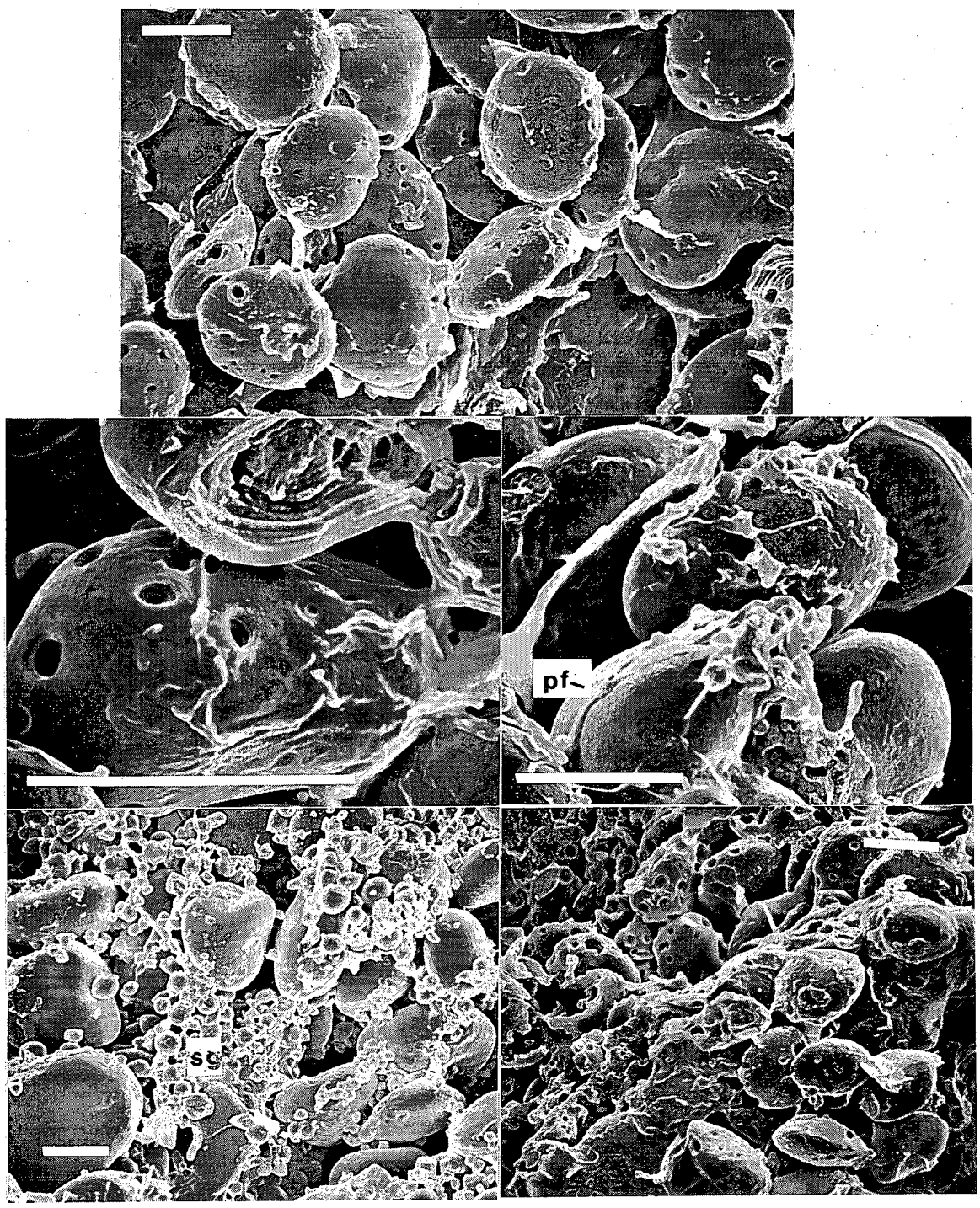
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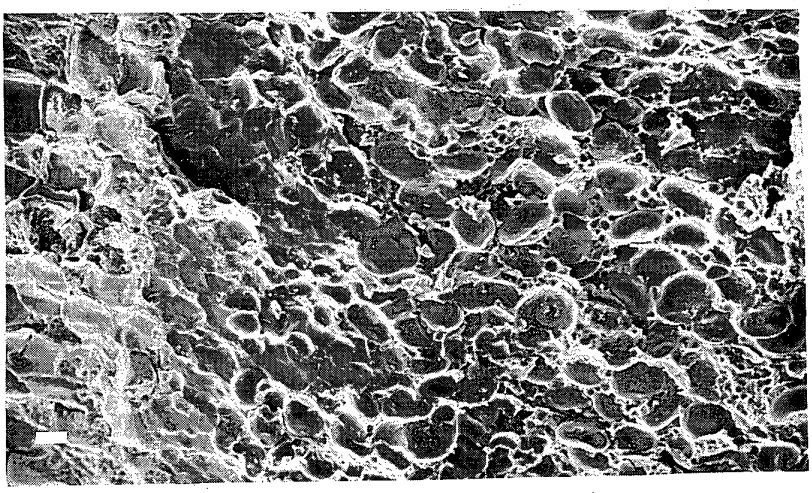

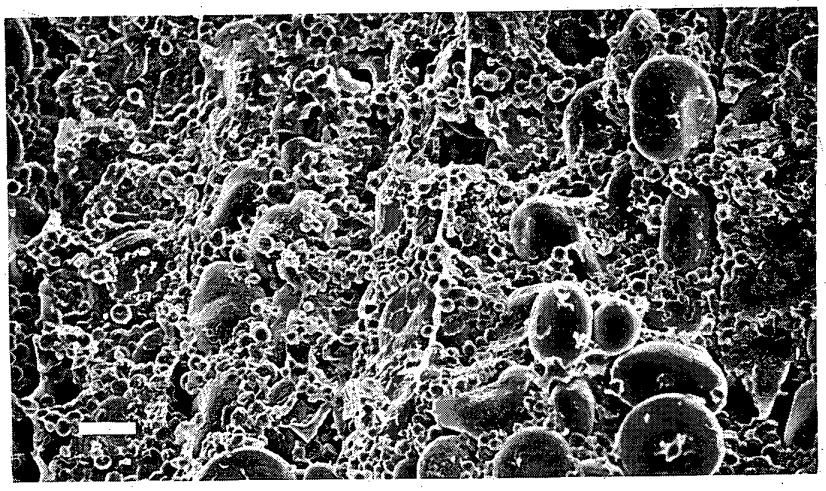

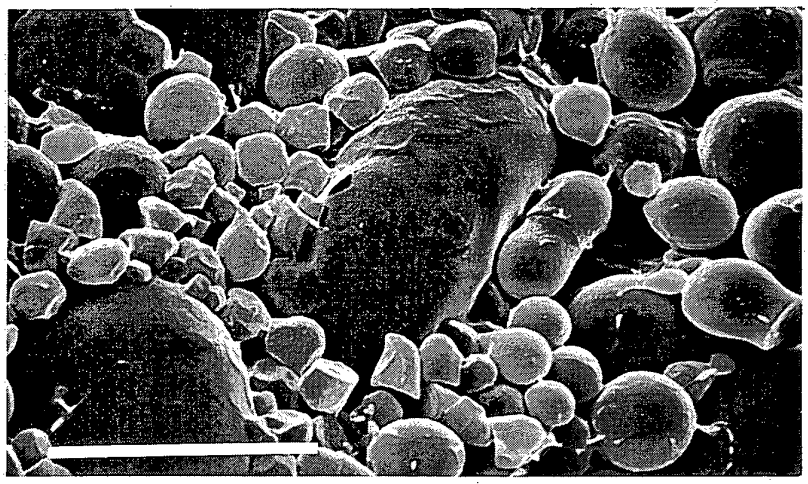

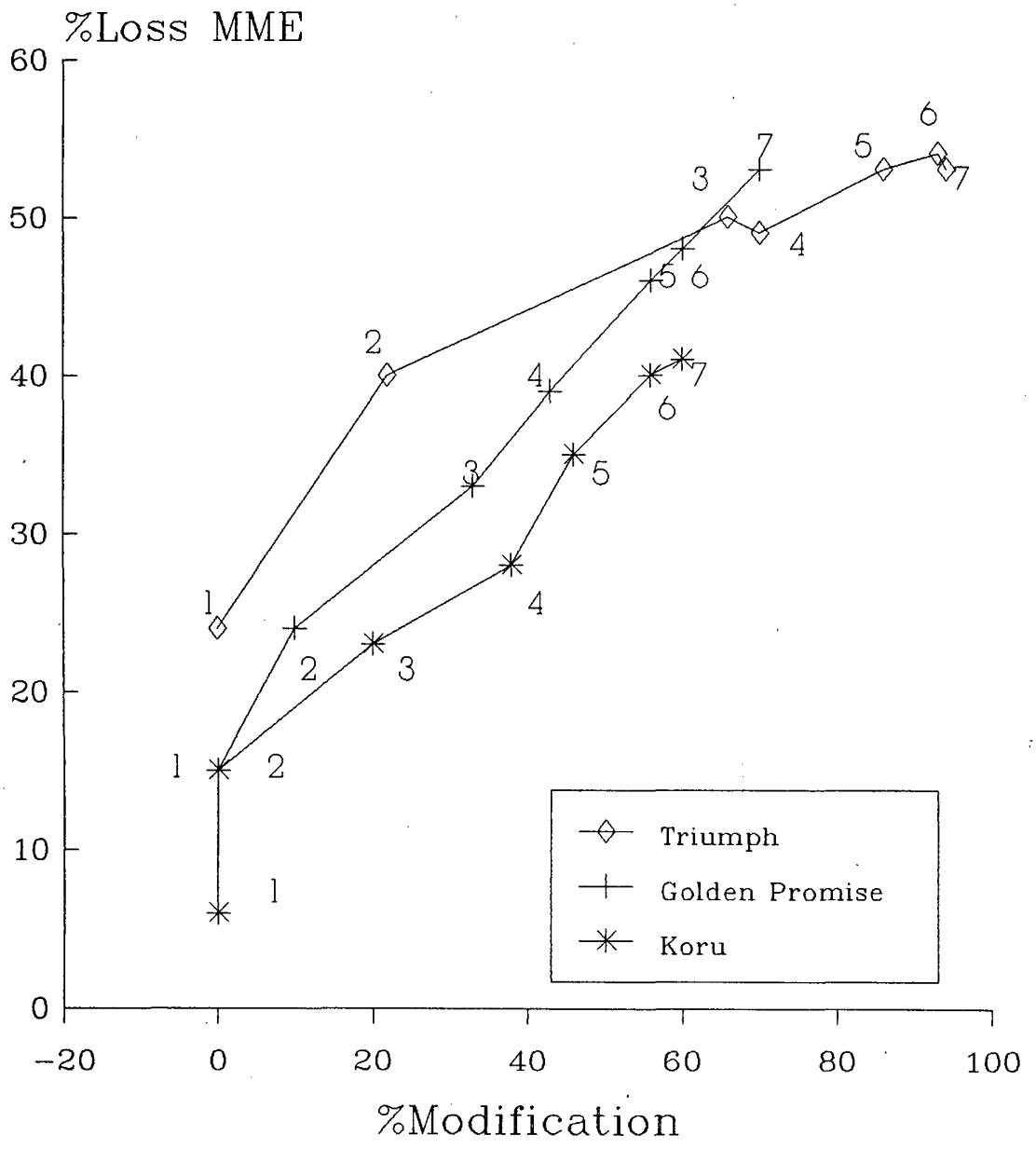

Figure 22. Loss of MME related to cell wall modification for 1–7 days of malting.

GME was significantly correlated with coarse and fine extracts (Table 14), but GME only accounted for about 25% of the variation. This indicates that although the physical structure of the endosperm is a significant determinant of extract, its biochemical composition and modification by enzymes are more important. GME was not correlated with grain nitrogen in this trial but was significantly, negatively, correlated with the ratio of soluble to insoluble nitrogen and Calcofluor fluorescence i.e. the degree of cell wall degradation ($P<0.001$). GME was also significantly correlated with the fine/coarse extract difference i.e. the extent to which large particles from the coarse grind are modified. These correlations suggests that the structure of the endosperm is important in determining the ease with which the protein and cell walls can be modified. MME showed a higher level of correlation than GME for the same parameters. In addition MME showed negative correlations with malt enzyme activity i.e. diastatic power and dextrinising units. The highest correlation was between MME and coarse extract indicating the effectiveness of this parameter for the description of the level of endosperm degradation.

Table 14. Correlations between milling energy and grain and malt characters.

		GME	MME
Grain texture	MME	0.53***	
Grain nitrogen		0.00	0.08
Malt nitrogen	Total	0.03	0.17
	Soluble	-0.40**	-0.71***
	SNR	-0.49***	-0.88***
Malt modification	ß-Glucan	-0.54***	-0.86***
Malt extract	Fine	-0.50***	-0.70***
	Coarse	-0.51***	-0.92***
	F/C diff	0.38**	0.89***
Wort properties	PSY	-0.44**	-0.87***
	DP	-0.26	-0.66***
	DU	-0.28	-0.71***

** = P < 0.01
*** = P < 0.001

SNR = Soluble Nitrogen Ratio
F/C diff = Fine/Coarse extract difference.
ß-glucan = Malt modification of cell wall ß-D-glucans assessed by fluorescence of Calcofluor.
PSY = Predicted Spirit Yield
DP = Diastatic Power
DU = Dextrinising Units

Constant Environment Experiment

In all four genotypes the largest grain size was achieved at 10°C. When the variation in grain size was taken into account it was obvious the GME was not affected by the temperature of growth and so not by any of the changes in starch composition reported by Tester *et al.* (1991).

Cultivar survey

Starch granule proteins were resolved by electrophoresis into 27 bands representing molecular weights from 12-45 kDa. Initial results using a single purification through CsCl on both spring and winter cultivars indicated that whilst some protein bands were common to different cultivars, genotypic differences were evident in the pattern of starch granule surface proteins molecular weights between 24-45 kDa where most banding occurred (Figure 23). Two bands were also present at about 20 - 14kDa i.e. on either side of the position occupied by the "friabilin" band in wheat patterns. However, no variation in these bands were found even in cultivars of widely differing GME.

Further experiments with centrifugation through CsCl revealed that an additional passage through CsCl resulted in appreciable variation in the low molecular weight protein bands (LMW) and this procedure was therefore adopted for the examination of RIL (Figure 24).

Genetic studies

Starch granule size was then measured in two spring cultivars, Doublet and Tyne, to examine any association with GME. This confirmed that Doublet, (GME 598J SE 10.2) had A-granules with a greater mean volume (1589 μm^3 SE 27) than Tyne (1458 μm^3 SE 39) which had a GME of 714J (SE 10.2). A more thorough analysis was therefore carried out on RIL.

The parents and progeny from RIL were classified according to the presence or absence of each electrophoretic band and the mean GME for each group was then calculated. This revealed (Table 15) that the group possessing bands of protein designated as having apparent molecular weights of 45 b and d and 12 had significantly higher GME than those without, whilst the group possessing bands 36c, and 36h exhibited lower GME. E224 had significantly higher GME (682) than Blenheim (665) and

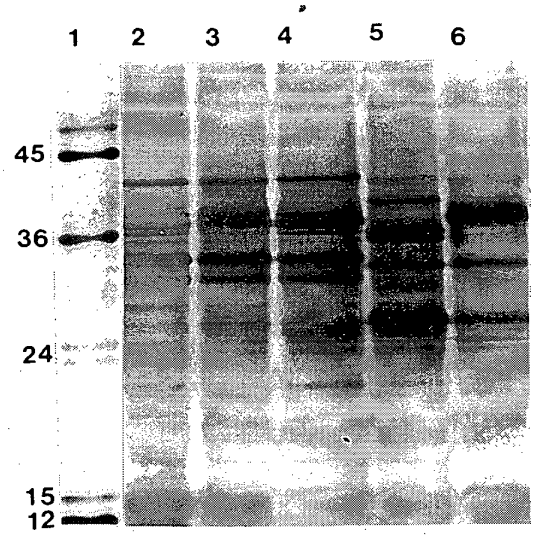

Figure 23. Variation in starch granule surface proteins of spring and winter barley cultivars. Proteins were separated on a polyacrylamide gradient gel (10-20%) and assessed relative to molecular weight markers.
1 Molecular weight markers, 2 Maris Otter, 3 Heriot, 4 Klaxon, 5 Tyne, 6 Gerbel.

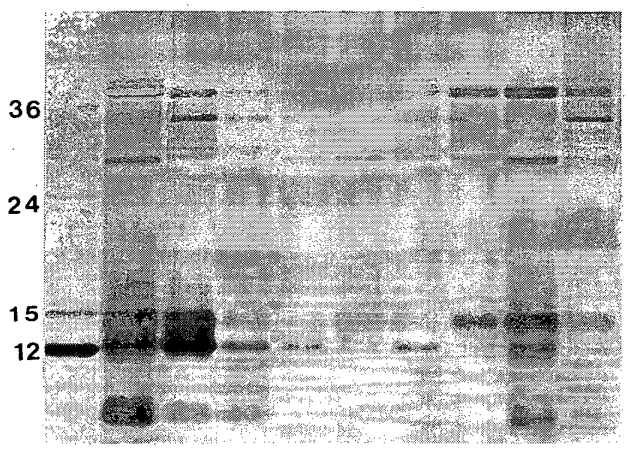

Figure 24. Variation in starch granule surface proteins of inbred lines from the cross Blenheim * E224.

the mean GME of the progeny generated from double haploids was intermediate between the parents.

Relationships between starch granule size, starch surface proteins, GME and HWE

The identification of starch granule surface proteins that showed potential as genetic markers for GME was followed by an examination of starch granule size. Starch granule surface proteins and granule sizes were also compared with HWE. Table 16 indicates that GME was significantly correlated with the surface area of the B-granules (Bsa) P 0.5-0.1, HWE ($P<0.01$), grain nitrogen ($P<0.001$) and TCW ($P=0.05$). Mean starch granule size, either of A (Asa) or B granules (Bsa), was not correlated with HWE but there was a significant correlation between HWE and the modal surface area of the B granules. Data given in this table also confirm the established relationship between HWE and nitrogen content.

Genotypes were classified according to the presence and absence of each starch granule surface protein band and malting quality parameters were recalculated for each group so formed. This revealed that proteins were significantly associated with a reduction in HWE whilst three were found in genotypes with higher HWE (Table 17). Three of these bands (45b,d & 36c) were the same as previously identified as associated with GME, and, where the presence of these bands was associated with an increase in GME (45b & d) these genotypes also showed a reduction in HWE. Conversely, 36c which was associated with a reduction in GME had genotypes with increased HWE.

Table 17 indicates that seven protein bands show significant associations with the surface areas of B-granules. However, only 45b & d were common to those affecting GME and HWE and these were both associated with an increase in B-granule size when present. Bands 24b and c also appeared to be associated with increasing B-granule size but 45 e, h & i were found in genotypes with smaller granules.

Table 15. Mean grain milling energy of groups derived by classifying RIL genotypes according to the presence or absence of starch granule proteins.

Band	Absent x	Absent se	Present x	Present se	P
45a	678	(4.5)	689	(11.0)	0.323
b	669	(5.1)	693	(6.5)	0.004**
c	676	(4.6)	693	(9.5)	0.110
d	676	(4.4)	700	(11.1)	0.034*
e	679	(4.2)	684	(28.6)	0.778
f	678	(6.5)	680	(5.3)	0.787
g	681	(7.6)	679	(5.0)	0.820
h	679	(5.0)	680	(7.0)	0.966
i	682	(5.4)	672	(11.8)	0.325
36a	681	(4.3)	659	(16.5)	0.140
b	679	(15.6)	679	(4.6)	0.985
c	684	(4.2)	651	(12.7)	0.004**
d	672	(13.6)	681	(4.4)	0.465
e	688	(14.6)	678	(4.2)	0.340
f	679	(6.6)	680	(5.4)	0.947
g	691	(13.6)	678	(4.1)	0.267
h	691	(4.9)	658	(6.2)	<0.001***
i	680	(4.4)	674	(15.2)	0.705
j	680	(4.3)	661	(19.6)	0.364
24a	680	(4.3)	656	(17.6)	0.245
b	680	(5.1)	679	(6.9)	0.960
c	679	(5.1)	679	(6.9)	0.960
d	678	(5.3)	682	(6.9)	0.599
e	678	(5.4)	682	(6.6)	0.634
19	687	(14.8)	679	(4.4)	0.567
15	684	(11.3)	679	(4.5)	0.681
12	675	(4.6)	699	(8.6)	0.027**

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

Table 16. Correlation matrix relating various starch granule characteristics to GME HWE nitrogen and TCW

	GME	%B	xBdia	xBsa	mBsa	xAsa	mAsa	HWE	N
xBsa	0.283**	0.298**							
mBsa	0.290**	0.452***							
xAdia	0.173	0.172	0.344**	0.451***	0.219*				
xAsa	0.194	0.188	0.368***	0.468***	0.228*				
mAsa	-0.209	-0.124	-0.077	-0.037	-0.197	0.583***			
SSA	0.106	0.580***	0.195	0.131	0.379***	-0.531***	-0.654***		
HWE	-0.288**	-0.145	-0.086	-0.119	-0.225*	0.141	0.272*		
Nit	0.423***	0.055	0.145	0.188	0.252*	-0.066	-0.303**	-0.440***	
TCW	0.217*	0.033	0.321***	0.313	0.239*	0.224*	-0.016	-0.044	0.263*

x = Mean, m = modal, sa = surface area, SSA = Specific surface area, B = B-granules, A = A-granules, N = nitrogen, HWE = Hot Water Extract L, TCW = Thousand Corn Weight

Table 17. Mean HWE and Modal surface area of starch B-granules attributed to groups of genotypes possessing or lacking electrophoretic bands 45-12Kd

Band	HWE Absent		HWE Present			Modal SA B-granules Absent		Modal SA B-granules Present		
	x	SE	x	SE	p	x	SE	x	SE	p
45b	294	(0.8)	289	(0.8)	<0.001***	39.1	(0.88)	43.4	(1.36)	0.008**
d	292	(0.7)	288	(1.4)	0.012*	40.2	(0.76)	44.6	(2.91)	0.042*
e	292	(0.6)	292	(3.4)	0.778	41.5	(0.81)	34.1	(3.11)	0.024*
f	290	(1.1)	292	(0.7)	0.057	40.4	(1.09)	41.2	(1.05)	0.629
h	291	(0.8)	293	(0.9)	0.127	43.0	(1.09)	38.6	(1.05)	0.006*
i	291	(0.7)	294	(1.0)	0.018*	43.0	(0.84)	34.0	(1.03)	<0.001***
36a	291	(0.6)	296	(2.0)	0.030*	41.1	(0.84)	39.6	(3.20)	0.608
c	291	(0.6)	295	(1.5)	0.022*	41.6	(0.88)	37.3	(1.57)	0.061
h	291	(0.8)	292	(1.0)	0.345	41.5	(1.09)	40.2	(1.18)	0.459
24b	292	(0.7)	290	(1.1)	0.189	39.9	(1.01)	43.4	(1.19)	0.047*
c	292	(0.7)	291	(1.3)	0.505	40.1	(0.95)	44.0	(1.28)	0.038*
19	294	(3.4)	292	(0.6)	0.441	45.1	(3.46)	40.8	(0.83)	0.237
15	294	(1.9)	291	(0.6)	0.234	44.6	(2.02)	40.6	(0.86)	0.134
12	292	(0.7)	289	(1.3)	0.064	40.5	(0.83)	43.1	(2.35)	0.192

*** = P <0.001
** = P <0.01
* = P <0.05

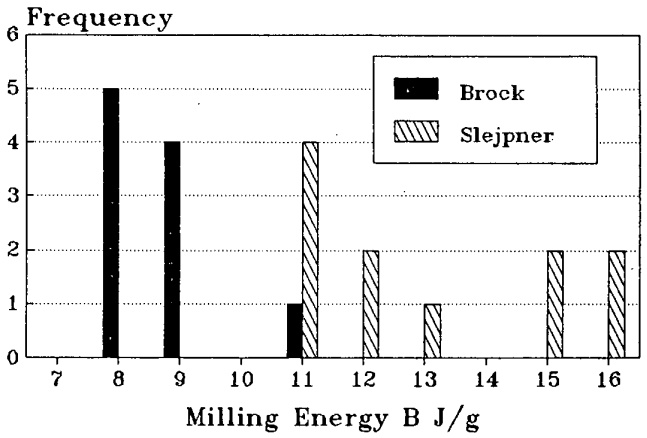

Figure 25b. GME of wheat, MEB values for Brock (soft) and Slejpner (hard).

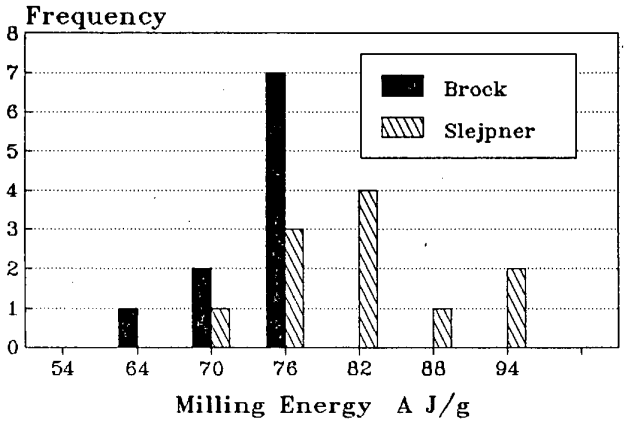

Figure 25a. GME of wheat, MEA values for Brock (soft) and Slejpner.

An examination of Brock, a soft milling wheat cultivar, and Slejpner, hard, showed that samples from several trials could be differentiated by their GME (Figure 26). However, the range of MEA values seen for Brock was well below that of all but the softest barleys (Figures 11 and 14). The highest value seen in Slejpner was 60% of the highest value for Tyne barley (Figure 14). Examination of the MEB values, i.e. the ME parameter that reflects the degradation of the particles created on the initial breakdown of grains, showed better differentiation of the cultivars than MEA. This indicates that the timing of the sampling of flywheel speed which is appropriate for barley GME requires re-optimisation for wheat measurement.

DISCUSSION

The starchy endosperm is the major tissue of the grain and is composed of starch (80-85%), protein (10-15%), and cell walls (3-5%). The starch is the raw material for the supply of sugars required during fermentation. However, the ease with which this supply can be utilised depends on the quantity and quality of the protein and cell walls which act as barriers to the starch-degrading enzymes. The ease with which the endosperm can be degraded by enzymes is reflected in the ease with which the endosperm can be physically broken down during milling (Allison *et al.* 1976). Soft milling cultivars have potential for malting which is rarely shown by hard milling types (Allison, 1986).

Malting quality depends on the composition of the endosperm while the whole grain is milled in the Comparamill. Consequently although the composition of the starchy endosperm is an important factor determining GME (at least 65%), it can be influenced by the husk and aleurone tissues, which we have shown have higher milling resistance than the starchy endosperm. This is particularly important in comparisons where the starchy endosperms of different cultivars have similar ME so that differences in GME would be attributable to the husk and aleurone layers. The influence of such "hard-milling" tissues can therefore have a

deleterious effect on predictions of malting quality as they can mask the true ME of starchy endosperm.

GME is significantly influenced by environmental effects such nitrogen dressing, sowing date and fungicide application. There were no significant correlations between either GME or MME and grain nitrogen across cultivars in the SAC malting trial nor in several trials in which grain nitrogen levels were higher than desired for malting (VNT86, VNT87). In contrast, at lower grain nitrogen levels, GME incresed with grain nitrogen suggesting that increasing levels of nitrogen could raise GME by an effect on the protein matrix. Later autumn sowing and fungicide treatment reduced GME because the early sown, untreated plants suffered stress due to infection by pathogens such as powdery mildew (*Erysiphe graminis*). This resulted in poor grain fill due to reduced starch accumulation and an increase grain nitrogen. The end result of all these of environmental contraints is an increase in the variation of grain size, which depends plant development and growth (Ellis and Kirby, 1980; Cottrell *et al.*, 1985). Biotic and abiotic stresses modify these processes beyond the limits that can be economically controlled on the farm. GME offers a means of monitoring the end result of crop husbandry and has been show to offer useful improvements in malting performance when barley lots of the same cultivar are segregated by GME levels (Maule, 1990).

An additional complication to studies of malting quality is that the environmental effects seen in the field are added to by the post-harvest environment. The conditions of storage, grain cleaning processes and malting regime all affect the outcome of malting. Storage for a period after harvest has been shown to result in improvements in malt extract (Gothard, 1984). This is an obvious difficulty in studies of the genetical control of malting quality because the difference between good and poor quality cultivars can be obscured. In turn the results of early malting can be over-influenced by dormancy. GME does not vary with storage and so is not related to the factors which can cause HWE to vary.

The high correlation between GME and hot water extract after coarse grinding reveals the importance of the physical properties of the grain to modification. GME was negatively correlated with the levels of nitrogen

that could be solubilised and with the degradation of cell walls. The influence of the protein matrix on GME is therefore related not just to the quantity of grain protein, but also to its structure, accessibility to enzymes and location, eg the proportion present in the ventral cheeks and distal tip.

When the rates of modification were compared milling energy revealed interesting differences between cultivars during the malting process. In Triumph, the rapid loss of MME is due to the widespread degradation of cell walls. This severely weakens the cell wall structure and facilitates access of the cell contents to proteases, without necessarily causing a loss of Calcofluor fluorescence. Subsequent modification of cell walls in Triumph progresses more rapidly than in Koru or Golden Promise as the remaining ß-D-glucans and proteins are removed but these have less effect on GME than the initial loss of endosperm integrity. In Koru and Golden Promise, where cell wall degradation was slower, the progressive loss of cell wall material resulted in a continuous fall in MME which was greater in Golden Promise than in Koru for the same degree of cell wall modification. The contrast in MME seen in Golden Promise and Koru is due to differential protein degradation.

Although cell wall modification reached between 70 and 90% the drop in MME reached a maximum of only 50-60%. This implies that residual, unmodified grain components may limit the reduction in MME or that other physical factors are important. As already demonstrated, the aleurone and husk are substantially "harder" than the starchy endosperm and together account for about 200 J/5 g. Fluorescence microscopy showed considerable changes in the cytoplasm of the aleurone cells. However, if the fibrous structure of these tissues retain their resistance to milling during malting, this would result in under estimates of endosperm modification. Assessments of modification between cultivars differing widely in GME are therefore more accurately determined from percentage loss of ME during malting than by MME alone, as the milling energy of the residual unmodified areas relate to that of the unmodified grain (Swanston, 1990).

The basis of wheat hardness has been extensively investigated (Anjum & Walker, 1991) and it has been concluded that the presence of particular proteins on the surface of starch granules is critical. Soft wheats are

characterised by the presence of a 15 kDa protein, friabilin, while in hard wheat and durum wheat this protein is absent. Barley and wheat show a considerable degree of genetic homoeology so it might be expected that a similar situation might apply in hard and soft barley. In fact the absense of glutenin type storage protein in barley appears to result in a weaker protein matrix. The result appears to be a more complex starch protein relationship in barley than in wheat. Even so it would appear that useful genetic markers can be developed from starch granule proteins to aid selection for malting quality components such as HWE.

CONCLUSIONS

Barley mills in a manner similar to a soft wheat with the lines of fracture predominantly through cells rather than along the cell walls. The comparamill completely mills a grain sample so that GME and, particularly MME, better represent the total grinding resistance of a sample rather than the friabilameter or an abrader.

Components of milling energy in barley, such as variation in grain size, the tissue composition of the grain and the interaction of protein and starch have been described. The largest component of milling energy lies in the endosperm.

Particular proteins, associated with the surface of starch granules, can be related to GME and HWE of the grain sample.

Milling energy was developed as a tool for selection in barley malting quality programmes. In each cross to which it is applied the outcome will depend on the contrasts between the parent cultivars because of variation in the factors which contribute to GME.

GME shows a strong enough relationship to HWE to be a useful selection tool in breeding programmes. The milling energy of malt or part malted grain can be used to predict HWE.

GME can be used at grain intake to reduce the environmental variability within grain lots by segregating lots before malting. MME can be used during the malting process to more accurately plan "flooring time" and manage the malting plant.

RECOMMENDATIONS

The work carried out in this project opens up a number of industrial development opportunities:-

1 Use of GME at intake control in maltings.

2 Application of MME to flooring process control at maltings.

3 Construction of a test kit to determine starch granule surface proteins.

4 Adapt the Comparamill for work with wheat.

In addition a there a number of areas that can be profitably exploited in further research projects, for example:-

1 Investigate the range of genetic variation in barley starch granule surface proteins (SGSP).

2 Characterise SGSP and elucidate individual relationships with malting characteristics.

3 Study grain development and determine the origin of starch and protein complexes in the barley grain.

4 Investigate the genetic control of proteolytic enzymes in barley and malt.

5 Determine the genetic control of dormancy in barley (this will depend on the development of a more precise germination test).

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