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ASSESSING MALTING QUALITY FOR BARLEY BREEDERS

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ABSTRACT

Selecting for malting quality is a major objective in barley breeding programmes. Strategies using micromalting and rapid tests to predict malting quality from measurements on ungerminated grain are described. The importance of a better understanding of the molecular and genetic basis of malting quality is discussed. Some research into the effect of qualitative variations in endosperm proteins and β-D-glucans as well as differential rates of synthesis of some enzymes are briefly described. The main conclusions are that the most effective current method of selection is by automated, labour saving methods of micromalting supported by a more detailed examination of prospective parental material using biochemical analyses. When more is known about the biochemistry and genetics of the major characters which affect quality, advances in our knowledge of molecular genetics may be exploited to improve quality in a more planned, rational manner.

KEYWORDS

Micromalting, automation, prediction tests, β-D-glucan, protein, enzymes.

INTRODUCTION

Eleven million tonnes of barley were grown in the UK in 1984 and more than 20% of this, valued at £ 250M, was used for the production of malt. Consequently, breeding for malting quality is an important objective for barley breeders in the UK, indeed it is the only quality criteria now considered because breeding for nutritional quality has virtually ceased. This paper reviews the methods and some research carried out at the Plant Breeding Institute (PBI) to assist barley breeders in selecting for malting quality.

The breeder can adopt one or more of three strategies to select amongst breeding lines; micromalt small samples of the lines and analyse the grain and malt; do predictive tests on the grain only; or test for individual characters which limit quality, and select for suitable combinations of these in grain and malt. The first option is currently adopted by most barley breeders, although predictive tests are sometimes used, especially for screening material from early generations.

MICROMALTING

Micromalting was first applied to barley breeding by Whitmore and Sparrow (1957) and there are currently many micromalting systems. The procedure used at PBI for advanced material still retains many features of the original system, particularly the use of test tubes to simulate a core through a bed of malting grain. Several of the operations have been streamlined and the grain and malt analyses have been modernised to take advantage of new technologies. The system is summarised in Fig. 1 and has been described in detail by Gothard *et al.* (1980). Micromalting systems adopted by breeders are rather different from those used by maltsters in that the former need a rigid, reproducible system to test variation between lines whereas the maltster needs a flexible system to obtain optimum conditions for a batch of grain. Micromalting has the advantage of selecting lines by assessing the interaction of all the characters which affect quality, most notably in terms of extract obtained. However, it has some disadvantages; it is very time consuming, labour intensive and not suited to screening the large numbers of samples which come from early generations. It also does not detect deficiencies or excellence in specific characters.

PREDICTION TESTS

Because of the restrictions imposed by micromalting, it became fashionable about a decade ago to search for more rapid tests to predict malting quality in early generations. This coincided with, and may well have been stimulated by, the introduction of the SDS test to select wheats for baking quality. Several predictive tests have been proposed and some are shown in Table 1, together with data from Gothard (1981). This list is by no means exhaustive but illustrates the diversity and empirical nature of some of these tests.

Bishop's equation is included as it has been used in the industry for almost 50 years and relies on nitrogen and 'insoluble carbohydrate (IC)' measurements. Although it accounts for over half the variation in extract, measurement of IC remains difficult and requires considerable technical expertise; it is not suited to rapid screening.

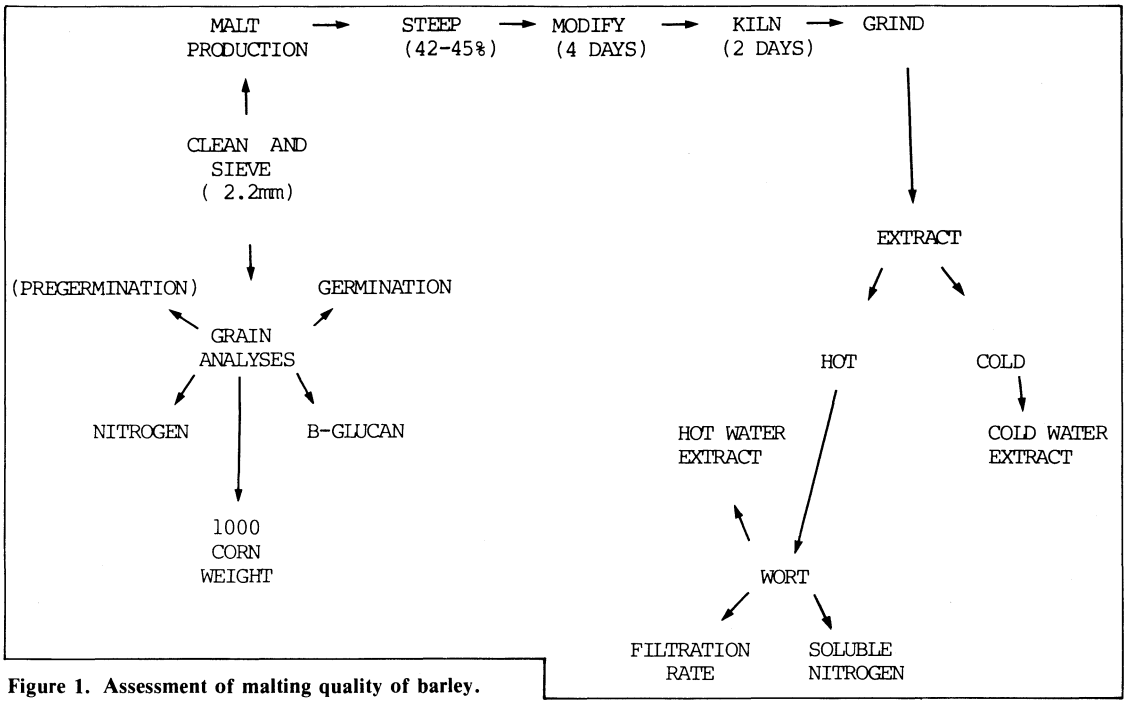

Figure 1. Assessment of malting quality of barley.

Table 1. Performance of some prediction tests using ungerminated grain (from Gothard, 1981).

Method	Units	Range	SED	%CV	r
Reference micromalt	% HWE	63.8-75.6	0.224	0.320	1.000
Bishop's equation	% predicted HWE	68.6-79.9	0.388	1.223	0.747***
Infrared reflectance	% predicted HWE	60.7-75.2	0.326	0.479	0.775***
Sedimentation in alcohol	% transmission	20.0-53.0	1.091	3.271	0.224*
Zelany sedimentation	cm^3 sediment	5.0-9.5	0.076	1.088	−0.670***
Milling energy	joules	343-573	3.570	0.758	−0.733***
Falling-time index	% predicted extract	69.0-79.0	0.144	0.193	0.682***

HWE = Hot-water extract; SED = Standard error of differences between replicate means; %CV = Coefficient of variation; r = Correlation coefficient with reference HWE; *** significant at 0.1%; * significant at 5.0%; n = 108.

Near infrared reflectance analysis (NIR) to predict extract, pioneered at PBI (Morgan and Gothard, 1979), was reported to give a high correlation with extract, but during our research we have observed large environmental effects, resulting in the need for frequent recalibrations. This is time consuming and retrospective and on balance we do not now consider NIR reliable enough to screen breeder's material.

Sedimentation in 70% alcohol (Palmer, 1975) has generally proved ineffective and an adaption of the Zelany test (Reeves *et al.*, 1979), although giving better correlation with hot-water extract (HWE), has also not been adopted by breeders. The milling energy test, proposed by Allison *et al.* (1976) is based on endosperm hardness (analagous to the Stenvert test) and has proved useful but its more widespread use has been restricted by slow production of the apparatus to conduct the test.

The falling-time index test (Morgan and Gothard, 1981), based on the viscosity of an acid extract of barley flour as a measure of soluble β-D-glucan, can account for about half the variation in extract. However it relies on one character only, which may not be the limiting character in many genotypes or environments.

Rapid tests on malted grains (data not shown) can give good results, particularly on the degree and evenness of germination (Gothard, 1981). However, they are not suited to screening early generations because the most time consuming part of the micromalting procedure, the production of the malt, is needed before these tests can be performed.

Figure 2. Computer-controlled micromalting apparatus.

It is therefore concluded that whilst some prediction tests may be useful (particularly if two or three are used), they are not dependable enough for routine testing, mainly because not enough is known about precisely what they are measuring, and each test measures something different.

Our philosophy has therefore been to select for other characters (yield and disease resistance) in early generations and to develop and build a micromalting system which is fully automated to process as many samples as possible, and to use this as early as possible in the breeding programmes. The system we have built is shown in Fig. 2 and is a fully automated, computer-controlled apparatus capable of producing about 100 micromalts per batch. Samples are placed in the apparatus and steep, air rest, modification times and temperatures are entered into the computer. The malts are prepared under these conditions completely automatically and a printed record of the actual conditions during each run is provided. The malted samples are then subjected to a simplified Institute of Brewing analysis to determine extract, soluble nitrogen, and filtration rate. Before malting, samples are scored for protein and acid-soluble β-D-glucan. Full details of this apparatus, its control and performance have been given by Gothard and Smith (1986).

SPECIFIC CHARACTERS

The third strategy available to breeders, testing for individual characters which affect quality, is complex and requires considerable research before it can be effectively put into practice. For example, we know that the quantity of starch, protein, β-D-glucan and perhaps lipid in grain affects malting and brewing performance, but are there also genetically controlled qualitative differences in these major components? In addition to these components of grain, during germination we might also consider variation in the rate of water uptake, rate of synthesis of gibberellic acid (GA), sensitivity of aleurone to GA, rates of enzyme synthesis and diffusion, and isozyme variation.

These are complex considerations and the questions we need to ask about them are: do they affect quality, is there variation, is any variation genetically controlled, will changes be compatable with agronomic characters, and what is the relative importance of these characters? Not only do we need to answer these questions if we are to select for the correct specific characters in conventional programmes but this knowledge is also necessary if we are to take advantage of emerging techniques in genetic manipulation. Massive investments are being made

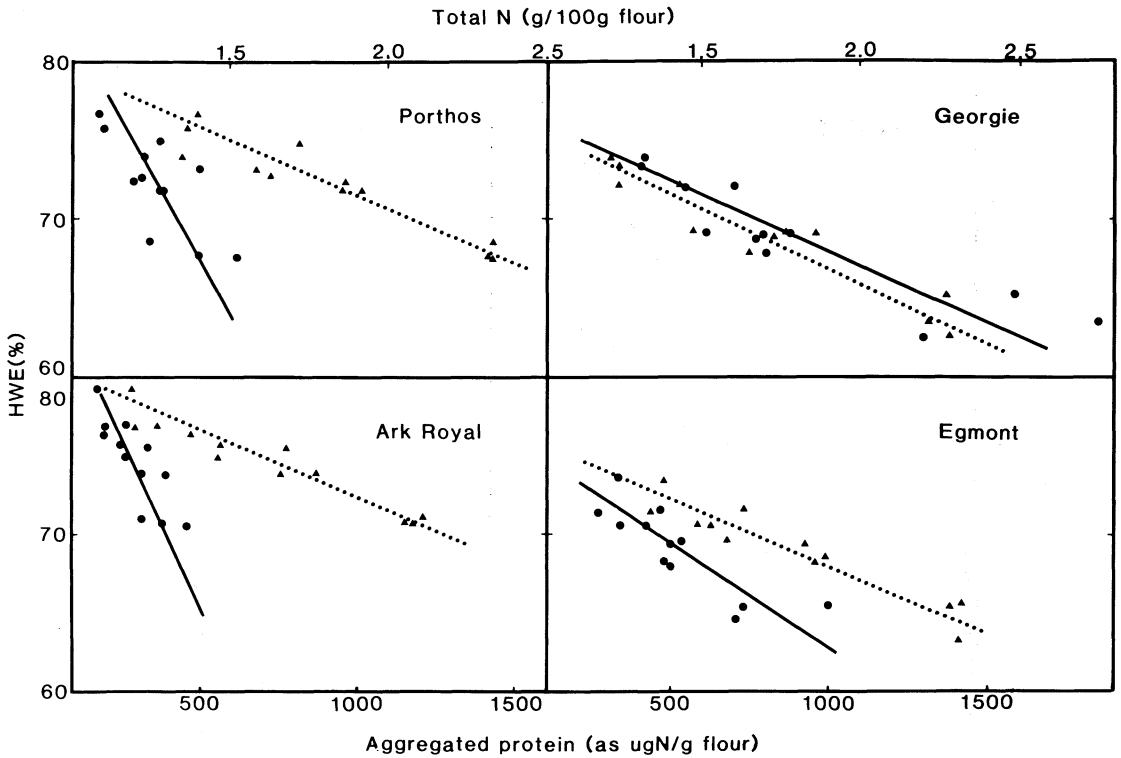

Figure 3. Relationships of total N (dotted lines) and aggregated proteins (solid lines) with hot-water extract (HWE) for good (left side) and poor malting varieties.

worldwide to develop such techniques and if these are to be used to improve malting quality we need to know which characters should be influenced and if they can be described in terms of single heritable units which can be quantitatively assayed.

We have tried, in a modest way, to answer some of these questions and summaries of three pieces of work are described to illustrate this approach.

Measuring protein

It has long been known that protein content is inversely related to malt extract. Fig. 3 is an illustration of this, but examination of this particular data shows that the regression lines for total N for different varieties have different intercepts, suggesting there may be a genetically controlled element of protein quality (Smith and Lister, 1983). Detailed examinations of protein banding patterns following electrophoresis revealed no relationship between polypeptide subunits and malting quality (Shewry *et al* 1980; Smith and Simpson, 1983) but measurements of the amount of protein involved in high-molecular-weight aggregates (formed by intra and intermolecular disulphide bonding) was found to be strongly related to malting quality (Smith and Lister, 1983). The conclusions we made were that the amount of aggregate is controlled by the content of hordein D, a protein of high molecular weight encoded by a locus on the long arm of Chromosome 5. The better malting varieties had low concentrations of hordein D, and consequently low concentrations of aggregate even when total protein content is high (Fig. 3). Subsequently, a search of the germplasm collection at PBI revealed a landrace cultivar from Ethiopia which lacked hordein D completely, and this has been introduced into the breeding programme to study the effect of lack of hordein D in a conventional genetic background. If this is shown to have the expected improvement on quality, it will be a character which can be rapidly and simply screened.

Enzyme synthesis studies

Morgan *et al.* (1983a, b) and Morgan *et al.* (1984) reported a study of genotype and environmental influences on the rates of synthesis of some enzymes and their effects on storage proteins and β-D-glucan during the germination of barley. An example of the kind of result obtained is shown in Fig. 4, which shows genetic variation in the rate of synthesis of endopeptidase, particularly at the embryo end of the grain in the later stages of malting. Similar variations were found for endo-β-glucanase and

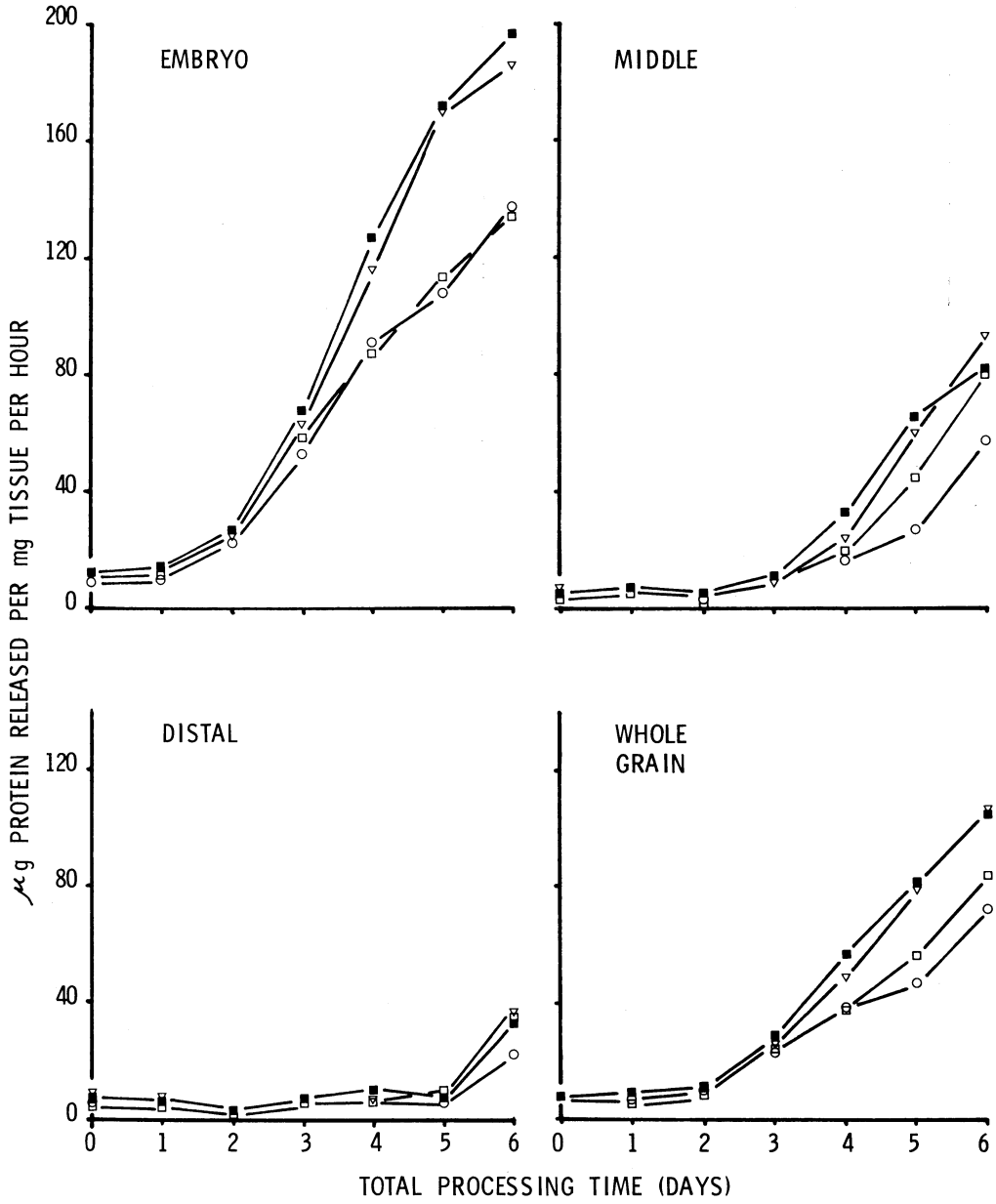

Figure 4. Variety means (average of four sites) of endopeptidase activity in whole grain and in embryo, middle and distal parts of the grains. Varieties shown are Ark Royal (■), Porthos (▽), Athos (□), and Georgie (○).

our main conclusion was that there were significant variety and site variations in the rate of synthesis of some enzymes, of which varietal variations were greatest and there is probably scope for selecting for high rates of enzyme synthesis. The percentage variance in extract accounted for in these samples by multivariate analysis suggested that characters related to β-D-glucans were more important than characters related to protein.

Cell wall components

Methods have been developed for the preparation of pure endosperm cell walls and for the rapid and accurate measurement of their main component, β-D-glucan (Ahluwalia and Ellis, 1985). No varietal differences were found in the composition of cell wall carbohydrate or protein although varietal differences in the solubility of cell wall β-D-glucan were observed when extracted at various temperatures in water and acid (Fig. 5), (Ahluwalia and Smith, 1985). Poor malting types tended to have more readily soluble β-D-glucan. Subsequent studies (unpub.) indicate that variations in ionic interactions and hydrogen bonding between β-D-glucans and, perhaps, arabinoxylans are responsible for these solubility differences. Ferulic acid was identified as the cross-linking agent which renders cell wall arabinoxylans largely insoluble (Ahluwalia and Fry, 1986) and which probably

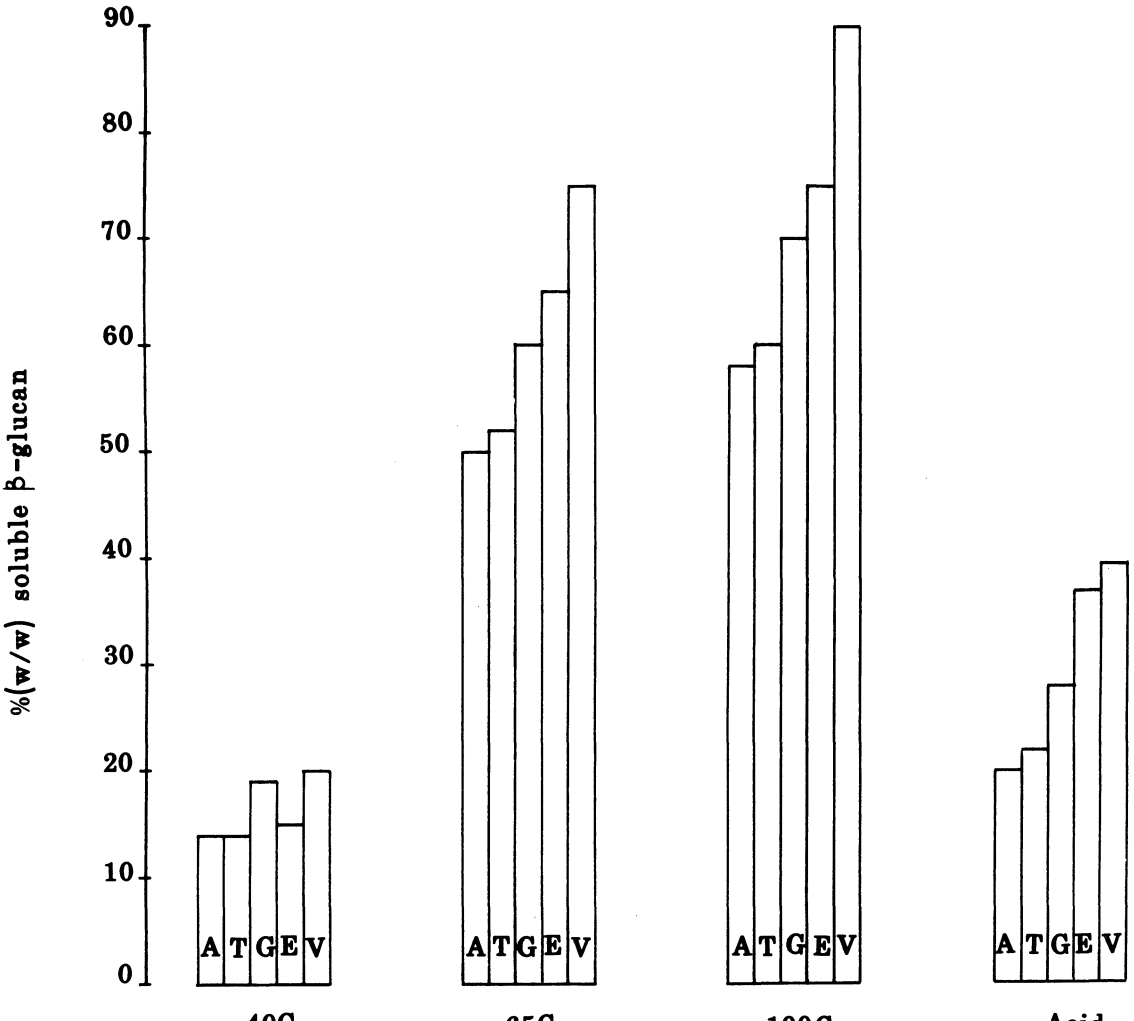

Figure 5. Solubility of cell-wall β-D-glucan in water at various temperatures and in dilute acid. Cell walls were prepared from endosperm of barley varieties Ark Royal (A), Triumph (T), Georgie (G), Egmont (E), and Varunda (V).

form the major part of the microfibrillar phase of cell walls (Fincher, 1975). It is suggested that breeders should select not only for higher rates of endo-β-glucanase synthesis and low concentrations of total β-D-glucan but also for lower levels of soluble glucan. Concentration of soluble β-D-glucan appears to be linked to poor malting performance and also contributes to filtration and haze problems in the brewery.

CONCLUSIONS

This research has reinforced the view that many characters influence malting quality. A study of quantitative changes in the major components of barley during malting and mashing (Smith and Gill, 1986) suggests that good malting barleys have moderate superiority in several characters rather than a fundamental difference in a single attribute. This supports the thesis that to further improve quality, breeders should select for several characters which are independantly inherited. It is clearly impractical to test for several such characters in all breeding lines but such tests should be applied for selecting parental material to enable complementary crosses to be made. Specific tests and micromalting can then be used to assess whether improvements in overall performance have been achieved. This approach is currently being introduced as part of the breeding programme at PBI.

In this paper, we have tried to briefly convey the current strategy for selecting for malting quality at PBI. This strategy is based on efficient and labour saving micromalting. We also conduct research into the relative importance of genetically distinct characters likely to be related to quality. As a result we hope to understand better the factors that limit quality, to breed barley more effectively and to exploit techniques in molecular genetics when they become available for cereals.

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SYMPOSIUM DISCUSSION

Dr W. Bushuk, University of Manitoba

Would you comment on the effect of grinding on the results for solubility of β-glucans? Is there any inter-relationship between kernel hardness and solubility with a constant grinding action?

Smith

We have a standard grinding technique. We have only used two grinding methods, and no difference has been found between them although we have not actually tested this. The grinding hardness test referred by Mike Ellis might well be related to this in an indirect way — he is looking at the character of protein and character of the glutenin in an indirect way by measuring the amount of energy required.