



Variation, across environments within the UK, in grain protein and grain hardness, in wheat varieties of differing distilling quality

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ABSTRACT

Twelve soft-milling wheat varieties were grown, over a total of 12 environments, trials being sown in three locations, in two seasons, both with and without nitrogen fertiliser (N) application. Grain protein and hardness levels were assessed on all samples and initial analysis showed highly significant effects of variety, site, season and N-rate on both characters. The environments differed widely for mean protein and hardness levels and the response of the individual varieties was assessed by linear regression of their protein and hardness values on the mean value of all twelve varieties, in each environment. For protein, there were very strong correlations between the environment means and the means of the individual varieties, but there were also slight differences in stability across environments, with varieties like Claire showing a tendency to accumulate more grain protein at sites characterised by higher protein levels. At sites with highest grain hardness, Ambrosia and Kipling, which both carry the 1B/1R translocation, had much harder grain than the other varieties. Correlations, across varieties, between grain protein and hardness were significant in only some environments. It was concluded that grain hardness could be a useful additional parameter for assessing both breeding lines and distillery intake samples, while further research should consider variation in grain texture and its genetic control.

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1. Introduction

In the 1980s, the Scotch Whisky industry began using wheat, in preference to maize, as the main cereal adjunct for grain distilling, largely for economic reasons (Brown, 1990; Riffkin et al., 1990). Within ten years this created a demand from the industry for almost half a million tonnes of grain (Taylor et al., 1993) and led to a near four-fold increase in wheat cultivation in Scotland (Swanston and Newton, 2009). The industry has not, subsequently, defined sample specifications for distilling quality, other than basic grain parameters such as nitrogen content and specific weight, but it has participated in the screening and selection of wheat varieties undergoing national trialling (Bringham et al., 2008). The major attribute required of wheat for distilling (Brosnan et al., 1999) is a high yield of alcohol from a given quantity of grain, but ease of processing in the distillery is also important. As a consequence of this, only certain soft milling varieties are classed as acceptable for distilling, while those which are hard milling, or which carry the 1B/1R translocation (Bringham et al., 2008) are rejected, as they have a high probability of processing problems.

However, alcohol yield is a quantitative trait (Sylvester-Bradley et al., 2010) and data from varietal trials (Bringham et al., 2008) showed a considerable effect of environment on the ranking order of varieties. Varieties that could deliver samples showing stable, consistently high alcohol yields over sites and seasons, would, therefore, be advantageous. As the UK is also establishing a wheat-based bioethanol industry, the economic value of such varieties would be considerable. Awole et al. (2009) postulated that cost savings from using grain with higher alcohol yield could exceed £3 million per annum for a plant processing 100,000 tonnes of wheat.

Breeding for distilling quality is hindered, as the testing procedure developed by the distilling industry (Agu et al., 2006; Brosnan et al., 1999) is not readily applicable to breeding programmes, due to the requirements of time and sample size (Swanston et al., 2005). Rapid, small-scale methods are, therefore, required. A further problem is that the most significant correlation with alcohol yield is generally observed with grain protein content (Riffkin et al., 1990) and, while there is some variation between varieties, for protein content, environmental variation has a considerably larger influence (Swanston et al., 2007). Several studies have attempted to include other factors such as starch content (Kindred et al., 2008); grain size (Agu et al., 2008; Awole et al., 2009; Swanston et al., 2005), or ease of starch release (Swanston and Smith, 2008), in addition to protein, to predict alcohol yield, but it was concluded that different varieties achieved

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high alcohol yields, through different mechanisms (Swanston et al., 2007), so the direct impact of specific attributes was not always clear.

As the enzymes for starch degradation come from malted barley, for grain whisky, or are extraneous, in the production of bioethanol, alcohol yield of wheat is essentially a property of the endosperm. This gives it similarity, in some respects, to malting quality of European barley (Turnbull and Rahman, 2002), which is characterised by high malt extract and low grain protein (Friedt et al., 2000), with enzymatic activity less critical (Elia et al., 2010). Allison (1986) demonstrated the value of grain hardness testing, in selecting for malting quality, as soft-grained types, originating from central Europe, were the progenitors of contemporary malting varieties. While hard- and soft-milling wheats differ due to the presence of specific proteins, in soft wheat, that interfere with the adhesion between the starch granules and the surrounding matrix protein (Greenwell and Schofield, 1986), there is also considerable variation within the two groups (Bettge and Morris, 2000). This variation in hardness has been linked with distilling quality (Agu et al., 2009; Misailidis, 2010). Misailidis (2010) developed an equation, to predict alcohol yield, based on hardness and grain diameter data from the Single Kernel Characterisation System (SKCS) (Martin et al., 1991), in addition to grain protein. When this was used to predict alcohol yield in a population of samples from the following season, it explained 80% of the variation observed when the alcohol yield of the samples was subsequently analysed (Misailidis, 2010).

Agu et al. (2009) considered ten soft-milling varieties, grown at four sites, at two of which they observed a highly significant correlation between grain hardness and alcohol yield. While hardness and nitrogen content were significantly correlated at all sites, the addition of grain hardness, along with nitrogen content, in a multi-variate regression, improved the prediction of alcohol yield, compared to that derived from nitrogen content, alone. However, Agu et al. (2009) assessed both protein and hardness by use of Near Infra-Red (NIR), so it is possible that the observed correlations, between protein and hardness, were enhanced through being obtained from the same spectral data (Sylvester-Bradley et al., 2010). However, Hong et al. (1989) also measured protein and hardness simultaneously, using NIR and found the correlation between the two traits to be of lower magnitude than those between hardness and both particle size and vitreosity. In addition, Bettge and Morris (2000) found very similar correlations between protein and both NIR and SKCS-derived hardness data.

Grain protein content is influenced by factors such as variety, site, season and fertiliser application and, given the significant association between protein and grain hardness (Agu et al., 2009), hardness is likely to be affected by similar factors. However, due to interactions between the factors, precise effects on protein content and hardness may differ. The association between the two parameters is therefore explored here, over two seasons and highly contrasting environments, both within and across individual varieties. Given the negative associations between alcohol yield and both protein content and grain hardness, it would also be expected that the best distilling varieties would show the lowest expression of these two traits. Consequently, selection of genotypes that had both a low and a stable expression of grain hardness and protein content, across sites and seasons, could represent a reasonable breeding strategy. In this paper, varieties are assessed for grain hardness and protein content, in contrasting environments and the stability of expression is considered in addition to the mean value for each variety. It is intended to determine whether varieties respond to environmental variation in different ways and whether this may effect or restrict their suitability for distilling.

2. Materials and methods

2.1. Field trials

Grain samples were obtained from 12 winter wheat varieties, which were common to six trials grown in two seasons (2004–2005) and (2006–2007). The varieties comprised ten soft-milling types that have been classed as suitable for distilling: Alchemy, Atlanta, Claire, Consort, Glasgow, Istabraq, Riband, Robigus, Wizard and Zebedee (Bringhurst et al., 2008; HGCA, 2006, 2010) and two other soft wheats, Kipling and Ambrosia, which carry the 1B/1R translocation (Smith et al., 2006). Trials were harvested at three sites in the UK in 2005 (sown autumn 2004) and in 2007, Terrington, Norfolk (trials subsequently referred to as TT05 and TT07) High Mowthorpe, Yorkshire (HM05 and HM07) and SCRI, Dundee (SC05 and SC07). Each variety was replicated three times in the Terrington and High Mowthorpe trials and twice at SCRI. In addition, to create a total of 12 environments, each trial, at each site, was grown with and without nitrogen (N) fertiliser application. Where N was applied, the amount used was determined by the aim of achieving an optimal alcohol yield per hectare, i.e. slightly sub-optimal for high yielding bread wheat. This ranged from 120 to 180 kg/ha (Sylvester-Bradley et al., 2010), depending on the existing N content obtained by soil analysis.

2.2. Data collection and analysis

Plots were combine-harvested when ripe and, after drying, a sub-sample of approximately 500 g was taken from each, cleaned and passed over a 2 mm sieve. These sub-samples were assessed for grain protein and hardness, using an Infra-Tec 1241 Food and Feed Analyser (Foss Instruments Ltd., Warrington, UK), as described by Agu et al. (2009). The calibrations used were as supplied by the manufacturer so, for grain hardness, would have been based on the SKCS method for hardness determination (Agu et al., 2009), with both hard and soft wheats included.

Estimates of stability for grain protein and hardness were obtained by plotting mean values for the individual varieties against the mean of all the varieties in each environment. This followed the approach described by Finlay and Wilkinson (1963) for assessing yield sensitivity, where the regression co-efficient was used as a measure of stability, with average stability, i.e. the mean of all entries, therefore being 1.0. Values higher than 1.0 were classed as below average stability, indicating varieties that were more sensitive to changes in the environment while those below 1.0 were associated with varieties that yielded consistently across a wide range of environments and were therefore classed as above average stability. A similar approach was used for malting quality characters in barley, although Sparrow (1970) did not use the logarithmic scale applied to yield (Finlay and Wilkinson, 1963), to increase the linearity, while Zheng et al. (2009) used regression across environments differing in nitrogen levels to compare the stability of grain protein in addition to grain yield. All statistical analyses were performed using GENSTAT version 11 (Lawes Agricultural Trust, Rothamsted, UK).

3. Results and discussion

3.1. Effects of variety, site and season

The statistical package permitted analysis of variance, despite the differences in replication between the sites. For both Grain Protein and Grain Hardness (Table 1) there were, as expected, highly significant effects of variety, site, season and N rate. There were also some changes in the ranking order of the varieties, for both traits,

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