Final Project Summary

Project title	A genome wide analysis of key genes controlling diastatic power activity in UK barley (DPGENES)		
Project number	21130001	**Final Project Report**	PR583
Start date	April 2015	**End date**	July 2017 (extension)
AHDB Cereals & Oilseeds funding	£207,967	**Total cost**	£217,967

What was the challenge/demand for the work?

Scotch whisky is divided into malt and grain whisky with the latter being larger in volume. Grain whisky is produced from a mash of a cooked cereal with malted barley added to provide a source of enzymes to digest the starch. The primary requirement of the malted barley is the ability to digest the cooked starch and is known as diastatic power (DP). This is recognised in the official testing process leading to varieties being identified as suitable for grain distilling under the 'IBD malting approval for grain distilling use' on the AHDB Cereals & Oilseeds Recommended List for spring barley. As the inclusion of high DP barleys in grain distilling is in the order of 10-15%, the annual demand for high DP barley is in the order of 100-120,000t, a relatively small amount for a commercial breeding programme. Varieties that are recommended, therefore, tend to arise more by chance than by deliberate selection. The current main variety, Belgravia, is now 10 years old and, in the North region, 14% lower yielding than the highest yielding varieties on the 2017 Recommended List, which equates to a loss of over 1t/ha in yield from the 2016 average treated yield.

Grain whisky production, like that of malt whisky, continues to increase and cannot rely on old varieties that are no longer agronomically suitable and the industry, therefore, identified a requirement for new barley varieties with enhanced DP that also provide a competitive grain yield. In the short term, this is best provided by using DNA fingerprinting techniques to identify genes within the current UK elite varieties that can then be used by plant breeders and testing authorities to select high DP lines. Currently, malting analyses are carried out on samples from sites that produce low grain nitrogen content and, as DP is positively correlated with grain nitrogen content, is not typical of the environments used to produce high DP barley. Thus, there was also a need to establish a better phenotypic screening regime for the character.

How did the project address this?

Whilst the major genes synthesising the enzymes that lead to DP are well known, our hypothesis was that these are likely to have been fixed in elite varieties by continued selection for good malting quality, especially in UK spring barley. Other unknown genes are, however, likely to affect DP as substantial phenotypic variation for the character is apparent from the results of the malting quality analyses that were used to establish whether or not the IBD would approve a variety for a specific malting use. We,

therefore, used existing DP data to identify pools of high and low DP varieties in spring and winter barley. This data has been collected from the National and Recommended List trialling process to evaluate suitability of varieties for malting and a BBSRC Crop Improvement Research Club project to study the processability of malting barley. By genome-wide comparison of high and low DP bulks within each crop type, we can identify genotypic differences between the bulks. Using a pool of at least 10 varieties would balance differences between the genetic backgrounds of individual varieties so that the vast majority of the remaining differences would reflect the differences in phenotype, i.e. DP, upon which the bulks were constructed. Whole genome sequencing of individual barley lines was not possible but a custom assay designed to capture representations of the gene content of barley was available and provided a most cost-effective means of sequencing DNA from each of the four pools, i.e. winter and spring barley high and low DP pools.

We then used bioinformatics analyses to identify over 80,000 polymorphisms at single nucleotide bases (single nucleotide polymorphisms – SNPs) in the DNA contrasts between the pools. The frequencies of the different sequence bases at each of these >80,000 locations were then used to determine if they were likely to be strongly associated with the difference in DP. For instance, if all the bases in the high pool were completely different from all the bases in the low pool at one SNP locus, then that locus is fixed and the absolute difference in variant frequency would be 1, strongly indicating that the locus was associated with differences in the phenotype. We, therefore, considered absolute differences of >0.75 as being indicative of association of a locus with DP, e.g. 80% of the lines in one pool had one variant at the locus and it was only present in 5% of the lines in the other pool. This reduced the number of potentially associated loci to 66 in winter and 32 in spring barley. Many of these were closely linked together and selecting the most probable amongst the linked loci reduced the numbers to test to eight in the winters and five in the springs. We then, further refined this list by validating the markers against the component varieties in the pools. A sample with pre-existing data from the same populations was used to construct the pools, and finally against an independent sample of unknown lines obtained from several breeding companies.

We used representative varieties from the DP pools in fertiliser x grain nitrogen, fertiliser management trials to study what would be the most appropriate to select for high DP in a screening programme. Because resources did not permit large scale malting analyses, we used a combination of yield and grain nitrogen measures to establish what would be the most appropriate regime.

What outputs has the project delivered?

1. The DP predicted from the 13 markers identified above was highly correlated (r=0.92) with the actual DP values of the component varieties in the bulks.

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2. Testing the allelic differences for each of the 13 SNPs against the historic DP values of 78 winter and 85 spring barley varieties showed significant differences in the character for all but 4, thus validating 9 of the 13 SNPs (6 winter and 3 spring).
3. These 9 SNPs were then used to predict the DP of a sample of 61 winter and 85 spring lines supplied without any information by breeding companies. The lines were grown under a high nitrogen management regime in one trial at JHI and samples sent for micro-malting and DP analysis at SWRI. Filtering the data out to eliminate samples that had not malted properly identified a significant correlation (R^2=0.48) between predicted and observed DP amongst the winter lines, but not the springs. The lack of correlation in the spring set probably reflects the fact that the high DP variant of one of the SNPs was fixed in all 85 lines and the high variants of the other two were in significant excess, indicating a lack of suitable variation due to continued selection for good malting quality.
4. Despite selection for good malting quality in the springs, the mean DP of the winter lines (155 °L) was significantly greater than that of the spring lines (133 °L) so winter varieties are the best source of high DP amongst current UK elite barleys.
5. Testing the selected spring lines from the DP pools showed that low grain N lines and N responsive lines were indistinguishable under normal malting barley N inputs, whereas the responsive lines were indistinguishable from the high grain N lines under a high N fertiliser regime. Even under the malting regime, the high grain N lines were higher than the others. This confirms the need to test lines with high DP potential under a high N regime, as the responsive lines might be of value to grain distillers but may not be considered under existing testing protocols. Timing studies demonstrated that delaying a split N application to GS31 increased the grain N content and thus, might result in higher DP.
6. Overall, we have identified markers that can be utilised on material coming through the current testing process to select the best lines and we have demonstrated that use of winter barley is a better way of improving DP. Phenotypically, screening for the character should be conducted under a higher N regime.

Who will benefit from this project and why?

- Growers will benefit as it provides a market outlet for winter barley at a relatively high N regime so that they can secure a high yield combined with a malting premium.
- Distillers will benefit as they will have a source of high DP barley that would meet their current annual demand of over 100,000t of barley. Note that maltsters purchased <25,000t of Belgravia from the harvest 2016 crop and it is not clear how much of the 108,000t of other varieties was made up of genuine high DP varieties. What is clear is that there is currently an under-supply of approved high DP varieties and the results from this project can be used to alleviate this.

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- Breeders and testing authorities will benefit as they can identify potential high DP varieties early in the official testing process and utilise the appropriate phenotypic screening protocol identified in the project to select out the best lines.
- Assuming a price of £125/t for high DP barley, a spend of £207k by AHDB is helping to secure an annual farm-gate crop worth over £13 million and contributing to a UK export market worth just under £3 billion in 2016 for grain and blended whisky.

If the challenge has not been specifically met, state why and how this could be overcome

1. Switching to winter barley as a source of high DP barley requires some interaction with the malting and distilling industry and could be achieved by presentations at appropriate technical meetings that could either be organised by SWRI, MAGB or AHDB.
2. A fuller survey of emerging UK spring barley varieties is required to determine whether or not there is any prospect to significantly increase DP through the use of the protocols identified in this project. Genotyping of spring barley lines added to the National List since 2014 through the IMPROMALT project may help to establish this and it could be confirmed by genotyping the lines with the markers identified from the project in a 'follow-on' project at relatively low cost.
3. Ideally, we would have liked to have collected DP data on the lines grown under the phenotypic screening part of the project. This could also be the subject of a follow-on project.

Lead partner	JHI
Scientific partners	SRUC
Industry partners	SWRI
Government sponsor	None