# FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

DAW00220

# Barley Grain Defects - Research And Screening Services

## PROJECT DETAILS

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| **PROJECT CODE:** | DAW00220 |
| **PROJECT TITLE:** | BARLEY GRAIN DEFECTS - RESEARCH AND SCREENING SERVICES |
| **START DATE:** | 30.06.2012 |
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## Summary

Grain defects due to black point (BP), kernel discolouration (KD) or pre-harvest sprouting (PHS) downgrade malting barley to feed category causing losses of millions of dollars across Australia. To limit losses, several barley germplasm sets were evaluated and tolerant genotypes were identified. Environments and genotypes were grouped into various risk groups of BP or KD. Genetic factors linked with tolerance to BP, KD or PHS were mapped on various chromosomes. This included grain colour linked quantitative trait loci (QTL) on chromosomes 2H, 3H, 4H, 5H, 6H and 7H. The key gene for PHS was identified. Results from this project will play key roles in developing new varieties with tolerance to grain defects and thus reduce losses.

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## Conclusions

1. Barley genotypes varied significantly for tolerance to barley grain defects.
2. Australian barley varieties and advanced breeding lines from Australian breeding programs varied substantially in risks associated with BP and KD.
3. Environmental variation played dominant roles on expression of grain defects in various barley germplasm sets.
4. Overall, coastal environments such as Esperance, South Stirling and Geraldton in Western Australia (WA) had a high risk associated with BP or KD.
5. Intact head misting was more indicative of and the most suitable test for PHS resistance than the post-harvest germination test.
6. QTL on chromosomes 2H, 3H, 4H and 6H strongly controlled BP in WA and Queensland (QLD) trial environments.
7. A fine-mapped gene at 0.3cM to a simple sequence repeat (SSR) marker Bmac0067 on chromosome 3H controlled pale or bright grain colour.
8. Grain colour in the Vlamingh(ϕ) and Buloke(ϕ) population was controlled by QTL mapped on chromosomes 2H, 4H, 5H, 6H and 7H.
9. Grain size and screening were controlled by QTL mapped on chromosomes 1H, 2H, 5H and 6H with QTL on 2H and 6H being negatively associated with grain colour.
10. A QTL on chromosome 5H was the most important QTL controlling grain plumpness.
11. A QTL linked with seed dormancy (SD) on chromosome 5HL was detected in nine environments and thus it was the most stable one followed by QTL on chromosomes 5HC, 4H and 6H.
12. Forty-five new INsertion/DELetion (InDel) markers and nine single nucleotide polymorphism (SNP) bacterial artificial chromosome (BAC) sequence based markers were developed to map the 5HC regions for SD. Two SNP marker combinations were identified with potential uses in selecting for SD and malting quality traits.
13. Nine diagnostic SNP markers of SD and six different haplotypes were identified from analysis of more than 400 lines with 38 new markers for the 5HL locus.
15. Four diagnostic molecular markers and candidate genes linked with SD were identified on chromosome 5HL. An allele from Gairdner(ϕ) was the best for SD and malting quality traits in most barley growing regions.

## Recommendations

Grain trait defects such as BP, KD and PHS vary strongly from location to location, and from season to season. Rainfall during the harvesting period in particular is one of the most important environmental factors that causes grain defects. Replication of trials across regions and sites has been adopted in this project. However, to cater for seasonal variation effects at the same or similar trial locations, studies on traits such as BP, KD and PHS affected by seasonal variability need to be replicated across seasons across four times.
Some high yielding Australian barley varieties, e.g. Keel and Hindmarsh$^{(\phi)}$, are susceptible to BP. Efforts should be made to eliminate the risk from these high yielding barley varieties. It is also worth understanding the relationship between BP tolerance and high yields.

## Outcomes

Several barley genotypes were identified with tolerance to BP, KD or PHS, collectively called grain defects. Although environmental variation played dominant roles in the observed variations, particularly in BP and KD, tolerance to grain defects was controlled by genetic factors. Genetic factors for grain defects were mapped as QTL. New molecular InDel markers and SNP markers were developed and delivered to the breeding programs. Genotypes including Australian barley varieties, advanced breeding lines from Australian barley breeding programs, as well as trial environments, were classified into different risk groups associated with BP or KD. Grain defect resistant genotypes and associated genetic factors, information on varietal, breeding lines and environmental vulnerability to grain defects will certainly play important roles in developing new barley varieties with resistance to grain defects.

The immediate benefit of this project includes the ability of barley growers to make informed decisions on which variety to grow in environments vulnerable to grain defects. The long term benefit of this project outcome includes incorporation of genotypes and genetic factors with tolerance to grain defects into breeding programs to develop new barley varieties. To facilitate uptake of this project's outcomes, seasonal results were disseminated by sharing data with Australian barley breeders, presenting results on Australian barley breeders' meetings, and industry meetings. The project outputs have provided efficient tools for the breeding companies to eliminate the risk of PHS in malting barley.

In summary, the outcomes of this project will have both short term, as well as long term benefits, in reducing losses of millions of dollars in the Australian barley industry as a result of downgrading of malting barleys to feed grade due to grain defects.

## Achievement/Benefit

BP and dark tip BP, KD and PHS, collectively called grain defects, downgrade malting barleys to feed category. BP is characterised by darkening of the grain tip which can be associated with, but is not caused by, fungal staining of the grain. KD is caramelisation of the whole grains as grain colour changes from a light straw colour of bright grain to a deep yellow or brown of badly weathered grain. Weather stained barley grains often have poor germination energy and vigour, and low post-harvest dormancy and steeps at a faster rate. Weather-bleached barley is also associated with grain defects and often exhibits PHS damage. PHS is germination of matured grain before harvesting. PHS has traditionally been at acceptable levels in several Australian barley varieties, unlike the Canadian barley varieties that typically experience PHS problems in Harrington and its derivatives. Canadian barley varieties are parents of two Australian barley varieties, Hamelin(Φ) and Flagship(Φ) that were released by the western and southern barley breeding programs, respectively. Both varieties have excellent malting quality standards, but they are susceptible to PHS. Gairdner(Φ) has also had PHS problems in the northern region and the southern barley growing areas. The problem of PHS in Australian barley varieties increased the likelihood of PHS susceptibility genes perpetuating in future barley varieties.

Annual economic losses due to grain defects amount to tens of millions of dollars in the Australian barley industry. Climate irregularities in recent years resulted in untimely rainfall during the harvest period. This untimely rainfall, coupled with temperature levels at the grain development stage, is believed to cause grain defects in barley and thus the associated economic losses. Traditionally, KD and PHS are the priorities for the southern barley growing regions, while PHS and BP are the priorities for northern barley growing regions. However, BP has now become a problem in a wider range of barley growing areas across Australia.

To limit economic losses of this magnitude due to grain defects, this collaborative project between Department of Agriculture and Food Western Australia (DAFWA) and Department of Agriculture and Fisheries Queensland (DAFQLD) was designed and implemented with the aims to:

(1) Identify germplasm with novel resistance to KD and BP, characterise them genetically and deliver the germplasm to Australian barley breeding programs with molecular markers.

(2) Identify QTL (non-5HL) conferring resistance to PHS, validate and characterise genetically, and deliver germplasm to Australian barley breeding programs with molecular markers.

(3) Develop efficient screening methods and nurseries, screen breeders' advanced breeding lines and current varieties to understand the grain defect risk for barley growers.

To achieve this project's objectives, more than seven different barley germplasm sets were evaluated for BP, KD and PHS across various environments. These seven barley germplasm sets were:

(1) International barley varieties from Europe, North America, China and Australian.

(2) Advanced breeding lines from Australian barley breeding programs.

(3) Mundah and Keel (MK) doubled haploid (DH) population.

(4) Flagship and ND (NF) DH population.

(5) Vlamingh(Φ) and Buloke(Φ) (VB) population.

(6) Barley entries in the National Variety Trials (NVT) system.

(7) Buloke and mutant line of pale-bright husk.

Each of the germplasm sets was evaluated in several trial sites with 34 different environments during the 2012 to 2014 growing seasons in QLD and WA. Phenotypic data of BP, KD or PS were recorded on samples collected from across environments. In addition, all the germplasm sets were studied genetically in order to identify genetic factors linked with BP, KD or PHS.

Overall, coastal environments such as Esperance, South Stirling and Geraldton in WA had high risk associated with BP or KD. A comparison of post-harvest germination tests and intact head misting in 10 barley varieties covering a range of dormancy

over a period of time showed that intact head misting was more indicative of and the most suitable test for PHS resistance. Mean and minimum temperatures at embryo development stage caused differences in extent of SD. When QLD and WA environments were compared, WA environments were more highly susceptible to BP than QLD environments in the MK and NF populations. In general, environments varied substantially in their vulnerability to BP and KD, and thus were classified into various risk groups. This observation has been included in the grains defect barley industry report (see attached Industry Report, Angessa et al. 2015).

When variations in tolerance of barley germplasm to BP, KD and PHS were considered, genotypes showed substantial variations for these grain defect traits. For instance in the NVT entries, Australian barley varieties varied significantly in BP and KD. Varieties such as Fathom$^{\phi}$, Flinders$^{\phi}$, Hindmarsh$^{\phi}$, La Trobe$^{\phi}$, Granger$^{\phi}$ , Westminster$^{\phi}$, Scope$^{\phi}$, Charger$^{\phi}$ and SY Rattler$^{\phi}$ had low kernel brightness compared with Baudin$^{\phi}$. This was in contrast to the variety Commander$^{\phi}$ which was brighter than Baudin. BP ranged from 8.6% to 54.2% in the Esperance environment in 195 lines, and from 0.6% to 59% in 120 lines of the MK population. In a single environment that MK and NF populations were commonly evaluated in, 50% of MK lines and 84% NF lines scored greater than10% BP. A SD test of 220 advanced breeding lines identified 12% PHS susceptible entries. In summary, genotypes with high KD and low BP identified in individual and across trial environments, and risk levels associated with trial environments has been summarised comprehensively and presented in the Industry Report 'Grains defect in barley due to kernel discoloration and black point' (see Attachment, Industry Report, Angessa et al. 2015).

Genetic assessment of barley germplasm identified several genetic factors associated with BP, KD or PHS. New QTL linked with BP, KD and SD were identified in 270 accessions, MK and NF DH populations. QTL linked with BP were identified on chromosomes 2H, 3H, 4H and 6H in WA and QLD trial environments. A mutant gene of bright grain colour was fine-mapped on chromosome 3H, and new germplasm with mutant gene developed from Australian barley genetic backgrounds. In the VB population, QTL linked with grain colour were mapped on chromosomes 2H, 4H, 5H, 6H and 7H. The QTL on chromosomes 2H, 4H and 6H were consistently detected in multiple environments. QTL linked with grain size and screening were mapped on chromosomes 1H, 2H, 5H and 6H. The QTL on 2H and 6H were negatively associated with grain colour. A QTL on chromosome 5H was the most important QTL controlling grain plumpness. A mutant gene linked with pale or bright grain husk was fine-mapped at 0.3cM to an SSR marker Bmac0067 on chromosome 3H in the Buloke and mutant population. This gene has been backcrossed into Buloke, Hindmarsh and Commander backgrounds.

A QTL linked with SD on chromosome 5HL was detected in nine environments and thus it was the most stable one followed by QTL on chromosomes 5HC, 4H and 6H. Among 45 new InDel markers designed to map the 5HC regions for SD, four closest linked markers were used to test 174 barley varieties that did not detect any clear haplotype. In addition, nine SNP markers were developed based on the BAC sequences. Again, no clear haplotypes were identified. Rather two SNP marker combinations were identified with potential uses in selecting for SD and malting quality traits. Analysis of more than 400 lines with 38 new markers identified nine diagnostic SNP markers and six different haplotypes. Four diagnostic molecular markers and candidate genes linked with SD were identified on chromosome 5HL. A candidate gene for PHS was identified and gene-specific molecular markers were developed. An allele from Gairdner was best for SD and malting quality traits in most barley growing regions.

## Additional Information

### Publications

Gong X et al. (2014). Seed dormancy in barley is dictated by genetics, environments and their interactions. Euphytica 197: 355.
Hua W et al. (2015). Study of genetic diversity of coloured barley. Genetic Res and Crop Evol 62: 395.
Fox G et al. (2015). QTL for black point (BP) in barley. 17th ABTS.
Huang Y et al. (2015). Effects of GA and ABA treatments on metabolite. Food Chem 192: 928.
Hua W et al. (2016). Identification and fine mapping of a white husk gene in barley. PLOS ONE.
Ye LZ et al. (2014). Haze activity of different barley trypsin inhibitors. Food Chem 165: 175.
Huang YQ et al. (2014). Genetic architecture of Ld inhibitor. BMC Plant Biology 14:117.

### Attachment

Angessa T et al. (2015). Barley grains defect due to kernel discoloration and black point. An Industry Report Prepared for GRDC. Murdoch University.