The Genetic Basis of Barley Black Point Formation

Timothy March

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The University of Adelaide

Faculty of Sciences

School of Agriculture, Food and Wine

Waite Campus

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Declaration

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Timothy March

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Abstract

Black point of barley grain refers to a discolouration of the embryo end of the grain. Historically black point has been proposed to be due to fungal colonisation of the grain. However, Koch's postulates have yet to be satisfied. The discolouration occurs during grain fill in response to high humidity or rainfall during the grain filling period. In wheat, which is also affected by black point, the discolouration has been proposed to be due to the oxidation of phenolic acids within the grain to form discoloured end products.

Within this study, two approaches were investigated in order to understand the proteins and genes associated with this disorder. Firstly, a proteomics approach enabled the identification of individual proteins associated with black point. Two-dimensional gel electrophoresis was used to compare the proteome of the husk and whole grain tissue of mature black pointed and healthy grain. Very little water-soluble protein was extracted from the husk tissue. However, a significantly larger amount of protein was extracted using a salt extraction buffer, indicating the husk proteins were mostly cell wall bound. Due to the effect of residual salt and low protein concentrations these proteins were not conducive to analysis using two-dimensional gel electrophoresis. Further experiments using acid hydrolysis of the husk tissue and subsequent amino acid analysis revealed that the proteins were bound to the husk cell walls via covalent bonds.

In contrast, large quantities of protein were obtained from the whole grain samples. This allowed statistically significant comparisons to be made between gels from healthy and black pointed grains. Two proteins were identified as being more abundant in black pointed grains. Mass spectrometry identified these as isoforms of barley grain peroxidase 1 (BP1). In addition, three proteins were identified as being more abundant in healthy grain. Mass spectrometry revealed these to be isoforms of the same protein with sequence similarity to a partial EST sequence from barley. Using 3' RACE the entire coding sequence of the gene was isolated which revealed that it encoded a novel putative late embryogenesis abundant (LEA) protein. Northern blot analysis was performed for *BP1* and *LEA* and showed that gene expression differences could not account for the differences seen in protein

quantities. Western blot analysis revealed that the LEA protein was biotinylated *in vivo* which is consistent with similar LEA proteins from other plant species. To further understand the role of these proteins in black point, antibodies were raised against the two proteins. Subsequent immunolocalisation studies indicated BP1 was present throughout all tissues of the grain whilst LEA was most abundant in the embryo and aleurone tissue.

The second major area of investigation within this thesis was to further delineate the previously identified quantitative trait loci (QTL) associated with black point in barley. Previous studies have reported QTL for black point and kernel discolouration in both barley and wheat. Comparison of the published QTL revealed a locus on the short arm of chromosome 2H to be of particular interest. To identify genes underlying this QTL the genomes of barley, wheat and rice were compared. An *in silico* approach showed that the QTL shared macro-synteny with rice chromosomes 4 and 7.

From the rice genome sequence, barley ESTs with sequence similarity were selected. In total, 20 ESTs were selected based on two main criteria: their putative role in black point and also being evenly spread across the region of the QTL length. These QTL were mapped within the Alexis x Sloop double haploid population. This approach revealed that there was some conservation of synteny but also identified clear boundaries where synteny between barley and rice had been lost since divergence. Significantly, the additional markers mapped to this region have enabled the initial black point QTL to be reduced from approximately 30cM to 20cM.

In conclusion, this study has added significant knowledge our understanding of the genetics of black point in barley through the use of two approaches. The proteomics approach has aided in understanding the biochemical processes occurring within the grain in response to black point. The comparative genetics approach has aided in understanding the genetic control of an important region of the genome influencing black point susceptibility. Combined, these findings will direct future research endeavours aimed at producing black point resistant barley cultivars.

Abbreviations

2-D	Two-dimensional
BAC	Bacterial artificial chromosome
BP	Black point
BP1	Barley grain peroxidase 1
CBB	Coomassie brilliant blue
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
cM	Centimorgan
DIGE	Difference in gel electrophoresis
DNA	Deoxyribonucleic acid
DPA	Days post anthesis
DTT	Dithiothreitol
EST	Expressed sequence tag
Fmoc	9-fluorenylmethyl chloroformate
g	Gram
g	Gravitational force
gDNA	Genomic deoxyribonucleic acid
GRP	Glycine-rich proteins
h	Hours
H	Healthy
HPLC	High performance liquid chromatography
IPG	Immobilized pH gradient
kDa	Kilodaltons
LDS	Lithium dodecyl sulfate
LEA	Late embryogenesis abundant
M	Molar
Mb	Mega-base
MES	4-morpholineethanesulfonic acid
min	Minute
mL	Milliliter
mM	Millimolar
MS	Mass spectrometry
NBT/BCIP	5-Bromo-4-Chloro-3'-Indolyphosphate p-Toluidine Salt/Nitro-Blue Tetrazolium Chloride
NCBI	National centre for biotechnology information
°C	Degrees Celsius
PAC	P1-derived artificial chromosome
PAL	Phenylalanine ammonia lyase
PBS	Phosphate buffered saline
PHS	Pre-harvest sprouting
pI	Isoelectric point
POPP	Protein or oligonucleotide probability profile
PRP	Proline rich protein
PVDF	Polyvinylidene fluoride
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Standard error
TAE	Tris-acetate-EDTA
TAL	Tyrosine ammonia lyase
TCA	Trichloroacetic acid
µm	Micrometer
V	Volume