Identification of Australian Barley Varieties

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Executive Summary

Twenty seven (27) Australian barley varieties have been analysed with the Agilent BioAnalyser using Protein 200 chips. The samples tested were genuine samples provided by the Australian Winter Cereals Collection (AWCC) in Tamworth. Unambiguous differentiation of 16 of these varieties is achieved with this system.

Three pairs of varieties - Lindwall and and Baudin, Parwan and Skiff, Barque and Grimmert - may be distinguished from the remaining varieties, but cannot be distinguished from the other variety in its pair.

Five varieties - Arapiles, Schooner, Sloop, Gairdner and Chebec - showed similar protein patterns and could not be distinguished from each other.

The 27 varieties tested, can therefore be classified into 20 groups, 16 containing an individual variety, 3 groups with a pair of varieties, and 1 group of five.

In addition, three of the varieties – Franklin, Mackay and Wyalong – existed in two ore more biotypes. The presence of these biotypes is unlikely to cause any problem in practice, as normal practice would be to ground many grains, and a representative mixture would be analysed.

Representative electrophoretograms of each variety have been placed into files which will serve as libraries. Thus, the identification of harvest samples of these varieties may be confirmed by comparison against this established library.
Background
The lab-on-a-chip method has been developed for quickly identifying wheat varieties on a Value Added CRC project carried out by Food Science Australia. Preliminary analysis of other grain species indicated that the method should also be applicable more widely than its initial use with wheat varieties described in the following two articles


Identification of barley varieties is important for the industry. Thus, GrainCorp proposed that the ability of the lab-on-a-chip method should be investigated for distinguishing barley varieties currently grown in Eastern Australia.

Aim: To determine the feasibility of creating a rapid and computer-assisted variety identification system for the identification of barley.

Scope of the project:
- Samples of authentic barley varieties to be obtained from the Australian Winter Cereals Collection to use in the variety identification process.
- The Agilent lab-on-a-chip bioanalyzer to be used to separate the protein bands extracted from barley using 1%SDS and 1%DTT. Five kernels (5 replicates) for each variety will be analysed. To test the reproducibility of the method five extracts of the same sample will be applied on to the bioanalyzer.
- The patterns obtained for each variety to be stored in a library in order to assist in variety identification of unknown barley samples.

Barley varieties to be identified:

Procedure:

1. Samples source
The above varieties were obtained from the AWCC, Tamworth, as the most authentic source of the varieties, except that the variety Quasar was provided to us by Neil Barker from GrainCorp. Nevertheless, there is the possibility that foundation seed and farm seed may differ slightly in genotype from the AWCC samples. All samples were analysed as 5 single grains, thus to obtain a preliminary indication of the possibility that the varieties exist as multiple biotypes (i.e. one variety being represented by more than one protein pattern). When multiple biotypes were identified in the initial set of grains, further grains were analysed along with wholemeal samples.

2. Sample analysis
**Requirements for sample extraction**

- **Grinder** – any simple type is suitable (electrically operated), preferably taking a small-sized sample of grain (say, 10 – 50 grams). Fine grinding is not essential.
- **Balance** – capable of weighing about 30 mg ground grain into a sample tube.
- **Extraction reagent** – 1% sodium dodecyl sulfate (SDS) together with 1% dithiothreitol (DTT). A stock solution of 1% SDS can be kept almost indefinitely at room temperature (1.0 gram SDS in water – distilled or demineralised – made to 100 mL). A fresh reagent should be made from this daily, by adding 100mg DTT to 10 mL of the 1% SDS solution.
- **Vortex mixer** or a **Thermomixer** – useful for quickly suspending the wheatmeal in the extraction solution. It is not essential; mixing can be done by hand shaking.
- **Centrifuge and sample tubes** – generally a multi-place small bench-top model that takes “Eppendorf-type” plastic tubes.
- **Water bath** – to heat extracts + Agilent reagents at 100°C for a few minutes
- **Pipettes** – capable of dispensing up to 1 mL of extraction solution. Preparation of extracts for the Lab-chip requires the use of an automatic pipette that can dispense about 20 μL.
- **Lab-on-a-chip equipment** – Agilent bioanalyzer model 2100. (Recently, alternative equipment has become available, marketed by BioRad, USA.) The Agilent equipment has a “footprint” on the bench of 15 X 40 cm. A laptop computer is placed beside it, but this could alternatively be some other form of computer.
- **Protein chips and reagents** – Agilent provides a packet of 25 LabChips (type Protein 200+), together with reagents.

**Likely costs (in Australian dollars)**

- **Grinder** (Mortar and pestle) – $30
- **Balance** – $2,700
- **Extraction reagent** – sodium dodecyl sulfate (SDS) - $170  Dithiothreitol (DTT) - $170
- **Vortex mixer** – $320
- **Thermomixer** - $3,000
- **Centrifuge and sample tubes** – $2,200
- **Water bath** – $1,055
- **Pipettes** – to dispense extraction solution (1,000 μL) and automatic pipette (20 μL) to prepare extracts for the Lab-chip with relevant pipette tips and boxes - $800
- **Lab-on-a-chip equipment** – Agilent bioanalyzer model 2100 - $39,000
- **Protein chips** – packet of 25 LabChips + reagents is $800-$900, equivalent to 2,500 tests
- **Other relevant items** (Beakers (10, 100 and 250 mL), Timer, Spatula) - $50

**PROCEDURE**

1. **Grind** a sample of grain, noting identification details for grain and wholemeal containers. The procedure is equally valid for flour samples. Alternatively, it may be necessary to crush and extract single grains individually – see comments below on interpreting results for samples containing a mixture of varieties. It is usual to include one or a few authentic samples relevant to those being tested.

2. **Weigh** out 20 ± 2 mg into a small plastic tube. Add 0.3 mL extraction solution (1% SDS + 1% DTT), and agitate vigorously, preferably by vortex mixing, for a few seconds. Shake by hand occasionally during 3 minutes' standing in a heating bath at 65°C or use the thermomixer at 65°C. The actual weights and volumes are important only to ensure that there is a constant ratio of wheatmeal to extracting solution, namely, 20 mg + 0.3 mL. It is usual to preform extractions in multiples of ten, to suit the ten-place capacity of the lab-chips.

3. **Centrifuge** the tubes, to provide a clear supernatant for application to the LabChip.

4. **Mix** 4 μL of clarified extract with 2 μL of Agilent sample buffer, and heat for 3 or 4 minutes at 100°C in a boiling water bath. Add 84 μL water to each, mix, and load extracts into the respective
sample positions of the LabChip, recording sample identities. Also load the “protein ladder” reagent, mixed with Agilent sample buffer as specified by the Agilent manual.

5. **Switch on the equipment and control running conditions from the software.**

6. **Interpret the results of identification using the PatMatch program.** Some typical results are shown in Figures 1 to 7. Mainly these appear as simulated gel-electrophoresis patterns, which may be more suitable for comparison purposes. However, it should be realised that these are derived from the basic elution profiles (shown as series of peaks for Figures 5 to 7), which provide the quantitative results used by the pattern-matching software.

**Results**

Almost all of the varieties were able to be distinguished using the Lab-on-a-Chip method (Figures 1-3). However, 5 varieties such as Arapiles, Schooner, Sloop, Gairdner and Chebec showed similar protein patterns (Figure 4). Barley varieties indicated in pairs also showed similar proteins bands for each pair. They are, Lindwall and Baudin; Parwan and Skiff; Barque and Grimmett.

![Figure 1: Comparison of varieties from left to right – Sloop, Weeah, Tilga, Arapiles, Sloop, Baudin, Chebec, Gairdner, Galaxy, Grimmett, Barque, Sloop and Binnalong](image1)

![Figure 2: Comparison of varieties from left to right – Dhow, Sloop, Parwan, Skiff, Wylong (biotype1), Wylong (biotype2), Mackay (biotype1), Mackay (biotype2), Sloop, Franklin (biotype1), Franklin (biotype2), Sloop and Quasar](image2)
Figure 3: Comparison of varieties from left to right – Sloop, Picola, Kaputar, Lindwall, Schooner Tantangara, Sloop, Clipper, O’Connor, Tallon, Galleon and Sloop

Figure 4: Comparison of varieties looking similar from left to right – Arapiles, Chebect, Sloop, Schooner and Gairdner

Three of the barley samples had biotypes. They are as follows:

- Franklin (Figure 5)
- Mackay (Figure 6)
- Wyalong (Figure 7)
Figure 5: Franklin showing biotypes (lanes 1 and 2) and a wholemeal sample of Franklin (lane 4).

Figure 6: Mackay showing biotypes (lanes 9 and 6). One single seed (from a total of 8 grains analysed separately) for this variety was missing one small peak as indicated on the picture.

Figure 7: Wyalong showing biotypes (lane 1 and 2) and a wholemeal sample of Wyalong (lane 3). One single seed (from a total of 8 grains analysed separately) for this variety was different from the others.

For the purpose of checking for authenticity of the barley samples, a CD containing the Lab-on-a-Chip runs of standard barley samples has been prepared for GrainCorp. The comparison files provided are given in Table 1.

These library files have been provided as "Read Only". It will be possible to add unknown samples to each to permit a comparison with known varieties (as detailed below), but the integrity of the library file will be maintained as the modified file could then only be saved under a new name.
<table>
<thead>
<tr>
<th>File Name</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal_Bin Wee_Dho Kap_Cli.xac</td>
<td>Galaxy, Binnalong, Weeh, Dhow, Kaputar, Clipper</td>
</tr>
<tr>
<td>Gall_OC_Tan_Tal_Til_Qua.xac</td>
<td>Galleon, O'Connor, Tantangara, Tallon, Tilga, Quasar</td>
</tr>
<tr>
<td>Lin_Bau_Gri_Bar_Par_Ski.xac</td>
<td>Lindwall, Baudin, Grimmett, Barque, Parwan, Skiff</td>
</tr>
<tr>
<td>Pic_Sch_Slo_Ara_Che_Gai.xac</td>
<td>Picola, Schooner, Sloop, Arapiles, Chebec, Gairdner</td>
</tr>
<tr>
<td>Fra_Mac.xac</td>
<td>Franklin, Mackay</td>
</tr>
<tr>
<td>Wyal.xac</td>
<td>Wyalong</td>
</tr>
</tbody>
</table>

A step-by-step guide to carrying out a comparison
1. Open the expert 2100 software
2. Click on Data icon in the contexts panel
3. Open the data file which contains the sample “unknown” which you want to compare
4. Click on Comparison icon in the contexts panel
5. Open the file (which is provided to you as .xac extension) which contains the variety “authentic” which you think matches your unknown sample. For example, if you think your sample is Sloop, open the comparison file which contains Sloop (Pic_Sch_Slo_Ara_Che_Gai.xac).
6. Once the comparison file is open, left click your mouse on the unknown sample now found under the heading “Select Data Files”, then right click the mouse and select “Add Sample to Comparison File”.
7. Your unknown sample will appear on the comparison file and matching can be done visually.
8. Your unknown sample should be deleted from the comparison file as soon as you finish identifying the unknown sample.
9. This can be done by right clicking on the unknown sample under “all comparison files” and selecting “Delete Sample from Comparison File”. Alternatively, close the file, discarding changes.
10. If it is necessary to kept the modified comparison file, this can be done by saving it under a different name.

Conclusion
The Lab-Chip system was shown to be effective in distinguishing between most of the 27 Australian barley varieties, using the extraction and analysis system developed for wheat varieties. Some barley varieties were more readily distinguished than others. The following combinations of varieties had similar electrophoretic patterns:
- Sloop, Schooner, Chebec, Gaidner and Arapiles
- Lindwall and Baudin
- Parwan and Skiff
- Barque and Grimmett

Biotypes were detected in three varieties, namely, in Franklin, Mackay and Wyalong. This means that these varieties are represented by two distinct patterns due to a small degree of polymorphism. The presence of biotypes for these varieties should present only minor complications provided wholemeal samples are analysed for both authentic and test samples.