



Routine Analysis of the Varietal Identity and Purity of Barley Using DNA based Approaches

Robert J Henry¹, Effie Ablett¹, Timothy A Holton¹, Gary Ablett¹, Michelle Ford¹ and Peter Portmann²

¹The Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, 2480

²The Grain Pool of WA, 172 St. George's Terrace, Perth, WA, 6000

DNA based varietal analysis has now moved from being a research tool to become a routine analytical method (Henry 2001). Cost effective DNA marker analysis requires high throughput facilities with a high level of automation. Microsatellite or simple sequence repeat markers (SSR's) provide a reliable method for routine analysis. Large numbers of microsatellites have been developed for barley and large sets of marker data have been collected on Australian and international barley varieties. More recently single nucleotide polymorphism (SNP) markers have become a useful alternative. A wide array of techniques is now available for SNP analysis. Detection of admixtures of barley is possible without the need to analyse individual grains. Issues associated with the transition from research to routine applications will be discussed.

DNA analysis of grain samples has often required germination of the sample so that DNA can be extracted from the grain. We now use ground grain samples directly as a source of DNA for analysis. Microsatellite analysis of DNA from a bulk sample has been shown to detect minor contaminants. Detection of alleles for the main variety establish the identity of the sample and minor peaks corresponding to alleles from contaminating varieties can reveal contamination levels of 1% or less. This avoids the need to test large numbers of samples individually as have been required for techniques such as protein electrophoresis.

We have employed DNA fingerprinting techniques to distinguish contamination of Harrington grain by other varieties, namely Stirling, Fitzgerald and Gairdner. Unlike morphological and protein-based tests, DNA techniques rely on a biochemical which is invariant in all tissues. The advantage of this is that the assays are not influenced by crop growth conditions, or seed treatment and storage environment. Also with the huge numbers of DNA markers available, DNA-based techniques particularly those involving microsatellite markers, are rapidly becoming the most widely accepted tools for plant variety identification.

Microsatellite markers have been developed that successfully distinguish Stirling, Fitzgerald and Gairdner from Harrington. PCR with primers to a microsatellite produced a band with Fitzgerald, and a band with Stirling, which are both markedly

different from the bands produced by Harrington and Gairdner. A second microsatellite can also be used to distinguish Stirling, Fitzgerald and Gairdner from Harrington. These band sizes are all highly reproducible, and comparison of both markers can be used to distinguish each of these varieties from the other three. Additional markers are available to distinguish these varieties from 22 barley cultivars tested so far.

Results from defined mixes of Stirling, Fitzgerald, and Gairdner with Harrington suggest we can detect as little as 1% contamination by Fitzgerald, 2.5 % contamination by Stirling, and 5% contamination by Gairdner. Further development is needed to produce a reliable assay for detecting lower levels of Gairdner. Initial screening of 57 samples supplied to us suggested all have less than 5% contamination with Stirling or Fitzgerald. Some samples had peaks corresponding to 2.5% contamination with Stirling. The 24 samples tested had no major contamination (>10%) with Gairdner.

References

1. Henry, R.J. (2001) Plant Genotyping: The DNA Fingerprinting of Plants, Henry, R.J. (ed) C.A.B. Int., U.K.
-