Epiheterodendrin in malting barley: molecular evidence for cytochrome P450-mediated production

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Cyanogenesis in barley: a quality problem for the Scotch Whisky industry. Many plant species produce cyanogenic glucosides which, through release of the breakdown product hydrogen cyanide (HCN), have been implicated in the natural defence response to herbivores and pathogens, and are also believed to have roles in nitrogen storage and osmoregulation. In certain cultivars of barley, known as 'producers', a leucine-derived cyanogenic glucoside, epiheterodendrin (EPH), is present at high levels in young seedlings (malt). Fermentation of malted barley leads to hydrolysis of EPH, via action of yeast-derived β-glucosidase and subsequent heating during distillation, to form HCN. A reaction within the distillate between HCN and ethanol, in the presence of copper and oxygen, leads to trace but significant levels of the potentially carcinogenic compound ethyl carbamate. Low ethyl carbamate varieties of barley are therefore high priority for the Scotch Whisky industry, leading to our detailed molecular characterisation of EPH pathways and development of an unambiguous marker for varietal selection by malting barley breeders.

Characterisation of EPH genes from barley Cytochrome P450 enzymes form the largest family of plant proteins, with over one thousand members identified, catalysing a wide array of both simple and complex reactions, generating a diverse range of natural plant products. EPH has been proposed to be derived from the amino acid leucine through action of two cytochrome P450 enzymes, cyp79 and cyp71. Barley ESTs with homologies to cyp79 and cyp71 were identified, representing partial 3' cDNA sequences, and the corresponding gene fragments were

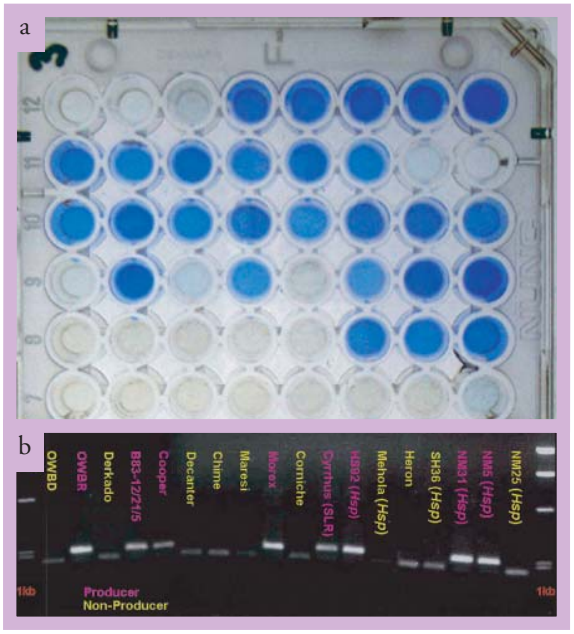

Figure 1 (a). Phenotypic assay of EPH activity: clear represents 'non-producers' and blue represents 'producers'; (b), Genotypic determination using multiplex PCR-based screen: 'producers' are in red and 'non-producers' are in yellow.

subsequently isolated using PCR. These gene probes were used to screen a barley (cv. Morex) bacterial artificial chromosome (BAC) library to isolate the full-length gene sequences. Fingerprinting of the BAC clones, utilising SNaP-shot-based fluorescent labelling of restriction-digested fragments, clustered the clones into two distinct groups. Single BAC clones for each gene were subcloned and sequenced revealing full length genes encoding cyp79 and cyp71, which contain two and three exons respectively, and also indicate the presence of putative retroelement sequences, which are a common feature of the barley genome. Expression of *cyp79* and *cyp71* genes were determined by real-time PCR, and both genes were clearly up-regulated in EPH 'producers' compared to 'non-producers' and showed similar patterns of temporal decrease in expression levels between 3-day and 4-day-old seedling leaf material. Using polymorphic barley populations, both genes genetically mapped to the same position, which was also confirmed as the EPH locus on chromosome 1H, first identified at SCRI.

Development of an EPH molecular marker Central to selection of barley varieties with low levels of potential ethyl carbamate generation is the development of a robust unambiguous molecular marker. Previously at SCRI, a simple-sequence repeat (SSR) marker (BMAC213) was generated which showed good, but not complete, association with the EPH phenotype, as determined by the biochemical assay. Oligonucleotide primers were designed to re-amplify regions of the *cyp79* and *cyp71* genes in a range of 'producer' and 'non-producer' barley accessions, including cultivated, landrace and wild barley germplasm. These primers only amplified in the 'producer' accessions, the *cyp79* gene product being absent in 'non-producers'. This complete association with the EPH phenotype, clearly distinguishing 'producers' from 'non-producers', was therefore used as the basis for development of a multiplex PCR-based screen (Fig. 1). This assay is currently being used for efficient, reliable and cost-effective marker-assisted varietal selection in commercial malting barley breeding programmes.

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