A large scale mutation grid in barley

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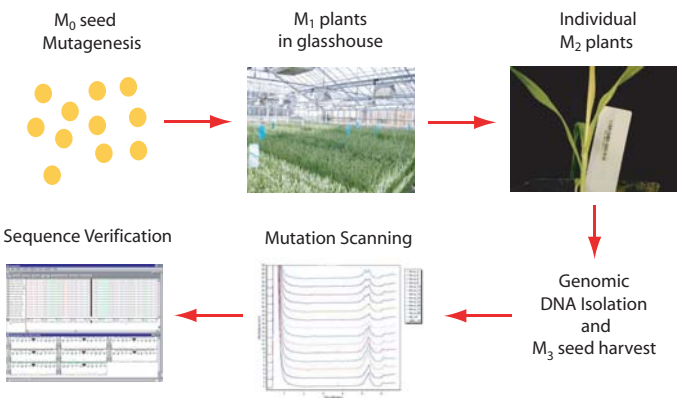
Introduction

Functional genomics in barley (*Hordeum vulgare* L.) is a relatively under-developed area of research due to the lack of structured reverse genetics populations. Here, we describe the creation of a large-scale mutant population derived from Ethyl-methanesulfonate (EMS) mutagenised barley (cv. Optic). Approximately 25,000 M_2 plants were obtained from three different treatments of EMS (20mM, 30mM, 40mM). AFLP analysis of mutation frequency indicates a range of one mutation every 40,000-100,000 nucleotides. Genomic DNA has been isolated from each plant and arrayed into one dimensional pools for identifying mutations in genes of interest. Currently, several mutation scanning approaches can be implemented for screening the population. The Cel I cleavage assay has been successfully used in barley, but is currently limited to pools of 8 plants using an unmodified protocol. Recent advances in dHPLC detectors (Transgenomic, Inc.), has shown the ability to discover SNPs in pools of 48 plants without the use of fluorescently-labeled primers. The multifunctional property (forward & reverse genetics) of this population is a valuable asset for the worldwide barley research community.

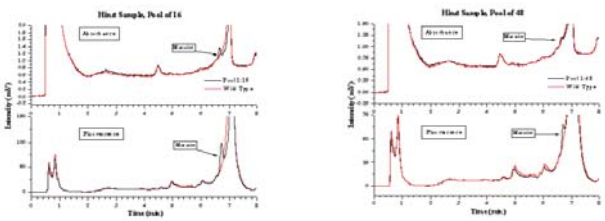
Overview of Creating the EMS Population



M_0 seed was mutagenised with EMS. The mutagenised M_0 seed gave rise to M_1 plants. The M_1 plants yielded M_2 seed. No more that two M_2 seed per one M_1 parent was taken forward, self polinated, and grown to maturity. Young leaf tissue was harvested for genomic DNA isolation and the M_3 seeds were harvested. Individual M_2 genomic DNA was 1-Dimensionally pooled and stored in 96 well titer plates. Regions of interest will be amplified with gene specific primers and scanned for mutations. Individual M2 lines will be identified and mutations confirmed.

Mutation Detection Techniques

A critical aspect in implementing our reverse genetics approach is the choice of a mutation detection method which will allow us to screen PCR-amplified target gene sequences with high sensitivity & specificity as well as low cost. Through a collaboration with Dr. Tony Yeung (Fox Chase Cancer Center) we have investigated the efficacy of enzymatic cleavage of heteroduplex DNAs using CEL I, a mismatch specific endonuclease. The CEL I system is a simple assay that requires PCR amplification of the target sequence, denaturation and annealing to allow formation of heteroduplexes between the wild type and the mutant allele, enzymatic mismatch cleavage, and analysis of the cleaved products by gel electrophoresis. Currently single mutations can be detected in pools of 8 plants (16 alleles).



dHPLC analysis of test pools

Pools of 16 and 48 alleles analysed on a standard UV and the new Fluorescence detector. Please note the difference in signal strength.

In addition, we have investigated the application of denaturing HPLC which is advantageous over other mismatch detection systems as it requires no post amplification template modification, is not gel based, and as a result is both inexpensive and HTP. Through a collaboration with Transgenomic, we have tested their next generation detector. In our test panels the new detector has shown the ability to consistently detect an individual SNP in pools of 48 alleles (24 plants). This increase in pooling depth will greatly reduce the cost and increase the speed of screening the population.

Mutant phenotypes

Over 23,000 M_3 families were scored in the field for visible mutant phenotypes. About 30% of the families exhibited variation, which covered the recorded mutation spectrum for barley. The most frequent classes (over 100 M_3 families) were short, late, early, prostrate, pale green, necrotic, narrow leaf and dense spike. Less frequent phenotypes (less than 100 M_3 families) included tall, GA-dwarf, extreme dwarf, glossy leaf, twisted leaf, broad leaf, awnless, lax ear, mulitiflorus, round grain and large grain. Rare (less than 10) mutants included elongated outer glume, elongated basal internode, awned palea, uni-culm, multi-noded and fragile stem. Novel mutations were also recorded. Comparisons with classic mutation desciptions allowed us to assigned putative genes symbols for many lines. Information is stored in a web accessible database:
http://bioinf.scri.sari.ac.uk/distilling/distilling.html

'Field of dreams'

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