Agarose-Based EcoTILLING as a Tool for Targeted Genotyping in Oryza Species

Introduction

EcoTILLING, a derivative of TILLING (Targeting Local Induced Lesions in Genomes), is a reverse genetics tool that is useful in identifying polymorphisms in natural populations at candidate genes of interest. We demonstrate the utility of agarose-based EcoTILLING as a fast and robust technique for detecting SNPs on a mini-core collection of 1536 diverse O. sativa and a panel of 95 wild AA genome Oryza species accessions. Candidate genes putatively involved in drought tolerance were identified through convergent evidence taking into account functional annotation, altered expression, co-localization with QTLs, and/or allelic shifts under selection. Locus-specific primers were designed using the Nicksonbare genomic sequence. Samples were contrasted against two reference lines, Nicksonbare (japonica type) and IR64 ( indica type). Mismatch patterns detected on agarose were confirmed by sequencing representative samples. The mini-core collection was phenotyped under vegetative drought stress (upland) and control conditions (lowland) field. Within isozyme groups there was large phenotypic variation related to drought stress, and significant associations established between traits and haplotypes of candidate genes within isozyme groups.

EcoTILLING was confirmed as a useful diagnostic tool for the taxonomic assignment of AA genome wild Oryza species. EcoTILLING individual accessions confirmed the taxonomic status of the non-Asian species, O. barthii, O. longistaminata, O. glumaepatula, and O. meridionalis but not the Asian species O. rufipogon and O. nivara which were not clearly differentiated from each other.

Candidate genes

- DNA pooling
- PCR primer
- PCR product
- CEL 1 components
- Gelatin extract
- CIE buffer
- SUP water
- Digestion
- Post digestion
- Electrophoresis

Plant materials

- Extended to 1536 O. sativa and 95 wild AA genome Oryza species accessions

Acrylamide vs. Agarose-based EcoTILLING

Mismatch detection for the MAP2 gene on acrylamide (via the LICOR system) and on agarose platforms. Complementary fragments indicating SNPs are highlighted by similar colored solid and broken lines viewed on the LICOR 700 and 900 channels, respectively. On agarose, corresponding bands are indicated by similarly colored arrows. Samples on agarose correspond to the last 24 samples on the LICOR image.

Results

- Pattern detection on agarose and confirmatory sequencing

- Left: mismatch variation patterns for 1536 O. sativa accessions contrasted against IR 64 and Nicksonbare (NB) detected by agarose-based EcoTILLING. Below, although adjacent SNPs (e.g., D17447 and D17448 in haplotype b) are not resolved on agarose (pattern 1), haplotypes generated from IR shows consensus tree with Rlp17447. A, B, C, D, E, F, G, H, I, J, and K indicates the 10 accessions. Sampled accessions with similar patterns detected on agarose (values in parentheses).

Association genetics

- Field screening of the mini-core collection was conducted over three consecutive dry seasons (2003-2006) under vegetative drought stress (upland) and well-watered conditions (lowland).

Association between sucrose synthase haplotypes and phenotypes under drought stress in the Japonica and indica subpopulations (1-way ANOVA, data for each subpopulation were analyzed separately). Bars with different letters significantly differ at the p<0.05 level. Error bars represent ±1 s.e.

Definition of biosystematic relationships in AA genome Oryza species

- Neighbor joining phylogenetic trees generated using Nei's Distance Method (PowerMarker v3.25) based on Mismatch patterns for 3 primer. Numbers on nodes correspond to bootstrap values. Species delineation among the non-Asian species supports current taxonomic classification.

Conclusion and future prospects

- Agarose-based EcoTILLING is an efficient genotyping tool for the Oryza germplasm. SNPs or indels detected on agarose are largely supported by sequence data and may prospectively be useful with associated phenotyping. Its utility as a diagnostic tool in Oryza classification can be further explored using primers for other low-copy nuclear genes with broader genome coverage.


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