TILLING for the Tomato functional genomics community

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Building a TILLING resource

**STEPS**

I. Produce mutant population
II. Harvest and bank DNA and seed
III. Develop mutation discovery system
IV. Set up service and stock distribution

**REQUIREMENTS**

need critical mutation density > 1/600 kb
must be efficient, cheap, reliable
cost effective, self-sustainable
Goal and Objectives

To establish a TILLING resource with a high-density of mutations (1/0.5 Mb or higher)

• Compare mutagens (MNU, EMS, UV), mutagenesis protocols, and varietal response
• Produce pilot and full size populations (6,000 plants)
• Establish a public service
Choice of variety

• VFNT Cherry
  – easy growth in greenhouse and field
  – TMV resistant
  – indeterminate growth, easy propagation
  – semi-glabrous stem marker
  – strong cleistogamy
  – high fertility of M1 after mutagen treatment

• Considered: M82, H1706, E6203
Choice of mutagen and protocol

• Seed mutagenesis
  – EMS: method of choice to date
    • killing curve
    • 07-08 M1 population, 12,000
    • 08-09 M2M1 double mutagenesis population, 6,000
  – MNU: strong phytoxicity, dosage difficult
    • killing curve

• Pollen mutagenesis
  – UV
    • killing curve
    • 07-08 population, 750, and pilot test
Killing curve: finding the range

M1 plants (from treated seed)

140  120  100  0
mM EMS
Example of EMS response

M1s after high concentration EMS

“Blindness” in E6203  Regularity in VFNT Cherry
Single and double mutagenesis

EMS

M1 plant

M2 seed from 12,000 lines

EMS

M2M1 plant

(M2)^2 seed from up to 6,000 lines

single seed
Mutation testing

EMS (M2)^2 -> Summer pilot on 768
5,000 (M2)^2 field growth in Summer 09

- TILLING library
  4,000 M3 lines ready in Fall 2009-Winter 2010
From mutant population to TILLING

EMS → seed → M1 → M2 → M3

Illumina sequencing → PCR of target genes → 2-D Superpools in 96-well plate → 8-fold pools → DNAs

mutant identification
TILLING by Sequencing

Plate of 96x8 = 768 individuals

12 column superpools of 64 individuals

8 row superpools of 96 individuals

PCR of 20 targets

12 libraries
Analysis of sequence changes

Os5g47850

high P candidate mutation

frequency of G>A change
Deconvolving 8x pool and confirming mutation

RE analysis

8 pool members

WT | Mut
---|---
994
645
462
347
14

Suc4 G830A digested O/N with HpaII
SB 10/18/08
Example: TILLING 3-08 experiment

• 19 genes TILLED
• 3 genes failed (bad primers)
• 16 genes
  – 83 candidates in 13 rice genes
  – 55 candidates in 3 camelina genes
  – 20 mutations deconvolved and confirmed
  – 6 not confirmed (=23% FDR)
Conclusions

The prospects for a well mutagenized population of tomato are good. A system is in place for mutation discovery and stock distribution.

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