Britt Lab: DNA repair, mutagenesis, recombination, damage response, accelerated breeding

Desiccation induced DNA double strand breaks (DSBs)

CRISPR-cas9 induced DSBs: Targeted mutagenesis without tissue culture “Editit” w’ Neelima Sinha

Haploid inducing lines via mutant centromeric proteins

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Why are CRISPRs revolutionizing plant breeding?

- **CRISPR/cas9** targets DNA double strand breaks and mutations to specific sequences. Thus targeted mutations can be induced without outcrossing.

- **CRISPR-generated mutations in tomato are not subject to regulation in the US.**

- **Licensing/ownership** of cas9 tech is currently a mess, but similar enzymes are in development.

- In crop plants, CRISPR/cas9 is usually introduced as a T-DNA (transgene). The transgene is then crossed out in the next generation (it is not linked to the target).
A rapid and simple method for CRISPR mutagenesis

**Formerly** plant breeding depended on existing randomly generated alleles

**Current** gene editing technologies (e.g., CRISPR) can target specific genes for mutagenesis, without affecting the rest of the genome. This process is extremely efficient in tomato.

**But** regenerating entire plants from single edited cells is:

- laborious
- expertise- and equipment intensive
- requires many months (4 to 24)
- for many crops/varieties impossible-

**We can fix that problem**- with a fast (2 mo), low-tech methodology for plant regeneration.
A bottleneck, to different degrees in different crops

- **Problem:** It’s an Art!!
- IN VITRO regeneration is slow, expensive, requires extensive experience, different for every species, and for many crops is impossible.
Our solution: EditIt

• The Editit process—so far tested only in tomato—produces heritable mutations quickly without sterile culture
• No protoplasts are involved
• The final product carries no transgenes

(Serving suggestion)
Shoots are easily/quickly regenerated in our model species (tomato)
Editing occurs frequently (10-20% of shoots)
Mutations generated are heritable, and transgene is lost in the next generation
Seed to seed generation of transgene-free mutants in 5-6 mo.
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