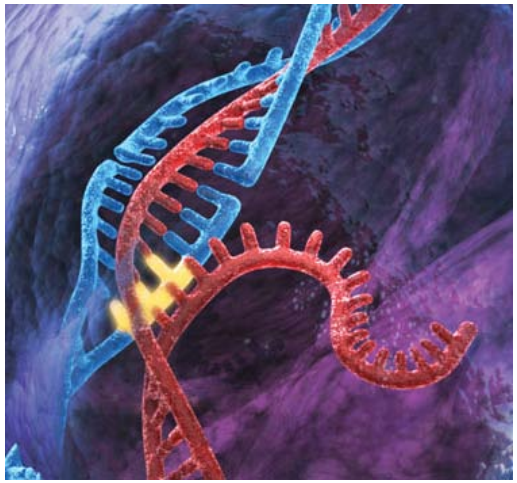
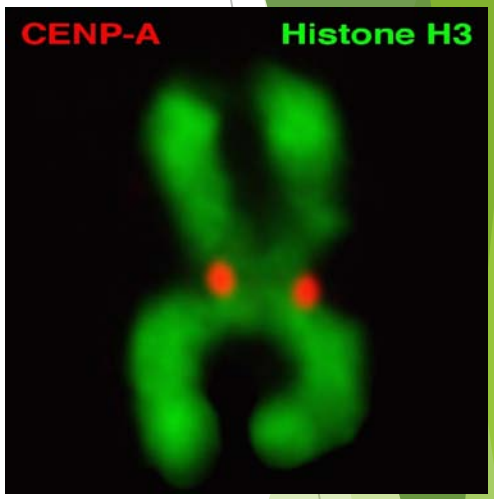
Britt Lab:
DNA repair, mutagenesis, recombination, damage response, accelerated breeding

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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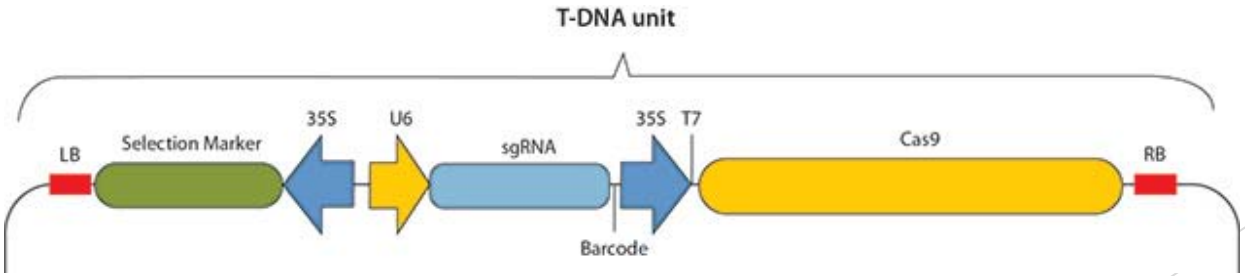
Why are CRISPRs revolutionizing plant breeding?

- **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).

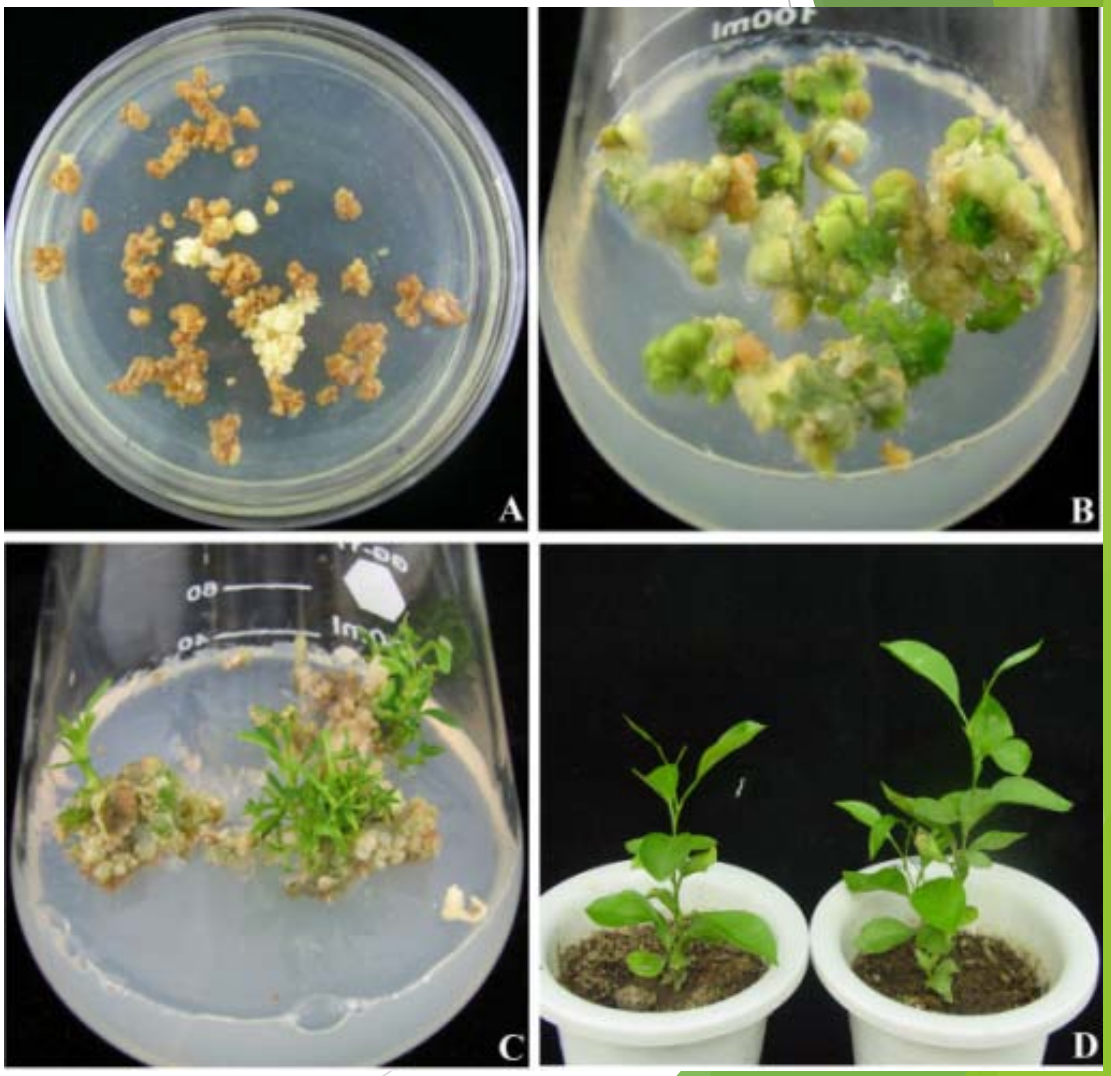


A rapid and simple method for CRISPR mutagenesis

- **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)
  - <u>for many crops/varieties impossible-</u>
  - **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

Our solution:

- The Editit process- so far tested only in tomato- produces heritable mutations quickly <u>without sterile culture</u>
- No protoplasts are involved
- The final product carries no transgenes

+

EditIt!

(Serving suggestion)

In tomato

- 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.

EditIt
Research and Development Team

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