Suspension cell genome editing in carrot using CRISPR/Cas9

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Summary

- Cultivated carrot (*Daucus carota* subs. *carota* L.; $2n = 2x = 18$) is a globally embraced root crop whose production has quadrupled over the last 40 years[1].
- This current boom is largely due to modern breeding efforts to increase orange (α- and β-carotene), yellow (lutein), red (lycopene) and purple (anthocyanins) pigmentation in roots[2].
- Carrot presents unique opportunities for genome editing due to the use of a suspension cell delivery system and single-cell regeneration via somatic embryos.
- CRISPR/Cas9 reagents were designed to target key genes controlling pigmentation in carrot roots and used to generate transgenic whole plants to study mutations.

Introduction/background

Somatic embryogenesis via carrot cell culture has been a unique and important model system for early plant development since the independent discoveries made by Reinert[3] and Steward[4] et al. in 1958. Proembryogenic masses (PEMs) are 10 to 20 small, highly cytoplasmic cell masses formed in carrot cell cultures when the auxin hormone, 2,4-D is applied. A single-cell acts as the progenitor to a PEM and can be regenerated as a somatic embryo if the 2,4-D hormone is removed from the culture.

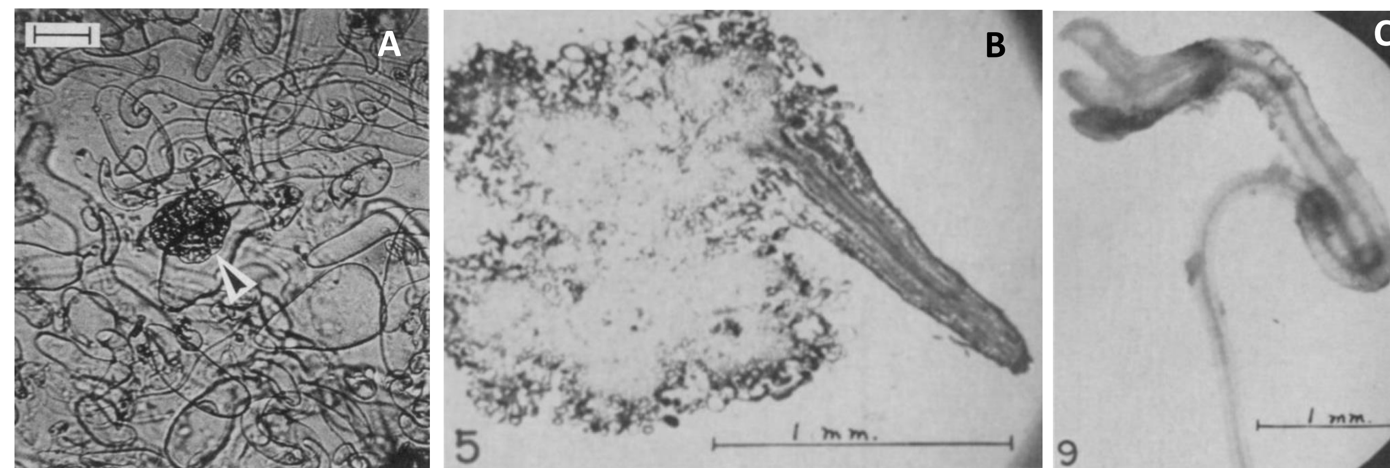

Figure 1. Carrot suspension cell culture and regeneration via somatic embryogenesis. A. PEM in carrot suspension cell culture. Bar = 48 µM. **B.** Hypocotyl development from PEM. **C.** Somatic embryo fully developed. Image for **A.** from de Vries[5] (1988). Images for **B.** and **C.** from Steward[4] (1958).

Genome editing via CRISPR/Cas9 and other Cas-like proteins has become a robust tool to target specific genes for modification. However, mutational chimerism remains an issue for most crop species that utilize callus intermediates for regeneration. In this study, CRISPR/Cas9 reagents were used to target key candidate genes controlling carrot root pigmentation to access their biological function and determine the efficacy of suspension cell transformation for reducing mutation chimerism in regenerated events.

1. **Food and Agriculture Organization of the United Nations (FAO) Statistics**, http://faostat3.fao.org/
2. **Simon P.W.** (2009) Plant Breeding for human nutritional quality. Plant Breeding Reviews 31:325-392
3. **Reinert J.** (1958) Morphogenese and ihre Kontrolle an Gewebekulturen aus Carotten. Naturwissenschaften 45:344-345
4. **Steward F.C., Mapes M.O., Hears K.** (1958) Growth and organized development of cultured cells: II. Organization in cultures grown from freely suspended cells. American Journal of Botany 45:705-708
5. **de Vries S.C., et al.** (1988) Acquisition of embryonic potential in carrot call-suspension cultures. Planta 177:196-204

Objectives

This proof-of-concept study was used to test the efficacy of suspension cell transformation to reduce mutational chimerism in transgenic carrots expressing CRISPR/Cas9 reagents targeting root pigmentation genes and test gene function:

1. Generate CRISPR/Cas reagents targeting genes controlling orange pigment (α- and β-carotene) accumulation in carrot roots.
2. Deliver CRISPR/Cas reagents to carrot cells via suspension cell transformation and regenerate transgenic cultures as somatic embryos.
3. Screen and characterize CRISPR/Cas9-induced targeted mutations in transgenic cultures and whole plants.

Suspension cell transformation

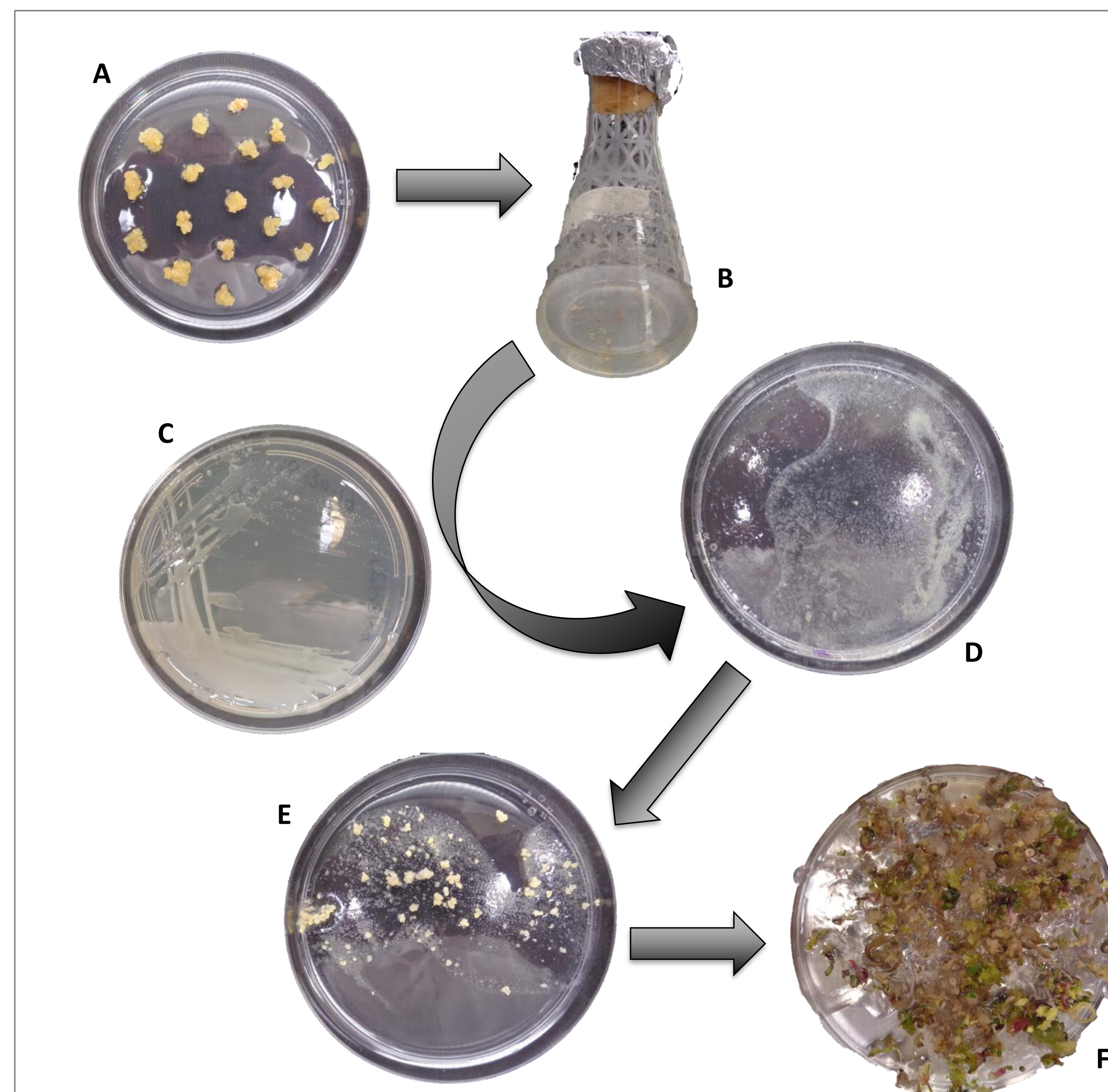

Figure 2. Suspension cell transformation and selection using carrot cell cultures. A. Carrot cell cultures are maintained as callus cultures on media containing 2,4-D and (**B.**) inoculated into liquid cultures for production of suspension cells. **C.** Suspension cells are collected and inoculated with *Agrobacterium* carrying CRISPR/Cas9 reagents and (**D.**) plated out to allow selection and transgenic callus growth. **E.** Transgenic callus is sampled and (**F.**) put on media without 2,4-D for regeneration and production of somatic embryos.

Mutation characterization

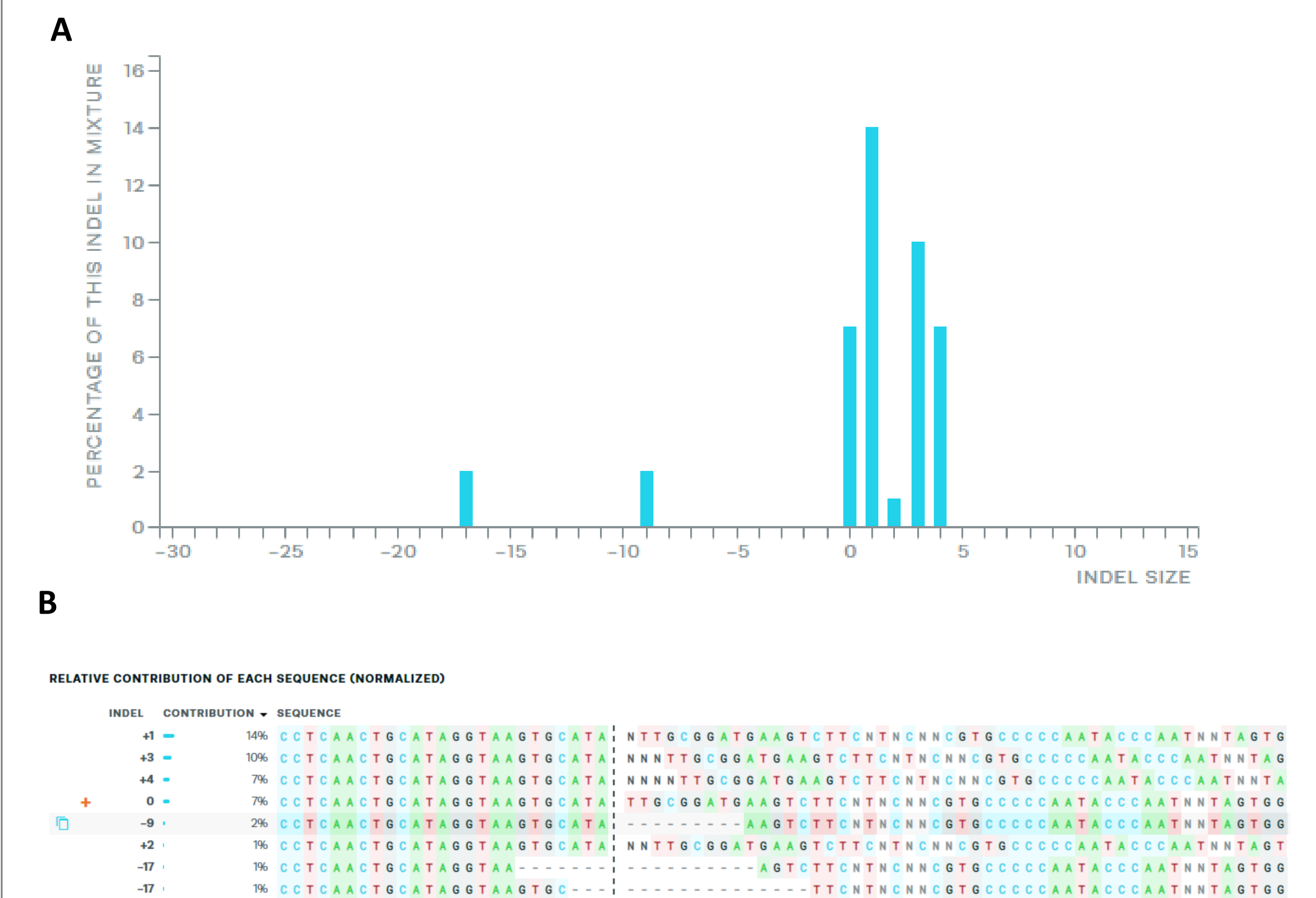

Figure 3. Frequency and sequence of targeted mutations in transgenic carrot cell cultures expressing CRISPR/Cas9. A. Percentage of indel mutations within a single transgenic carrot cell culture expressing CRISPR/Cas9 reagents targeting a gene controlling orange pigmentation accumulation in roots. **B.** Sequence of indel mutations from **A.** Analysis conducted using the ICR v2 CRISPR Analysis Tool.

Table 1. Transformation and targeted mutation frequencies from suspension cell transformation experiments delivering CRISPR/Cas9 reagents.

Experiment #	# of selection resistant calli	# of transgenic calli	% of transgenic calli	% of transgenic calli with targeted mutations	Average frequency of targeted mutations within calli
Experiment 1	75	21	28%	19%	19%
Experiment 2	60	9	15%	22%	8%
Experiment 3	59	11	19%	27%	13%

Conclusions

Suspension cell transformation is an effective method of delivering CRISPR/Cas9 reagents to carrot cells and generating targeted modifications. Cell cultures carrying targeted mutations will be regenerated to determine stablity of mutations in whole plants and the effects targeted mutations have on pigmentation accumulation in roots.

Acknowledgements

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