Annual Biotechnology Congress
July 23-24, 2018 | Vancouver, Canada

The use of hairy roots for validation of CRISPR/Cas plasmid constructs and genome functional analysis in composite plants

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Statement of the Problem: Since the inception of CRISPR/Cas based plant genome engineering, *Agrobacterium tumefaciens*-mediated plant transformation procedures have been frequently used for crop improvement. Once CRISPR/Cas-sgRNA plasmid constructs are synthesized and before commencing plant transformation, validation of the constructs is indispensable. So far, sequence analysis sometimes coupled with agro-infiltration procedures are being employed for the same. However, in recalcitrant crops such as the cucurbitaceae, agro-infiltration procedures are either ineffective or impossible. Alternatively, validation of CRISPR/Cas-sgRNA cassettes and characterization of edited gene/promoter/ sequences in *A. rhizogenes* induced hair roots seem reliable and time saving.

Methodology & Theoretical Orientation: The CRISPR/Cas-sgRNA plasmid constructs were mobilized into ATCC15834 or K599 *A. rhizogenes* strains to transform tomato and potato, or melon and cucumber explants, respectively. Genomic DNA was extracted from kanamycin resistant roots, PCR amplified with gene specific primers, PCR products were restriction digested, cloned and Sanger sequenced.

Findings: Mostly, we observed deletion of DNA sequences located in the sgRNA target regions, upstream of the proto spacer adjacent motif (PAM). Sometimes, deletions/substitutions/ of nucleotides both up- and downstream to the PAM or removal of the whole DNA sequence between two sgRNA target sites were observed. Although the same CRISPR/Cas-sgRNA cassette was used in tomato and potato, the types of insertions/deletions (indels) showed significant variations. In cucumber, where two independent CRISPR/Cas-sgRNAs were designed to target promoter sequences, with an assumption that the downstream gene would be up-regulated, more than two-fold transcript increment of the gene were observed, in the promoter-edited roots compared with the WT.

Conclusion & Significance: Before implementing whole plant transformation procedures, validation of CRISPR/Cas-sgRNA plasmid constructs in *A. rhizogenes* induced hairy roots could save time. Validation of CRISPR/Cas-sgRNA plasmid constructs and genome engineering/functional/ analysis in *A. rhizogenes* has induced hairy roots and/or composite plants seems vital and efficient.

Biography

Bekele Abebie has his expertise in Horticulture, especially postharvest physiology of cut flowers and plant molecular biology. Since Sep 2017, as a postdoctoral student, he has been working on developing methodologies for validation of CRISPR/Cas-sgRNA plasmid constructs and promoter functional studies in *A. rhizogenes* induced hairy roots, in some vegetable crops of the solanaceae and cucurbitaceae. In addition, he used A. tumifeciens mediated plant transformation protocols for developing virus resistance plants via CRISPR/Cas-genome editing, and the regenerated plants are being evaluated. Currently, he is developing transformation protocols for evaluating the effectiveness of CRISPR/Cas-genome editing technology in banana composite plants, which is expected to give a way for developing banana plants resistant to Fussarium wilt disease, via genome editing.

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