Engineering Herbicide Resistance in TK with CRISPR/Cas9

Kyle Benzle and Katrina Cornish

Department of Horticulture and Crop Science, The Ohio State University, Wooster, Ohio 44691

ABSTRACT

Taraxacum kok-saghyz (TK or Buckeye Gold) is being developed to supplement supplies of *Hevea brasiliensis* (the Brazilian rubber tree) rubber, currently the leading source of this natural product. Weed management is the main challenge facing commercial scale production of TK, and so herbicide resistance is essential. *ALS* catalyzes the biosynthesis of valine, leucine, and isoleucine and is inhibited by a class of herbicides called ALS inhibitors. We have generated a series of TK CRISPR/Cas9-mutated *ALS* (*mALS*) genes and introduced *aadA* (aminoglycoside 3′-adenyltransferase gene) by particle bombardment for use as a selection marker. Transplastomic *mALS* plants selected for resistance to spectinomycin will be assessed for herbicide resistance.

INTRODUCTION

Natural rubber (NR) is an important commodity that remains in high demand with many industries and governments requiring its use in countless applications such as tires, medical devices, construction, etc. [1] TK is under development to help supplement the current main source from *Hevea brasiliensis*, because it makes rubber very similar in quality and can be grown in the northern US. TK is not yet domesticated and a full production chain, from seed to processing, is being developed. Herbicide resistance is crucial, but weeds quickly adapt to herbicides of a single mode of action, thus this research aims to introduce resistance to multiple herbicides and modes of action. The specific goal of this work has been to produce non-transgenic herbicide resistant lines using the CRISPR/Cas9 system [2]. CRISPR/Cas9 is a powerful genome-editing tool that cuts at a specific DNA site using guide RNA. Site-specific gene modification and homology-directed repair [3] allows for site-specific modifications to be made to modify the endogenous plastidic TK ALS gene [4] without adding a permanent “transgene”. Non-homologous end-joining (NHEJ), a more common repair method after DNA, is broken by the Cas9 enzyme. However, in this work we are also using Homology Directed Repair (HDR), which can be directed with the addition of DNA molecules homologous to the DNA break region, along with a DNA repair template in an expression cassette.

MATERIALS AND METHODS

Cas9-guide RNA technology is being used in TK with four different plant codon-optimized vectors containing *Streptococcus pyogenes* Cas9 endonuclease and co-introduced guide RNAs with and without repair templates using biolistic transformation targeting the gene, acetolactate synthase (*ALS*). TK calli were generated from line TK-17 regenerated on hormone containing medium (Fig.1). Roots were removed from the plantlets, sectioned and then dipped into *A. rhizogenes* cell suspensions for about 10 seconds. *A. rhizogenes* cell suspensions were prepared as previously described [5]. Plantlets were then placed on 1/2 MS medium [6] with 200μM acetosyringone. After 3-days of co-culture and a 7-day recovery period using established protocols, plantlets were transferred to 1/2 MS medium with 400mg L^{-1} Timentin.

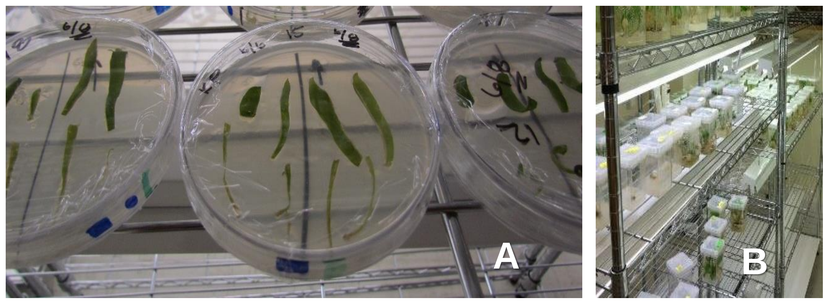

Fig. 1 Agrobacterium-mediated transformation using hydroponically grown TK roots as target tissue. (A) Sterile TK plants were gown in sterile conditions, dissected and roots and crowns were used as target tissue for transformation, (B) spectinomycin was used as a selection agent.

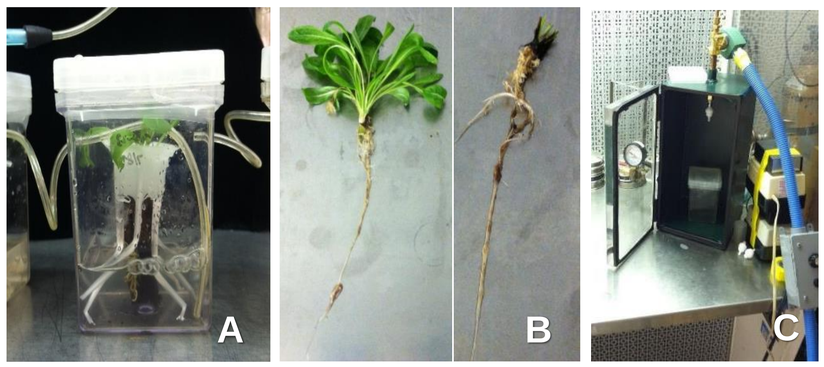

Fig. 2 Biolistic transformation using hydroponically grown TK roots as target tissue. (A) Sterile TK plants were gown in ½ MS liquid media in sterile conditions; (B) Plants were dissected and roots and crowns were used as target tissue for transformation; (C) A helium powered gene gun was used to accelerate DNA coated tungsten particles into plant tissue.

RESULTS AND DISCUSSION

Constructs (Fig.3) have been produced and introduced into TK. While *Cas9* and gRNA are expressed in TK, we have not yet seen successful mutation of the targeted sites.

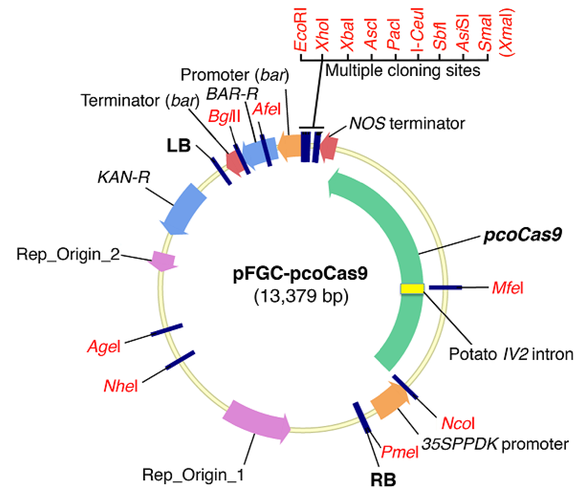

Fig. 3 Plant pFGC CRISPR/Cas9 plasmid being used in this research.

CONCLUSIONS

Full integration of TK natural rubber into the industrial supply chain is dependent on producing herbicide resistant TK crops and a TK line that has this property while remaining “non-GMO” is highly desirable.

ACKNOWLEDGEMENTS

Funding was provided by the PENRA Consortium, OARDC, the Center of Applied Plant Sciences, Ohio Third Frontier, and USDA National Institute of Food and Agriculture (Hatch project 230837).

REFERENCES

1. Cornish, K. (2001). Biochemistry of natural rubber, a vital raw material, emphasizing biosynthetic rate, molecular weight and compartmentalization, in evolutionarily divergent plant species. Nat. Prod. Rep. 18, 182–189.
2. Sander, J. D., & Joung, J. K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. Nature biotechnology, 32(4), 347-355.
3. Maliga, P. (2002). Engineering the plastid genome of higher plants. Curr. Opin. Plant Biol. 5, 164–172.
4. Jin, S., and Daniell, H. (2015). The Engineered Chloroplast Genome Just Got Smarter. Trends Plant Sci. 20, 622–640.
5. Zhang, Y., Iaffaldano, B., Xie, W., et al. Rapid and hormone-free *Agrobacterium rhizogenes*-mediated transformation in rubber producing dandelions *Taraxacum kok-saghyz* and *T. brevicorniculatum*, Industrial Crops and Products, 66, 4, 2015.
6. Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.