



Enhancing rubber dandelion germplasm by increasing mevalonate pathway activity

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INTRODUCTION

- *Taraxacum kok-saghyz* (TK, a.k.a. rubber root dandelion) produces high-quality natural rubber (NR) in its roots.
- Global NR demand solely dependent on the *Hevea brasiliensis* rubber tree, a threatened crop.
- TK could supplement NR but is not economically viable due to low rubber yield.

AIM

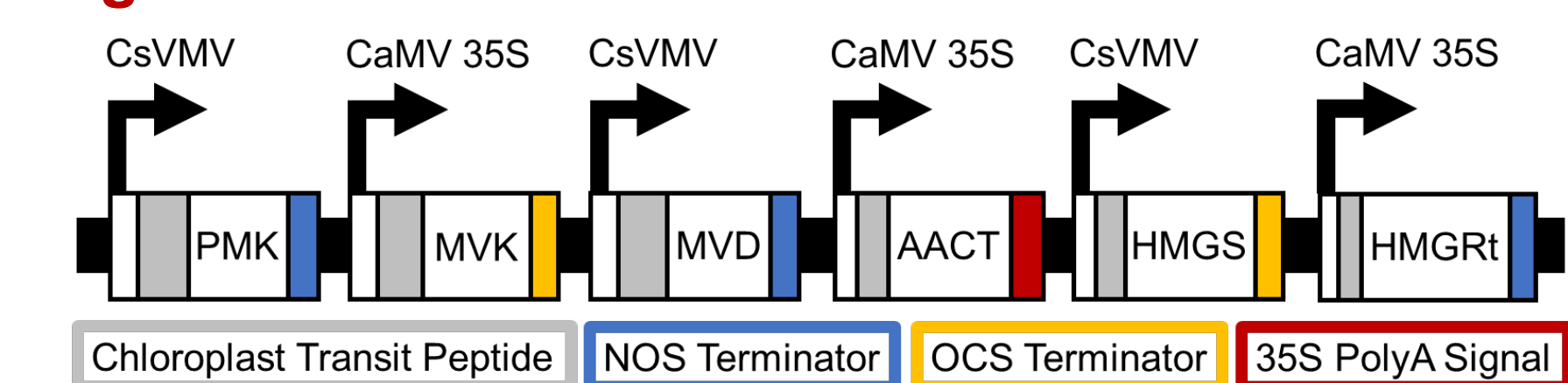
Improve TK germplasm through biotechnology.

- The mevalonate (MVA) pathway produces much of the isopentenyl pyrophosphate (IPP) utilized in NR production.
- *Agrobacterium rhizogenes* was used to insert a multigene construct containing MVA pathway genes targeted to chloroplasts.
- The *Ri* gene co-inserted by *A. rhizogenes* causes hairy root disease and limits plant growth.
- *Ri* is easily identifiable and can be confirmed by PCR. Further study of *Ri* phenotype could result in selecting *Ri* plants without PCR.
- Breeding T_3 MEV6 TK while excluding *Ri* may produce progeny with increased IPP pool and consequently NR production, making TK more valuable as a crop.

METHODS

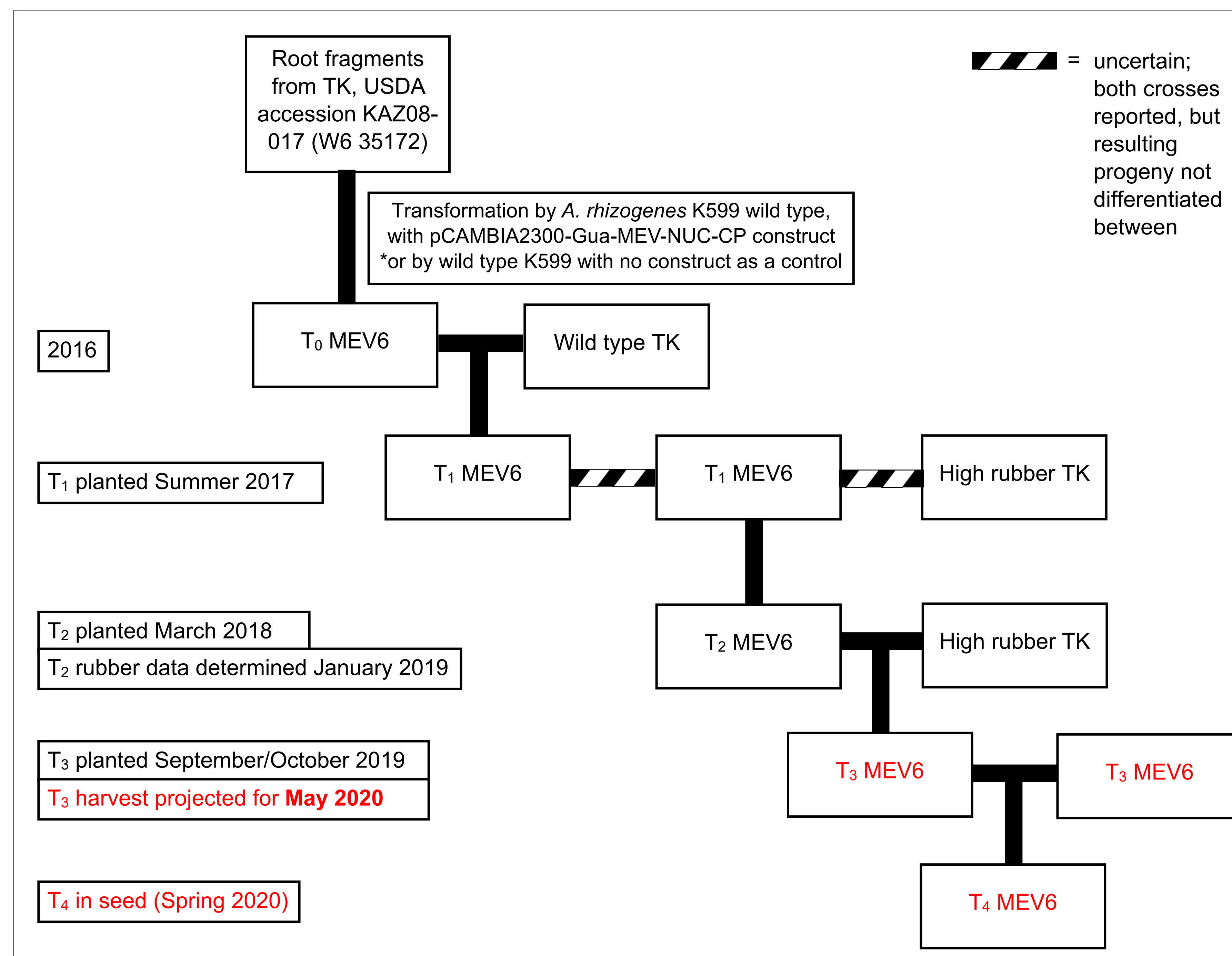
- TK transformed in 2016 by Yingxiao Zhang.
- T_1 – T_2 generations propagated by Kyle Benzle.
- T_3 planted September/October and are growing to maturity.
- Crosses performed to exclude *Ri* and produce TK homozygous for the construct.
- Plants being tested for construct and *Ri* by PCR.
- Plants will be harvested, and rubber content will be determined.
- Targeted metabolomics may be performed to confirm that IPP pool size increased in high rubber plants.

Figure 1 – MEV6 cassette



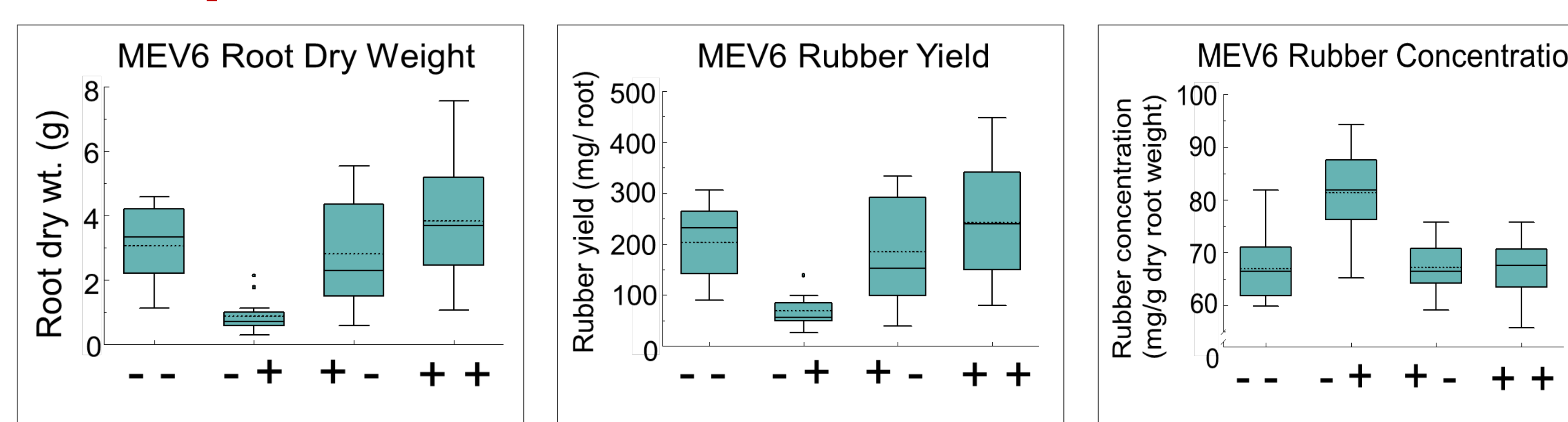
Cassette of MEV6 construct, from Yingxiao Zhang's dissertation. PMK is 5-phosphomevalonate kinase; MVK is mevalonate kinase; MVD is mevalonate-5-pyrophosphate decarboxylase; AACT is acetoacetyl CoA thiolase; HMGS is HMG-CoA synthase; HMGRt is an N-terminal truncated 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. CsVMV is a cassava vein mosaic virus promoter; CaMV 35S is a cauliflower mosaic virus 35 S promoter.

Figure 2 – MEV6 project flow chart



Flow chart of MEV6 project in the Cornish lab. Text in red was start of my involvement in the project. Crosses of $T_1 \times T_1$ and $T_1 \times$ High Rubber TK performed, but which progeny were used as T_2 (or a mixture of both) was not reported.

Figure 3 – T_2 rubber data



Rubber data collected by Kyle Benzle from T_2 plants. (--) is negative [-] for MEV6 and positive [+] for Ri; (-+) is - for MEV6 and + for Ri; (+-) is + for MEV6 and - for Ri; (++) is + for MEV6 and + for Ri. This work suggests that presence of both the MEV6 construct and Ri gene (++) can overcome the negative effects of Ri. Rubber concentration is measured as mg rubber per gram of dried root weight.

Figure 4 – *Ri* and wild type phenotypes



Ri phenotype (left) compared to wild type phenotype (right). Phenotypes not yet confirmed by PCR but is hypothesized based on observable phenotype. Notable features are high number of small leaves and smaller flowers in *Ri*-containing plants.

CONCLUSIONS

- Previous work suggests MEV6 insertion can overcome the effects of *Ri*. Future analysis of T_3 and T_4 TK will support or refute this suggestion.
- Analysis of T_3 and T_4 plants will determine if MEV6 plants produce more IPP and NR.

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