Cannabis Genetic Engineering

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Abstract: Meristem-based transformation systems have been established at the Wisconsin Crop Innovation Center (WCIC) for soybean (*Glycine max* L.), cow pea (*Vigna unguiculata* L.), dry bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), and Chickpea (*Cicer arietinum* L.) among others. DNA delivery in these systems is primarily via *Agrobacterium tumefaciens*, however a biolistics approach has also been demonstrated. Germline transformation with transmission of transgenes to T1 progeny have been confirmed empirically via RFP and GUS staining of T_1 progeny and further validated via PCR assays. We have modified these methods to successfully transform Cannabis sativa, an otherwise recalcitrant species. Numerous T0 Events have been produced along with their T1 progeny. We believe this to be the first example of stable, germline transformation of C. sativa.

Introduction:

An "optimal" plant transformation system has several key characteristics including genotype independence, efficient transformation frequency, short culture/selection period with no intervening callus phase, and easy to obtain, storable target explants. To date several reports of T0 C. sativa transient, cell, tissue and plant transformations have been published including, embryogenic based, hairy root, and VIGS RNAi methodology. Unfortunately, none of these methods were able to produce a germline event that successfully produced T1 transgenic progeny. We are please to report here that we have successfully modified a meristem-based transformation approach resulting in the generation of stable T1 transgenic C. sativa.

Figure 1. From left to right: seeds of a Cherry Wine Hybrid C. sativa cultivar, seed imbibed overnight, isolated embryo explants with intact meristems

Materials and Methods:

- **Embryonic axis explants are isolated from imbibed seeds by hand separation.**
- **Fresh explants are targeted for DNA delivery via Agrobacterium (strain 18r12) or by particle bombardment (0.6*u*m gold particles)**
- **Plasmid vector used in the experiment was Dicot Binary 19**
- **Following DNA delivery, targeted explants were placed on spectinomycin containing selection medium**
- **After 4-6 weeks on selection medium, putative T0 transgenic shoots (green, expanding shoots with leaflets) are transferred to rooting medium containing spectinomycin.**
- **Following 2-3 weeks on rooting medium, rooted plantlets are transferred to soil and grown in the greenhouse until plants are mature and produce T1 seed.**
- **T0 plantlets and T1 seeds and seedlings are assayed for screenable marker genes (RFP, GUS) expression and molecular confirmation of transgene presence, integration, and inheritance**

Results:

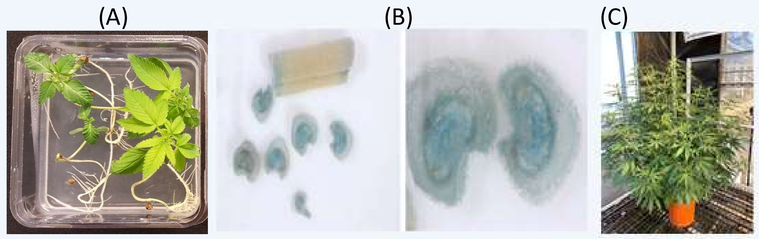

Figure 2. Development of (putative) transgenic, green shoots in soybean during selection on spectinomycin containing medium (A). Stable expression in the vascular region of leaf petiole sections which indicate probable germline transformation (B). First transgenic T0 C. sativa event (WP421-1) (C).

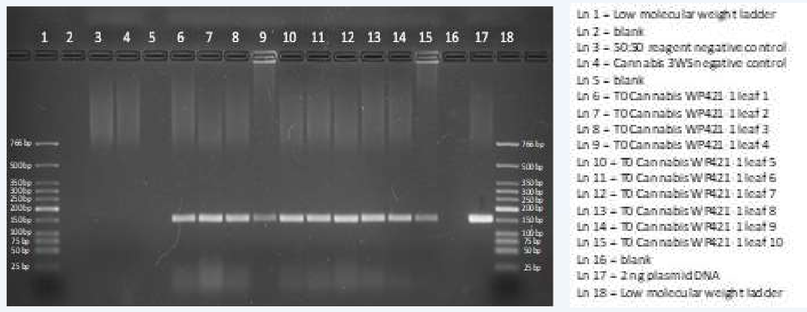

Figure 3. PCR amplification of antibiotic resistance gene (aaDa), in multiple leaves of the T0 WP421-1 event, showing that plant is non-chimeric.

Validation of Transgene Presence in the T_1 Progeny

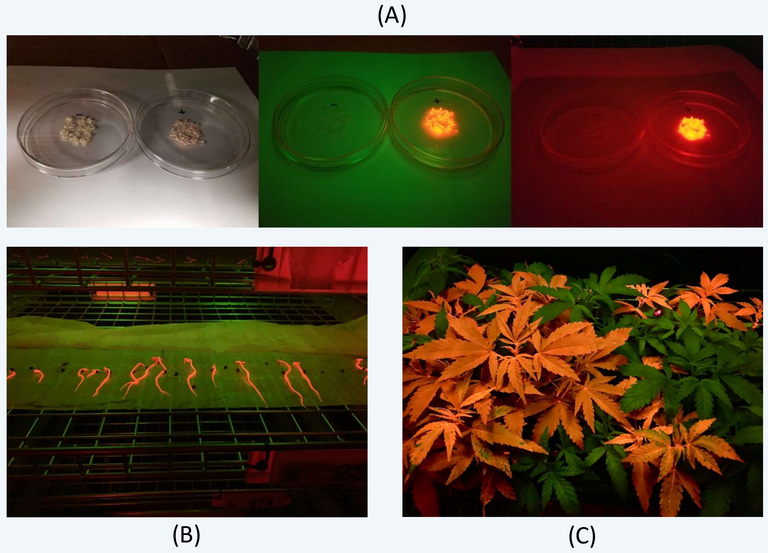

Figure 4. T_1 red fluorescence assay. T1 WP421-1 C. sativa seeds under white light and two different lights/filters showing red fluorescence in positive seeds (A). T1 WP421-1 C. sativa germinating seeds showing positive red fluorescence in the elongating roots in an expected 1:1 genetic ratio (B). T1 WP421-1 C. sativa plantlets showing red fluorescence in the leaves in an expected 1:1 genetic ratio. Green plantlets are null segregants (C).

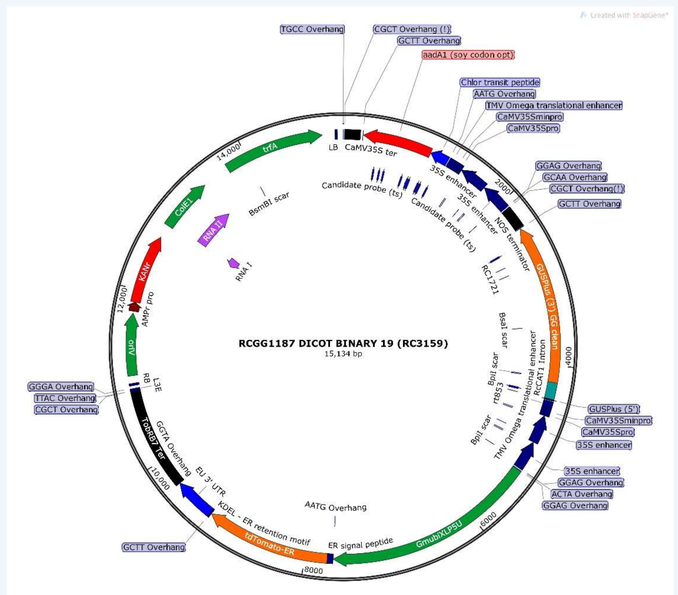

Figure 5. Dicot Binary 19 map. Binary vector used to produce WP421-1 C. sativa events.

Discussion:

C. Sativa is very recalcitrant to regeneration in tissue culture and many attempts to produce transgenic or edited plants have been unable to produce true T1 expressing progeny. The success of our transformation methodology will open the needed avenues for key modern breeding techniques such as editing as well as giving the community the ability to introduce novel traits into C. sativa. Our method is likely genotype flexible as we have shown this to work in multiple genotypes.

The WCIC and the University of Wisconsin are actively pursuing research that leverages this new technology to produce traits of interest through both genetic engineering and gene editing, and to improve the efficiency of our system. We currently offer transformation services for both public and private entities and would welcome collaborations with others on projects of interest.

Summary:

- **1st known germline transformation of C. sativa**
- **Methodology uses mature seed embryo explants and spectinomycin selection to organogenically produce engineered plants**
- **Multiple genotypes have been transformed**
- **Actively pursuing relationships/collaborations with others**

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