Genetic transformation of sweet cherry cultivars and root-stocks using sweet cherry *Flowering locus T* gene-expressing and *Terminal flower* 1 gene-silencing constructs: phenotype evaluation of transgenic trees.

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Abstract

The use of transformation technologies for *Prunus* crops has been rather modest when compared to the major commercialized genetically engineered crops, and the lack of efficient biotechnology platforms is considered to be the 'bottleneck' preventing the improvement of these species by through genetic transformation. In addition, dormancy and cold requirement are also relevant traits for an industry extremely impacted by climate change effects every season. Recently, we have designed several procedures for massive micropropagation of sweet cherry genotypes and their rootstocks using temporary immersion systems; these procedures accompanied the establishment of an organogenesis and transformation platform in some of these genotypes, which now is presented under a scenario in which *Flowering locus T* (FT) and *Terminal flower* 1 (TFL1) genes from *P. avium* L. were the experimental target. Several seed-derived explants were evaluated as starting point of transformation/regeneration procedures, and those corresponding to hypocotyls, epicotyls, embryo segments, and cotyledons, led to successful generation of transgenic Maxma-14, 'Bing', and 'Rainier' trees. Experiments included two transformation plasmids: a "FT-GFP" fusion, in which the flowering inductor previously evaluated in *Arabidopsis* plants led to Pav-FT overexpression; and "amiR-TFL1", an artificial microRNA targeting the sweet cherry TFL1 gene, which generates a RNAi process against this flowering inhibitor. Currently, whereas none of the transformed trees have shown deregulated or increased flowering rate (as formerly expected) after a first cold cycle, several important architecture changes have been obtained in these transgenic materials. The results suggest the involvement of these genes in plant shape and also indicate that flowering process could be more complex than expected and that are probably associated or influenced by/to additional processes taking place in these trees, such as juvenility.

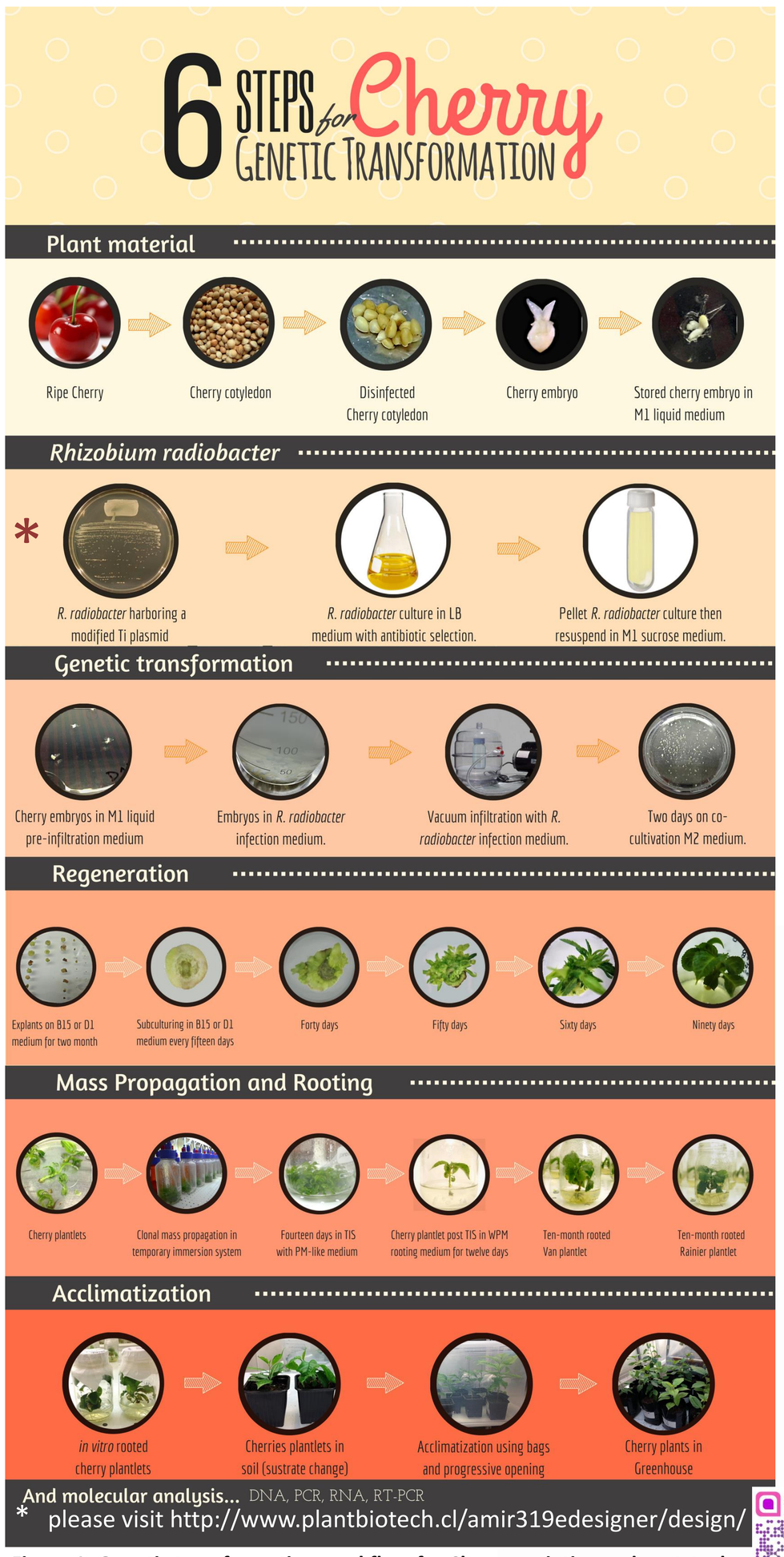

Figure 1: Genetic transformation workflow for Cherry varieties and rootstocks.

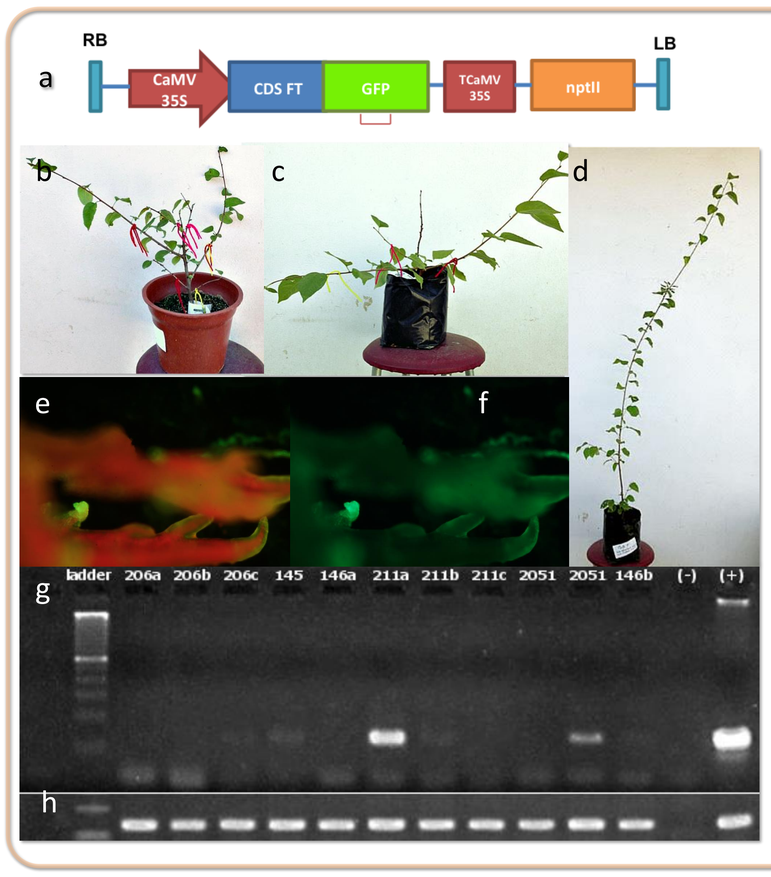

Figure 2. Characterization of Maxma-14 trees transformed with a sweet cherry FT gene-GFP fusion gene expression plasmid. Genetic transformation experiments were carried out using the sweet cherry FT gene fused to the GPF reporter gene (a). PCR positive individuals were evaluated according to UPOV's phenotype descriptors for the species. Apical dominance was one of the main differences found (b and c) compared to a wild-type individual (d) of the same age. Functional analysis of the FT-GFP fusion gene was evaluated by GFP fluorescence using epifluorescence microscopy (e and f). Transgene transcript detection (g) was carried out on cDNAs from selected transgenic lines and judged by RT-PCRs focused on GFP primers (g) and TEF II primers (h).
TEF II, *transcription elongation factor II* based on *P. persica* sequence; *ladder,* 100 bp molecular weight standard; +, positive control PCR using sweet cherry FT gene-GFP fusion gene expression plasmid ; -, no template PCR control.

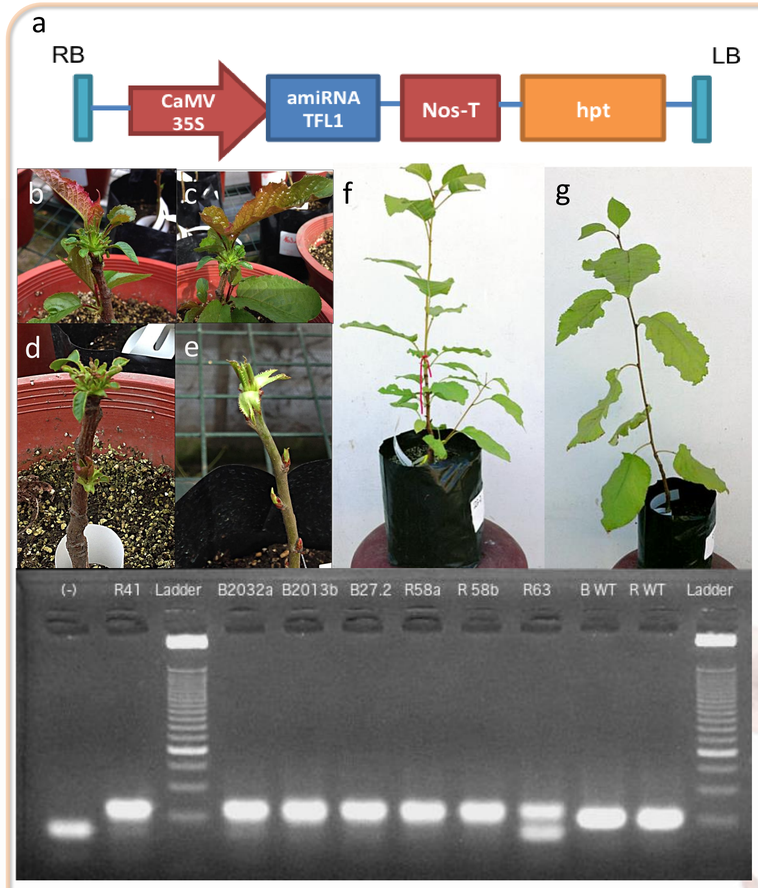

Figure 3. Characterization of 'Rainier' trees transformed with an artificial microRNA targetting the sweet cherry TFL1 gene. Genetic transformation experiments were carried out using the amiR-TFL1 based on sweet cherry sequences (a). PCR positive individuals were evaluated according to UPOV's phenotype descriptors for the species. Results showed shape differences between apical segments from transgenic (b, c and d) and wild type plants (e) of the same age. Differences in tree branching between amiR-TFL1 plants (f) and wild type (g) were also found. Cherry RNA was obtained from selected transgenic lines and subjected to RT-StemLoop PCR reactions using amiRTFL1 primers (h). Transformed 'Rainier' lines show 61 pb PCR products, unlike WT and no template negative control. *ladder,* 25 bp molecular weight standard (Lanes 3 and 12)

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