GenFun

Exploring a gene inducible system for functional studies related to flowering phenology in apple.

ABSTRACT

Genetic transformation has been successfully applied to evaluate the function of candidate genes (CG) related to agronomic traits in many economically important crops. However, using genetic transformation for the evaluation of gene function is not practical for fruit trees, such as apple, with low transformation efficiency, long regeneration time and long life cycle. This is an important bottleneck for scientists working with these species on fundamental and apply research programs. As alternative to the generation of adult transgenic trees, the function of CG can be addressed through transcriptomic studies. Figure 1. Genetic transformation of apple leaf explants. Leaf explants were agroinfiltrated and transformed with p35S::GFP. On the left, shoots regenerated in organogenesis medium and observed under bright field. On the right, the same shoots observed under the fluorescent microscope showing GFP signal.

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GOAL

We propose a collaborative project between the AFEF and HoNuDe teams to explore the use of a new method to decipher CGs function in apple. This method will allow us to misexpress CGs through an inducible system in apple calli. In a pilot experiment, global changes in gene expression associated to the misexpression of the candidate genes involved in apple flowering phenology will be captured by RNA-seq. In particular, we will: (1) establish a protocol for the Agrobacterium tumefaciens-mediated transformation of apple calli, (2) optimize a gene inducible system for CG transient expression studies and (3) characterize the function of CGs related to apple phenology by a transcriptomic approach.

RESULTS

We are planning the following actions:
1. Establishment of a protocol for the A. tumefaciens-mediated transformation of apple calli. Different protocols for A. tumefaciens-mediated genetic transformation will be tested. In a first protocol, we will transform leaf explants with a binary vector containing a reporter gene (GFP) to generate and select transgenic pool of cells. Then, we will induce callus formation from the transformed tissue to obtain transgenic calli. In a second protocol, we will perform the genetic transformation directly on calli derived

from leaf explants. The protocol that allow us to produce transgenic calli in the most rapid and efficient way will be selected for further studies. 2. Optimization of a gene inducible system for CG transient expression studies. A binary vector containing for rapid selection in plant calli and conditional gene induction will be constructed. The CGs will be cloned in this plasmid and the resulting molecular constructs will be transformed into apple calli by making use of one of the protocols assayed in the objective 1. Transgenic calli will be used to optimize the CG inducible expression system. The optimal conditions allowing a strong and reproducible induction of CG mRNA expression will be chosen to develop the next objective. 3. Characterization of the function of CGs by a transcriptomic approach. In this objective, the inducible system developed in the previous objectives will be used to characterize the function of four CGs. These CGs will be misexpressed in transgenic calli and samples for RNA studies will be collected as defined in the objective 2. RNA-seq experiments will be performed in order to monitor the transcriptomic changes associated to the induction of each tested CGs. The results of these experiments will be analyzed in order to identify genes and genetic pathways regulated by the CGs. Moreover, these data will be compared with known gene networks controlled by their Arabidopsis thaliana orthologues to contribute to their functional characterization.
The method developed in GenFun and its application to new projects will contribute to the better understanding of the CGs function and thus, it is expected to boost our studies on the control of flowering phenology.