FUNCTIONAL GENOMICS OF PHOTOSYNTHETIC GENES OF SUGARCANE

Helaine CARRER
Luiz de Queiroz Agriculture School / University of São Paulo (ESALQ/USP)

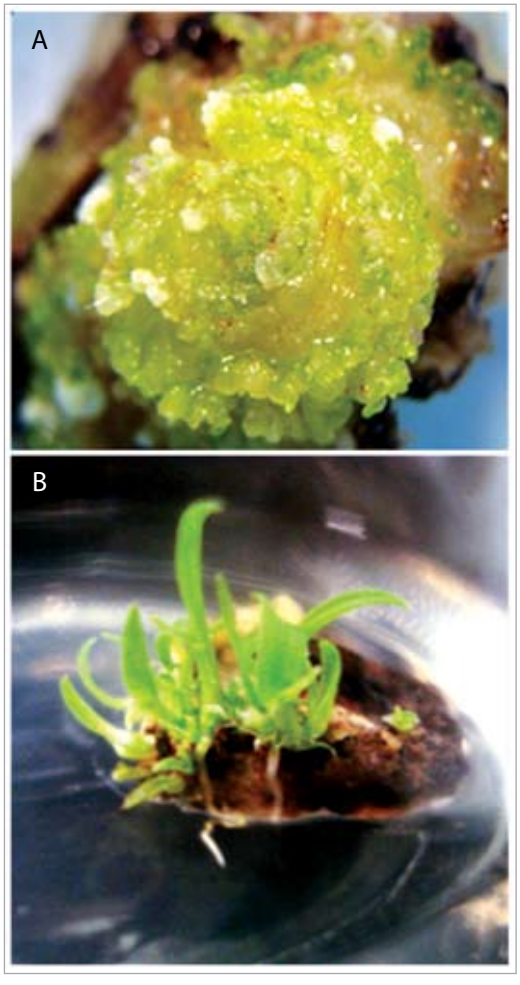

Figure 1. Regeneration of transgenic sugarcane; (A) Somatic embryogenesis from immature leaf-disc; (B) Leaf-disc with transgenic sugarcane

Sugarcane (*Saccharum officinarum*) is one of the most important feedstock sources for biofuel. In Brazil, sugarcane has been a prominent cultivated species undergoing accelerated expansion. This crop has launched Brazil as the major and most relevant country for exporting ethanol, as well as it became an important source of world bioenergy. In order to sustain and develop this enlarging agricultural and commercial sector, in a long-term, it becomes mandatory a continued quick release of increasingly productive sugarcane cultivars carrying specific advantageous traits, including increased sucrose content. Adversely, the breeding of sugarcane has been naturally limited by its low fertility, complex genome, narrow genetic basis, and long periods of 12 to 15 years to create a new variety. The development of efficient systems of molecular biology and genetic transformation are fundamental, and often the only way, to rapidly introducing new valuable agronomic and commercial traits into sugarcane elite germplasm.

Increase of sucrose content in elite sugarcane cultivars may be a main point to be addressed by using genetic transformation, and is directly dependent of increasing photosynthetic efficiency. The vast majority of photosynthetic proteins is nucleus-encoded and require N-terminal presequences, named chloroplast transit peptides, to target them to the chloroplast. About 2100 to 3600 distinct chloroplast proteins are nuclear-encoded, while about 100 to 120 are encoded by the organelle genome. The present project aims to develop efficient methods of sugarcane *in vitro* culture as well as methods of nuclear and chloroplast genetic transformation, applying them to modify photosynthetic genes in order to incorporate new photosynthetic traits in already productive Brazilian cultivars. The sugarcane photosynthetic efficiency is expected to be improved upon manipulation of photosynthetic genes (i.e. ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, carbonic anhydrase) generating novel knowledge in this research field as well as leading to increased synthesis of triose phosphates and, ultimately, increased sucrose content in the transgenic cultivars.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Genomic and genome sequencing bring significant advances in chloroplast research to understand how chloroplast functions and communicates with other cellular compartments. The vast majority of chloroplast proteins are nuclear-encoded and require N-terminal pre-sequences, termed "chloroplast transit peptides", which target them to the chloroplast. Therefore, bioinformatics tools were used to identify genes associated with photosynthesis and with transit peptide sequences that, likely, are transported to the chloroplast for expression and function. Initially, we performed a keyword search for Arabidopsis chloroplast transit peptides in the UniprotKB database. Arabidopsis gene orthologous were then identified in sugarcane with TBLASTN using Arabidopsis protein sequences as queries against the SUCEST database. Around 650 sugarcane sequences with significant similarity (1e-10 e-value cutoff) were retrieved. The TargetP prediction of subcellular localization of the products of sequences showed that 245 are potentially targeted to chloroplast. In addition, we identified eight putative orthologous of known Arabidopsis and maize carbonic anhidrases (CA) by BLAST searches in sugarcane database using the most highly conserved regions of the CA amino acid sequences.

Concomitantly, we are establishing and optimizing direct plant regeneration and callus-based propagation methods in sugarcane. MS medium with different concentrations of 2,4-D and kinetin were tested to obtain highly embryogenic calli and to induce cellular dedifferentiation in the immature leaf discs prior plant regeneration. Results showed that immature leaf disc-based approach is a more feasible as well as cheaper and faster method to obtain directly plant regeneration as compared to embryogenic callus.

Genes associated with photosynthesis identified in the SUCEST database will be main targets to nuclear and plastid transformation. It is expected that analyses of these transgenic plants will shed light on sugarcane genetics, biochemistry and physiology and, furthermore, it is anticipated to accomplish significant improvements in specific agronomic and commercial traits within short time and at reduced cost.

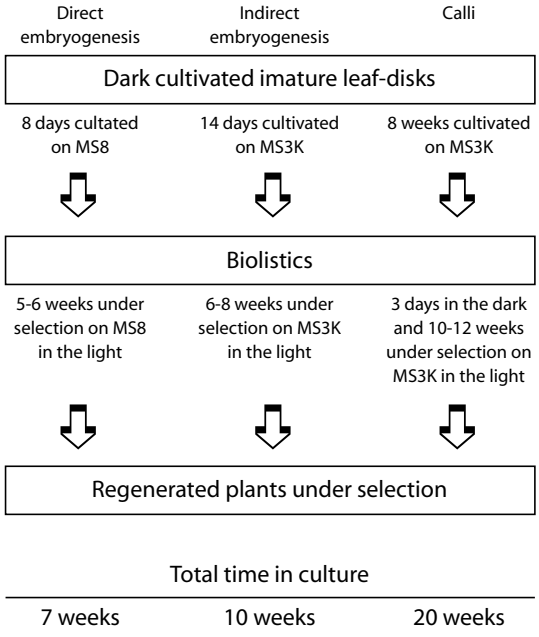

Figure 2. Relative timeframe to generate transgenic sugarcane plants from immature leaf-discs. Somatic embryogenesis was induced through three distinct regeneration processes: direct and indirect embryogenesis and calli

Helaine Carrer

Escola Superior de Agricultura Luiz de Queiroz (Esalq)
Universidade de São Paulo (USP)
Departamento de Ciências Biológicas
Av. Pádua Dias, 11 – Caixa Postal 9
CEP 13418-900 – Piracicaba, SP – Brasil

+55-19-3429-4344 r. 32
hecarrer@esalq.usp.br