Scientia Horticulturae 229 (2018) 65–76

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Insights into the conversion potential of *Theobroma cacao* L. somatic embryos using quantitative proteomic analysis

Liliana Alexandra Pila Quinga[a], Angelo Schuabb Heringer[b,c], Hugo Pacheco de Freitas Fraga[d], Leila do Nascimento Vieira[a], Vanildo Silveira[b,c], Douglas André Steinmacher[e], Miguel Pedro Guerra[a,f,*]

[a] Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis, SC, 88034-001, Brazil
[b] Laboratório de Biotecnologia, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 28013-602, Brazil
[c] Unidade de Biologia Integrativa, Setor de Genômica e Proteômica, UENF, Campos dos Goytacazes, RJ, Brazil
[d] Laboratório de Micropropagação Vegetal, Departamento de Botânica, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR, 81531-970, Brazil
[e] Vivetech Agrociências, Marechal Cândido Rondon, PR, 85960000, Brazil
[f] Tree and Timber Institute, National Research Council of Italy, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Keywords:
Micropropagation
Somatic embryogenesis
Cacao
Embryo quality
Label-free proteomics

ABSTRACT

Somatic embryogenesis (SE) has been routinely used as mass micropropagation technique, and as a model system for investigating the structural, physiological, and molecular events occurring during somatic embryo development. Successful *in vitro* SE is related to the quality and yield of somatic embryos obtained. In cacao (*Theobroma cacao* L.) SE, the efficiency of somatic embryo production has improved with secondary SE. However, the low number of somatic embryos able to conversion into viable plantlets is still low. Then, two morphological types of normal mature somatic embryos can be identified during cacao secondary SE. The first type shows white appearance and high conversion potential (75%), while the second type shows translucent appearance and exhibit low conversion potential (15%). In order to investigate the proteins that can be associated to conversion potential in cacao somatic embryos, the mass spectrometry HDMS^E proteomic approach was used. At least 60 proteins showed differences in abundance levels in cacao white somatic embryos, when compared to translucent. An increased abundance of Beta-glucosidase, NAD(P)-linked oxidoreductase and Electron transfer flavoprotein proteins were observed in white somatic embryos. Moreover, in translucent somatic embryos were observed an increased abundance of Cytochrome P450 and Pathogenesis-related proteins. Using white somatic embryos as a model, we suggest that carbohydrate metabolism process and the redox regulation are involved in the control/regulation of somatic embryo quality. These new findings may improve cacao SE protocol, as well as the understanding of the role of pivotal metabolic pathways associated to this *in vitro* morphogenetic route.

1. Introduction

Cacao (*Theobroma cacao* L.) is a tropical plant, which play a relevant role in both the stability of tropical ecosystems and in the economy of millions of small-holder farmers (Araújo et al., 2011). Cacao beans are rich source of polyphenols and represent the main raw material for the multi-billion-dollar chocolate industry (Maximova et al., 2014).

Plant propagation through somatic embryogenesis (SE) is an effective method to large-scale clonal propagation (Jin et al., 2014; Li et al., 1998), which can be incorporated into breeding programs. Cacao SE is well characterized (Alemanno et al., 1997; Li et al., 1998; Maximova et al., 2008; Maximova et al., 2002), and somatic embryo-derived plants have been tested under field conditions, revealing similar growth patterns to those from plants propagated by seeds (Maximova et al., 2008).

Secondary SE protocol for efficient somatic embryos production have been established (Minyaka et al., 2008). However, somatic embryos still exhibit very low conversion potential into plantlets, which is

* Corresponding author at: Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis, SC, 88034-001, Brazil.
E-mail addresses: miguel.guerra@ufsc.br, mipguerra@gmail.com (M.P. Guerra).

http://dx.doi.org/10.1016/j.scienta.2017.10.005
Received 21 July 2017; Received in revised form 2 October 2017; Accepted 3 October 2017

a limitation for the commercial application of this technique.

Considering that somatic embryo developmental patterns are similar to zygotic embryos in terms of morphology, biochemistry, desiccation tolerance, and germination (Alemanno et al., 1997), several studies have been carried out comparing cacao zygotic and somatic embryos, using zygotic embryos as a reference model (Niemenak et al., 2015; Noah et al., 2013).

Conceptually, developmental stages of somatic and zygotic embryo are divided into two main metabolic stages: the first is a morphogenetic stage that is characterized by cell division and the onset of cell differentiation; the second is a metabolic stage or maturation phase that is characterized by biochemical activities, which involves the accumulation of major storage products and the preparation for desiccation, dormancy, and germination/conversion (Leljak-Levanić et al., 2004; von Arnold et al., 2002; Harada et al., 2010). In this last phase, somatic embryos achieve both morphological and physiological maturity, which, guarantee satisfactory post-embryonic performance. Therefore, the conversion potential is considered to be programmed during embryo maturation (Dodeman et al., 1997).

The early conversion steps in somatic embryos depends both on activation of enzymatic system that mobilizes nutrient elements, and the storage compounds accumulated during maturation phase (Stasolla and Yeung, 2003). Insufficient accumulation of storage compounds and enzymatic imbalances in somatic embryos were earlier suspected to cause poor conversion potential in cacao (Alemanno et al., 1997).

Previous studies of comparative proteomic analysis of somatic and zygotic embryos in cacao showed that the most important difference between the two types of embryos is related with carbohydrate metabolism. Thus, zygotic embryos display a high glycolytic activity while somatic embryos showed the important increased of TCA (tricarboxylic acid) cycle proteins, which is related with intensive aerobic/respiration pathway activity (Noah et al., 2013). On the other hand, it was observed high expression of stress-/defense- related proteins in somatic embryos, the authors suggest that they are resilient to the stress imposed by *in vitro* culture (Niemenak et al., 2015).

Despite the similarities between these two types of embryogenesis, some key differences exist, and, zygotic embryos may be nourished via phloem and simultaneously development of a normal endosperm tissue. In SE, embryos are dependent on exogenous carbohydrate supply and morphological stages occurs without the surrounding embryo sac and the simultaneous development of a normal endosperm tissue. In addition, one marked difference between somatic and zygotic embryos is the availability of storage compounds such as carbohydrates, lipids and proteins (Rode et al., 2012).

During cacao SE it is possible to recognize two different types of normal mature somatic embryos with well-defined and developed hypocotyl and cotyledons: the white somatic embryos type which show enhanced conversion potential, and translucent embryos type that show limited conversion potential (Li et al., 1998). Previous studies performed by our research group with EET 103 and EET 111 cacao genotypes, showed that white somatic embryos conversion rate was 90% and 76% respectively, while translucent embryos conversion rate was 17.8% and 15%. In addition, the proportions of two somatic embryos types during SE is about 50% in EET 103 cacao genotype, despite this is genotype-dependent feature (data no show).

Thus, in the present study we used a proteomic approach, involving 2D-nanoESI-HDMS^E technology, in order to compare normal somatic embryos with white and translucent morphological appearances in cacao at the equivalent developmental stage (cotyledonary-staged).

2. Material and methods

2.1. Plant material

The present study was performed with the cacao genotype “EET 103”, which is classified into the ‘Nacional’ genetic group. This genotype is known for its high productivity and is considerate as “Cacao de Aroma” fine flavor (Seguine and Meinhardt, 2014).

2.2. Somatic embryogenesis

Secondary SE was obtained from cotyledons of somatic embryos previously established *in vitro* as described by Maximova et al. (2002), using cacao genotype EET 103. All culture media were composed by DKW (Phytotechnology Lab, Overland Park, KS, USA) basal salts, as described by Driver and Kuniyuki (1984) The embryo development (ED) culture medium was supplemented with $MgSO_4$, as described by Minyaka et al. (2008).

Cotyledons from mature somatic embryos were excised and subcultured in SCG (secondary callus growth) culture medium for 14 days. This culture medium was supplemented with DKW vitamins, 20 g L^{-1} glucose, 9 µM 2,4-dichlorophenoxyacetic acid (2,4-D; Sigma-Aldrich), 1.2 µM kinetin (Kin; Sigma-Aldrich) and 0.2% (w/v) Phytagel® (Sigma-Aldrich, St. Louis, MO, USA). Cultures from SCG culture medium were transferred to ED culture medium plant growth regulators-free, and subcultured every 21 days. After 45 days in culture, normal cotyledonary somatic embryos were classified into two types: white and translucent (Fig. 1). The weight of a normal somatic embryo was about 40–50 mg. There was no significant difference between weight of white and translucent somatic embryos (data not shown). Three independent experiments were carried out with three biological replicates. Samples (500 mg, about 12 normal somatic embryos) of two types of mature somatic embryos per biological replicate were frozen in liquid nitrogen and stored at −80 °C for 2 months until protein extraction.

2.3. Proteomic analyses

2.3.1. Total protein extraction

Proteins extractions for each somatic embryo type were carried out in biological triplicate (500 mg) according to Carpentier et al. (2005),

Fig. 1. Somatic embryogenesis of cacao genotype EET 103. (A) Somatic embryos. (B) White somatic embryo at cotyledonary stage. (C) Translucent somatic embryo at cotyledonary stage (bar = 2.0 mm).