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Responses of Arabica coffee (*Coffea arabica* L. var. Catuaí) cell suspensions to chemically induced mutagenesis and salinity stress under in vitro culture conditions

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Abstract

The objective of the present study was to induce mutation for salt tolerance using sodium azide and ethylmethanesulphonate (EMS) in embryogenic cell suspensions of coffee (*Coffea arabica* L. var. Catuaí), followed by cell line selection and subsequent plant regeneration. Determination of the optimal growth conditions for culture of embryogenic calli in liquid medium was the first step, three culture media were evaluated (CP ;van Boxtel & Berthouly, 1996), Teixeira *et al*, 2004 and Silva *et al.*, 2000). It was determined that culture in flask with Teixeira liquid medium promoted the fastest calli proliferation and that the embryogenic regeneration was successfully achieves by culturing the calli in RITA ® systems with regeneration medium (van Boxtel & Berthouly, 1996). The medium lethal doses (LD_{50}) were determined for NaN_3 (5mM for 15 minutes) and for EMS (185.24 mM for 120 minutes). These doses were implemented in an *in vitro* selection protocol to determine the NaCl concentration that facilitated the identification of putative mutant cell lines. The NaCl concentration ended up being 150 mM. Finally, genetic variability was assessed and evaluated with RAPD markers; 50 bands were amplified and 22% of those bands were polymorphic. To our knowledge, this is the first report of in vitro selection of salt tolerant variants following sodium azide and EMS treatment of embryogenic coffee cell suspensions.

Introduction

Coffee represents the most important non-alcoholic beverage in the world economy. In the list of largest commodities in the international markets, it is currently ranked second, only behind oil. Coffee production sustainability and profitability are a growing problem. This is worsened by the complicated road that must be traveled to achieve successful conventionally bred varieties.

Crop improvement via mutagenesis is a powerful tool that adapts fairly well to the needs of a lot of coffee breeding programs. Mutagenesis can induce variability in genetically homogenous populations.

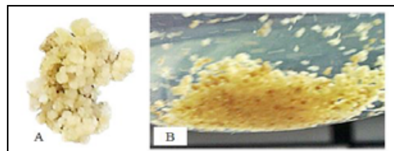

Figure 1. Embryogenic calli of coffee (*C. arabica* L. var. Catuaí). **a** Embryogenic friable calli obtained in C20 medium. **b** Embryogenic cell suspension culture growing in TEX liquid medium (Teixeira *et al.* 2004)

Material and methods

Embryogenic suspension cultures (Fig. 1) were incubated with 0.0, 2.5, 5.0, or 10.0 mM NaN3 or 0.0, 185.2, 370.5, or 741.0 mM EMS. Then, embryogenic suspension cultures treated with NaN3 or EMS were cultured on selective medium supplemented with 0, 50, 100, 150, 250, or 300 mM NaCl. Random amplified polymorphic DNA (RAPD) analysis was performed to determine whether the NaN3 or EMS treatments could induce genetic variability and resulted in identifiable polymorphic markers

Results/ Disussion

As the concentration of NaN3 or EMS increased, the survival of embryogenic suspension cultures decreased compared to controls. The median lethal dose (LD50) for NaN3 was 5 mM for 15 min and for EMS it was 185.2 mM for 120 min (Fig. 2 and 3).

Plantlet growth and total amino acid content were affected by NaCl stress; some mutants had longer shoots and higher amino acid content than controls

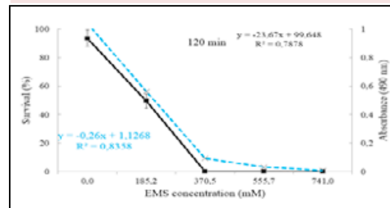

Figure 3. Effect of EMS concentration on survival and viability of coffee (*C. arabica* L. var. Catuaí) embryogenic calli. **a** Survival percentage (solid line) and absorbance (490 nm) (dotted line) after 60 min of exposure time to different EMS concentrations.

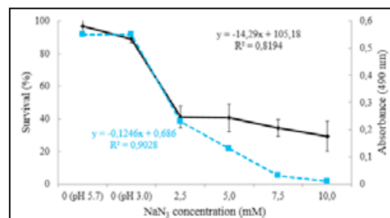

Figure 2. Effect of NaN_3 concentration on survival and viability of coffee (*C. arabica* L. var. Catuaí) embryogenic calli. **a** Survival percentage (solid line) and absorbance (490 nm) (dotted line) versus NaN_3 concentrations.

Plantlet growth and total amino acid content were affected by NaCl stress (50 mM); some mutants had longer shoots and higher amino acid content than controls (Fig 4).

A total of 18 10-mer primers were used to amplify genomic DNA of putative mutant and non-mutant arabica coffee embryogenic cultures and produced 50 scorable bands, of which 22% were polymorphic (Fig. 5)

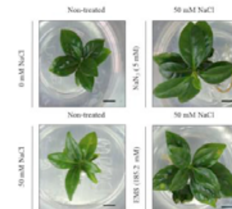

Figure 4. Coffee (Coffea arabica L. var. Catuaí) plantlets regenerated from embryogenic cell suspensions treated with sodium azide (NaN3; 5 mM) or ethyl methanesulfonate (EMS; 185.2 mM) growing on R semisolid medium supplemented with 100 mM NaCl (Bolívar-González et al. 2018. https://doi.org/10.1007/s11627-018-9918-x)

Figure 5. Band patterns obtained from the amplification of decameric primer OPB-02 in 7 genomic DNA samples of coffee (*C. arabica* L. var. Catuaí) embryogenic cell suspensions.

Conclusion

Our results indicate that embryogenic suspension cultures are suitable for sodium azide and EMS mutagenesis and provide the basis for the improvement of agriculturally important traits and to study gene function in coffee.

References:

Bolívar-González, Valdez-Melara, Gatica-Arias (2018) In Vitro Cellular & Developmental Biology – Plant https://doi.org/10.1007/s11627-018-9918-x