In vitro tissue cultures from *Coffea arabica*: from callus to cell suspension

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INTRODUCTION

Coffee plant has potential applications in human therapy and cosmesis as a major source of phytochemicals whose biological activities could be exploited against critical diseases [1]. Cell suspension cultures (CSCs) provide a technological option to increase the production of high-value secondary metabolites (SMs) of plant origin [2]. Friable callus is most suitable for the establishment of CSCs, favoring the disintegration of the cell mass into small and homogeneous aggregates. Fine cell suspension is critical for the development and maintenance of productive cell lines for molecular farming purposes.

MATERIALS & METHODS

A. Callus induction. Leaf discs of *Coffea arabica* cvs. Bourbon Red and Castillo were placed on MS medium supplemented with PGRs according to the experimental design. Trials were arranged in three steps aiming at defining: i. AUX-CK concentration, 5x3 factorial experiment with 5 levels of KT (0, 0.5, 1, 2 and 4 mg L^{-1}) and 3 levels of 2,4-D (1, 2 and 3 mg L^{-1}); ii. CK type and iii. AUX type. A total of 17 PGRs were tested. Callogenesis was evaluated by measuring the fresh explant+callus weight (FW).

B. Cell suspension. After stabilization into fine suspension, CSCs growth was monitored by determining the cell fresh (FW_{csc}) per mL culture. Cell viability was evaluated at the inoculation time and at weekly intervals by fluorescein diacetate (FDA) staining.

CONCLUSIONS & PERSPECTIVES

The large-scale production of valuable SMs first of all requires the establishment of healthy and viable CSCs. The combination of 1 mg L^{-1} of both KT and 2,4,5-T was found to be the best for this purpose. These procedures could be investigated and extended to other *Coffea* cultivars and species. Several elicitation conditions will be applied on CSCs to induce the synthesis of bioactive compounds.

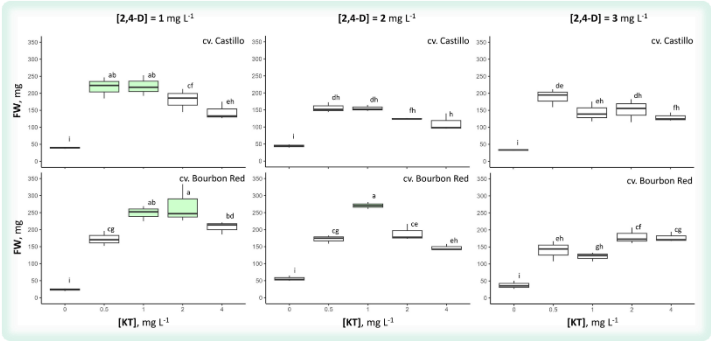

Fig. 1. Fresh weight (FW, mg) of explant+callus measured at different concentrations of 2,4-D and KT in cvs. Castillo and Bourbon Red. PGRs combination reaching the highest FW values are highlighted in green.

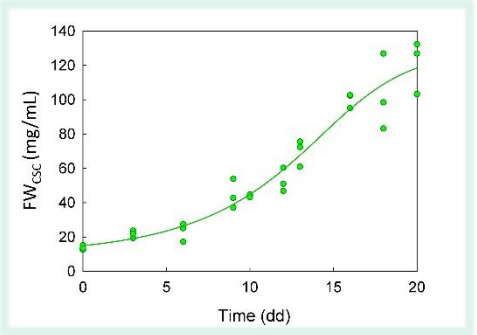

Fig. 2. Growth pattern of Bourbon Red cell suspensions.

Source of variation	MS	Df	F value	p-value
Cultivar	0.1270	1	1.38	ns
Cytokinin	0.8366	8	9.11	**0.001**
Cultivar x Cytokinin	0.1454	8	1.58	ns
Residuals	0.0918	36		

Tab. 1. Two-way ANOVA outcome to test the relevance of cultivars and type of cytokinin as variability factors for callogenesis.

Source of variation	MS	Df	F value	p-value
Cultivar	0.0067	1	0.14	ns
Auxin	3.9958	7	84.86	**0.001**
Cultivar x Auxin	0.1524	7	3.24	**0.05**
Residuals	0.0470	32		

Tab. 2. Two-way ANOVA outcome to test the relevance of cultivars and type of auxin as variability factors for callogenesis.

RESULTS

A. The presence of KT was found fundamental for callus induction. In KT-provided media, the PGRs combination of 1 mg L^{-1} 2,4-D and KT turned out to give more consistent results (Fig. 1), also in terms of callus texture (highly friable). The effect of CKs and AUXs was significant (Tab. 1 & 2). Amongst the CKs giving the highest response, KT was found superior both in terms of callus induction level and callus texture. FW reached the highest values and a highly friable texture in the presence of 2,4,5-T.
B. The growth pattern of CSCs displayed a typical sigmoidal shape, with an initial lag, followed by exponential and stationary phases (Fig. 2). At the end of the experiment, FW/mL increased over 7-fold compared to the value at the inoculation time.
Cell viability remained stable at high percentages (> 90%).

References: [1] Munyendo *et al.*, 2021 – doi: 10.1016/j.jfca.2021.103995
[2] Yue *et al.*, 2016 – doi: 10.3109/07388551.2014.923986