# DEVELOPMENT OF A REGENERATION PROTOCOL, VIA SOMATIC EMBRYOGENESIS OF *Dioscorea* spp.

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**Introduction.** The yam (*Dioscorea)* habitat is mainly in tropical or subtropical areas (1). The tuber yam is valuable wild food and economic resource for the people of the region (2). In México, the tuber is harvested from plants that grow naturally in the tropical forest and its obtaining generates a high degree of difficulty (3).To establish yam cultivation, propagation is asexual (tuber), because the sexual via presents difficulties (plant dioica). Moreover, any improvement in this species is important to encourage sustainable agriculture in these regions (4). *In vitro* somatic embryogenesis is a protocol that can be used in many biotechnological tool for genetic improvement.
Therefore the goal of this research is to develop a protocol for regeneration via somatic embryogenesis of *Dioscorea* spp.

**Methods.** Two types of explants (root and nodal stem) were used to induce callus formation. For callus induction MS medium (5) was used with 1mg/L 2,4-Dichlorophenoxyacetic in absence of activated charcoal (medium IA) and presence of 2mg/L charcoal activated (medium IB), in dark and photoperiod condition. After 2 months, the calluses were transferred in medium II (somatic embryo induction), MS medium, 1.0 mg/L 2,4-D and 0.5 mg/L benzylamino purine, in dark condition. After 2 months, the calluses were transferred to medium III (somatic embryo maturation), MS medium containing 0.1mg/L of abscisic acid and 100mg/L glutamine in photoperiod condition. In this study we evaluated the formation of callus embryogenic callus and number of somatic embryos per callus. Results were subjected to an analysis of variance (ANOVA). Followed by Tukey multiple media comparison test ($P<0.05$ were considered significant).

**Results.** In medium IB was obtained 13.3% of callus formation of the explants of nodal stem (both in photoperiod as in darkness condition) and root explants (darkness condition), at 60 days of culture. However, in medium IA, under dark condition, the response of callus formations from the explants of roots increases 80%. The best treatment was in medium IA that was obtained 100% of callus formation from nodal stem tissue, both in photoperiod as darkness condition, with significant differences. Only callus from nodal stem in medium IA, dark condition, formed embryogenic callus (EC) and somatic embryos (SE), figure 1A-D, in medium II. However, the formation of EC was low at 6.6% and formed 1 SE per EC, this mainly due to the high degree of tissue oxidation. By transferring the EC to medium III in photoperiod condition, tissue continued its process phenolization and the subsequent cell death.

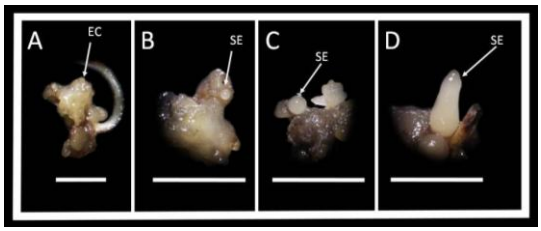


**Fig.1** Embryogenic callus and development of Dioscorea spp. SE after transferring callus to medium II. (A)Embryogenic callus, (B-C) Globular stage SE, (D) Scutellar stage SE (bar size 5mm)

**Conclusions.** Achieved the formation of embryogenic callus and somatic embryos, but did not achieve their development by the high degree of tissue phenolization, so it is important to find alternatives to help prevent tissue phenolization which could maybe improve embryogenic response.

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