Establishment of *in vitro* cultures of *Linum scabrellum* for the production of cytotoxic lignans

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Introduction. Cancer is a disease that is treated by rural communities in Mexico with the use of higher plants. *Linum scabrellum* is a Mexican plant, with a very significant cytotoxic activity in two human cancer cell lines in culture (1), deserving detailed scientific studies. However, natural populations of *L. scabrellum* are very scarce in Mexico. Plant tissue and organ culture techniques allow to propagate and grow plants and plant cells under uniform and controlled conditions. The objective of this work is to micropropagate and to develop somatic embryos of *Linum scabrellum* able to produce cytotoxic secondary metabolites.

Methods. Seeds from *Linum scabrellum* collected in the state of Querétaro (voucher number 27193) were sterilized and germinated *in vitro.* Plantlets were subjected to different phytohormonal treatments. Hypocotyls of 7-15 days old additioned with different combinations of 2,4-D (2,4-dichlorophenoxyacetic acid) and Zeatin (Table 1),were evaluated to induce embryogenesis internodes and leaf explants were obtained and supplemented with combinations of 2,4-D and BAP (benzilaminopurine) (Table 2) for micropropagation. The explants were placed in Petri dishes with Murashige and Skoog medium, and additioned with the hormonal combinations. Three explants were placed in each dish with a total of 3 dishes per experiment for each hormonal combination. Each experiment was conducted by triplicate. The explants were cultured at 25 ° C under 16 h photoperiod, and the formation of embryogenic calluses was registred.

Results. Neither shoot formation or adventitious roots were observed with hypocotyl explants; however, there was little callus formation from hard to friable in treatments number 5,6,7 and 8 (Table 1). These hormonal combinations have been reported for the formation of somatic embryos in other species of *Linum sp*. (Gomes *et al.*, 1996). In the micropropagation experiments, after 2 weeks in culture, apparent visible changes in color were observed for intermodal explants an leafs, with swollen or elongated and rough appearance. Calluses emerged in treatments 5, 6 and 8. In cultures with leaf explants callus started emerging at 15 days in treatments 1,2,3,4 and 7. Until now there has been no shoot formation, however, there are interesting structures that give indications of shoot formation in the explants (Fig. 1) (treatments 4, 6, 2 and 5). In a preliminary experiment, treatments (1, 2, 3,4,5,7 and 8) have been employed in this plant observing organogenesis or other structures in explants of leafs and internodes at 4 weeks.

Treatment	C	1	2	3	4	5	6	7	8
2,4-D (mg/L)	0	0.2	0.4	2	5	0.2	0.2	0.4	0.4
Zeatin (mg/L)	0	0	0	0	0	0.4	0.8	0.4	0.8

Table 1. Combination of plant growth regulators used in induction of somatic embryogenesis. C=control culture

Treatment	C	1	2	3	4	5	6	7	8
2,4-D (mg/L)	0	0.4	0	0	0	0.2	0.2	0.4	0.4
BAP (mg/L)	0	0	0.2	0.5	1	0.5	1	0.5	1

Table 2. Combination of plant growth regulators used in micropropagation of *Linum scabrellum*. C=control culture

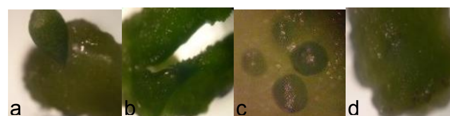

Fig.1 Structures formed in response to different regulators. a) and b) leaf explant, c) and d) internode explant

Conclusions. So far there is incipient callus generation of some treatments, interesting morphological changes observed in leaf explants and internodes that give us clues to the formation of structures such as shoots, roots and possibly somatic embryos in hypocotyl explantes as well as in leaves and internodes.

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References.

1. Lautié E, Quintero R, Fliniaux M-A, Villarreal M-L. 2008. *J. Ethnopharmacol.*: 402-412.
2. Gomes DA, Cunha AC, and Ferreira MF. 1996. *Plant Cell, Tissue and Organ Culture* 47: 1–8.