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Genetic transformation of a highly abiotic stress tolerant moss.
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Plagiomnium cuspidatum is one of the models we have been studying in our laboratory. In previous work, we have shown that its spores can germinate under high salt and osmotic stress conditions (200 mM NaCl and 600 mM sorbitol, respectively). In addition, its gametophores can survive after more than two years under desiccation conditions (30 % relative humidity). In order to transform this moss, we have standardized a system to get protoplasts from chloronema tissue (5, 10 and 15 days of age) testing different driselase concentrations (1, 2 and 3 %) and exposure times (1, 1.5 and 2 hours); besides, we evaluated their capacity to regenerate new protonemal tissue. The best treatment to obtain protoplasts (5.35x10^6 protoplasts g^-1) was: age 10 days, concentration 2 % and exposition 2 hours; however, the best regeneration rate (72.08 %) was found at: age 5 days, concentration 2 % and exposition 1 hour. We have carried out genetic transformation of P. cuspidatum protoplasts through PEG-mediated plasmidic DNA uptake. Antibiotic resistant colonies were recovered at high transformation efficiency (82 colonies µg^-1 DNA), yielding approximately 1500 colonies per genetic transformation event, using the plasmid pPGX8; however, we have only obtained resistant unstable colonies, though this kind of colonies can be used to produce recombinant proteins\(^1\). Our ultimate goal is to develop a stable genetic transformation system that will allow us to study gene function and to explore the production of recombinant proteins. We thank SIP and CONACYT for student fellowships and grant financial support.