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Development of a Methodology for In Vitro Tissue Culture and Callus Generation of *Polypogon Australis* Brong. (Poaceae)

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Extended Abstract

Phytoremediation technologies uses the ability of certain plants to absorb, accumulate, metabolize, volatilize or stabilize contaminants such as metals, that are present in the soil. *Polypogon australis* (Brong.) a native Chilean grass (Gramineae), is a facultative metallophyte that spontaneously colonizes abandoned mine tailing dumps. Genetic edition may be used to improve its metal tolerance and accumulation capabilities and thus to enhance its soil metal phytoextraction potential. At present, there are no protocols to carry out in vitro somatic embryogenesis and callus induction in *P. australis* which may lead to obtaining a complete gene-edited plant. In this study methodologies for massive in vitro plant tissue culture, callus formation and regeneration were studied by germinating and growing *P. australis* seeds in plates with Murashige and Skoog (MS) medium supplemented with sucrose and MS vitamins under controlled conditions (24°C, 4LS, 16/8; light/dark). A methodology for callus formation and plant tissue regeneration was carried out by cutting the seedlings in segments that were placed in plates with callus induction medium (CIM) and kept in darkness for 3 weeks, under control conditions (24°C, 0/24; light/dark). Results showed that both the medium and the growth conditions were effective for seed germination and early development of *P. australis*. A germination potential of 44-51% for *P. australis* seeds in the first 15 days was observed. After 7 days, callus development began only in the first segment corresponding to the hypocotyl. Therefore, for obtaining calluses of *P. australis* seedlings the optimal tissue was the hypocotyl. The percentage of callus formation was up to 38.9%. Nevertheless, 45.5% of total calluses could regenerate vegetative tissue (both leaves and radicle) without the need of additional hormones (i.e., synthetic auxins, cytokinins) to stimulate organogenesis. Positive results obtained in this work enabled us to develop an effective methodology for the growth and development of plant tissue culture and the generation and regeneration of calluses from hypocotyls of *P. australis* seedlings. Development of a methodology for massive in vitro callus generation and regeneration will allow us to apply genetic edition and plant tissue culture techniques for the enhancement of metal tolerance and accumulation of a native grass like *P. australis* for its use in soil phytoremediation.