

Resource Summary Report

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Root-to-shoot organogenesis in Citrus jambhiri Lush.

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Protocol Information

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Summary: A protocol for high-frequency direct organogenesis from root explants of Citrus jambhiri Lush. was developed. Full-length roots (~3cm) were isolated from the in vitro grown seedlings and cultured on Murashige and Skoog basal medium supplemented with Nitsch vitamin (MSN) with different concentrations of cytokinin [6-benzylaminopurine, (BAP)] and gibberellic acid (GA3). The frequency of multiple shoot proliferation was very high, with an average of 34.3 shoots per root explant when inoculated on the MSN medium supplemented with BAP (1.0 mg L⁻¹) and GA3 (1.0 mg L⁻¹). Optimal rooting was induced in the plantlets under half strength MSN medium supplemented with indole-3-acetic acid (IAA, 0.5-1.0 mg L⁻¹). IAA induced better root structure than 1-naphthaleneacetic acid (NAA), which was evident from the result of scanning electron microscopy (SEM). The expressions of growth-regulating factor genes (GRF1 and GRF5) and GA3 signaling genes (GA2OX1 and KO1) were high in the regenerants obtained using MSN+BAP (1.0 mg/L)+GA3 (1.0 mg/L). The expressions of auxin regulating genes were high in roots obtained in ½ MSN+IAA 1.0 mg L⁻¹. Furthermore, the virus indexing of the regenerants confirmed that there were no virus amplicons detected for Huanglongbing and Citrus tristeza virus. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers detected no polymorphic bands amongst the regenerated plants. The high-frequency direct regeneration protocol in the present study provides an enormous significance in Citrus organogenesis, its commercial cultivation and genetic conservation.

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