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INTERACTION IN PLANT GROWTH REGULATOR AND PHYSIOLOGICAL PROCESSES TO CONTROL PLANT REGENERATION AND IN VITRO DEVELOPMENT OF TULBAGHIA SIMMLERI

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he endogenous auxin and cytokinin contents of in vitro regenerated Tulbaghia simmleri maintained on applied plant growth regulators in Murashige and Skoog (MS) medium were investigated using UHPLC-MS analysis. The highest number of shoots (27.6 per leaf) were produced in MS medium supplemented with 2.5 µM thidiazuron. A higher number of these shoots were rooted with 10 µM 6-(2-hydroxy-3-methylbenzylamino) purine (PI-55, cytokinin antagonist). Production of somatic embryos (SEs: 16.4 – 4.6, globular to cotyledonary stages) improved significantly with liquid MS medium containing 2.5 µM picloram, 2.5 µM phloroglucinol (PG) and 1.5 µM gibberellic acid or 1.5 µM PI-55 and 1.0 µM trans-zeatin. SEs (torpedo and cotyledonary stages) germinated (100%) in plant growth regulator free MS medium. The plantlets were acclimatized, and all survived in the greenhouse. Higher levels of endogenous auxin, 2-oxindole-3-acetic acid (oxIAA, 371.52 pmol/g DW) and indole-3-acetylaspartate (IAAsp, 141.56 pmol/g DW) were detected in shoots from PG treatments. The roots of garden-grown-mother plants possessed the highest level of indole-3-acetic acid (IAA, 630.54 pmol/g DW) and oxIAA (515.26 pmol/g DW). Cytokinins [CKs: trans-zeatin-O-glucoside (tZOG), cis-zeatin (cZ) and N6-isopentenyladenosine-5⁻-monophosphate (iPRMP)] levels were relatively high in shoots and roots of plantlets in vitro. However, PI-55 treatments influenced the development of plantlets promoting a higher biosynthesis level of iPRMP (418.06 pmol/g DW in root) and cZRMP (904.61 pmol/g DW in roots and 1427.83 pmol/g DW in shoots). The reported protocol highlights the significance of exogenous and endogenous hormonal effects on large-scale production of in vitro plant development and improves the understanding of physiological processes of CK metabolism, signalling and transport in in vitro development.

