Optimization of a Liquid Culture System for Shoot Regeneration and Achieving an Enriched Level of Scopadulcic Acid B in the Leaf Organ Cultures of *Scoparia dulcis* L. by Response Surface Methodology

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Abstract

Response surface methodology (RSM) approach was utilized in the present investigation to optimize the constituents of Murashige and Skoog's (MS) liquid medium to enrich the scopadulcic acid B (SDB) content in *Scoparia dulcis* L. RSM approach using the central composite design (CCD) was employed to identify the precise concentration of growth regulators of medium and substantiated in shake-flask cultivation. CCD-RSM model revealed a strong agreement, with a predicted coefficient of determination (R^2) values of 0.881 and 0.872 for the shoot regeneration percentage (Y_1) and the average number of shoots per explant (Y_2), respectively. RSM predicted augmented conditions of MS liquid medium considerably influence the shoot proliferation (Y_1 and Y_2) in the leaf explants of *S. duclis*. The medium was fortified with 3.59 µM kinetin (KN; X_1), 6.00 µM 6-benzylaminopurine (BAP; X_2), 3.93 µM indole-3-acetic acid (IAA; X_3), and 25.84 g L^{-1} of sucrose (X_4). The present investigation conquered a maximal response of Y_1 and Y_2 with 91.28 ± 3.85 and 82.26 ± 2.13, respectively. The experimentally detected values are in close agreement with the predicted values of 90.07 and 79.70, respectively. This proposed that the developed design using CCD had efficacy in the optimization of medium components. The enhanced level of SDB, 9.89 ± 0.98 mg g^{-1} FW (*ca.* 5-fold), in the plantlets grown on liquid medium was significantly greater than those accomplished in the other tested plant tissues, comprises of field-grown parent plant leaves and *in vitro* developed callus and micropropagated plants on MS solid medium. For the first time, the methodology was developed effectively using RSM to promote the shoot proliferation and enhance the SDB production in the leaf organ culture of *S. dulcis* on MS liquid medium.