UNIVERSITI TEKNOLOGI MARA

ENHANCED PRODUCTION OF ADVENTITIOUS ROOT BIOMASS AND SELECTED FLAVONOIDS OF *Boesenbergia rotunda* (L.) MANSF. VIA LIQUID CULTURE SYSTEM

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ABSTRACT

Boesenbergia rotunda (L.) Mansf. is a unique species of Zingiberaceae (ginger) family which possesses valuable flavonoids, highly potential for pharmaceuticals. Selected flavonoids; pinostrobin, cardamonin and panduratin A in *B. rotunda* are renowned to exhibits pharmacological properties such as antimicrobial, antiviral, anti-inflammatory, antioxidant, anticancer, anti-HIV and anti-dengue. Limitation of these phytochemicals in nature have make the alternative approach through plant tissue culture as the most preferable route to obtain desired bioactive compounds. In this study, a tissue culture protocol emotively to proliferate *in vitro* adventitious roots and enhance selected flavonoids via liquid suspension system was established. The adventitious roots of *B. rotunda* were successfully induced from *in vitro* hypocotyl explants as early as on the 7^{th} day of culture with 95% induction rate on solid medium of half (1/2) strength Murashige and Skoog (MS) supplemented with 0.5 mg/L 1-Naphthaleneacetic acid (NAA). The establishment of adventitious roots in liquid cultures via shake flask system was achieved under optimized (physical and chemical) culture conditions. The highest root biomasses; fresh weight (FW) and dry weight (DW) with high production of selected flavonoids were obtained when cultured in liquid half strength MS medium + 0.5 mg/L NAA with addition of 5% of maltose at initial pH 5.8. The adventitious roots were cultured using initial inoculum density of 1.5 g per 50 mL medium under total (24 hours) darkness while continuously agitated on orbital shaker at the speed of 100 rpm for five weeks. Root biomass produced in the optimized culture conditions was found to be 2.3 folds higher than in the control conditions. In addition, the selected flavonoid contents in the optimized culture conditions were found almost 5 folds higher of pinostrobin content, 2.7 folds higher of cardamonin and 1.4 folds higher of panduratin A compared to the control. Elicitation and precursor feeding were applied to further enhance flavonoids productions in the adventitious roots. Salicylic acid (SA) was found to be the most ideal elicitor for *B. rotunda* adventitious root cultures compared to methyl jasmonate (MeJA) and silver nitrate ($AgNO_3$). Elicitation of 200 µM/L SA significantly enhanced the selected flavonoids (pinostrobin, cardamonin and panduratin A) production in the adventitious roots of *B. rotunda* with 32.7, 25.5 and 12.4 folds higher respectively. The selected flavonoids production in *in vitro* adventitious roots-fed precursor of 20 mM/L phenylalanine were significantly improved with 4.7 folds higher for pinostrobin, 11.1 folds higher for cardamonin and 20 folds higher for panduratin A compared to the control root samples. This successful outcome, is potential prior to obtain higher selected flavonoids, particularly panduratin A, as this compound exist low in nature and reported to be found only in trace amount in cell suspension cultures of *B. rotunda*. *In vitro* cultivation of *B. rotunda* adventitious roots in larger vessels was proven to be attainable through successful *in vitro* cultivation of *B. rotunda* adventitious roots via balloon type bubble bioreactor (BTBB) and SETIS bioreactor (SB). The *in vitro* adventitious roots grew well in BTBB compared to in the SB. Results showed significantly higher root production in BTBB with 11.1 folds average increment of root biomass compared to the roots cultured in SB. Production of selected flavonoids also were significantly enhanced in BTBB with 1.4 folds increment compared to the SB. The overall findings showed that the *in vitro* adventitious roots of *B. rotunda* elicited and precursor fed in the optimum culture conditions were promising for high production of the selected flavonoids. This study could also serve as a platform for larger scale production of similar bioactive compounds in the future.

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