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Effect of Plant Growth Regulators on in vitro Biosynthesis of Antioxidative Compounds in Callus Culture and Regenerated Plantlets Derived from Taraxacum officinale

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Abstract : Taraxacum officinale Weber or dandelion (Asteraceae) is an important Indian traditional herb used to treat liver detoxification, digestive problems, spleen, hepatic and kidney disorders, etc. The plant is well known to possess important phenolic and flavonoids to serve as a potential source of antioxidative and chemoprotective agents. Biosynthesis of bioactive compounds through in vitro cultures is a requisite for natural resource conservation and to provide an alternative source for pharmaceutical applications. Thus an efficient and reproducible protocol was developed for in vitro biosynthesis of bioactive antioxidative compounds from leaf derived callus and in vitro regenerated cultures of Taraxacum officinale using MS media fortified with various combinations of auxins and cytokinins. MS media containing 0.25 mg/l 2, 4-D (2, 4-Dichloro phenoxyacetic acid) with 0.05 mg/l 2-iP [N6-(2-Isopentenyl adenine)] was found as an effective combination for the establishment of callus with 92 % callus induction frequency. Moreover, 2.5 mg/l NAA (α-Naphthalene acetic acid) with 0.5 mg/l BAP (6-Benzyl aminopurine) and 1.5 mg/l NAA showed the optimal response for in vitro plant regeneration with 80 % regeneration frequency and rooting respectively. In vitro regenerated plantlets were further transferred to soil and acclimatized. Quantitative variability of accumulated bioactive compounds in cultures (in vitro callus, plantlets and acclimatized) were determined through UPLC-MS/MS (ultra-performance liquid chromatography-triple quadrupole-linear ion trap mass spectrometry) and compared with wild plants. The phytochemical determination of in vitro and wild grown samples showed the accumulation of 6 compounds. In in vitro callus cultures and regenerated plantlets, two major antioxidative compounds i.e. chlorogenic acid (14950.0 µg/g and 4086.67 µg/g) and umbelliferone (10400.00 µg/g and 2541.67 µg/g) were found respectively. Scopoletin was found to be highest in vitro regenerated plants (83.11 µg/g) as compared to wild plants (52.75 µg/g). Notably, scopoletin is not detected in callus and acclimatized plants, but quinic acid (6433.33 µg/g) and protocatechuic acid (92.33 µg/g) were accumulated at the highest level in acclimatized plants as compared to other samples. Wild grown plants contained highest content (948.33 µg/g) of flavonoid glycoside i.e. luteolin-7-O-glucoside. Our data suggests that in vitro callus and regenerated plants biosynthesized higher content of antioxidative compounds in controlled conditions when compared to wild grown plants. These standardized cultural conditions may be explored as a sustainable source of plant materials for enhanced production and adequate supply of oxidative polyphenols.

Keywords : anti-oxidative compounds, in vitro cultures, Taraxacum officinale, UPLC-MS/MS
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