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Strategies to enhance antioxidant potential and biosynthesis of essential oils in Ajuga bracteosa

Essential oils are the plant-derived natural products and are called “essential” due to the essence of the distinctive fragrance they contain, specific to the fragrance of the respective plant from which they are derived. A large portion (10%) of the known plant essential oils has important commercial applications. Essential oils are of interest in many industrial applications, including food, fragrance, cosmetic and pharmaceuticals. These volatile organic compounds have strong anti-bacterial, anti-fungal and anti-tumor potentials and can act as suitable alternatives to synthetic drugs. These volatiles are mainly produced in different parts of medicinal and aromatic plants. However, their limited productivity due to geographic variability and environmental fluctuations in natural plants does not meet the emerging industrial demands.

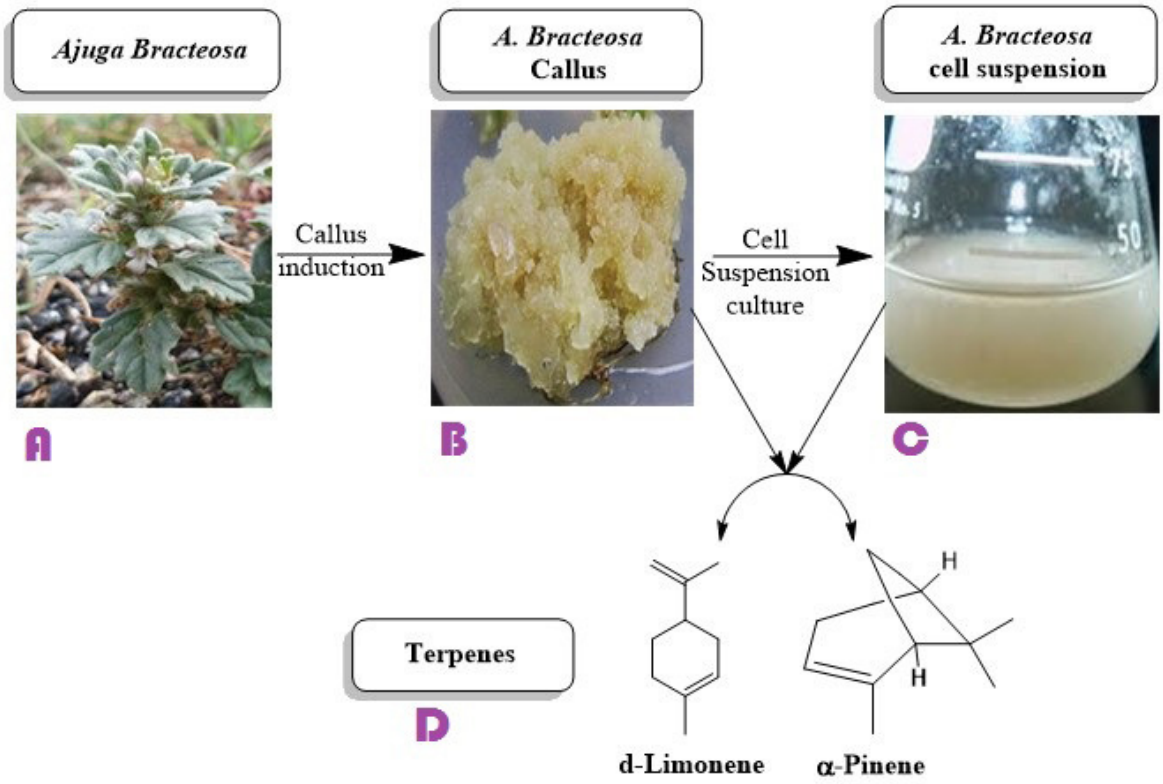

Fig. 1. Development of plant callus and cell suspension cultures for production of essential oils in Ajuga bracteosa. A: Wild grown plants used for explant preparation, B: Application of different types of elicitors such as Methyl jasmonate and Phenyl acetic acid under the effects of light or dark on the explants for induction and formation of callus biomass, C: Establishment of cell cultures for selection of suitable growth time and bioprocessing of callus tissues for hyper accumulation of antioxidants and essential oils, D: Biosynthesis of essential oils in callus tissues quantified through analysis of gas chromatography mass spectrometry.

Compared with the conventional cultivation procedures, plant cell cultures provide faster growth rate, healthy and uniform plant material, stable production of metabolites with no environmental restrictions and a promising source for synthesis of novel bioactive products which are usually not produced in the native plants. Cell cultures might also serve as suitable model systems for studying the physiology, molecular biology and biochemistry of plant cells to better understand the underlying mechanisms. Through the process of elicitation, cell cultures can be manipulated further for the enhanced production of many high valued secondary products. Different elicitation strategies can be employed through application of hormonal or physical elicitors in plant cell cultures, for increasing the yield of secondary metabolites production. Hormonal elicitors such as methyl jasmonate (Me-J) and phenyl acetic acid (PAA) have been used to increment the yield of different bioactive compounds in the cell cultures of a variety of MAPs . Similarly, physical elicitation through in vitro manipulation in the routinely employed photoperiod i.e. 16h. light & 8h. dark in plant cell culture laboratories, has been found to significantly influence the production of pharmacologically important secondary metabolites . Continuous provision or absence of light can differentially regulate plant cell growth and secondary metabolism through activation of distinct light responsive metabolic pathways, those can modulate the plant cell to cope with the varying stress conditions. Thus designing an elicitation strategy through in vitro employment of Me-J and PAA under the effects of different photoperiod regimes in Ajuga cell cultures can influence biomass formation and production of medicinally important secondary products. In this study, effects of different photoperiod regimes and hormonal elicitors were investigated on the accumulation of biomass, antioxidant potential and biosynthesis of secondary volatiles in the cell cultures of Ajuga bracteosa. Maximum accumulation of biomass (13.2g/L) was recorded in cell cultures established at 1.0mg/L benzylaminopurine (BA) in exposure to continuous dark. Biochemical assays showed that in the presence of 0.5 methyl jasmonate (Me-J), cell cultures grown under continuous dark had the higher activities of superoxide dismutase (SOD: 4.5U/mg), peroxidase (POD: 3.1U/mg), total phenolic content (TPC: 8.1mg GAE/g of DW) and total flavonoid content (TFC: 5.2mg QE/g of DW) respectively. Nonetheless, the free radical scavenging activity (FRSA) was found correlated with the phenyl ammonia lyase (PAL) activity in the dark grown cell cultures. Analysis through gas chromatography-mass spectrometry (GC–MS) showed, biosynthesis of 29 compounds in the in vitro raised cell cultures. The major identified compounds consisted of monoterpene hydrocarbons such as β-pinene (2.1–9.5%), β-ocimene (1.4–8.3%), 1-terpinene-4-ol (5.8–9.6%), caryophyllene (1.3–6.2%), β-farnesene (0.82–7.8), oxygenated monoterpenes including myrtenal (2.2–8.4%), citronellyl acetate (2.1–7.3%) and sesquiterpenes such as caryophyllene oxide (1.5–5.5) and β-elemene (2.2–8.8%). This protocol has the potential for commercial production of important secondary volatiles.

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