Abstract

The present investigation is targeted to characterization of spent media during sandalwood somatic embryo production in bioreactor. This study has been conducted in suspension culture, particularly in airlift bioreactor. Earlier studies in our laboratory were devoted to optimization of somatic embryogenesis of sandalwood in solid media by Dr. Surajit Das (1999); followed by liquid media and bioreactor by Dr. Susobhan Das (2001). In those processes large amount of embryogenic biomass goes waste along with the spent media. They play important role in somatic embryogenesis performance. Some of their constituents are commercially important. Hence metabolite profiling was done to explore those compounds systematically. Two important cell wall molecules (Arabinogalactan protein and peroxidase) involved in differentiation were characterized biochemically. Effects of stress on somatic embryo induction vis-à-vis those molecules were studied.

Embryogenic suspension was established from germinated seed hypocotyls and other explants. The auxin 2,4-D at 1.0 mg/L was the best auxin in establishing the embryogenic suspension culture of sandalwood yielding maximum embryogenic clumps (EC). At 1.0 mg/L of 2,4-D maximum embryo induction efficiency (EIE) value of 4.48% was obtained. An equimolar concentration of the auxins IAA and cytokinin BAP at 0.5 mg/L each was required for further embryo development in suspension. Maximum globular embryo differentiation with an EE value of 3.71% was obtained at these combination and concentration. An initial inoculum dose of 1.2% (w/v) was best performing in terms of globular embryo production, for which embryogenesis efficiency (EE) was 3.92% .The specific growth rates and doubling time obtained in shake flasks and bioreactor were 0.104 day^{-1}, 6.6 days and 0.09 day^{-1}, 7.7 days respectively. The highest biomass level obtained by cell suspension culture in airlift bioreactor culture was 6.19 g/L (dry weight).

Metabolite profiling was done with embryogenic biomass and spent media with the help of different analytical techniques like TLC, HPLC, NMR, IR etc. Principal component analysis (PCA) of FTIR fingerprint seems to be a useful chemometric technique to analyze biochemical features of cell/tissue, which dynamically change with cellular differentiation.

The yield of peroxidase, arabinogalactan proteins, phenolics and sandal oil indicate them as potential by-products for further value addition to the process. The advantage lies in their easy downstream processing, as these are mostly extracellular product secreted in the spent medium.

AGP and peroxidase (two important molecular marker of various physiological processes) have been characterized biochemically. AGP seems to play important role in somatic embryogenesis. AGP content in various tissues (unorganized to differentiated) and crossed electrophoresis pattern indicated that AGP could be used as a marker for differentiation. MTT and NO production assays in splenocytes revealed AGP's possible immunomodulatory use. HPLC, ^{1}H NMR and FTIR confirmed typical chemistry of AGP. The characteristic features of the isolated peroxidase were: Km 10.91 mM;Vmax 14.88 μM/min; temperature optimum 50 ^{0}C. Yield was 32,200 U/litre of spent medium with specific activity of 1.3417 U/μg protein.

Different chemical stress agents were found to induce somatic embryogenesis, increase total peroxidase activity and decrease in AGP quantity. The reason might be increased oxidative stress leading to increase activity of peroxidase which along with other enzymes might catalyze increased cross-linking of AGP with other cell wall polymers.

Key words: Spent media, Sandalwood, Metabolite profiling, Arabinogalactan protein (AGP), Peroxidase (POX), Stress, Embryogenesis, Bioreactor