Abstract

The primary source for food, shelter, and pharmaceutical products are primarily derived from plants, continuous consumption of plants for its medicinal and other uses leads to the extinction of the species from the wild. Plant tissue culture may be an alternate and ultimate solution to address this issue, in this technique any plant cell, tissue, or organ are used to grow in the culture media supplemented with plant growth regulators inside a controlled and aseptic environment. This technique is very important for mass propagation and rapid production of uniform, and disease-free plants; it is also very useful for the preservation of endemic plants and plant genome transformation. It is also very much effective tool for production of secondary metabolites and bioactive compounds from plants. Plant genome modification, plant improvement, transformation for vaccine production, and production of secondary metabolites are widely performed using this technique.

In the present experiment five medicinal plants were selected from Tamulpur and Kokrakjar district of Assam, those includes- *Torenia crustacea, Lindernia pusilla, Phlogacanthus thyrsiformis, Enydra fluctuans,* and *Hygrophila auriculata.* This experiment aimed to standardize *in vitro* mass propagation technique, study of genetic stability in micropropagated plants using RAPD assay, comparison of antioxidant activity using different antioxidant assays, comparison of gallic acid and quercetin content in the wild and *in vitro* plant using HPLC analysis, and identification of bioactive constituents in wild and micropropagated plants using GC-MS analysis.

The explant surface sterilization for *in vitro* propagation was conducted using 0.1% mercuric chloride for 1-5 min followed by incubation of the sterilized explants inside the growth chamber under a controlled and aseptic condition in the growth medium supplemented with growth regulators (BAP, IAA, NAA). During the entire process of tissue culture, the *in vitro* plant genome may undergo somaclonal variation due to the different *in vitro* conditions and growth regulators. For the detection of the somaclonal variation RAPD technique was conducted using 14 RAPD primers (OPC 01 to OPC 10, OPA1, OPA 02, OPA 12 and OPA 13).

Different nutrient sources and *in vitro* conditions in the micropropagated plants may vary in the production of the secondary metabolites and active compounds from the wild plant. Therefore, the comparative antioxidant capacity experiments (total phenol content, total flavonoid content, total antioxidant capacity, and DPPH free radical scavenging activity) in wild and *in vitro* plants were conducted. Again, the quantitative gallic acid and quercetin content in the wild and *in vitro* plants were studied using HPLC analysis, and finally the bioactive compounds in the wild and *in vitro* plants were identified using GC-MS analysis.

In the *in vitro* propagation experiment of *T. crustacea,* most effective explant surface sterilization was observed in 2 min treatment (63.33±4.71%) using 0.1% mercuric chloride, after 21 days of culture. In case of *L. pusilla,* 2 min and 3 min of treatment showed the maximum explant survival rate (76.67±4.71%) after 21 days of culture. In case of *P. thyrsiformis,* 2 min and 3 min treatment showed maximum survival rate (76.67±4.71%) of the explants after 21 days of explant culture, in case of *E. fluctuans,* 3 min and 4 min showed highest explant survival rate (76.67±4.71%) was observed after 28 days of the culture initiation, finally in case of *H. auriculata,* 4 min showed highest (56.67±4.71%) explant survival rate after 21 days of explant culture.

Most effective shoot proliferation and multiplication of *T. crustacea* and *P. thyrsiformis* were observed in the MS media supplemented with 1mg/L BAP and 0.2 mg/L NAA and the highest average shoot length was observed and maximum average number of rooting was observed in MS media supplemented with 1mg/L IAA. In case of *L. pusilla* most effective shoot proliferation and multiplication were observed in the MS media supplemented with 1mg/L BAP and 0.2 mg/L NAA and the highest average shoot length was observed and maximum average number of rooting were observed in the MS media supplemented with 0.5mg/L IAA. Most efficient shoot multiplication of *E. fluctuans* explants were observed in the MS medium with 2 mg/L BAP (BM6), highest root formation was obtained in the MS medium supplemented with 1mg/L IAA. Finally in case of *H. auriculata m*ost effective shoot proliferation and multiplication were observed in the MS media supplemented with 1mg/L BAP and 0.5 mg/L NAA and the highest average shoot length was observed. Maximum average number of rooting were observed in the MS media supplemented with 1mg/L IAA.

Somaclonal variation was observed in all the genome of *in vitro* propagated plant genomes from the RAPD experiment. From the comparative antioxidant study of wild and *in vitro* plants, the *in vitro* propagated plants showed higher antioxidant potential as compared to the wild plant. Also, from the comparative quantitative detection of gallic acid and quercetin in the wild and *in vitro* plant, the *in vitro* plant produced higher gallic acid and quercetin content than the wild plant.

The active compounds in the wild and *in vitro* plants were identified using GC-MS analysis based on the peaks observed on the chromatograms. Diverse group of active compounds were identified with important characteristics including antimicrobial, antioxidant and antiviral activities were exhibited in the *in vitro* and wild plants.

The study concludes that 0.1% mercuric chloride is effective for the explant surface sterilization of all the explants. Again, MS medium supplemented with higher concentration of BAP (1mg/l BAP) and lower concentration of NAA (0.2-0.5mg/L NAA) was most effective for the shoot multiplication and elongation, finally rooting was observed best in the MS medium supplemented with IAA (0.5mg/L to 1mg/L IAA). RAPD assay suggested that the presence of somaclonal variation in the *in vitro* plants. The antioxidant study suggested that the *in vitro* plants possessed higher antioxidant activity as compared to the wild plant and the comparative quantitative gallic acid and quercetin content analysis using HPLC suggested that the *in vitro* plants carried higher concentration of gallic acid and quercetin content as compared to the wild plant. In the screening of bioactive compounds some important bioactive compounds were identified in different medicinal plants, and the active components were varied in the *in vitro* plants than the wild plant. The somaclonal variation in the *in vitro* plants and the variation in the antioxidant properties and active compounds in the plant extracts may be caused due to the different *in vitro* conditions during the tissue culture process and use of different growth regulators in the culture medium.

The performed research will help in commercial production of *Lindernia crustacea, Lindernia pusilla, Phlogacanthus thyrsiformis, Enydra fluctuans,* and *Hygrophila auriculata*. Production of secondary metabolites, and extraction of bioactive compounds form these plants are possible using the performed experiment.