

0.03, 0.04, 0.1, 0.5, 0.1, 0.2, 2.5 mg/L), BAP (0.0, 0.01, 0.02, 0.03, 0.04, 0.1, 0.5, 0.1, 1.5 mg/L) in different concentration and combination to promote callus formation. The pH of the culture media was maintained between 5.4-5.7, adjusted with NaOH or HCl, and solidified with 0.8% agar.

Cultures were kept at a controlled temperature of (22±2) °C with a 16-hour photoperiod, with sub culturing performed every two weeks. Callus induction involved sub culturing leaf explants in different concentrations of BAP and NAA to enhance the shoot formation. In regular intervals of two weeks were maintained for monitoring and promoting the number of shoots generated from the callus (Dodds and Roberts, 1982). The study found that MS medium supplemented with BAP and 2,4-D was more effective for callus induction compared to NAA and BAP. Callus exhibited a range of colours (Lisowska and Wysokinska, 2000) and showed significant genetic variability, leading to successful multiple shoot formation from the leaf explants.

4.2 Effective propagation method for salt tolerance

Using apical tip as meristem as explant it resulted in quicker plant growth than Nodal Segment. This Medicinal coleus explant grown in the NaCl Medium of different Concentration from 0.1% to 0.4%. Later they found that the explant of medicinal coleus was able to withstand and grow at the low concentration of 0.2% of NaCl with the support of BAP and NAA (Sharan et al., 2014).

Generally, the use of BAP is considered to be most suitable for promoting large-scale multiplication and micropropagation of various plant species (Shrivastava and Banerjee, 2008). In *C. forskohlii*, BAP alone has shown best result in promoting multiple shoots formation from the nodal segments and shoot tips (Sen and Sharma, 1991).

4.3 Effect of brassinosteroid in rooting

Swamy et al. (2021) found that the application of brassinosteroid significantly increased root numbers in medicinal coleus plants. The application of 28-homobrassinolide at 100 µM resulted in an 85% increase in root numbers by day 15, indicating its effectiveness in promoting rooting. The findings suggest that brassinosteroid could be pivotal in commercial cultivation practices for medicinal plants, enhancing both rooting and vegetative growth (Rao et al., 2010). Terminal cuttings from healthy coleus plants were treated with BRs, monitored for 160 days, and found to increase carbohydrate levels and forskolin content in roots, suggesting a link between growth regulators and secondary metabolite production.

4.4 Effect of cytokinin combined elicitors in coleus

In vitro culture techniques using cytokinins combined with elicitors can enhance both the propagation efficiency and secondary metabolite production of *Coleus* spp., a plant group with notable medicinal value. Cytokinins promote cell division, shoot differentiation, and tissue proliferation, while elicitors stimulate defense-related physiological responses and activate secondary metabolic pathways. Their combined application may therefore improve the accumulation of bioactive compounds associated with antioxidant, anti-inflammatory, and pharmacological activities.

Compared with conventional cultivation, tissue culture provides controlled growth conditions, stable nutrient availability, and a shorter production cycle. These factors create a favorable environment for secondary metabolite biosynthesis in *Coleus* spp. Previous studies have shown that controlled culture conditions and nutrient supply can increase secondary metabolite levels in tissue-cultured plants (Govindaraju et al., 2018). Therefore, optimizing cytokinin types and elicitor combinations is an effective strategy for improving both in vitro propagation and medicinal compound production in coleus.

4.5 Shoot regeneration from proximal, middle and distal segment of *Coleus forskohlii* leaf explants

Rapid shoot regeneration was examined in Three different segments of coleus leaf explant (Proximal, Middle and Distal) where cultured on Murashige and Skoog (MS) Medium with different concentrations of BAP, KIN, NAA, and IAA were tested for shoot elongation. Although callus proliferation was observed, KIN was found to be ineffective in the present study. However, Sharma et al. (1991) previously reported that KIN-enriched media