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DETERMINATION OF EFFECTIVE PARAMETERS IN PARTICLE BOMBARDMENT METHODOLOGY FOR SUCCESSFUL DWARFING GENE INTEGRATION AND TISSUE REGENERATION OF *OSBECKIA OCTANDRA* (L.) DC

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Osbeckia octandra (L.) DC is a perennial endemic shrub and it has attractive pale pink or pinkish purple flowers. There is a high potential to introduce it as a flowering potted-plant if the height and branching habit are manipulated. In this study, particle bombardment methodology along with a dwarf-phenotype conferring gene was used to fulfill this objective.

Fully expanded one month old 3rd and 4th leaves from the *in vitro* grown shoots of *O. octandra* were cut into approximately 1 cm^2 and 0.5 cm^2 leaf pieces and they were cultured with the abaxial sides touching the Murashige & Skoog (MS) medium supplemented with 100 mg/L myo-inositol, 6 % sugar and 0.25 M mannitol. The setup was incubated in dark at 25 ^{0}C for 10 days. Biolistic PDS-100/He instrument was used for the bombardment on the explants. Fifty leaf explants of 1 cm^2 size and 100 explants of 0.5 cm^2 size were maintained per Petri dish. Hundred and fifty samples were used per treatment combination. Bombardments were performed at a 900 psi, at a vacuum of 25 Hg inch and 2.5 cm gap distance with gold microcarriers. For microprojectile bombardment, the plasmid pJIT 60 was used, which has been constructed expressing *gai* under the control of the 35S CaMV promoter containing ampicilin resistant marker and the *gus-A* reporter gene. Three target distances of 6 cm, 9 cm and 12 cm were investigated. Bombarded explants were cultured on five MS media combinations supplemented with 2, 4, and 6 mg/L kinetin and 3 mg/L Benzyle Amino Purine in combination with 0 and 0.5 mg/L Naphthalene acetic acid after 7 days of bombardment. The leaves then incubated in dark until the regeneration signs appeared. Then these were transferred to the light after about 5 weeks from the bombardment. After one week, shoot regenerated explants were screened with 100 µM ampicilin and the surviving explants were tested with ß-glucoronidase (GUS) assay.

Bombarded leaf tissues showed differences in callus and shoot formation in five different media combinations. According to non parametric categorical data analysis, highest number of callus and shoots per explants were observed in the MS medium supplemented with 4 mg/L kinetin, without NAA or BAP ($P<0.05$). Leaf sizes were not significantly affected by the shoot regeneration. In ampicilin screening, control samples died after one week. From treated samples, 178 viable shoots and callus remained, and from that, one sample treated with 6 cm target distance was positive for the GUS assay. According to the results of the GUS assay, the best gene integration was achieved at 6 cm target distance (microcarrier flight distance) of the Biolistic PDS-1000/He particle delivery system.