

In vitro mutagenesis and characterization of mutants through morphological and genetic analysis in orchid *Aerides crispa* Lindl.

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Mutation breeding techniques combined with tissue culture and molecular marker methods provide a powerful tool for improvement of slow growing plants such as orchids. In this study, we developed a protocol for *in vitro* mutagenic studies in a medicinal orchid of Western Ghats, *Aerides crispa* Lindl., commonly called the Curled Aerides. *In vitro* grown 60 day old protocorms treated with low concentrations (0.025–0.03%) of colchicine, ethyl methane sulphonate (EMS), and low doses (1–4Gy) of gamma radiations, and 27 combinations resulted in healthy multiple shoot bud formation with 40–60% survival frequency. Higher singly or combined dose treatments caused protocorm or shoot initials death, after 20–30 days. Healthy 2500 *in vitro* seedlings were screened for phenotypic changes in shoots and roots owing to a mutagenic effect. In total, 206 *in vitro* seedlings of 52 variant lines were identified on the basis of their unique leaf shape, colour, white stripes, thickness, length, and width and their root length and thickness. These variant lines were multiplied, established in an orchidarium, and compared with the control for genetic variability by using Random Amplified Polymorphic DNA method. Only 15 genetically distinct mutant lines were identified, which exhibited disparity in growth rate, leaf shape, leaf length, width, chlorophyll variegation, stomatal density, and/or pigment contents.

Keywords: Colchicine, Ethyl methanesulfonate, Gamma radiation, *In vitro* mutation, Orchidarium, Protocorms, Western Ghats