Research Article

GAMMA RADIATION INDUCED PHENOTYPIC DIVERSITY IN DENDROBIUM 'EMMA WHITE'

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ABSTRACT

Dendrobium, one of the largest genera of family *Orchidaceae,* is economically important both as an ornamental and medicinal crop. Varietal improvement of Dendrobium through the conventional breeding methods is difficult and challenging due to existence of incompatibility among the respective primary gene pool. Gamma irradiation technique is useful to induce variability and generate mutants with improved traits in a short period. In our study, protocorm-like-bodies (PLBs) of Dendrobium hybrid 'Emma White' were exposed to gamma rays (10-80 Gy) and *in-vitro* propagated up to M1V5 generation to raise irradiated plant population. Phenotypic variability was recorded among the plantlets during the *ex-vitro* semi-hardening stage. Leaf variations in terms of shape, size, structure, surface, tip and colour were observed in 10 Gy irradiated plants. Significant variations were observed in leaf structure and arrangement at 40 Gy. The putative mutants were analyzed for molecular characterization. Among some of the desirable mutants isolated, Mutant line 10/46 was found to exhibit early flowering. The results suggest significant potential of gamma radiation on enhancing genetic variability of both vegetative and flowering traits of Dendrobium hybrid.

INTRODUCTION

Orchid is known to be one of the most beautiful, diverse and complex flowering plants in the earth. It belongs to *Orchidaceae*, the largest family of angiosperms, with nearly 600-800 genera and 20,000-30,000 species (Goh and Kluge, 1989). In India, orchid resources represent nearly 9% of flora (Deb, 2013). Among all, the genus Dendrobium exhibiting largest diversity in terms of size, shape, colour and structure was established by Olof Swartz in 1799 (Nova Acta Regiae Societatis Upsaliensis) comprises 1400 species distributed to India, Japan, Malaysia (South), Indonesia, New Guinea (East) and Australia (Devadas *et al*., 2009). In the world of floriculture, Dendrobium is regarded economically important and a huge contributor of foreign exchange earner. As per the United States National Agricultural Statistics (2017), it has a total estimated value of $4.6 million, with potted plant contributing $3.2 million and cut flower $1.2 million in Hawaii, the major Dendrobium growing region. Thus, the development of new varieties with enhanced floral traits has become important to compete in the international floriculture market. The conventional breeding method is difficult due to existence of incompatibility among the respective genetic pool (Devadas *et al*., 2016). Mutagenesis as an alternative technique can be adopted to develop new varieties in short period of time with maximum diversity. Through gamma irradiation on PLBs of Dendrobium Sonia, 11 stable mutant varieties of different flower morphology, having 4 lines with commercial potential was identified (Ariffin *et al*., 2010). The effect of radiation on differentiation of PLBs (Emma white x Banyat pink) was found to be decreasing with increased dose (Velmurugan *et al*., 2010). Similarly, gamma irradiation on PLBs of scented orchid, *Zygopetalum maculatum*, had exerted various effects on survival rate, growth and differentiation (Sherpa *et al.,* 2018). Morphological variations with regard to plant height and leaf structure was also reported in plantlets generated from irradiated PLBs of Dendrobium hybrid Sonia x Emma (Velmurugan *et al*., 2010). Growth pattern of irradiated shoot cultures of Dendrobium Sonia under various monochromatic light had significant influenced on growth and survival (Billore *et al.*, 2019). The present work on Dendrobium hybrid 'Emma White' describes the gamma

radiation induced morphological variations at tender *ex-vitro* hardening stage.

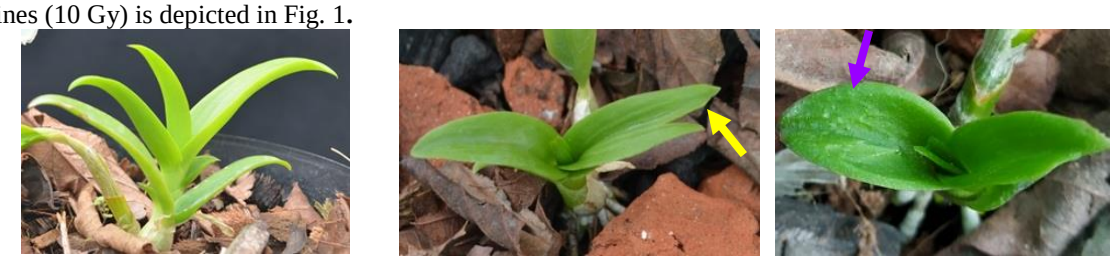
MATERIALS AND METHODS

Protocorm-like-bodies (PLBs) of Dendrobium hybrid 'Emma White' were exposed to gamma radiation of different doses (10, 20, 40, 60, 80 Gy) using cobalt-60 gamma irradiator- Gamma Chamber 5000 (dose rate: 32.54 Gy/min) at Bhabha Atomic Research Centre, Trombay, Mumbai. Post irradiation, PLBs were transferred onto fresh Gamborg medium supplement with PGRs and propagated up to M1V5 generations. Well-developed *in-vitro* plantlets (4-5 leaves; 6-7 roots) were selected for semi-hardening and acclimatization. Phenotypic variations in terms of leaf shape, size, structure, surface, apex and colour were regularly monitored and recorded.

RESULTS AND DISCUSSION

Approximately 200 hardened plants and 500 *in-vitro* plantlets (rooting stage) were regenerated from treated PLBs (10, 20, and 40 Gy) within 24 months (2017-2019) of irradiation. Among 120 putative mutants, 12 lines were selected for further evaluation. The mutants were characterized based on vegetative parameters such as leaf structure, size, shape, apex, margin and colour. The differences in phenotypic variations in traits were compared with control plants (un-irradiated) during *ex vitro* screening process. The observed variations of putative mutants are described below and, the comparison of control versus mutants lines (10 Gy) is depicted in Fig. 1.

- Mutant line **10/4**: Asymmetrical leaf with notch forming two uneven apex/tip
- Mutant line **10/5**: Closed leaf with marginal tooth or serrations in and around apex 2 leaves emerged from 4th leaf base
- Mutant line **10/17**: Asymmetric ovate leaf with 2 midribs, slightly sickle-shaped, 2 leaves emerged from 4th leaf base
- Mutant line **10/21**: Linear leaves (long and narrow like a blade of glass),Yellowish green
- Mutant line **10/35**: Bilobed leaf (02 leaves fused forming two separate midribs)
- Mutant line **10/36**: Uneven leaf, rounded base, shaped like a lance tip, elevated midrid
- Mutant line **10/60**: Broad leaves
- Mutant line **10/66**: New shoot emerged from top node of main stem, elliptic/oval shaped leaves, short or no tip, one tip slightly notched
- Mutant line **10/75**: Rough, uneven leaf surface (doted type)
- Mutant line **10/76**: Uneven sickle-shaped leaf, midrib on left side, 02 leaves emerged from 1st leaf base
- Mutant line **10/82**: Fused nodes and leaves, 3D structured type with pointed tip
- Mutant line **10/85**: Deformed leaf emerged from base of other leaf, Oval shaped with wider base and tip, 2 midribs.



Control **10 Gy mutants**

Fig. 1. Morphological variations of observed mutants @ 10 *Gy*

Extremely variations and deformity of plant & leaves were observed in *ex-vitro* semi-hardened plantlets at 40 Gy from *in-vitro* stage to hardening stage. Most of them were deformed leaf structure with multiple tooth or serrations, deep notches, asymmetric leaf arrangements were recorded in generated 40 Gy lines (Fig. 2).

Control **40 Gy**

Fig. 2. Morphological variations of selected lines of mutant @ 40 *Gy*

Preliminary observations have indicated an early flowering mutant among the10 Gy treated plants. The regenerated plant exhibited flower buds within nine and half months from the date of ex-vitro semi-hardening. Significant morphological changes with regard to shape of petals, sepals and lip were also reported in gamma irradiated PLBs of Dendrobium Sonia (Ariffin *et al.,* 2010). In our initial study, different doses of gamma radiation showed the reduction in the proliferation and differentiation efficiency of PLB's in 'Emma White' (Sherpa *et al.,* 2018). Stomata size was decreased with cellular damage at radiation higher doses (Dehgahi *et al.,* 2017), which can influence pattern of growth and proliferation.

Control Flower **10/46**

Fig. 3. Early flowering mutant (10/46)

CONCLUSIONS

Results from the present study indicated significant effects of gamma radiation induced variations in both vegetative and flowering characters of Dendrobium hybrid. Selected mutant lines based on morphological variations can be used for further molecular characterization and plant improvement.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest within themselves and others including the funding agency and the agency where the research was carried out.

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