

## Research Article

# GAMMA RADIATION INDUCED PHENOTYPIC DIVERSITY IN DENDROBIUM 'EMMA WHITE'

Sherpa, R.<sup>1</sup>, R. Devadas<sup>1\*</sup>, R. K. Pamarthi<sup>1</sup>, T.D. Nikam<sup>3</sup>, S.N. Bolbhat<sup>4</sup>, P. Suprasanna<sup>2</sup>

<sup>1</sup>ICAR-National Research Centre on Orchids, Pakyong-737106, Sikkim, INDIA

<sup>2</sup>Bhabha Atomic Research Centre, Trombay, Mumbai-400085, Maharashtra, INDIA

<sup>3</sup>Department of Botany, Savitribai Phule Pune University, Pune-411007, Maharashtra, INDIA

<sup>4</sup>A.A. College, Manchar, Tal. Ambegaon, Pune-410503, Maharashtra, INDIA

\*Corresponding author's E-mail: ramgopal.devadas@icar.gov.in

## KEYWORDS:

Dendrobium,  
gamma radiation,  
mutagenesis,  
phenotypic variation

## 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.

## ARTICLE INFO

### Received on:

01.09.2019

### Revised on:

08.12.2019

### Accepted on:

09.12.2019

## 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 (Ariiffin 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 4<sup>th</sup> leaf base
- Mutant line **10/17**: Asymmetric ovate leaf with 2 midribs, slightly sickle-shaped, 2 leaves emerged from 4<sup>th</sup> 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 midrib
- 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 (dotted type)
- Mutant line **10/76**: Uneven sickle-shaped leaf, midrib on left side, 02 leaves emerged from 1<sup>st</sup> 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 Flower

10/46

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

Preliminary observations have indicated an early flowering mutant among the 10 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



40 Gy

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.

## ACKNOWLEDGEMENT

The research is being funded by the Board of Research in Nuclear Sciences (BRNS), Bhabha Atomic Research Centre (BARC), Mumbai (project code: 35/14/22/2016-BRNS/35061) and also thank Director, ICAR-NRCO for providing support.

## REFERENCES

- Ariffin, S., A. Mohamad, A. A. Hassan, Z. Ahmad and M.N. Basiran. 2010. Flower morphology of *Dendrobium Sonia* mutants. *Research and Development Seminar*, Malaysia.
- Billore, V., Shriram J., Mirajkarb, Penna Suprasanna and M. Jain. 2019. Gamma irradiation induced effects on *in vitro* shoot cultures and influence of monochromatic light regimes on irradiated shoot cultures of *Dendrobium Sonia* orchid. *Biotechnology Reports*, **24**. doi.org/10.1016/j.btre.2019.e00343
- Deb, C. R. 2013. Orchids of Nagaland, propagation, conservation and sustainable utilization: A review. *East Himalayan Society for Spermatophyte Taxonomy, Pleione*, **7**(1): 52 – 58.
- Dehgahi, R and A. Joniyasa. 2017. Gamma Irradiation-Induced Variation in *Dendrobium Sonia*-28 Orchid Protocorm-Like Bodies (PLBs). *Fungal Genomics and Biology*, **7**: 151. doi:10.4172/2165-8056.1000151
- Devadas, R., P. Khatiwara, D. Barman and S. P. Das. 2009. Breeding *Dendrobium phalaenopsis*-cane type hybrid in India: NRCO-42 (Den. "Emma White" x Den. "Pompadour"). *Indian Journal of Genetics and Plant Breeding*, **69**(3): 237-242.
- Devadas, R., S. L. Pattanayak and D. R. Singh. 2016. Studies on cross compatibility in *Dendrobium* species and hybrids. *Indian Journal of Genetics and Plant Breeding*, **76**(3): 344-355.
- Goh, C.J. and M. Kluge. 1989. Gas Exchange and Water Relations in Epiphytic Orchids. In: Lüttge U. (eds) *Vascular Plants as Epiphytes*. Ecological Studies (Analysis and Synthesis), **76**: 139-166.
- Sherpa, R., R. Devadas, P. Suprasanna and P. R. Kishore. 2018. Effect of gamma irradiation on PLBs of *Dendrobium*. *National Conference on Floriculture for Rural and Urban Prosperity in the Scenario of Climate Change*, CAE & PHT (CAU), Sikkim, February 16 – 18.
- Sherpa, R., R. Devadas, P. Suprasanna and P. R. Kishore. 2018. Effect of Gamma Radiation on Asymbiotic Seed Germination of Scented Orchid *Zygopetalum Intermedium*. *International Conference on Climate Change, Biodiversity and Sustainable Agriculture (ICCBSA-2018)*, Jorhat, Assam, December 13-16, Volume II; pp.64.
- Velmurugan, M., K. Rajamani, P. Paramaguru, R. Gnanam, J.R. Kannan Bapu, C. Harisudan and P. Hemalatha. 2010. *In vitro* mutation in horticultural crops- a review. *Agricultural Reviews*, **31**(1): 63 – 67.

### How to cite this article?

Sherpa, R., R. Devadas, R.K. Pamarthi, T.D. Nikam, S.N. Bolbhat and P. Suprasanna. 2019. Gamma radiation induced phenotypic diversity in *Dendrobium* 'Emma White'. *Innovative Farming*, **4**(4): 204-206.