

## Study on the Effects of Gamma Irradiation on Protocorm-Like Bodies of *Dendrobium* Hybrid against *Fusarium proliferatum* and *Fusarium oxysporum*

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Running title: Gamma Radiated PLBs Against *Fusarium* sp.

**Abstract.** *Dendrobium*s orchids are prone to diseases caused by *Fusarium proliferatum* and *Fusarium oxysporum*. Therefore, gamma irradiation was utilised on *Dendrobium* hybrid, D5 to produce cultivars that are resistant towards these fungi. Gamma doses of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gray (Gy) were radiated on thin cell layer (TCL) of protocorm-like bodies (PLB) and were observed for four weeks. Gamma doses of 20 and 30 Gy were optimum for survivability and shoot regeneration of treated PLB. The radio sensitivity (LD<sub>50</sub>) of the PLB was approximately 63 Gy. Histology and scanning electron microscopy (SEM) analysis showed prominent cell damage and alteration in surface morphology in gamma irradiated PLB. The number of stomata and stomatal aperture decreased where it was  $15.47 \pm 8.16 \mu\text{m}$  at 10 Gy but reduced to only  $8.24 \pm 3.91 \mu\text{m}$  at 90 Gy. Direct amplification of minisatellite DNA (DAMD) showed high degree of polymorphism in these PLB. When challenged with spore suspension of these fungi, plantlets radiated with 20 and 30 Gy of gamma irradiation showed the least disease symptom, thus, proving resistance towards these fungi. Therefore, this study is a preliminary screening study where the optimum doses of gamma irradiation were selected based on the reaction of radiated TCL of PLB towards the mutagen.

**Keywords:** Disease resistance, Leaf-bridge bioassay, Pathogen, Resistance, Radio sensitivity

## INTRODUCTION

Orchids are the most diverse botanical family in the world with nearly 3736 genera around the world (Chase et al. 2015). There is an increased demand for orchids due to their beneficial uses in the horticulture market and in the medicine field (Singh and Duggal 2009). However, high demands for the orchids causes overexploitation of this plant. Therefore, the horticulture industry relies mainly on artificial propagation where several tissue culture techniques have been developed for orchids. In recent years, thin cell layer (TCL) is used as another option of explant

for micropropagation of orchids. TCL is a thin layer of cells which is totipotent and able to produce a greater number of explants compared to the whole protocorm-like bodies (PLBs) (Teixeira da Silva 2013).

When working with plants *in vitro*, somaclonal variation is inevitable. In addition to the basic genetic implications of this phenomenon, the variation has proven advantageous in breeding programs of various crop plants (Krishna et al. 2016). These advantages of somaclonal variation can be used to overcome the problem of diseases and pests in orchids. *Dendrobium* orchids are prone to disease caused by *Fusarium proliferatum* and *Fusarium oxysporum* (Swett and Uchida 2015). Previous studies have reported that it is very difficult to eradicate these fungi because they are resistant to most fungicide (Masiello et al. 2018). The typical symptoms of infection on plants are discolouration which indicates rotting of tissues (Latiffah et al. 2009).

Hence, a valuable approach for improving the productivity of *Dendrobium* hybrid, *D5* (*Dendrobium* Waipahu Beauty × *Dendrobium* Burana White Big Flower) in this research was to select regenerated clones which are resistant or tolerant to fungal diseases. Induced mutation using gamma ray irradiation is commonly used to develop new species in plant breeding (Oladosu et al. 2015). It can rapidly create the variability of inherited traits in crops that have contributed to increased yield potential or disease resistance (Gaswant et al. 2016).

Therefore, the objective of this research was to determine *in vitro* selection of disease resistant *Dendrobium* hybrid orchid PLBs using different doses of gamma irradiation on the TCL of PLBs by investigating its morphology and direct amplification of minisatellite region DNA (DAMD) analyses. To test the treated plantlets for resistance or tolerance towards *F. proliferatum* and *F. oxysporum*, a leaf-bridge bioassay was conducted.

## **MATERIALS AND METHODS**

### **Gamma Irradiation Treatment on *In Vitro* Cultures of *Dendrobium* Hybrid**

*In vitro* cultures of *Dendrobium* hybrid were used for selection study. Stock cultures were maintained in half-strength semi-solid Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 2 % (w/v) sucrose, 2.80 g/L, Gelrite™ [Duchefa Biochemie] and 1 mg/L of 6-benzylaminopurine (BAP). The pH value was adjusted with a pH meter [Eutech instruments pH700] to 5.7 to 5.8 before autoclaving [Tony high pressure steam steriliser ES315]. The cultures were incubated at 25 ± 2 °C under 16 hours photoperiod using cool white fluorescent lamps. The TCL of healthy, four weeks old PLBs were used as explants. Longitudinal TCL (ITCL) of PLBs were prepared using Protocol 3 (Teixeira da Silva 2013).

The explants were irradiated with acute gamma irradiation at the Agrotechnology and Bioscience Division, Malaysian Nuclear Agency, Bangi, Selangor. The source of gamma rays was <sup>60</sup>Co. Ten TCLs of PLBs were inoculated in each Petri dish and six replicates were conducted for each gamma ray dose. The TCL of PLBs was acutely irradiated with 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 Gy. The control TCLs were not irradiated. After irradiation, the treated TCLs of PLBs were immediately transferred to fresh half-strength semi-solid MS medium.

The PLBs were observed for four weeks where the survival rate and number of shoots produced by the irradiated TCL of PLBs were scored based on colour of PLBs. Survived PLBs remained green, whereas dead PLBs turned dark brown or white with no green patches. The survival percentage of PLBs was calculated as follow:

$$\text{Survival (\%)} = \frac{\text{(Number of survived PLBs)}}{\text{total PLBs cultured}} \times 100 \%$$

These results were statistically analysed using one-way ANOVA and differentiated with Tukey's test with the probability value set at 5 % with the SPSS 22 program. For further propagation of tolerant PLBs and to build up sufficient materials for the leaf-bridge bioassay experiment, survived PLBs were multiplied on MS medium.

### **Histological Analysis of PLBs**

Control samples and PLBs that survived gamma ray irradiation were selected for histology analysis. The histological procedure applied in this treatment was modified from Vyas et al. (2010). Imaging, photography and observations of the slides were carried out with the aid of a light microscope [Olympus BX50, Olympus Optical Co. Ltd., Japan] fitted with a JVC K-F55B colour video camera [JVC Victor Company of Japan, Limited, Japan].

### **Scanning Electron Microscopy Analysis of PLBs**

The stomata behaviour between gamma ray irradiated PLBs with control treatment were determined using scanning electron microscope (SEM) observation via the freeze drying method. The samples prepared were then viewed with the aid of a Leo Supra 50VP Field Emission scanning electron microscope [Carl Zeiss SMT, Germany]. Thirty (30) stomata were randomly chosen from the PLBs and mean width of stomata were recorded [CellSens Program]. Data was analysed using one-way ANOVA and differentiated with Tukey's test with the probability value set at 5 %.

### **DAMD-DNA Analysis of PLBs**

#### **DNA extraction**

For genomic DNA amplification and analysis, untreated control and PLBs treated with gamma radiation were collected. DNA was extracted from these PLBs using Promega Wizard® Genomic DNA Purification Kit, based on the manufacturer's instructions.

#### **Amplification reaction with DAMD primers**

The treatments were examined using 19 primers based on the primer list reported (Devi et al. 2014; Bhattacharyya et al. 2015). The primers were synthesised by Integrated DNA Technologies Company and is listed in Table 1.

PCR amplification was modified from a study by Zhou et al. (1997) using [BioRad T100] thermal cycler. The reaction mixture of 20 µL was prepared and contained 50 ng genomic DNA, 0.2 mM of dNTP mix [Next Gene Scientific], 2.5 mM MgCl<sub>2</sub>, 1× PCR buffer, 1.0 U *Taq polymerase* [My

TACG Bioscience Enterprise] and 0.3 mM of primers. PCR amplification was performed by initial denaturation at 94 °C for 2 min and 40 cycles of denaturation for 1 min at 92 °C, annealing for 2 min at 5 °C below each primer's melting temperature (TM), extension for 2 min at 72 °C and a final extension at 72 °C for 10 min.

### **Gel electrophoresis**

DAMD bands were visualized in 1.5 % (w/v) agarose gel. The gel was casted in the 15 × 7 cm Mini Gel Caster [Bio-Rad Laboratories, Inc., USA]. 1.5 % agarose gel was prepared by dissolving 0.60 g agarose in 40 mL 1×Tris-Borate-EDTA (TBE) buffer using a microwave oven set at medium heating for four minutes. For electrophoresis, 3 µL of 1 kb marker [Thermo Fisher Scientific] and a mixture of 3 µL of the amplified DNA and 3 µL of 6×DNA loading dye (total 6 µL) were loaded into the wells.

A PowerPac™ Basic Power Supply [Bio-Rad Laboratories, Inc., USA] was attached to the electrophoresis system. The electrophoresis was allowed to run at 90 V for 90 minutes until the loading dye passed two-thirds of the gel's length. Then, the gel was viewed immediately under 302 nm UV transilluminator [Molecular Imager® Gel Doc™ XR+ System with Image Lab™ and Quantity One 1-D Analysis Softwares, Bio-Rad Laboratories, Inc., USA] for visualisation of the bands.

### **Determination of polymorphisms analysis**

The determination of the DNA fragment patterns of each treated sample was performed by determining the similarity indices (SI) of the groups as compared to the untreated control. The visible bands were manually scored as 1 or 0 for the presence or absence of similar band. Similarity index was calculated according to previous report (Nei and Li 1979).

### **Leaf Bridge Bioassay**

Three (3) months after the survived gamma irradiated PLBs were transferred to MS medium, leaflets of size 1 to 1.5 cm were harvested. MS medium was filled into one of the two compartments in a two compartment Petri dish. Then, two (2) leaflets were placed far apart from each other on the medium. The petioles were embedded in the medium whereas; the leaves were raised above the partition and faced the other compartment of the Petri dish. The Petri dish was sealed with Parafilm with caution taken not to touch the leaflets with the lid of the Petri dish to avoid contamination. The incubated leaflets remained healthy for two weeks.

Spore inoculum of 10,000 spores/mL was prepared for *F. proliferatum* [VCG 1380] (Dehghi 2016). The tip of the leaflets was wounded with a sterilised needle. Then, two (2) leaflets were inoculated by rubbing the conidial suspension on the tip of leaflet surfaces using a cotton bud. Control leaflets were not inoculated. Three replicates were conducted. Subsequently, the Petri dishes were sealed with Parafilm and kept in an incubator at 25 ± 2 °C with the photoperiod of 16 hours light and eight hours dark. The same procedure was applied for *F. oxysporum*. After four weeks, infected regions of leaflets which were indicated by discolouration or appearance of necrotic symptoms of the diseased tissues, beginning at the inoculation point was recorded. The leaflets were divided into five sections along the midrib. Each leaf section was numbered in

increasing order from the leaf apex to the base and disease progress was scored using a 0 to 5 scale according to the absence or presence of disease symptom on each section (Dehgahi 2016). Leaflets were deemed as resistant, intermediate and susceptible with scores of 0 to 2, 2 to 3 and more than 3, respectively.

## RESULTS

Irradiation with doses of 10 to 100 Gy produced significant effects on survival rate of TCL of PLBs after four weeks. Initially, there was a steady rise in the survival rate of PLBs from 0 to 30 Gy. Then, the survival rate decreased at 40 Gy and remained constant till 50 Gy. From 60 Gy onwards, there was a gradual decrease in survivability of PLBs. At the end of the selection cycle, there was a significant difference between the survival rate of gamma radiated PLBs and the control. The control PLBs have 83 % of survival rate whereas, PLBs radiated with 100 Gy had only 33 %. Besides, only PLBs treated with 10 to 50 Gy produced shoots. The LD<sub>50</sub> graph for the effect of various doses of gamma irradiation on the survival rate of *Dendrobium* hybrid PLBs after four weeks was approximately at 63 Gy (Figure 1).

Histology analysis of non-irradiated PLBs cells was octahedral in shape and the cell walls were intact. The nucleus and cytoplasm were present in the cells. Besides, the arrangement of cells was compact and packed together. PLBs radiated with 10 to 50 Gy had less damage in the cells. There was presence of meristematic cells in these PLBs. The cells were packed together but cell wall rupture was visible in some cells. The cells appeared much damaged in 60 to 100 Gy treated PLBs. Cell rupture was more prominent and arrangement of cells was also disturbed whereas, intact cells had plasmolysed and was irregularly shaped. Besides, darkly stained cells showed dense cellular material accumulation from broken cell membranes (Figure 2a-f).

In the SEM analysis, the size of stomatal aperture decreased as gamma radiation doses increased. PLBs treated with 10 and 20 Gy had surface morphology which is comparable to the control. The cells were compactly arranged and the octahedral shape was maintained. However, PLBs treated with 30 to 100 Gy had cells that had lost their turgidity. These cells also had stomata that appeared burst and ruptured cells. There were no visible stomata found on the PLBs surface radiated with 100 Gy (Figure 3a-d). One way ANOVA analysis of mean stomatal aperture of 30 stomata of PLBs proved that there was a definite decrease in the width of stomatal aperture with increasing dose of gamma radiation (Table 2).

Based on the results obtained by using 19 DAMD primers (Table 1), only 10 primers generated reproducible and clear bands from the gamma irradiated and non-irradiated PLBs. A total of 673 bands were generated in the entire DAMD molecular analysis. Total number of polymorphic bands is 275 bands (41 %). Similarity index (SI) for control and irradiated plants ranged from 0 to 1. The treatments which were completely monomorphic to the control were PLBs irradiated with 10 Gy (6.2H (+) and HBV5), 20 Gy (6.2H (+), URP6R, and 6.2H (-)), 30 Gy (HBV5), 40 Gy (6.2H (-)), 50 Gy (HBV5), 60 Gy (HBV5), 80 Gy (HBV5), 90 Gy (HBV5) and 100 Gy (HBV3 and HBV5). Meanwhile, the PLBs irradiated with 40 Gy (URP2F) and 50 Gy (URP30F) showed total polymorphism compared to the control (Table 3).

When challenged with spore suspension of these fungi, leaflets radiated with 20 and 30 Gy of gamma irradiation showed the least disease symptom, thus, proving resistance towards these fungi (Tables 4 and 5).

## DISCUSSION

Several researchers have confirmed that low doses of gamma irradiation increase growth and germination rate in plants (Iglesias-Andreu et al., 2012). It was reported that at the lowest dose (100 Gy) of gamma ray, maximum seed germination (90.47%) of cumin cv. Gujarat Cumin-4 was obtained while the highest dose (500 Gy) resulted in minimum seed germination (9.52%) (Verma et al. 2017). Also, plant height, seedling survival, stem diameter, number of leaves, leaf length, number of branches, and leaf width of okra decreased significantly with increasing doses of gamma rays (Asare et al. 2017). Furthermore, Singh et al. (2013) stated that gamma radiation at a low dose of 25 Gy or lower stimulates, while high dose of 100 Gy and above inhibits plant growth and development of wheat cultivar PBW-343.

Reduction in seed germination with gamma radiation might be due to an increase in the production of active radicals responsible for seed lethality (Fulzele et al. 2015). Reactive oxygen species (ROS) causes the plants to be more fragile and easily degraded than the non-irradiated controls (Kim et al. 2015). Apart from that, the rate of seed germination depends on the level of chromosomal damage caused by high dose of radiation (Nurmansyah et al. 2017). Besides, due to irradiation, plant health is affected by poor carbon and nitrogen assimilation efficiency and plant uptake of mineral nutrients (Singh et al. 2013).

Apart from that, stomata closure is induced by the stress caused by gamma irradiation. This constitutes an instant defence response to prevent further damage to plants. Abscisic acid (ABA) is a major plant hormone involved in stress responses. Stress-activated ABA synthesis causes stomata closure and prevents opening to reduce water loss and cell dehydration. ABA acts directly on guard cells and induces stomatal closure via the efflux of potassium and anions from guard cells and the removal of organic osmolytes (Zhu et al., 2012).

In the current research, there was a certain degree of polymorphism in the gamma irradiated PLBs. Similar results were obtained where gamma irradiated seeds of cowpea produced a high degree of polymorphism than the non-radiated control (Gaafar et al. 2016). There was also presence of newly appeared bands and disappearance of some bands as compared to the control in specific gamma rays induced mutants. The results are also in agreement with gamma irradiated potato (Wendt et al., 2008) and soybean (Mudibu et al., 2011). Besides, treatment with highest gamma irradiation promoted the highest variation in genetic distance in okra plants (Hegazi and Hamideldin 2010).

It has been proposed that these effects of gamma rays may be due to structural rearrangements in DNA caused by different types of DNA damages (Hoeck et al. 2015). Apart from that, the rate at which mutation occur in plants vary according to the dose of gamma irradiated on the plants. At low doses, the anomalies do not trigger repair mechanism and is transferred to accumulate in the successive cell generations. However, at high doses, the damages are sufficient

to trigger repair systems which transmit the mutation from the irradiated mitotic cells to the meiotic cells. Thus, the mutation is inherited by the next generation through fertilisation (Li et al., 2016).

Previous studies have also reported that gamma irradiation reduces disease incident from various pathogens such as virus, fungi, nematodes and bacteria (Ali et al. 2015). Gamma radiation has been used for reduction of Aflatoxin B<sub>1</sub> in corn (*Zea mays*) used in the production of feed for broiler chickens (Serra et al. 2018). Besides, it was reported that gamma irradiation along with heat treatment had synergistic effect in the reduction of bacterial and fungal spores during storage of cereals (Dikkala et al. 2018). Similar results were obtained using combined effects of *Cananga odorata* essential oil with gamma irradiation on growth and mycotoxins production by *Fusarium graminearum* in corn (Kalagatur et al. 2018).

## **CONCLUSION**

In this research, *in vitro* selection of disease resistant *Dendrobium* orchid PLBs using different doses of gamma irradiation was determined. This study has successfully produced a cultivar of D5 which is 100% resistant to *F.proliferatum* when irradiated with 20 Gy and 62.5 % resistant to *F. oxysporum* when irradiated with 30 Gy. PLBs radiated with 0 to 100 Gy showed decline in survival and shoot regeneration rate as the irradiation dose increased. The highest survival and shoot regeneration rate were obtained in PLBs radiated with 20 and 30 Gy. Besides, histology analysis proved that severe changes in the cell morphology have occurred when the PLBs were increasing amount of gamma was irradiated. These damages in the cell influence many processes and functions which are vital for plant survival. Evidence from SEM analysis also verified the significance of damage caused by high doses of gamma radiation to the PLBs. The changes in surface morphology of the PLBs and decrease in the width of stomatal aperture could be related to the decline in survival rate of the PLBs. DAMD analysis showed that there was a high degree of polymorphism when the PLBs were treated with high doses of gamma radiation. Although 20 and 30 Gy have induced promising resistance towards the fungi, it has still caused morphological and DNA structural damage to the PLBs. This is due to the destructive nature of gamma irradiation itself, but the damage caused by low doses of gamma radiation is not severe and almost negligible. Thus, this research is useful as a stepping stone for further researches be done on determining the exact gene which was mutated through this radiation and how to further manipulate it to produce inherited resistance of the PLBs towards these fungi. The findings of this project are also significant for orchid breeders to propagate orchids which are resistant towards these fungi.

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**Table 1.** List of DAMD primers

Primers	Sequence (5'-3')	G+C Content (%)	TM (°C)
M13	GAGGGTGGCGGCTCT	73.3	57.9
URP4R	AGGACTCGATAACAGGCTCC	55.0	56.1
6.2H (+)	AGGAGGAGGGGAAGG	66.7	52.4
URP6R	GGCAAGCTGGTGGGAGGTAC	65.0	60.6
URP17R	AATGTGGGCAAGCTGGTGGT	55.0	60.1
URP2F	GTGTGCGATCAGTTGCTGGG	60.0	59.1
HBV3	GGTGAAGCACAGGTG	60.0	50.0
YN73	CCCGTGGGGCCGCCG	93.3	67.2
URP9F	ATGTGTGCGATCAGTTGCTG	50.0	56.0
YNZ22	CTCTGGGTGTCTGTC	66.7	52.8
URP38F	AAGAGGCATTCTACCACCAC	50.0	54.5
HBV5	GGTGTAGAGAGGGGT	60.0	49.0
URP1F	ATCCAAGTCCGAGACAACC	55.0	56.8
URP2R	CCCAGCAACTGATCGCACAC	60.0	59.1
URP13R	TACATCGCAAGTGACACAGG	50.0	54.9
URP32F	TACACGTCTCGATCTACAGG	50.0	53.0
URP25F	GGACAAGAAGAGGATGTGGA	50.0	53.9
URP30F	GGACAAGAAGAGGATGTGGA	50.0	53.9
6.2H (-)	CCCTCCTCCTCCTTC	66.7	50.4

**Table 2.** Effect of various doses of gamma radiation on mean stomatal aperture ( $\mu\text{m}$ ) of *Dendrobium* hybrid PLBs.

Gamma radiation doses (Gy)	Mean stomata aperture ( $\mu\text{m}$ )
Control	13.39 $\pm$ 8.31 <sup>bc</sup>
10	15.47 $\pm$ 8.16 <sup>ab</sup>
20	20.17 $\pm$ 9.66 <sup>a</sup>
30	9.46 $\pm$ 4.40 <sup>cd</sup>
40	8.56 $\pm$ 3.46 <sup>cd</sup>
50	6.68 $\pm$ 3.38 <sup>d</sup>
60	9.98 $\pm$ 4.22 <sup>cd</sup>
70	7.77 $\pm$ 4.66 <sup>d</sup>
80	10.40 $\pm$ 6.53 <sup>cd</sup>
90	8.24 $\pm$ 3.91 <sup>d</sup>

Note: Letters a-d represents significant differences using Tukey's range test at  $P \leq 0.05$ .

**Table 3.** DAMD banding profiles of DNA samples obtained from irradiated PLBs

Primer	SI for control & 10 Gy irradiated PLBs	SI for control & 20 Gy irradiated PLBs	SI for control & 30 Gy irradiated PLBs	SI for control & 40 Gy irradiated PLBs	SI for control & 50 Gy irradiated PLBs	SI for control & 60 Gy irradiated PLBs	SI for control & 70 Gy irradiated PLBs	SI for control & 80 Gy irradiated PLBs	SI for control & 90 Gy irradiated PLBs	SI for control & 100 Gy irradiated PLBs
M13	0.73	0.29	0.77	0.77	0.77	0.77	0.77	0.92	0.50	0.67
6.2H (+)	1.00	1.00	0.67	0.83	0.67	0.83	0.77	0.77	0.71	0.77
URP6R	0.73	1.00	0.66	0.67	0.67	0.50	0.55	0.50	0.60	0.80
URP2F	0.22	0.22	0.22	0	0.29	0.20	0.33	0.20	0.33	0.29
HBV3	0.83	0.77	0.86	0.83	0.86	0.86	0.55	0.91	0.71	1.00
HBV5	1.00	0.73	1.00	0.86	1.00	1.00	0.86	1.00	1.00	1.00
URP13R	0.71	0.80	0.50	0.71	0.80	0.71	0.71	0.80	0.62	0.71
URP32F	0.50	0.50	0.22	0.50	0.29	0.29	0.33	0.33	0.33	0.67
URP30F	0.63	0.59	0.57	0.59	0	0.67	0.59	0.43	0.17	0.31
6.2H (-)	0.90	1.00	0.95	1.00	0.86	0.84	0.80	0.50	0.95	0.84

**Table 4.** Response of regenerated leaves from gamma irradiated PLBs when tested with *F. proliferatum* spore suspension (%).

Treatment (Gy)	Percentage of susceptibility response of regenerated leaves when tested with <i>F. proliferatum</i> spore suspension (%)		
	Resistant	Intermediate	Susceptible
Control	0	12.5	87.5
10	0	37.5	62.5
20	100.0	0	0
30	12.5	87.5	0
40	37.5	25.0	37.5
50	0	0	100.0

**Table 5.** Response of regenerated leaves from gamma irradiated PLBs when tested with *F. oxysporum* spore suspension (%).

Percentage of susceptibility response of regenerated leaves when tested with <i>F. oxysporum</i> spore suspension (%)			
Treatment (Gy)	Resistant	Intermediate	Susceptible
Control	0	12.5	87.5
10	0	12.5	87.5
20	50.0	37.5	12.5
30	62.5	37.5	0
40	0	37.5	62.5
50	0	25.0	75.0

**Fig. 1.** Effect of various doses of gamma irradiation on the survival rate of *Dendrobium* hybrid PLBs after four weeks and the LD<sub>50</sub> level

**Fig. 2.** Cell condition of control and gamma irradiated *Dendrobium* hybrid PLBs (scale bar = 20 µm). (a) Non-irradiated PLB with intact cell walls, (b) PLB irradiated with 20 Gy and, (c) 40 Gy with meristematic cells, (d) PLB irradiated with 60 Gy with ruptured cell wall, (e) PLB irradiated with 80 Gy and, (f) 100 Gy with dense cellular material accumulation, ic (intact cells), rc (ruptured cells), mc (meristematic cells)

**Fig. 3.** Stomata of control and gamma irradiated *Dendrobium* hybrid PLBs (scale bar= 1.00K ×). (a) Non-irradiated PLB, (b) PLB irradiated with 30 Gy, (c) PLB irradiated with 60 Gy, (d) PLB irradiated with 90 Gy