TROPICAL LIFE SCIENCES RESEARCH

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

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Highlights

- TCL of *Dendrobium* orchid Protocorm-Like Bodies (PLBs) were gamma irradiated to induce mutation.
- Induced mutation causes phenotypic, morphological and genotypic changes in PLBs.
- Mutated PLBs show resistance towards *Fusarium proliferatum* and *F. oxysporum*.

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

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Abstrak: Orkid *Dendrobium* mudah terdedah kepada penyakit yang disebabkan oleh *Fusarium proliferatum* dan *Fusarium oxysporum*. Oleh itu, penyinaran gamma digunakan pada hibrid *Dendrobium*, D5 untuk menghasilkan kultivar yang rintang terhadap kulat ini. Dos gamma 10, 20, 30, 40, 50, 60, 70, 80, 90 dan 100 Grey (Gy) dipancarkan pada lapisan sel nipis (TCL) badan seperti protokm (PLB) dan diperhatikan selama empat minggu. Dos gamma 20 dan 30 Gy adalah optimum untuk kelangsungan hidup dan penjanaan semula pucuk PLB yang dirawat. Sensiviti radio (LD50) PLB adalah kira-kira 63 Gy. Analisis histologi dan pengimbasan mikroskop elektron (SEM) menunjukkan kerosakan sel yang ketara dan perubahan morfologi pada permukaan PLB yang dipancarkan dengan sinaran gamma. Bilangan stomata menurun daripada15.47 ± 8.16 µm pada 10 Gy sinaran gamma kepada hanya 8.24 ± 3.91 µm apabila dipancarkan dengan 90 Gy. Penguatan langsung DNA minisatelit (DAMD) menunjukkan tahap polimorfisme yang tinggi dalam PLB ini. Apabila diinokulasi dengan suspensi spora kulat ini, pucuk PLB yang dipancarkan dengan 20 dan 30 Gy penyinaran gamma menunjukkan simptom penyakit yang paling kurang, oleh itu, membuktikan kerintangan terhadap kulat ini. Oleh itu, kajian ini adalah kajian saringan awal di mana dos optimum penyinaran gamma dipilih berdasarkan tindak balas TCL yang dipancarkan PLB terhadap mutagen.

Kata kunci: Ketahanan Penyakit, Biocerakinan *Leaf-bridge*, Patogen, Ketahanan, Sensitiviti Radio

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-

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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_{50}) 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 ± 8.16 µm at 10 Gy but reduced to only 8.24 ± 3.91 µm 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 3,736 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 & 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 & 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.* 2019). 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.

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 & 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 ± 2ºC under 16 h photoperiod using cool white fluorescent lamps. The TCL of healthy, four weeks old PLBs were used as explants. Longitudinal TCL (lTCL) 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 ^{60}C. 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.

Polymerase chain reaction 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 $MgCl_2$, 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.

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	CTCTGGGTGTCGTGC	66.7	52.8
URP38F	AAGAGGCATTCTACCACCAC	50.0	54.5
HBV5	GGTGTAGAGAGGGGT	60.0	49.0
URP1F	ATCCAAGGTCCGAGACAACC	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

Gel electrophoresis

DAMD bands were visualised in 1.5% (w/v) agarose gel. The gel was casted in the 15 × 7 cm Mini Gel Caster [Bio-Rad Laboratories, Inc., USA]. The amount of 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 4 min. 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 min 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 & Li 1979).

Leaf Bridge Bioassay

Three 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] (Dehgahi *et al.* 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 h light and 8 h 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 *et al.* 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_{50} 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 (Fig. 1).

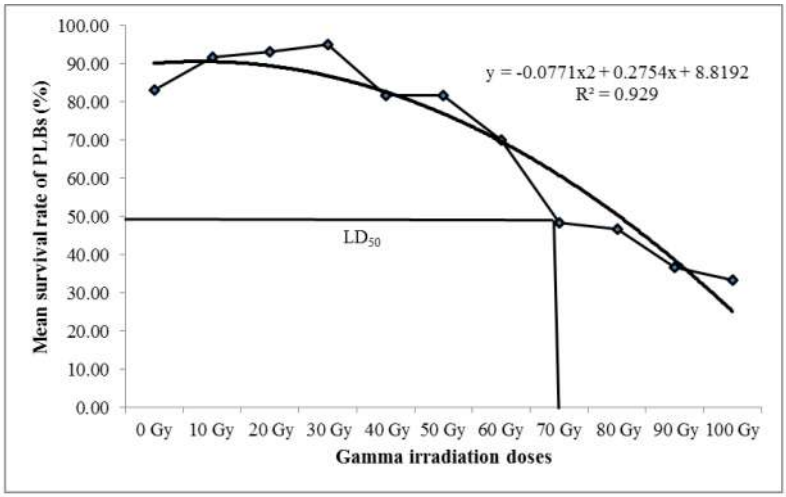

Figure 1: Effect of various doses of gamma irradiation on the survival rate of *Dendrobium* hybrid PLBs after four weeks and the LD_{50} level.

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 (Fig. 2).

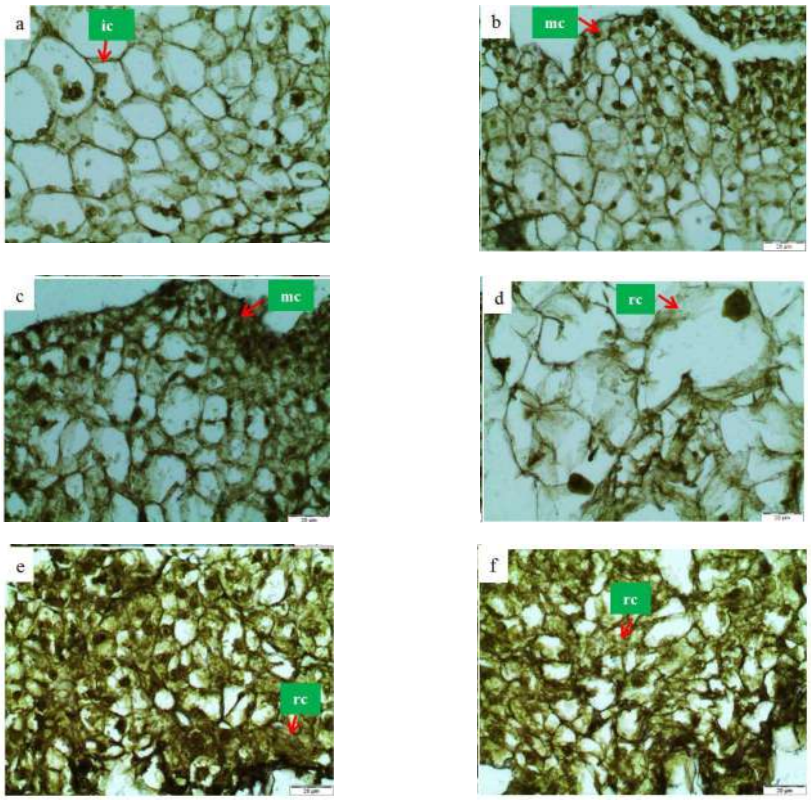

Figure 2: Cell condition of control and gamma irradiated *Dendrobium* hybrid PLBs (scale bar= 20 µm). (a) Non-radiated PLB with intact cell walls, (b) PLB irradiated with 20 Gy and, (c) 40 Gy with meristematic cells, (d) PLB irradiated with 60 Gy with ruptured cell wall, (e) PLB irradiated with 80 Gy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

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 (Fig. 3). 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).

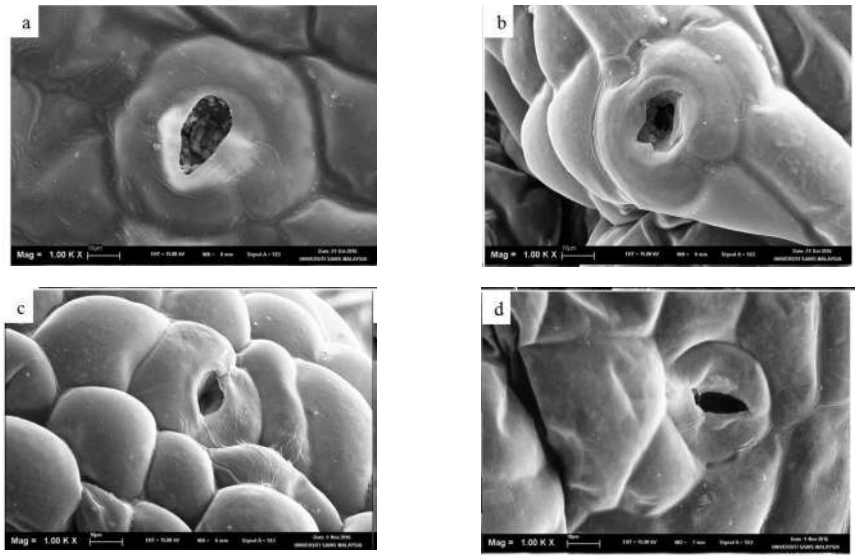

Figure 3: Stomata of control and gamma irradiated Dendrobium hybrid PLBs (scale bar= 1.00K ×). (a) Non-radiated PLB, (b) PLB irradiated with 30 Gy, (c) PLB irradiated with 60 Gy, and (d) PLB irradiated with 90 Gy.

Table 2: Effect of various doses of gamma radiation on mean stomatal aperture (µm) of *Dendrobium* hybrid PLBs.

Gamma radiation doses (Gy)	Mean stomata aperture (µm)
Control	13.39 ± 8.31bc
10	15.47 ± 8.16ab
20	20.17 ± 9.66a
30	9.46 ± 4.40cd
40	8.56 ± 3.46cd
50	6.68 ± 3.38d
60	9.98 ± 4.22cd
70	7.77 ± 4.66d
80	10.40 ± 6.53cd
90	8.24 ± 3.91d

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

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).

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

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).

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

Percentage of susceptibility response of regenerated leaves when tested with *F. proliferatum* spore suspension (%)			
Treatment (Gy)	**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 *F. oxysporum* 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

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 & 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_1 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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