3. Thailand

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Introduction

Dendrobium hybrids are the major commercial orchid plants grown for cut flower and potted plant in Thailand. The *Dendrobium* Sonia 'BOM' is a popular hybrid, fast growing, floriferous, bright color and has long vase life. The original clone of *Dendrobium* Sonia 'BOM' has red-purple with white color at the central. After successive propagation by tissue culture, it produced many mutants. The selected mutants for cutflower had developed clones such as *Dendrobium* Sonia 'BOM 17K', *Dendrobium* Sonia 'BOM 17 Red', *Dendrobium* Sonia 'BOM 28' (large size flower), *Dendrobium* Sonia 'Kalya', *Dendrobium* Sonia 'Miss world'. And the second cross of the same parent produced the *Dendrobium* Sonia 'BOM Jo' which similar to *Dendrobium* Sonia 'BOM'. and also produced mutant clones after successive propagation : *Dendrobium* Sonia 'BOM Jo Red', and *Dendrobium* Sonia 'Earsakul' which having superior dark color and obtained higher price than the original clone.

Thailand exported quantity of orchid flowers in 2003 was 439.86 million inflorescences or 17,411 metric tons, and of orchid plants was 27.12 million plants. The flowers were exported 26.59, 17.04, 16.53, 13.56, 6.57, 6.06 % to Japan, America, Italy, Hong Kong, China and Taiwan, respectively and only 10.29 % were shipped to other countries.

Thrips

Thrips are the important insect pest to the orchid industry especially *Dendrobium* orchids. Thrips feed inside newly expanding leaves and the developing young inflorescence. Their feeding damage is not seen until leaves expanded and deformed flowers, leaving plant unmarketable. Thrips have a wide host range and active all year in heat greenhouse.

Since the habitat of thrips is a flower petal, the plant quarantine agencies of many countries will not allow to entry until all of the thrips are completely killed. The fumigation costs in imported countries are very expensive, time consuming and reduce the flower quality. It is almost impossible to completely eradicate them in the growing area. To create resistance clone will be the most valuable in the orchid industry.

Thrips are classified in Order Thysanoptera, Family Thripideae. Thrips being found on orchid plants in Thailand were recorded as *Dichromothrips corbetti* (Priesner) and *Thrips palmi* Karny by Kamjaipai (1984). Kajita *et al* (1992) recorded of *Thrips sumatrensis* Priesner, *Tusothrips teinostomus* Okajima, *Franklinella schultzei* (Trybon) and *Microcephalothrips abdominalis* (Crawford). Beside these species, *Thrips hawaiiensis* (Morgan), *T. tabaci* Lindeman, *Taeniothrips eucharii* (Whetzel), *Frankliniella intonsa* (Trybom), and *Selenothrips dorsalis* Hood were found by the Japanese plant quarantine from orchid imported from Thailand at the Japanese ports (Hayase, 1991; Itoh, 1990). Yano and Napompeth (1995) reported that they collected and identified only 2 species of thrips; 1) *Dichromothrips corbetti* (Priesner) on genus *Dendrobium, Mokara, Vanda* and *Oncidium* and 2) *Taeniothrips eucharii* (Whetzel) on *Dendrobium* orchid. In 2001, Kienmesuk *et al*. reported that only *Thrips palmi* Karny is the major insect pest in Thailand.

Normal flower

Infested flower by thrips

Characteristics of thrips

Thrip is a tiny yellow (young) or black (adult) insect with 0.8-1.0 mm in length. The adult moves fast by flying while young thrips with short wing-pads can walks very quickly. They hide from their enemies or sunlight in corners within the flowers. Thrips can be seen walking on petals when they are over crowned. When they suck sap from the flower, the wounds will

be dry strips which make the flower look burnt. Thrips can spread very fast due to the high fecundity of female and short life cycle 2 - 3 weeks in 25 - 30 °C. The survival rate from egg to adult is about 45 - 50 %. *Thrips palmi* Karny can not survive under 10 °C condition. Thrips become active and reproduce in dry weather, in the hot season and in the rainy season. When it does not rain, they reproduce very quickly. High population of thrips was found during dry season in January to April and in October to December. It is almost impossible to completely eradicate them (Kienmesuk *et al*., 2001). Yano (1995) estimated that thrips probably occur at almost all nurseries. The percentage of *Dendrobium* flowers attacked by thrips was 74 % (84 from 113 flowers surveyed) in the nursery where no insecticide was applied. But most of the orchid nurseries were under heavy and regular application of insecticide resulting few insect collection.

Damaged leaves of Dendrobium infested by thrips

Cost for control thrips

Approximately 2,240 hectares of growing area, the estimation of insecticide cost is about 1,500 US$/hectare/year. The most important insect pest is thrips.

The farmers control thrips by applying insecticide at 7 days interval. The recommended 5 groups of insecticides for eliminate thrips are

1) Imidacloprid (Confidore 100 SL 10% 20 ml/l, 1250 l/hectare)
2) Acetamiprid (Molan 20% SP 5 gm/20 l, 1250 l/hectare)
3) Abamectin (Jacket, Vertimec 1.8 % EC, 20 ml/20 l)
4) Fipronil (Ascent 5 % SC 20 ml/20 l, 1250 l/hectare)
5) Cypermethrin/phosalone (Parzon 28.75 % EC 40 ml/20 l, 1250 l/hectare)

Problems of thrips on orchid export

Reports from the Plant Quarantine Section in Bangkok showed number of shipments that found thrips at the imported countries (Komson, 2003). The shipment that found thrips will manage for fumigation or fire burn. The fumigated flower attains reduced vase life.

Year	No. of exported shipments	No. of flower spikes (millions)	No. of shipments with thrips	
			number	%
1997	30,776	239.4	107	0.36
1998	35,708	302.9	90	0.25
1999	34,441	317.7	61	0.18
2000	38,573	283.1	70	0.18
2001	38,759	386.0	32	0.08
2002	39,907	421.6	26	0.06

Thrips control and eradication

The Department of Agriculture, Ministry of Agriculture has many projects to eradicate thrips:

1) Research on irradicated thrips in the farms by insecticides.
2) Research on physical control using sticker pad. The results stated that the white or blue sticker pad could trap more number of thrips than other colors. The suitable level for hanging was 40-60 cm above ground and 4 m apart.
3) Research on integrated thrips control by insecticide, sticker pad and counting number of thrips on flowers. It needs not to apply insecticide if the number of thrips on random sampling flower is lower than 10/40 flower spike/1600 m^2. This method can reduce half cost of insecticide (Kienmesuk *et al*., 2001).
4) Produce booklet of GAP (Good Agricultural Practice) for recommending the farmer to irradicate thrips in the farm.
5) Postharvest research on fumigation of orchid cut-flowers with methyl bromide. All thrips die when fumigate with methyl bromide at 20-22 g/m^3 for 90 min.

   The random sampling number of thrips by counting from 40 flower spikes/rai. If they have more than 10 thrips/40 flower spikes, it needs to spray insecticide for irradicated (Kienmesuk *et al*., 2001).

Study on irradiation by gamma rays of orchids

Gamma irradiation was carried out to obtain the optimum doses on inducing mutation of *Dendrobium* PLBs (Vajrabhaya, 1977; Angamnuasiiri, 2001), *Brassolaeliocattleya* PLBs (Thammasiri, 1996); protocorm derived from seed of many orchids species (Piluek, 2002). The results showed that most of the orchid protocorms or PLBs were tolerant to chronic irradiation, they could survive and develop into normal plantlets.

The aims of this research work are:

1. To get the optimum doses of gamma rays for mutation induction of different growth stages of *Dendrobium*
2. To develop *Dendrobium* lines/varieties resistant to thrips using gamma radiation as a mutagen

Materials and Methods

Eight experiments were carried out in this project.

Experiment 1 Evaluation and identification of breeding materials

Four clones of commercial cut flower orchids: *Dendrobium* Sonia 'BOM 17 red', *Dendrobium* Sonia 'Earsakul', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* hybrid 'White Sanan' were evaluated.

***Den.* Sonia 'BOM 17 Red'**

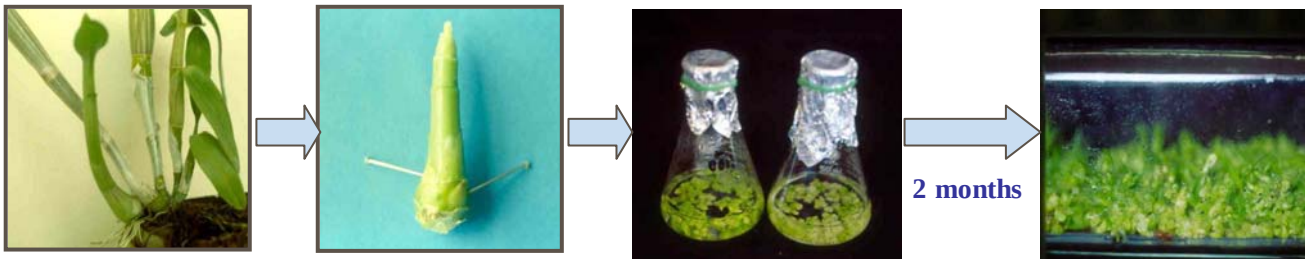
***Den*. Pinky Sem 'Rinnapa'**

***Den.* 'White Sanan'**

***Den.* Sonia 'Earsakul'**

Experiment 2 Initiation of tissue culture of clones

Young shoots of *Dendrobium* Sonia 'Earsakul', *Dendrobium* Sonia 'BOM 17 Red', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* hybrid 'White Sanan' were collected from commercial orchid nursery for tissue culturing. The lateral buds and terminal buds excised from sterilized shoots were cultured in liquid Vacin-Went medium.

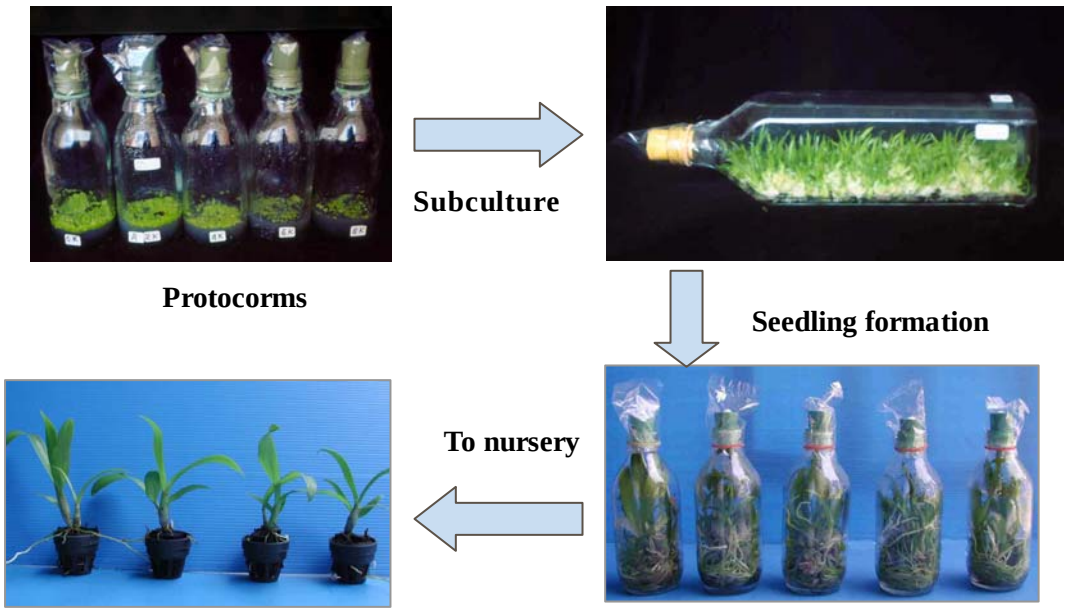


Experiment 3 Exchange of material clones

The material clones are exchanged among Thailand, Malaysia and Indonesia.

Experiment 4 Determination of radiation sensitivity and optimum dose

4.1 Radiation effects on protocorm developed from seed of *Dendrobium* hybrid



4.2 Radiation effects on PLBs of *Dendrobium* hybrids

Experiment 5 Irradiation of PLBs for insect resistant clones

5.1 Effects of irradiation on plantlet growth of *Dendrobium* Sonia ' BOM 17 red'

Protocorms like bodies (PLBs) of *Dendrobium* Sonia 'BOM17 Red' were irradiated with acute gamma rays at 0, 60, 70, 80, 90 and 100 Gy and subcultured. Large size seedlings were taken out from aseptic culture and were evaluated for seedling weight and size before growing in the nursery.

5.2 Effects of irradiation on plantlet growth of *Dendrobium* Sonia 'Earsakul'

Protocorms like bodies (PLBs) of *Dendrobium* Sonia 'Earsakul' were irradiated with acute and chronic gamma rays and then subcultured.

1. High dose acute gamma irradiation 0, 60, 70, 80, 90 and 100 Gy
2. Low dose acute gamma irradiation 0, 2, 4, 6, 8, 10 Gy
3. Split dose acute gamma irradiation 0, 20, 20+20, 40 Gy
4. Chronic gamma irradiation 0, 400, 800 Gy

Large size plantlets taken out from aseptic culture and were evaluated for seedling weight and size before growing in the nursery.

Experiment 6. Study on the effects of single and split doses of acute and chronic irradiation on *in vitro* plantlets and PLBs of *Dendrobium* Sonia 'Earsakul' and *Dendrobium* Sonia 'BOM 17 Red'

6.1 The *in vitro* plantlets

A. *Dendrobium* Sonia ‘Earsakul’

8 treatments of

Single doses (Gy) 0
50
100
200

Split doses(Gy) 50+50
100+50
100+100
200+100

B. *Dendrobium* Sonia ‘BOM 17 Red’

6 treatments of chronic gamma irradiation of 0, 50, 50+50, 100+50, 100+100 and 200+100 Gy

6.2 The PLBs of *Dendrobium* Sonia ‘Earsakul’ and *Dendrobium* Sonia ‘BOM 17 Red’

5 treatments of 0 Gy

acute 0 + chronic 50+50 Gy

acute 10 + 50+50 Gy

acute 20 + 50+50 Gy

acute 30 + 50+50 Gy

1. *In vitro* plantlet cultures of *Dendrobium* Sonia ‘Earsakul’ and *Dendrobium* Sonia ‘BOM 17 Red’ were chronic gamma irradiated in the Gamma Room at Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University.
2. For split doses, the interval between the first and the second irradiation was 1 month.

Experiment 7. Radiosensitivity study on acute gamma irradiation for determination of LD_{50} of *Dendrobium* Sonia ‘Earsakul’ at 3 growth stages

Three different growth stages of *Den*. Sonia “Earsakul”, protocorm like bodies (PLBs), plantlets and back bulbs, were acute gamma irradiated using Mark I Gamma Irradiator at Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University. Radiation doses were 0, 40, 80, 160, 320, 640 and 1280 Gy. After irradiation, each material was undertaken as follows:

7.1. Protocorm like bodies (PLBs)

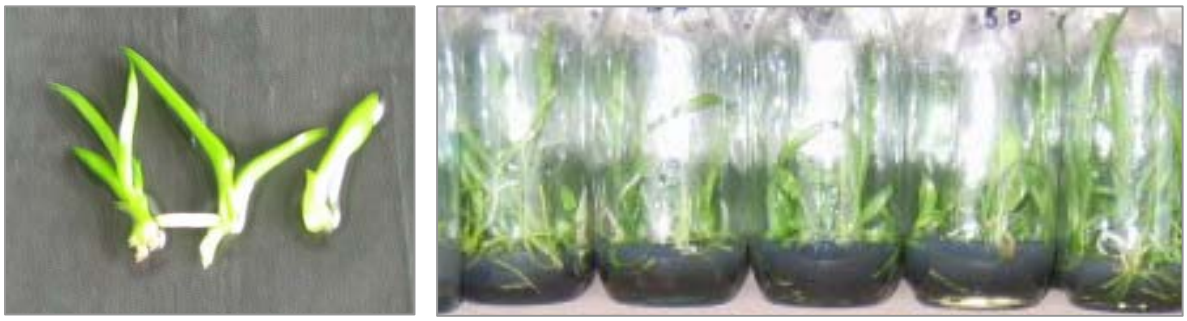
Two replications of PLBs were irradiated. For the first replication, irradiated PLBs were subcultured to the new medium, 20 pieces of PLBs were transferred to each bottle whereas only 10 pieces/bottle was used in the second replication.

Survival of PLBs was recorded after two months of subculturing. LD_{50} was determined from relationship between radiation dose and survival percentage.

Protocorm like bodies (PLBs) to be irradiated

7.2. Plantlets

In vitro plantlets were irradiated and were transferred to the new medium. Four months after irradiation, a number of survived seedlings was recorded, and LD_{50} was determined.



Plantlets to be irradiated

7.3. Back bulbs

Twenty back bulbs of *Dendrobium* Sonia "Earsakul" were irradiated for each treatment and were kept in nursery for investigation. Two months after irradiation, back bulbs producing new shoots were checked as survival. Percent survival of each treatment was calculated to get LD_{50}.

Back bulbs to be irradiated

Experiment 8. Evaluation of natural infestation of thrips in the nursery

1. The irradiated and the control plantlets from Experiments 5 and 6 were transplanted from the medium and grew in nursery to be naturally infested by thrips for 6 months.
2. A number of plantlets infested by thrips was recorded and percent infestation was calculated. Infested and non-infested plantlets were tagged and were planted in pots for further investigation.
3. Irradiated plants as well as the controls were kept in the nursery until flowering for further observation on morphological characters and would be selected for thrips resistance.

Results

Experiment 1. Evaluation and identification of breeding materials

Four clones of commercial cut flower orchids: *Dendrobium* Sonia 'BOM 17 red', *Dendrobium* Sonia 'Earsakul', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* hybrid 'White Sanan' were evaluated. (Table 1-1 and Table 1-2)

Table 1-1. Plant height and number of flower spikes of cut flower orchids

Clone	Bulb	Bulb characteristics			Number of spikes/bulb	
		Height (cm)	% of bulb with leaves	No. of leaves/ bulb	% of bulb produced flowers	No. of spikes/ bulb
1. *Den*. Sonia 'BOM 17 Red' Culture period: 2 years	front bulb	57.5	100	7.2	100	3.0
	1st back bulb	47.5	100	7.2	100	3.3
	2nd back bulb	39.8	100	5.4	100	2.4
	3rd back bulb	31.3	100	4.2	90	1.5
2. *Den*. Sonia 'Earsakul' Culture period: 2 years	front bulb	42.4	100	7.6	100	2.2
	1st back bulb	41.2	100	6.6	100	4.4
	2nd back bulb	36.0	100	5.8	100	3.2
	3rd back bulb	32.0	100	4.6	100	3.0
3. *Den*. Hybrid 'White Sanan' Culture period: 2 years	front bulb	62.3	100	10.1	100	2.7
	1st back bulb	48.7	100	6.6	100	3.1
	2nd back bulb	27.6	70	2.0	60	0.9
	3rd back bulb	20.2	10	0.1	30	0.4
4. *Den*. Pinkysem 'Rinnapa' Culture period: 3 years	front bulb	45.8	100	6.1	100	3.5
	1st back bulb	43.8	100	4.7	100	3.6
	2nd back bulb	32.7	80	3.4	100	2.9
	3rd back bulb	24.0	30	0.6	70	1.2

Table 1-2. Flowering behavior, number of flower spikes/plants of cut flower orchids

Flowering behavior	Clone			
	BOM 17 Red	**Earsakul**	**White Sanan**	**Rinnapa**
% of bulb which have 2 flowers spikes from terminal buds				
front bulb	70	100	70	60
1st back bulb	30	40	30	80
2nd back bulb	20	80	20	50
Average number of flower spikes/plant	10.2	12.8	7.1	9.9
-from terminal bud	5.2	7.4	4.1	5.8
-from lateral bud	5.0	5.4	3.0	4.1
Average number of flower spikes from lateral bud				
-1st lateral bud	2.6	2.6	1.4	2.7
-2nd	1.3	1.8	0.7	1.5
-3rd	0.7	0.8	0.4	0.5
-4th	0.3	0.2	0.3	0.3
-5th	0.1	-	0.1	0.1
-6th-9th	-	-	0.1	0.2

Experiment 2. Initiation of tissue culture of clones

Young shoots of *Dendrobium* Sonia 'Earsakul', *Dendrobium* Sonia 'BOM 17 Red', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* 'White Sanan' were collected from commercial orchid nursery for tissue culture. The lateral and terminal buds were excised from sterilized shoots and were cultured in liquid Vacin-Went medium. After two months under agitation condition, explants produced mass of protocorm like bodies (PLBs). The PLBs were multiplied in liquid medium and were transflasked to solid Vacin-Went medium for PLBs prolification.

Experiment 3. Exchange of material clones

Material clones, PLBs of *Dendrobium* Sonia 'BOM 17 Red', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* hybrid 'White Sanan' were exchanged among Thailand, Malaysia and Indonesia. At the Mid term evaluation meeting, every country agree to research on their varieties. Thailand selected *Dendrobium* Sonia 'Earsakul' and *Dendrobium* Sonia 'BOM 17 Red'.

Experiment 4. Determination of radiation sensitivity and optimum doses

4.1. Radiation effect on protocorm developed from seeds of *Dendrobium* hybrids

Seeds of *Dendrobium* hybrid were *in vitro* germinated on modified Vacin-Went solid medium. After germination for 20 days, seeds developed into tiny green protocorms. These protocorms were irradiated with acute gamma rays of 0, 20, 40, 60, and 80 Gy. Two months after irradiation, protocorms were subcultured to the new medium. All protocorms survived and shoot tips were developed. The second and third subcultures were done for seedling formation. Six months after irradiation, the seedlings were taken out from sterile condition and the results were recorded. The results show that the irradiated seedlings have shorter plant height and leaf length but have more fresh weight, leaf number and root number than the control plants (Table 4-1). The percentages of abnormal seedlings: 0, 21.33, 15.33, 11.33 and 9.33 are found in the treatments of 0, 20, 40, 60 and 80 Gy, respectively (Table 4-2).

Table 4-1. Effects of gamma rays on seedling growth of *Dendrobium* hybrids derived from *in vitro* acute irradiation

Radiation dose (Gy)	Fresh weight (g)	Plant height (cm)	Leaf number	Leaf length	Root number
0	1.29 c	7.90 a	4.59 c	5.76 a	8.12 b
20	2.20 a	6.46 b	5.27 a	3.81 b	12.87 a
40	1.63 b	5.29 c	4.86 bc	3.21 c	12.65 a
60	1.73 b	4.97 cd	5.13 ab	2.90 c	12.05 a
80	1.43 bc	4.69 d	5.28 a	2.89 c	11.53 a
F-test	**	**	**	**	**
CV (%)	26.79	14.81	10.69	16.30	26.84

Table 4-2. Abnormal seedlings (%) and survival (%) of *Dendrobium* hybrid seedlings

Radiation dose (Gy)	Survival rate (%)		*Abnormal seedlings (%)
	in vitro	*in vivo*	
0	100.00	100.00	0
20	100.00	60.16	21.33
40	100.00	47.37	15.33
60	98.67	73.10	11.33
80	98.67	65.32	9.33

*Abnormal seedlings: rosette, thick leaf, malformation leaf, variegated leaf

4.2. Radiation effects on PLBs of *Dendrobium* hybrids

The PLBs of 4 commercial cut flower clones: *Dendrobium* Sonia 'Earsakul', *Dendrobium* Sonia 'BOM 17 Red', *Dendrobium* Pinky Sem 'Rinnapa' and *Dendrobium* hybrid 'White Sanan' were irradiated with acute gamma rays at 0, 60, 70, 80, 90 and 100 Gy.

The irradiated PLBs were subcultured to the new medium. One month after irradiation, the number of survived and dead PLBs were inspected. The results show that all PLBs of *Dendrobium* Sonia 'Earsakul' and *Dendrobium* hybrid 'White Sanan' were survived, while PLBs of *Dendrobium* Pinky Sem 'Rinnapa' were tolerated to gamma rays than *Dendrobium* Sonia 'BOM 17 Red'. The non irradiated PLBs of *Dendrobium* Sonia 'BOM 17 Red' could survived 97.74 % and after irradiating with 60, 70, 80, 90 and 100 Gy, the survived PLBs were 77.45, 60.41, 62.39, 61.29 and 58.93 % respectively (Table 4-3). The PLBs were subcultured for shoot multiplication and seedling formation, respectively.

Table 4-3. Survival (%) of *Dendrobium* hybrids after irradiated with gamma rays

Radiation dose (Gy)	*Dendrobium* Pinky Sem 'Rinnapa'	*Dendrobium* Sonia 'BOM 17 red'	*Dendrobium* Sonia 'Earsakul'	*Dendrobium* hybrid 'White Sanan'
0	98.42	97.74	100	100
60	88.04	77.45	100	100
70	84.48	60.41	100	100
80	85.26	62.39	100	100
90	84.09	61.29	100	100
100	78.38	58.93	100	100

Experiment 5. Irradiation of PLBs for insect resistant clones

5.1. Effects of radiation on plantlets growth of *Dendrobium* Sonia 'BOM 17 Red'

The survived PLBs were subcultured for 4 times to the new medium for multiplication and seedling development.

Subculture	Period (month)	Growth
1st	1	PLBs
2nd	3	multiplication of PLBs
3rd	3	micro shoot formation with 3 - 4 leaves, 2 - 2.5 cm high
4th	5	culture 3 seedlings in one bottle until the seedling developed to large size with roots for transplanting to grow in the nursery

The PLBs of *Dendrobium* Sonia 'BOM 17 Red' were 1st subcultured after irradiation and were continued to subculture for multiplication, seedling formation with 3-4 leaves, 2-2.5 cm. high and for developing plantlets to large size with roots ready to be transplanted to grow in the nursery.

After the 4th subculturing for 5 months, the plantlets were taken out from aseptic media for evaluation of weight, number of pseudobulb / plant, pseudobulb height and diameter, leaf number and size. No significant difference in weight, pseudobulb diameter and leaf width. The number of pseudobulb/plant, pseudobulb height, leaf number and leaf length of irradiated plants were lower than the control. Irradiated treatments with 90 and 100 Gy reduced pseudobulb height and leaf length (Table 5-1) and the abnormal rosette plantlets were found 15.71 and 15.79 %, respectively (Table 5-2).

The irradiated plantlets were reduced in plant height. When the plantlets were ranked in height for 4 levels, it was found that 49 % of non-irradiated plantlets had 6.1 - 11.0 cm high with 51 % of 11.1 - 16.0 cm high. While the irradiated plantlets were in 6.1 - 11.0 cm. high. These small plantlets had normal shape and large size plantlets but the growth rate was very slow (Table 5-3).

The plantlets were grown in a rainproof nursery for one month. The 80 - 100 Gy irradiated plantlets survived at 88.09, 85.71 and 86.84 %, respectively (Table 5-4). The normal plantlets were alive while the rosette plantlets could not survive. The survived plantlets were cultured for growth determination.

Table 5-1. Seedling weight and plant characteristics of *Dendrobium* Sonia 'BOM 17 Red'

Radiation dose (Gy)	Weight (g)	Plant Height (cm)	Pseudobulb			Leaf		
			number /plant	size (cm)		number /plant	size (cm)	
				height	width		length	width
0	1.62	12.18a	2.85a	3.14a	0.29	10.9a	8.99a	0.55
60	1.19	8.669b	2.15b	2.40b	0.33	7.60b	6.50b	0.58
70	1.37	8.49b	1.85b	2.32b	0.33	6.40b	6.15b	0.55
80	1.11	7.92b	1.85b	2.23b	0.34	6.80b	5.51bc	0.62
90	1.42	6.77bc	1.98b	1.93b	0.37	7.05b	4.60cd	0.58
100	1.21	5.76c	2.20b	1.87b	0.37	7.20b	3.93d	0.62
F-test	ns	**	**	**	ns	**	**	ns

Table 5-2. Abnormal plantlets affected by radiation

Radiation dose (Gy)	Abnormal plantlets	
	%	Characteristics
0	0	non
60	7.51	rosette, thick leaf, short stem
70	8.57	rosette, thick leaf, short stem
80	11.91	rosette, thick leaf, short stem
90	15.71	rosette
100	17.79	rosette

Table 5-3. Percentage of seedlings ranked in 4 levels of plant height of *Dendrobium* Sonia 'BOM 17 Red'

Radiation dose (Gy)	Plant height (cm)				
	Abnormal	Slow growth (small size)	Normal growth		
	1.8-3.0	3.1-6.0	6.1-11.0	11.1-16.0	Total
0	0	0	49	51	100
60	2	14	61	22	83
70	2	16	69	13	82
80	2	19	66	13	79
90	4	25	64	7	73
100	4	33	60	3	63

Table 5-4. Total of survived plants after growing in the nursery for 1 month

Radiation dose (Gy)	Survival (%)
0	97.60
60	94.62
70	98.57
80	88.09
90	85.71
100	86.84

5.2. Effects of radiation on plantlet growth of *Dendrobium* Sonia 'Earsakul'

PLBs of *Dendrobium* Sonia 'Earsakul' were irradiated with

a) high doses of acute gamma rays : 0, 60, 70, 80, 90, 100 Gy

b) low doses of acute gamma rays : 0, 2, 4, 6, 8, 10 Gy

c) split doses of acute gamma rays : 0, 20, 20+20, 40 Gy

d) chronic gamma rays : 0, 200, 400, 800 Gy

The PLBs were 1st subcultured after irradiation, then continued to subculture for multiplication, seedling formation with 3 - 4 leaves, 2 - 2.5 cm high and for developing the plantlets to large size with roots ready to be transplanted to grow in the nursery.

After the 4th subculturing for 3 months (8 months from irradiation), the plantlets were removed from aseptic media for evaluation of weight, number of pseudobulb / plant, pseudobulb height and diameter, leaf number and size. The irradiated plantlets at high doses reduced in weight, height, pseudobulb height and number, number of leaf and leaf length. No significant difference in number of pseudobulb/plant and leaf width (Table 5-5). The percentage of plant height separate in 5 ranks were shown in Table 5-6. The low doses irradiation had no effect on growth of plantlets (Table 5-7 and Table 5-8)

5.2.1 High doses of acute gamma irradiation

Table 5-5. Weight and plant characteristics of *Dendrobium* Sonia 'Earsakul'

Radiation dose (Gy)	Weight (g)	Plant height (cm)	Pseudobulb			Leaf		
			number /plant	size (cm)		number /plant	size(cm)	
				height	width		length	width
0	2.0a	11.06a	2.8a	3.10a	0.42	8.9a	7.98a	0.60
60	1.2b	8.61b	1.7c	2.52b	0.41	6.3b	6.13b	0.61
70	1.4b	7.53bc	2.3b	2.33b	0.44	7.2b	5.53bc	0.67
80	1.4b	6.91cd	2.0bc	2.31b	0.44	7.2b	4.67cd	0.69
90	1.1b	6.86cd	1.9bc	2.16b	0.43	6.8b	4.59cd	0.63
100	1.0b	5.69d	1.6c	2.01b	0.44	7.1b	3.70d	0.74
F-test	**	**	ns	**	ns	**	**	ns
CV (%)	67.5	36.4	38.8	36.9	36.4	33.4	38.6	27.0

Table 5-6. Percentage of plant height separate in 5 ranks of *Dendrobium* Sonia 'Earsakul'

Radiation dose (Gy)	Plant height (cm.)				
	Abnormal	Slow growth	Normal growth		
	1.8-3.0	3.1-6.0	6.1-11.0	11.1-16.0	Total
0	0	0	52	48	100
60	1	4	74	21	95
70	4	30	57	9	66
80	3	29	65	3	68
90	1	31	64	4	68
100	4	33	62	1	63

5.2.2 Low doses acute gamma irradiation

Table 5-7. Weight and plant characteristics of *Dendrobium* Sonia 'Earsakul'

Radiation dose (Gy)	Weight (g)	Plant height (cm)	Pseudobulb			Leaf		
			number /plant	size (cm)		number /plant	size(cm)	
				height	width		length	width
0	1.9	10.84	2.0	2.75	0.42	7.5	8.00	0.71
2	2.0	11.04	2.2	2.77	0.40	7.3	8.39	0.70
4	2.0	10.83	2.3	2.68	0.40	8.3	8.24	0.74
6	1.8	10.44	2.3	2.67	0.39	8.3	8.03	0.69
8	1.8	10.15	2.0	2.66	0.41	7.2	7.64	0.72
10	1.8	10.11	2.1	2.56	0.39	8.1	7.51	0.70
F-test	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	40.9	23.0	31.6	23.7	66.2	26.5	23.5	18.1

Table 5-8. Percentage of seedlings ranked in 4 levels of plant height of *Dendrobium* Sonia'Earsakul'

Radiation dose (Gy)	Plant height (cm.)				
	Abnormal	Slow growth	Normal growth		
	1.8-3.0	3.1-6.0	6.1-11.0	11.1-16.0	Total
0	0	0	74	26	100
2	0	0	72	28	100
4	0	0	74	26	100
6	0	0	80	20	100
8	0	2	80	18	98
10	0	1	85	14	99

5.2.3 Split doses acute gamma irradiation

Table 5-9. Seedling weight and plant characteristics of *Dendrobium* Sonia'Earsakul'

Radiation dose (Gy)	Weight (g)	Plant height (cm)	Pseudobulb			Leaf		
			number /plant	size (cm)		number /plant	size(cm)	
				height	width		length	width
0	1.9	10.84a	2.0	2.75a	0.42	7.5b	8.00a	0.71
20	1.9	9.37b	2.3	2.40b	0.39ab	9.1a	7.06ab	0.67
20+20	1.5	7.49c	2.1	2.27b	0.37b	9.8a	5.08c	0.64
40	2.0	8.88b	2.4	2.39b	0.42a	8.9a	6.56b	0.70
F-test	ns	**	ns	*	*	**	**	ns
CV (%)	41.3	27.8	37.1	26.1	74.7	30.4	32.2	19.4

Table 5-10. Percentage of seedlings ranked in 4 levels of plant height of *Dendrobium* Sonia'Earsakul'

Radiation dose (Gy)	Plant height (cm)				
	Abnormal	Slow growth	Normal growth		
	1.8-3.0	3.1-6.0	6.1-11.0	11.1-16.0	Total
0	0	0	74	26	100
20	0	6	85	9	94
20+20	0	26	73	1	74
40	0	11	82	7	89

5.2.4 Chronic gamma irradiation

Table 5-11 Seedling weight and plant characteristics of *Dendrobium* Sonia Earsakul'

Radiation dose (Gy)	Weight (g)	Plant height (cm)	Pseudobulb			Leaf		
			number /plant	size (cm)		number /plant	size(cm)	
				height	width		length	width
0	1.9a	10.84a	2.0	2.75a	0.42	7.5	8.00a	0.71
400	1.6ab	10.33a	2.0	2.45ab	0.37	8.2	7.85a	0.65
800	1.3b	8.28b	1.8	2.35b	0.38	7.5	5.64b	0.68
F-test	*	**	ns	*	ns	ns	**	ns
CV (%)	49.3	24.0	25.9	25.3	75.5	23.0	27.4	22.4

Table 5-12. Percentage of seedlings separate in 5 ranks of plant height of *Dendrobium* Sonia 'Earsakul'

Radiation Dose (Gy)	Plant height (cm)				
	Abnormal	Slow growth	Normal growth		
	1.8-3.0	3.1-6.0	6.1-11.0	11.1-16.0	total
0	0	0	74	26	100
400	0	3	78	19	97
800	0	20	74	6	80

Experiment 6

Study on the effects of single and split doses of acute and chronic irradiation on *in vitro* plantlets and PLBs of *Dendrobium* Sonia 'Earsakul' and *Dendrobium* Sonia 'BOM 17 Red'

6.1 The *in vitro* plantlets

A. *Dendrobium* Sonia 'Earsakul'

Table 6-1. Pseudobulb height of *Dendrobium* Sonia 'Earsakul' after grew in nursery for 6 and 12 months

Radiation dose (Gy)		Pseudobulb height (cm)	
		6 months	12 months
Single doses	0	6.63	9.98
	50	6.47	8.10
	100	6.16	7.52
	200	5.63	6.37
Split doses	50+50	6.14	7.60
	100+50	6.11	8.41
	100+100	6.06	8.24
	200+100	5.45	6.22

B. *Dendrobium* Sonia 'BOM 17 Red'

Table 6-2. Pseudobulb height of *Dendrobium* Sonia 'BOM 17 Red' after grew in nursery for 6 and 12 months

Radiation dose (Gy)		Pseudobulb height (cm)	
		6 months	12 months
Single doses	0	6.95	11.07
	50	6.44	9.55
Split doses	50+50	6.21	8.89
	100+50	6.36	8.91
	100+100	6.60	9.06
	200+100	6.12	8.62

6.2. The PLBs of *Dendrobium* Sonia 'Earsakul' and *Dendrobium* Sonia 'BOM 17 Red'

A. *Dendrobium* Sonia 'Earsakul'

Table 6-3. Plantlets characteristics of *Dendrobium* Sonia 'Earsakul' after transplant from aseptic condition

Radiation dose (Gy)	Fresh weight (g)	Pseudobulb		Leaf length (cm)
		Number of plant	Height (cm)	
0	2.01	2	3.42a	6.75a
acute 0 + chronic 50+50	1.98	2.1	3.30ab	5.53b
acute 10 + chronic 50+50	1.92	1.9	3.31ab	5.59b
acute 20 + chronic 50+50	2.12	2.2	3.25ab	5.17b
acute 30 + chronic 50+50	1.87	2.0	3.06b	5.11b
F-test	ns	ns	**	**
CV (%)	31.70	26.41	32.78	24.87

Table 6-4. Pseudobulb number/plant and height of *Dendrobium* Sonia 'Earsakul' and percent natural infestation of thrips on seedlings after growing in nursery for 6 months

Radiation dose (Gy)	Pseudobulb	
	Number/plant	Height (cm)
0	4.67a	11.51a
acute 0 + chronic 50+50	4.47ab	8.81b
acute 10 + chronic 50+50	4.73a	9.29b
acute 20 + chronic 50+50	4.03b	9.04b
acute 30 + chronic 50+50	4.33ab	9.55b
F-test	**	**
CV (%)	13.93	22.86

B. *Dendrobium* Sonia 'BOM 17 Red'

Table 6-5. Plantlets characteristics of *Dendrobium* Sonia 'BOM 17 Red' after transplant from aseptic condition

Radiation dose (Gy)	Fresh weight (g)	Pseudobulb		Leaf length (cm)
		Number/plant	Height (cm)	
0	2.11a	2.2	3.61a	6.39a
acute 0 + chronic 50+50	1.91ab	2.3	3.36ab	6.33a
acute 10 + chronic 50+50	1.87ab	2.0	3.33ab	6.01ab
acute 20 + chronic 50+50	1.85ab	2.1	3.30ab	5.64ab
acute 30 + chronic 50+50	1.73b	2.2	3.03b	5.21b
F-test	*	ns	**	**
CV (%)	34.92	29.74	23.72	30.02

Table 6-6. Pseudobulb number/plant and height of *Dendrobium* Sonia 'BOM 17 Red' and percent natural infestation of thrips on seedlings after growing in nursery for 6 months

Radiation dose (Gy)	Pseudobulb	
	Number/plant	Height (cm)
0	4.70b	13.32a
acute 0 + chronic 50+50	4.80ab	11.01b
acute 10 + chronic 50+50	4.97ab	11.11b
acute 20 + chronic 50+50	5.23a	11.57b
acute 30 + chronic 50+50	5.10ab	10.99b
F-test	*	*
CV (%)	17.89	27.40

Experiment 7. Radiosensitivity study on acute gamma irradiation for determining LD_{50} of *Dendrobium* Sonia 'Earsakul' at 3 growth stages

7.1 Protocorm like bodies (PLBs)

The result showed that no survival of PLBs of *Den.* Sonia 'Earsakul' at the doses higher than 320 Gy (Table 7-1). Relationship between radiation dose and percent survival gave LD_{50} (at 2 months after irradiation) of 70 Gy (Figure 1).

Table 7-1. Percent survival of *Den.* Sonia 'Earsakul' PLBs treated with different acute gamma radiation doses (2 months after subculturing)

Radiation dose (Gy)	Survival (as % of control)		
	Rep. 1	Rep. 2	Ave.
0 (control)	100	100	100
40	100	100	100
80	47	40	43.5
160	17	10	13.5
320	0	0	0
640	0	0	0
1280	0	0	0

7.2 *In vitro* plantlets and back bulbs

In commercial orchid growing, the gardeners do vegetative propagation of sympodial orchid by dividing. After culturing for 4 years the *Dendrobium* orchid plants produced 7-9 pseudobulbs per plant. The gardeners separate each mature psuedobulb or back bulb and left them produce new shoot from lateral bud before growing on coconut medium. After culturing for 8 months these shoots produce the inflorescences.

Three months after irradiation, *in vitro* plantlets treated with 0 and 40 Gy produced new shoots while plantlets in the other treatments stopped to grow and die in the 4th month. For the back bulbs, all of non-irradiated pieces produced new shoots from lateral buds at rhizomes in 2 weeks whereas the buds treated with radiation still dormant. After 2 months, only 40 % of buds treated with 40 Gy produced small shoots. The shoots could not develop when treated with radiation doses at 80 - 1280. The results are shown in Table 7-2.

Table 7-2. Plantlets and back bulb survival of *Den.* Sonia "Earsakul" treated with different doses of acute gamma irradiation

Radiation dose (Gy)	Survival (as % of control)	
	Plantlets (4 months after irradiation)	Back bulb (2 months after irradiation)
0	100	100
40	70.0	40
80	62.5	0
160	61.8	0
320	50.9	0
640	26.2	0
1280	0	0

Plantlet survival decreased as radiation dose increased. LD_{50} of plantlet at 4 months after irradiation was 330 Gy (Figure 1).

For back bulb, radiosensitivity was higher than plantlets. Radiation doses over 40 Gy prohibited shoot formation of irradiated materials. LD_{50} of back bulb at 2 months after irradiation was 33 Gy (Figure 2).

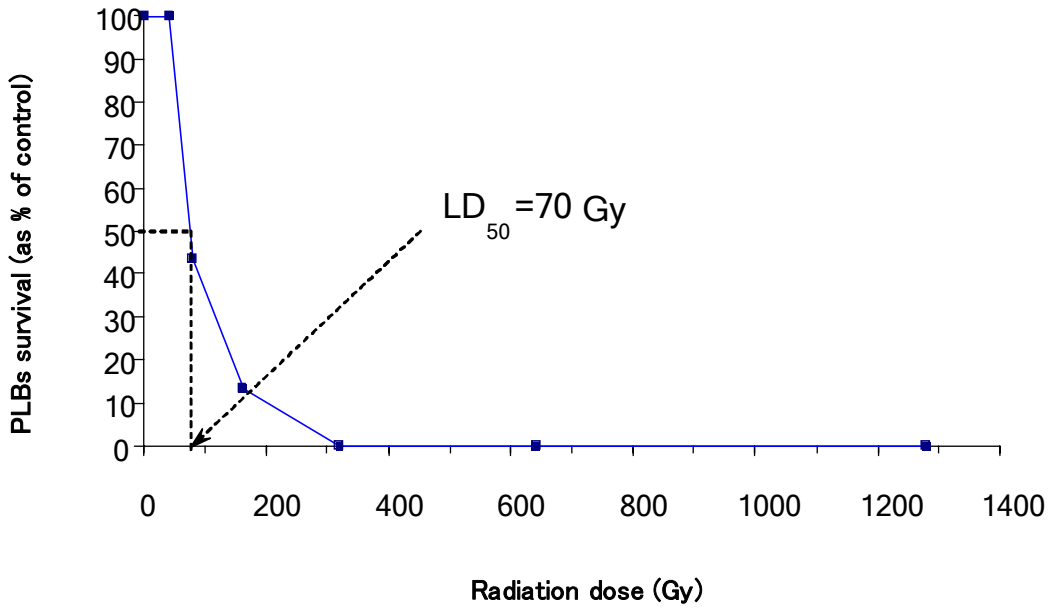


Figure 1. Relationship between radiation dose and survival of protocorm like bodies (PLBs) 2 months after irradiation

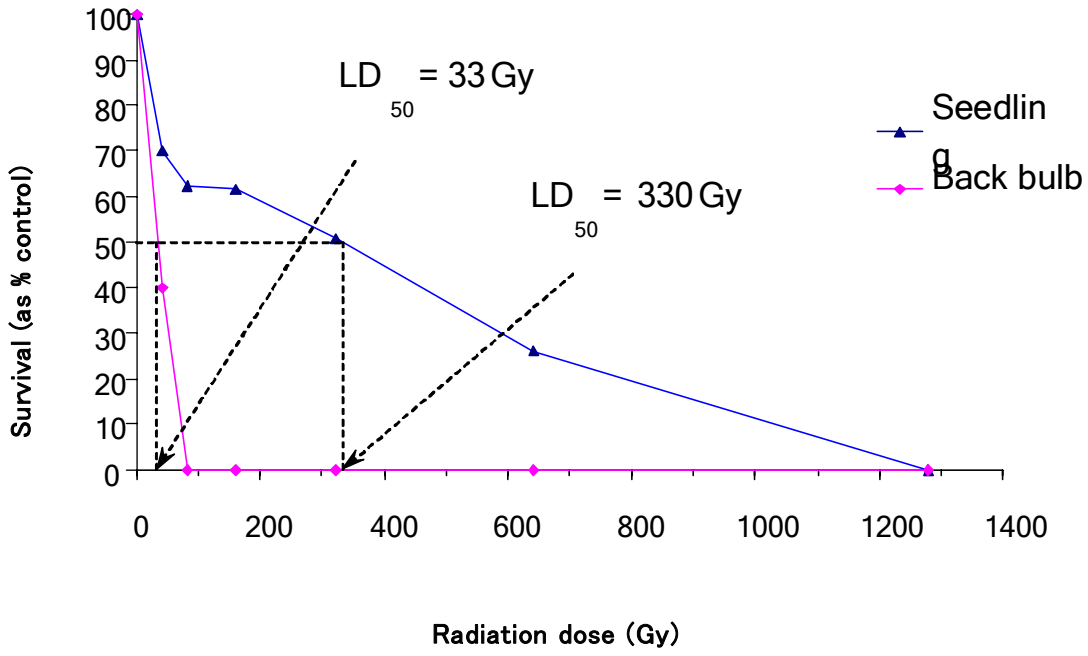


Figure 2. Relationship between radiation dose and survival of plantlet and back bulb 4 and 2 months after irradiation, respectively.

Experiment 8. Evaluation of natural infestation of thrips in the nursery

8.1 Plantlets from Experiment 5

After transplanting of plantlets from aseptic culture, the plantlets were grown in 1 inch pot with coconut fiber as medium under the rainproof nursery. Water was applied in every morning and fertilizer was sprayed once a week. No application of insecticide and fungicide.

The first evaluation was carried out for 4 months during November 2004-February 2005. The plantlets of *Dendrobium* Sonia 'BOM 17 Red' and *Dendrobium* Sonia 'Earsakul' were grown in the nursery for natural infestation of thrips. The results showed that only 0 - 17.14 % of plants were damaged by thrips. The non-irradiated plants showed 16.85 and 17.14 % damaged which more than irradiated plants, since the control plants started to produce new shoots earlier than irradiated plants. Thrips could damage more number of the control plants (Table 8-1).

Table 8-1. Percentage of seedlings which the first new shoots were damaged by thrips

Radiation dose (Gy)	% of thrips damaged seedlings	
	***Den.* Sonia 'BOM 17 Red'**	***Den.* Sonia 'Earsakul'**
0	16.85	17.14
60	4.55	5.00
70	5.26	5.56
80	7.14	5.81
90	0	3.57
100	2.04	3.27

After 10 months in the nursery during November 2004 - August 2005, a number of plants died, whereas the living plants produced the 2nd and 3rd shoots. The data was recorded again from the living plants. The results showed that 67.27 - 92.31 % of plants were damaged by thrips (Table 8-2).

The number of damaged seedling were classified into 2 types:

1) damaged on the 2nd new bulbs with no damaged on the 3rd new bulbs

2) damaged on the 2nd and the 3rd new bulbs (Table 8-3).

In the non-pesticide with hot temperature and high humidity condition, thrips gave no problems to the plants. In rainy season, plants died by the infestation of worms, fungi and bacterial diseases. A lot of insect larvae were feeding on most of leaves during dry season, thus the plants had no leaves for thrips.

The non-damaged plants by thrips were transplanted to grow in large size pots for evaluation of growth and flower size. The experiments on selection of thrip resistant clones in flowering stage will be designed.

Table 8-2. Survival and percentage of the 3rd new shoots damaged by thrips

Radiation dose (Gy)	*Den.* Sonia 'BOM 17 Red'		*Den.* Sonia 'Earsakul'	
	Survival (%)	Damaged seedlings (%)	Survival (%)	Damaged seedlings (%)
0	42.0	92.31	45.0	86.40
60	30.5	83.78	33.0	87.88
70	10.5	80.77	43.5	88.51
80	3.0	83.33	40.5	87.78
90	0	--	27.5	67.27
100	3.0	83.33	45.0	85.66

Table 8-3. Percentage of seedlings which the 2nd and the 3rd new shoots were damaged by thrips

Radiation dose (Gy)	Damaged seedlings (%)		
	Damaged on the 2nd new shoots and no damaged on the 3rd new shoots	Damage on the 2nd and the 3rd new shoots	Total
0	37.60	48.80	86.40
2	37.06	56.65	93.71
4	36.89	45.08	81.97
6	40.63	53.12	93.75
8	40.79	40.79	81.58
10	39.89	38.95	76.84

8.2. Natural infestation of thrips on chronic gamma irradiation of *Dendrobium* Sonia 'BOM 17 Red' and *Dendrobium* Sonia 'Earsakul' plantlets (plantlets from Experiment 6).

1) The irradiated plantlets

The numbers of *Dendrobium* Sonia 'BOM 17 Red' and *Dendrobium* Sonia 'Earsakul' plantlets infested by thrips of each treatment are shown in Table 8-4 and Table 8-5, respectively.

Table 8-4. Percent natural infestation of thrips on plantlets of *Dendrobium* Sonia 'BOM 17 Red' treated with different doses of chronic gamma irradiation (6 months after growing in the nursery)

Radiation dose (Gy)	Number of plantlet		Infested (%)
	Total	Infested	
0 (control)	320	14	4.38
50	50	11	22.00
50+50	295	95	32.20
100	94	16	17.02
100+50	245	41	16.74
100+100	153	24	15.69
200	79	22	27.85
200+100	444	97	21.85

Table 8-5. Percent natural infestation of thrips on plantlets of *Dendrobium* Sonia 'Earsakul' treated with different doses of chronic gamma radiation (6 months after growing in the nursery)

Radiation dose (Gy)	Number of plantlet		Infested (%)
	Total	Infested	
0 (control)	127	34	26.77
50	219	34	15.53
50+50	191	26	13.61
100+50	234	43	18.38
100+100	142	22	15.49
200+100	140	21	15.00

Natural infestation of thrips was more severe on *Dendrobium* Sonia 'Earsakul' than *Dendrobium* Sonia 'BOM 17 Red'. Percent infestation reduced on the irradiated treatments of both varieties.

For *Dendrobium* Sonia 'Earsakul', the lowest infestations were found in doses of 100+100, 100+50 and 100 Gy. Lower or higher doses seemed to be susceptible to thrips. However, the infestation of irradiated plantlets was higher than the control.

The opposite result was noticed in *Dendrobium* Sonia 'BOM 17 Red'. It appeared that radiation doses gave no different effects on infestation of thrips but all radiation treatments were shown to be resistant than the control.

2) Plantlets derived from irradiated PLBs

Table 8-6. Percent natural infestation of thrips on seedlings of *Dendrobium* Sonia 'Earsakul' and *Dendrobium* Sonia 'BOM 17 Red' after growing in nursery for 6 months

Radiation dose (Gy)	Infested (%)	
	'Earsakul'	'BOM 17 Red'
0	6	6
acute 0 + chronic 50+50	8	10
acute 10 + chronic 50+50	4	8
acute 20 + chronic 50+50	8	6
acute 30 + chronic 50+50	6	8

For growth and morphological characters, it was found that at the beginning, plantlets growth decreased as radiation doses increased, but later, they returned to normal growth. At the same total dose, split dose showed less effect than single dose. Some abnormal leaves and flowers, as well as some other morphological changes e.g. flower color, flower size were observed. Selection for thrips resistance needs to be followed at flowering stage.

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