FNCA／MEXT
Technical Material

Achievement

Sub-Project on Insect Resistance in Orchid

(2003 – 2009)

Mutation Breeding Project

Forum for Nuclear Cooperation in Asia (FNCA)

March, 2010

Foreword

Orchid is one of the largest families of flowering plants comprising an estimated 20,000 to 25,000 species. Orchids such as *Dendrobium* have been the major cut-flower export for Thailand, Malaysia and Indonesia as well as for other Southeast Asian countries. Thailand is the major orchid producer with an export value of US$ 80 million in 2006, or approximately 80 % of the country's total ornamental export value. The export value of orchids from Malaysia is estimated at RM 150 million per year, representing approximately 40 % of the total floriculture production. Problems regarding the infestation of insects in orchid flowers have caused considerable losses to exporters due to strict quarantine regulations. Among them, mites and thrips are the most devastating pests of the orchid. Spraying with insecticides and mitecides are another option when dealing with orchid pests, especially when populations are high and spraying is the only effective means of controlling mite attacks. This practice leads to heavy use of chemicals which requires government review and regulation. One of the strategies that can be adopted to overcome the problem is to induce insect resistance in orchid through radiation breeding. The Mutation Breeding Project recently focused on the improvement of insect resistance in orchid and represents one Sub-Project developed through the Forum for Nuclear Cooperation in Asia (FNCA), organized by Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), in 2003. This Sub-Project was established with the collaboration of member countries such as Indonesia, Malaysia, and Thailand. Mutation breeding through gamma-ray and ion beam irradiation is one of very useful methods to induce favorable changes in germplasm when required breeding materials are not otherwise available.

Activities of this project are focused on a wide array of important agronomic topics; of which the following were deemed of highest importance: (1) exchange of breeding materials and promising mutants; (2) exchange of information regarding efficiency of screening methods for insect-resistance; (3) establishment of breeding techniques such as in vitro culture and identification of optimum irradiation dose to plant parts. Each year, results and encountered problems within this project are discussed at the FNCA Workshop held in the member countries as well as two meetings of specialists. In this project, promising lines have been selected in Malaysia with the support of Indonesia and Thailand and evaluation of performance and yield trials for the registration are being conducted. Since the cooperative research activities fulfilled the project goals, the project was terminated in 2009. However, several additional years of efforts are required for the plant breeders to release new cultivars to the farmers. Following the identification of promising lines, yield trials, spanning at least 3

growing seasons, are required for a new cultivar registration.

It is our hope that the promising lines developed in this project will benefit the orchid farmers of Asia. We also hope that this book will be useful not only for the breeders interested in gamma ray and ion beam induced mutation breeding, but also for the breeders of orchid.

I would like to state my appreciation to the contributing authors for their achievements in this project and the submission of their final reports.

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March, 2010

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1. Indonesia

1. Indonesia

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Introduction

In Indonesia, demand for orchid cut flower is increasing due to the growth of population and their income. According to Soeroyo (1992), the world consumption value for orchid cut flowers was US $ 1.935 billion, if Indonesia could contribute 2.5 %, it needs the availability of 129.43 million flower stalk for export quality. Whilst for those of 5 % for export quality, it needs 32,357,500 plants (supposed each plant produced 8 flower stalk). Orchid industry needs the support from science and technology based on the use of optimal natural resources. Development of technology in culturing orchids is an opportunity to create desirable characters of the flowers such as bright colour, beautiful shape and longer vase life.

Dendrobium is the most popular orchids in Indonesia (Figure 1) followed by *Catleya*, *Vanda* and *Phalaenopsis.* Recently *Dendrobium* is mostly grown in Java and Bali islands. Both sexual and asexual propagation are common for multiplication of *Dendrobium*. Orchid consumers in Indonesia tend to prefer the new type. Micropropagation in orchid is very common since the demand for potted plants and cut flowers increased.

Figure 1. *Dendrobium Jayakarta* for cut flowers is widely grown in Indonesia

Breeding of the genus *Dendrobium* has been gaining sufficient appreciation and development in Southeast Asia as the most popular source of tropical cut flowers. A large number of different cultivars of distinct colours and shapes of this species have been created and grown exclusively in farms in this area. Commercial plants produce cut flowers and cooled inflorescences are exported throughout Europe and Japan.

The fact that orchids are, in terms of their development, a very young family of plants and their genetic instability. Therefore they tend to form mutation by means deviations. Usually the frequency of mutation was very low and lack of desirable traits. In the wild, these deviations are suppressed and gradually disappear. Orchid growers are able to stabilize them by further appropriate hybridisation and maintain the desirable traits such as colour and size of the flower (Jezek, 2003).

Sutater (1997) reported that exported orchid flower from Indonesia decreased from US $ 2,1 million (1993) to US $ 1.41 million (1995), whereas imported orchid flower increased from US $ 28,831 (1994) to US $ 312,767 (1995). Based on the data mentioned above, it seems that the demand was higher than the supply. Therefore, the opportunity for growing orchid in Indonesia is very promising.

Demand of orchid especially *Dendrobium* is increasing because of its unique, beauty, frequent flowering, longevity and easier to be cultivated. These characters are leading compared with other orchids. *Dendrobium* could be used as indoor and outdoor potted plants, cut flowers or corsages. However, *Dendrobium* is very susceptible to pets and diseases. Flower damage caused by Thrips is one of the obstacles faced by orchid growers.

An effort to solve this problem had been initiated in FNCA Mutation Breeding Project Workshop held in Yogyakarta, Indonesia from 30 August – 3 September 2004 (Hutabarat et al, 2004 and Piluek, 2004). The Orchid Group agreed to use *Dendrobium* Sonia 'BOM 17 Red', a leading variety from Thailand as the main material, *Den*. Sonia 'BOM 17 Red' is a popular hybrid, fast growing, floriferous, bright colour and has long vase life.

In FNCA Mutation Breeding Project Workshop held in Bangkok from 5 - 9, September 2005, the Orchid Group decided to irradiate different growing stages of young seedlings or shoots, plantlets and PLBs of local variety from each country, such as *Dendrobium Jayakarta* from

Indonesia should be irradiated in order to obtain the optimum dose for *Dendrobium* sp. from different member countries.

The most severe damage caused by irradiation was showed by PLBs compared with plantlets and shoots. Three months after irradiation, survival rates from the dose 1,280 Gy up to the dose 40 Gy of irradiated PLBs, plantlets and shoots were 0 - 50 %, 0 - 78 % and 60 - 95 % respectively. Survival rate of untreated PLBs, plantlets and shoots remained 100 % after three months irradiation. Explants of *Den*. Sonia 'BOM 17 Red' cultured in VW enriched with BAP was not able to form roots, whilst those enriched with coconut water and active charcoal could form 2.2 - 4.7 roots (Sutarto et al 2006).

Materials and methods

Irradiated PLBs of *Den*. *Jayakarta* cultured in VW medium were regenerated to form plantlets and each plantlet was planted in a single jar. While irradiated plantlets formerly grown in community pots had been transferred to single pots. Irradiated potted plants started blooming were grown under lath house. Potted plants were grown in the lath house and fertilized every week by applying 0.2 % liquid fertilizer.

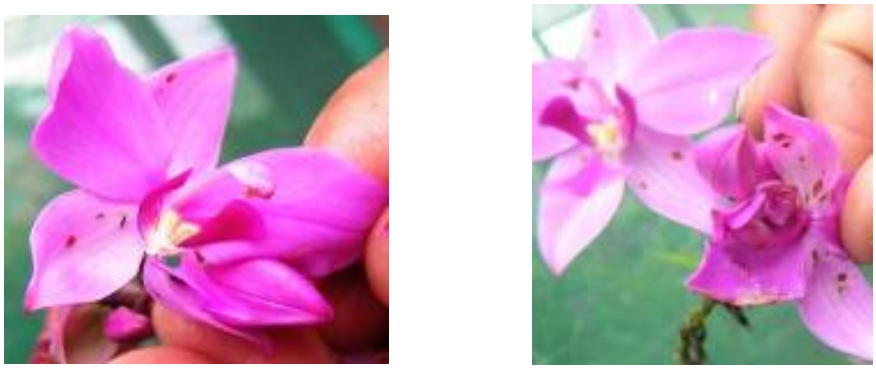
Irradiated explants of *Den.* Sonia 'BOM 17 Red' grown at CAIRT were cultured by applying modified VW medium enriched with coconut water (15 %), charcoal (0.1 %) and BAP (1 ppm). Whereas those grown at IOCRI were cultured in VW medium. The last accession of *Den.* Sonia 'BOM 17 Red' was Ac 10 + 50 +50 (Acute irradiation 10 Gy + chronic irradiation 50 + 50 Gy) and Ac 20 + 50 +50 (acute irradiation 20 Gy + chronic irradiation 50 + 50 Gy).

The use of different modified Vacin and Went (VW) media such as application of coconut water, active charcoal and Benzyl Amino Purine (BAP) was also carried out to improve the growth and propagation of irradiated plantlet of *Den.* Sonia 'BOM 17 Red' and *Den. Jayakarta*.

Application coconut water (Widyastoeti and Syafril, 1993) and active charcoal (Widyastoety and Bahar, 1995) could stimulate the growth of *Dendrobium* plantlets. Kano and Fukuoka (1996) reported that coconut water contained complex organic materials such as plant growth regulators that promoted plant growth.

Mass rearing Thrips was done at Entomology Laboratory of IOCRI by placing mature Thrips into the flask with pumpkin inside (Figure 2), and let the Thrips lay eggs and multiply its population, 25 - 30 days later mature Thrips were put on the flower of *Spathoglottis* sp (Figure 3) in order to see how the Thrips attack the flower since irradiated *Dendrobium* had not bloomed yet. When the plants started blooming, insect tolerance trial will be conducted *in vivo* in the green house.

Figure 2. Thrips were put in the flask with the pumpkin inside



(a) **(b)**

Figure 3. a. Mature Thrips were placed on the flower of Spathoglottis sp., b.Thrips attacked the flower of Spathoglottis sp.

The parameter observed were number of shoots per plant, number of leaves per shoots, plant height, number of plantlet or plant per pot and number of potted plants. The data were taken 12 months after irradiation.

Results

After 12 months irradiation, PLBs of *Dendrobium Jayakarta* grew to form plantlets. The highest number of plantlets and number of shoots per plant were found from untreated PLBs followed by irradiated PLBs at the doses of 40 and 80 Gy. In contrast the highest number of leaves per shoot was obtained from irradiated PLBs at dose 80 Gy followed by irradiated

PLBs at the dose 40 Gy and untreated PLBs, while the highest plant was showed by untreated PLBs followed by irradiated PLBs at the doses 80 and 40 Gy (Table 1 and Figure 4).

Irradiated plantlets of *Dendrobium Jayakarta* grown in the lath house showed better tolerance to gamma rays than irradiated PLBs. Plantlets were able to grow well up to the dose 160 Gy (Table 2 and Figure 5), whereas irradiated young plants could only grow at the dose 40 Gy (Table 3 and Figure 6).

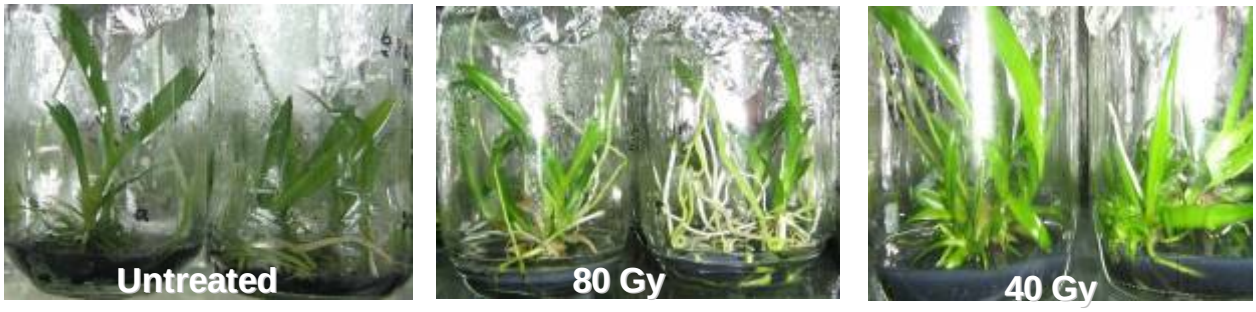


Figure 4. Untreated (0 Gy) and irradiated PLBs of *Den. Jayakarta* at the doses 40 and 80 Gy (12 months after culturing)

The most plantlets and clusters of PLBs formed were obtained from irradiated plantlets of *Den*. Sonia 'BOM 17 Red' at the dose 70 Gy. PLBs was not found from those at the dose 30 Gy and untreated plantlets (Table 4 and Figure 7). Abnormalities were found in irradiated PLBs of *Den.* Sonia 'BOM 17 Red' (Figure 8).

Acute irradiation 10 Gy followed by chronic radiation 50 + 50 Gy indicated better number of plants and leaflets, whereas acute irradiation 20 Gy followed by chronic radiation 50 + 50 Gy indicated higher number of shoots per plants and plant height (Table 5 and Figure 9).

Irradiated plantlets of *Den.* Sonia 'BOM 17 Red' at the dose of 90 Gy grown in the lath house of IOCRI for acclimatization showed better performance compared to those at the dose 80 Gy (Table 6)

Figure 5. Untreated (0 Gy) and irradiated plantlets of *Den. Jayakarta*

Compared to those after 3 and 6 months irradiation, all the parameter observed such as survival rate, number of shoots per plant, number of leaves per shoot and plant height on irradiated PLBs of *Den. Jayakarta* were gradually decreased as the doses of gamma rays increased. After 3 months irradiated plantlets were able to grow up to the dose 640 Gy although the survival rate was only 39 %. Irradiated young plants grew well until 3 and 6 months after irradiation up to the dose of 1,240 Gy (Sutarto et al, 2006).

According to Broertjes and Van Harten (1988) the optimum dose for orchids varies from approximately 10 to 40 Gy. However, in view of the great number of very different genera and species, and of the material to be irradiated, the optimum dose should be determined in every case before starting a mutation breeding program.

Figure 6. Untreated (0 Gy) and irradiated shoot of *Den. Jayakarta* (12 months after planting)

Table 1. Growth of irradiated PLBs of *Den. Jayakarta* (12 months after irradiation) cultured in jars at growth room of CAIRT

Dose of Gamma rays (Gy)	Number of plantlets	Number of shoots/plantlet	Number of leaves/shoot	Plantlet height (cm)
0	63	2.97	4.67	5.95
40	38	2.69	6.05	4.62
80	3	2.67	6.97	4.75

Table 2. Irradiated plantlets of *Den. Jayakarta* grown in the lath house of CAIRT (12 months after irradiation)

Dose of Gamma rays (Gy)	Number of plants	Number of shoots/plant	Number of leaves/shoot	Length of leaf (cm)	Width of leaf (cm)	Plant height (cm)
0	26	3.54	2.46	5.39	1.50	14.30
40	16	2.69	2.26	4.23	1.23	8.89
80	20	3.00	2.50	6.38	1.83	13.92
160	6	3.17	2.50	5.20	1.40	13.90

Table 3. Irradiated shoots (young plants) of *Den. Jayakarta* grown in the lath house of CAIRT (12 months after irradiation)

Dose of Gamma rays (Gy)	Number of plants	Number of shoots/plant	Number of leaves/shoot	Length of leaf (cm)	Width of leaf (cm)	Plant height (cm)
0	12	4.33	4.11	10.38	2.99	51.96
40	9	5.33	3.32	6.17	2.81	35.50

Table 4. Growth of irradiated plantlets of *Den.* Sonia 'BOM 17 Red' cultured in modified VW medium with coconut water and active charcoal (CAIRT)

Parameters	Dose of Gamma rays (Gy)			
	0	30	70	80
Number of plantlets	15	12	77	5
Number of shoots/plantlet	2.00	1.58	2.43	1.80
Plantlet height (cm)	2.05	5.00	2.64	1.96
Number of leaflets (avrg)	2.75	3.11	3.86	2.61
Number of PLBs (clusters)	0	0	13	2

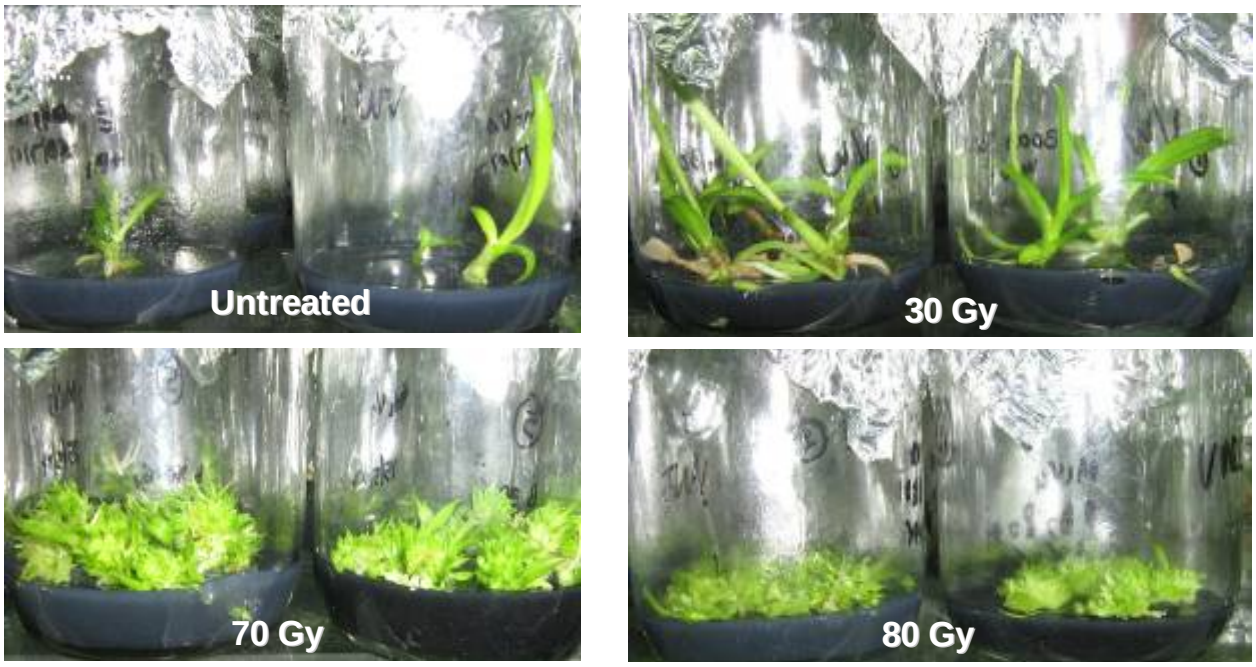


Figure 7. Untreated (0 Gy) and irradiated PLBs of *Den.* Sonia 'BOM 17 Red'

Table 5. Growth of irradiated plantlets of *Den.* Sonia 'BOM 17 Red' grown at the lath house for acclimatization (CAIRT)

Parameters	Dose of Gamma rays (Gy)	
	Ac 10 + Chr (50 + 50)	Ac 20 + Chr (50 + 50)
Number of plants	27	24
Number of shoots/plants	4.59	5.33
Plantlet height (cm) (avrg)	1.38	2.28
Number of leaflets (avrg)	2.17	1.13

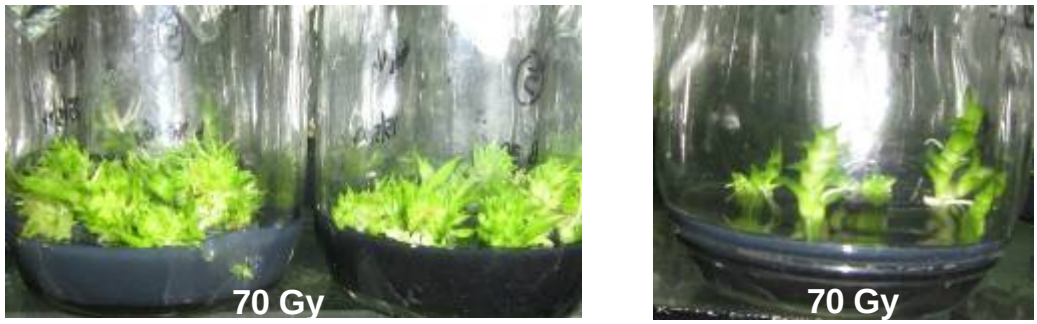


Figure 8. Abnormality found in irradiated PLBs of *Den.* Sonia 'BOM 17 Red'

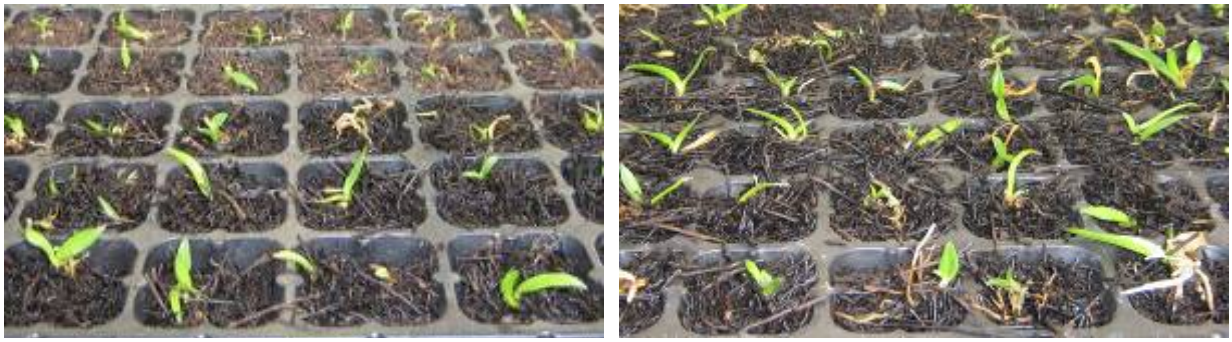


Figure 9. Acute and chronic irradiation of *Den.* Sonia 'BOM 17 Red'

Left (acute 10 Gy + chronic 50 + 50 Gy), Right (acute 20 Gy + chronic 50 + 50 Gy)

Table 6. Irradiated plantlets of *Den.* Sonia 'BOM 17 Red' grown in the lath house of IOCRI for acclimatization.

Dose of Gamma rays (Gy)	Number of plants	Number of shoots/plant	Number of leaves/shoot	Length of leaf (cm)	Width of leaf (cm)	Plant height (cm)
80	27	1.21	2.22	3.12	0.53	4.29
90	44	2.48	3.61	4.33	1.60	8.72

Figure 10. Irradiated plantlets of *Den.* Sonia 'BOM 17 Red' grown in the lath house of IOCRI (12 months after irradiation)

Conclusion

The results of this observation can be concluded that PLBs and young shoots of *Den. Jayakarta* were very sensitive to gamma rays. The optimum dose for PLBs and plantlets of *Den. Jayakarta* was 40 Gy since there was no PLBs and young shoots could grow at the doses more than 40 Gy after 12 months of irradiation. Mass rearing Thrips can be done by using pumpkin as artificial feeding. The most plantlets and clusters of PLBs formed were obtained from irradiated plantlets of *Den.* Sonia 'BOM 17 Red' at the dose 70 Gy. PLBs was not found from those at the dose 30 Gy and untreated plantlets. Irradiated plantlets of *Den.* Sonia 'BOM 17 Red' at the dose of 90 Gy grown in the lath house of IOCRI for acclimatization showed better performance compared to those at the dose 80 Gy

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2. Malaysia

2. Malaysia

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Introduction

Orchid is one of the largest families of flowering plants in the world comprising of an estimated 20,000 to 25,000 species and untold number of hybrids of some 700 different genera. Some estimates have put the registered number of hybrids at more than 50,000 entries, with additional new ones at a rate of 1,000 per year. Orchids can be found growing at almost every latitude and stratosphere, where plants can normally grow. The range of different characteristics in shapes, sizes and colors have made orchids the plant for all types of interests and sought for by collectors.

The successes of hybridization technology in producing vast number of orchid hybrids with attractive characteristics and the introduction of the *in vitro* technology to mass-propagate clonal planting materials have been a tremendous boost for the orchid growing industry. Wide range of successful cultivars with attractive combinations of spray length, bud number, flower color and form, vase life, fragrance, seasonality, and compactness have been produced through hybridization. Meanwhile tissue culture has been the standard method of germinating seeds and propagating seedlings for the industry. Through the meristem cloning technique, thousands of plants can be grown in a relatively short period of time and every regenerated plant is exactly similar to the parent plant in every aspect. These two technologies have so far been very reliable in supporting the orchid industry in the region. Commercially attractive hybrids and varieties have been able to be mass propagated and supplied to the growers for the market.

Orchids such as *Dendrobium* have been the major orchid cut-flower export for Malaysia as well as for other Southeast Asian countries like Thailand and Philippines. Thailand is the major orchid producer with an export value of US$ 80 million in 2006, or approximately 80 % of the country's total ornamental export value (www.reedexpo.com/images/100266 /documents/OrchidPavilion.doc). The export value of orchids from Malaysia is estimated at

RM 150 million per year, representing approximately 40 % of the total floriculture production.

Problems regarding the infestation of insects in the orchid flowers have caused a lot of losses to exporters due to strict quarantine regulations. Presence of even one insect can cause the whole export consignment to be rejected by importing countries. There were cases when the whole orchid consignment had to be shipped back to the exporting countries due to the presence of insect pests. Spraying with insecticides and miticides are the options when dealing with orchid pests, especially when populations are high. This practice however leads to heavy use of chemicals but the problems with insects still persist. Studies have been conducted for post-harvest disinfestations of cut flower using irradiation. However, the post-harvest irradiation treatment has shown detrimental effect on the quality of the cut flowers. The strategy that can be adopted to overcome the problem is to breed for insect resistance in orchid. Insect resistant orchid hybrid may minimize the use of chemicals and overcome the strict quarantine requirements of importing countries.

Mutagenesis is considered as an appropriate approach to induce resistance as hybridization is limited by the unavailability of a resistant genotype and problem of sexual compatibility. Insect resistance has been induced by using mutagenesis approach in varieties of plants such as mungbean and rice. Mutation induction by irradiation has effectively changed certain characteristics of the plants to be 'unattractive' to insects.

Through biotechnological techniques, the insect resistance can be achieved via two approaches. The first used genes that synthesize products, which are toxic to feeding insects (eg Bt insecticidal protein and the cowpea trypsin-inhibitor protein) (Perlac et al. 1991; Hilder et al. 1987). The second approach used a cytokinin-synthesis gene 9ipt), which under the control of a specific promoter will induce the synthesis of one or more insecticidal metabolites (Smigocki et al. 1993). Several reports have shown that transformation of orchids is possible using the particle gun (biolistic) and the *Agrobacterium*-mediated method (Knapp et al. 2000; Wee et al 1999). Thus it is possible to introduce the genes for insect resistance into orchids.

Following consultations with the local orchid growers, we have identified that mites and thrips are the main pests for orchids in Malaysia. Other pests include aphids, whiteflies,

mealybugs, slugs and snails. At present, more than 30,000 mites have been identified all over the world. Most of them are parasitic on animals and humans, with only a few feeding on plants. Among the most common mites for orchids are false spider mites or flat mites (Gough, 1988). Flat spider mite or *Tenulpalpus orchidarum* is from the family of Tenuilpalpid. Flat mites are native to tropical and subtropical habitats and hosts, and are moved globally by the plant trade. Flat mites are very difficult to see without magnification although they move very slowly and they have a great resemblance with other mite species. Generally, adult flat spider mite can only reach up to 0.03 mm in length. These mites are pale yellowish-green to orange-red color. Flat spider mites have a flat appearance somewhat resembling spider mites and are often reddish colored with patterns of dark pigmentation.

Another common mite pest for orchids in Malaysia is *Tetranychus urticae* (twospotted spider mite). The pest is commonly found in the lower surface of leaves and sucks the sap of the leaf. The leaves become pale yellow with numerous small spot which reduces the quality of the leaves. The pest can be controlled with 0.03 % sulphex spray.

Mites normally feed by sucking the sap from individual cells on the surface of the leaves. Because of their feeding style, they may also be potential vectors for various diseases. Leaves infested by mites appear silvery, especially on the underside, where the cells of the surface layer were damaged. From the top, the leaf often has many tiny yellow spots. The false spider mites do not make silk, and are extremely tiny and hard to see, but they produce the same silvery structures on the leaves. A good way to know the presence of mites is to wipe the leaf with a white tissue; if the tissue has red smears, then the plant has mites.

Thrips is another important insect pest for orchids in many countries including Malaysia, and causes a lot of problems in flower production. Thrips normally infest young flower buds and newly expanded leaves. Blooms of infested plants may become prematurely brown, whilst the infested petals may either become spotted, streaked, silvery or discolored. Symptoms on leaves include chlorotic spots, wilting and eventually dropping. Plant growth can also be stunted, and in a severe infestation case, the whole plant will die (Jones 2008). Examples of mite and thrips infestation symptoms commonly found on infested orchid plants are shown in Figure 1.

Figure 1 Common infestation symptoms by mites (A) and thrips (B) on orchid plants

Three orchid species used in this project were *Dendrobium mirbellianum* (Malaysia), *Dendrobium* Sonia 'BOM 17 Red' (Thailand) and *Dendrobium jayakarta* (Indonesia). *Dendrobium mirbellianum* is a species commonly found in the Pacific region. It is easy to grow, robust and produces long spray (up to 45 cm) with up to 30 flowers. It is flowering throughout the year and the flowers last for about 4 weeks. Each flower measures between 3.5 - 5.5cm across. This species is lacking in several qualities such as attractive color and long lasting shelf life, thus making it as a good candidate for the mutagenesis project.

Dendrobium Sonia 'BOM 17 Red' is a very popular commercial hybrid in the region and widely grown for the cut flower export market. This hybrid has purple flowers that can last up to 14 days as cut flower. This commercial hybrid was chosen as it is one of the most popular cut flower *Dendrobium* hybrids grown in the country. Several variations of this hybrid differing in petal and sepal pigmentation are available such as Sonia and Sonia BOM Jo. Meanwhile, *Den. jayakarta* is a popular commercial hybrid in Indonesia with white colored flowers. Figure 2 shows the three orchid species used in this project.

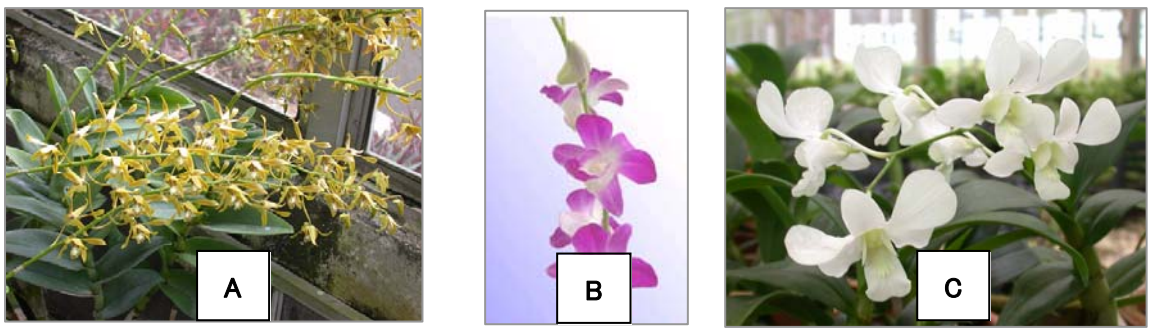

Figure 2 : Orchid species used in the project;
***Den. mirbellianum* (A), *Den.* Sonia 'BOM 17 Red' (B) and *Den. jayakarta* (C)**

Objective of the Project

The main objective of this project is to produce orchids which are resistant / tolerant to insect pests, mainly mites and thrips.

Approach and Technology

In this project three main technologies were employed and developed. They were:

1. Mutagenesis using gamma irradiation and ion beam - Gamma and ion beam irradiation technology were used to generate mutant lines with potential resistant to insects.
2. Selection of insect resistant orchids - The techniques of screening were developed in this study to select plants resistant to insect infestation at both *in vitro* (tissue culture) as well as *in vivo* (flowering) stages.
3. *Agrobacterium*-mediated genetic transformation was also used and optimized to transfer genes that may confer insect resistance in *Dendrobium* species/hybrids used in this study.

Materials and Methods

Plant materials

Irradiated PLBs of *Dendrobium* Sonia 'BOM 17 Red' were obtained from Thailand (courtesy of Associate Professor Dr. Chitrapan Piluek, Kasetsart University, Bangkok), whilst non-irradiated PLBs of *Den. jayakarta* was obtained from Indonesia (courtesy of Ms Ismiyati Sutarto, BATAN, Indonesia). For *Den. mirbellianum,* PLBs were initiated from mature seeds of self-pollinated flowers. The seed capsules were surface sterilized by dipping them in 90 % ethanol and flaming. They were cut open with sterile tools and the seeds were germinated on half-strength (½ MS) medium (Murashige and Skoog, 1962) at 26 °C with 16h photoperiod until PLBs were formed. These PLBs were maintained in culture on ½ MS medium at the same temperature and photoperiod, and subcultured every 4 weeks on fresh media.

Ion beam irradiation

PLBs of *Den. mirbellianum* and *Den. jayakarta* that were uniform in size were transferred into sterile 6.0 cm Petri dish, covered with a sterile 8 µm-thick polyimide film (Kapton^{R} Du Pont-Toray, Japan) and sealed with Nescofilm. These PLBs were then irradiated with 220MeV $^{12}C^{6+}$ ion beam at Takasaki Ion Accelerators for Advanced Radiation (TIARA), JAEA, Takasaki at doses 0, 0.4, 0.8, 1.0 and 2.0 Gy for *Den. mirbellianum* and 0, 1.0 Gy and 2.0 Gy for *Den. jayakarta*. Two to three days after irradiation, PLBs were transferred onto fresh ½ MS medium.

The PLBs were allowed to proliferate and multiply by subculturing onto fresh medium every four weeks. After four periodic subcultures, the irradiated PLBs were allowed to regenerate into whole plantlets, transferred into pots and grown to maturity until flowering. For *in vitro* insect resistance study, plantlets were placed individually in culture vials.

Gamma irradiation

Dendrobium mirbellianum

Den. mirbellianum plantlets with 3 to 4 expanded leaves and almost similar in size were placed on petri dishes containing ½ MS Agar. Each petri dish contains 5 plantlets. They were irradiated at doses of 0, 20, 40, 60, 80, 160, 320, 640 and 1,280 Gy using a gamma chamber (JL Shepherd). Irradiated plantlets were subsequently removed from agar, cleaned under running tap water and transferred onto wet tissue in the lab for one week to allow the plantlets to be hardened before being planted in the nursery. Assessment on the survival rate of the plantlets was done every week for 4 consecutive weeks.

Gamma chamber in Malaysia

Dendrobium Sonia 'BOM 17 Red'

PLBs of *Den.* Sonia 'BOM 17 Red' from Thailand have been irradiated with gamma ray at doses of 0, 60, 70, 80 and 90 Gy .The PLBs were multiplied, regenerated into complete plantlets, and transplanted in the glasshouse.

Dendrobium '*Jayakarta*'

Irradiation of *Den. jayakarta* PLBs using gamma rays (JL Shepherd) was carried out using two approaches;

i. A single dose of 30 Gy (acute radiation)
ii. Fragmented dose (10 Gy every week for 3 weeks)

The irradiated PLBs were cultured on ½ MS agar. Surviving PLBs were multiplied on the same medium until the fifth generation. After five periodic subcultures, the irradiated PLBs were allowed to regenerate into whole plantlets and transferred to glasshouse.

Mites culturing / rearing

Initially, mites were propagated in petri dishes containing clean *Arachis pintoi* leaves and wet cotton, to study their morphology and reproductive behavior. They were segregated according to their life stages; adult, deutonymph, protonymph, larvae and egg. Segregation according to sex was done to adult mites. Female and male adults were allowed to mate and the duration of the development of different life stages produced were monitored and recorded.

The mites used for screening of irradiated orchid plantlets were later reared and multiplied *in vitro*. Adult mites (*Tenuipalpus pacificus*) collected from nursery-grown orchid plants were placed in tissue culture jars containing *Den. mirbellianum* plants having at least two open leaves. The jars were subsequently placed at room temperature (approximately under 10 hours photoperiod), under rearing temperature at 27 °C and approximately 70 % relative humidity to allow the mites to multiply (Krantz 1978). Adult mites were transferred to new tissue cultured *Den. mirbellianum* plants after 2 weeks

***In vitro* mites infestation and screening**

Irradiated orchid plantlets used for *in vitro* screening were put in individual vials. Plantlets of about 4 cm with 4 open leaves were used in this study. Three female and two male adult mites were then put in the vials containing the plantlets. Observations on the pattern of infestation and the multiplication of mites were made every week according to Zhang (2001). Plantlets showing no or less symptom of infestation will be isolated and mass propagated.

For symptom analysis, each leaf was divided into five regions (Figure 1). Infestation was detected using a dissecting microscope. Scoring was done for the first 3 consecutive weeks and after 9 weeks, by estimating the area of infestation as shown in Figure 1. The infestation levels were scored as follow; 1: no infestation, 2: less than 5 % infestation, 3: 5 - 10 % infestation, 4: 11 - 25 % infestation and 5: more than 25 % infestation. In cases where the infestation occurred on different leaves, the areas of infestation were combined to get the total infestation area.

After 3 months of infestation, surviving plants were transferred into small pots for hardening and left to grow in the greenhouse until flowering. Fertilizers were applied to the plants for growth, but not pesticides. Secondary screening at the flowering stage by infestation with the target insects were carried out on these potential mutants.

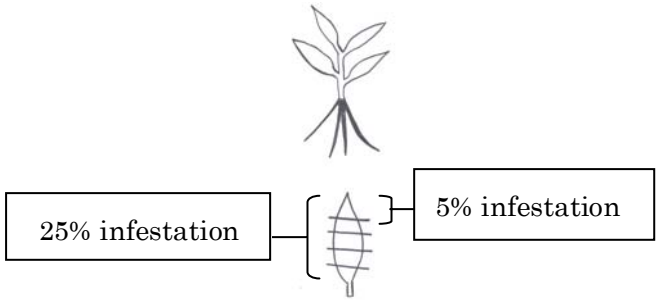

Figure 1 Illustrated scoring index for mite infestation on orchid leaf

***In vitro* thrips infestation and screening**

In vitro screening on irradiated orchids for tolerance towards thrips was carried out using the same procedure for mite screening (as discussed above). Thrips were collected directly from heavily infested orchid plants.

***In vivo* thrips infestation**

In vivo thrips infestation studies were carried out on flowering plants in a nursery dedicated for insect rearing and screening. Flowering plants of potential insect tolerant mutants identified at tissue culture stage, as well as randomly selected irradiated plants were placed alongside heavily thrip-infested plants in the nursery. Observations on the flowers were made for 30 days from the date of infestation. Non-irradiated plants were used as controls.

Orchid transformation and regeneration

Genetic transformation work has been initiated on *Den. mirbellinium* in 2005, using *Agrobacterium tumefaciens* LBA4404 strains, carrying plasmid with a marker gene (hygromycin resistance), a reporter gene (GUS) and a chitinase gene. The *Agrobacterium* was maintained on LB agar plate containing 100 mg/L kanamycin. A single colony was inoculated overnight in liquid LB medium at 28 °C with 100 mg/L kanamycin and 200 uM acetosyringone. For transformation, the bacterial pellet was diluted in MS liquid in a ratio of 1:10. Protocorm-like bodies (PLBs) of *Dendrobium mirbellianum* were generated as described above.

PLBs were pre-cultured for 5 days on ½ MS agar-solidified medium and incubated at 25 °C ± 2 with 16 hours photoperiod. Prior to inoculation, 1 ml of overnight grown *Agrobacterium* was centrifuged at 5,000 g and the pellet was dissolved in 10 ml ½ MS liquid medium (1:10 dilution). PLBs were inoculated by immersing in the diluted bacterial solution for 30 minutes

with occasional shaking, blot dried and were placed back on ½ MS agar-solidified medium. The inoculated PLBs were co-cultivated at 25 ± 2 °C in the dark for 3 days to allow the transfer of genes from *Agrobacterium* into plant cells.

Following 3 days of co-cultivation, the PLBs were washed with sterile water and transferred into conical flasks containing ½ MS liquid medium supplemented with 100 mg/L cefotaxime. The flasks containing PLBs were placed on an orbital shaker for 4 hours to remove traces of *Agrobacterium.* Subsequently, the PLBs were collected and blotted dry on sterile tissue paper before being placed on ½ MS agar-solidified medium containing 100 mg/L cefotaxime for a week. After one week, the PLBs were transferred again to ½ MS agar-solidified medium containing 10 mg/L hygromycin for selection. Surviving PLBs were subcultured onto fresh selective medium every 4 weeks until the formation of plantlets.

Results and Discussion

Orchid tissue culture

Plantlets of all varieties were successfully regenerated and planted in the glasshouse. Of these, *Den. mirbellianum* and *Den. Jayakarta* plants have been flowering, whilst *Den.* Sonia 'BOM 17 Red' plants are still in growing stage and have not flowered.

Ion beam irradiation

Initial radiosensitivity data has shown that the optimum dose for ion beam irradiation of orchid PLBs was in the range of 0.8 to 1.0 Gy (Sakinah et al. 2005). This data was obtained through studies on orchid mutagenesis using ion beams under bilateral project between Nuclear Malaysia and JAEA. Therefore, for *Den. mirbellianum*, PLBs irradiated at doses of 0.4, 0.8, 1.0 and 2.0 Gy were used for this project, whilst those from other doses will be used for *in vivo* screening of other characteristics such as flower color and morphology. For ion beam irradiated *Den. jayakarta*, a number of plantlets from all doses (0, 1 and 2 Gy) were also infested with mites and thrips.

Gamma irradiation

Gamma irradiation of *Den. mirbellianum* plantlets showed that the plantlets were sensitive gamma ray above 600 Gy. Most plantlets irradiated below 640 Gy were still viable after 1 week. However after four weeks, those irradiated at higher doses could not survive; i.e only 47.5 % plantlets at 640 Gy were viable and only 2.5% at 1,260 Gy. Table 1 shows detailed

results on gamma irradiation of *Den. mirbellianum* plantlets. Gamma irradiated *Den.* Sonia 'BOM 17 Red' and *Den. jayakarta* were also been planted in the glasshouse and used for *in vivo* screening of insects.

Table 1. Effect of gamma irradiation on the viability of *Den. mirbellianum* plantlets

Dose (Gy)	Viability after 1 week		Viability after 4 weeks	
	No. of viable plantlets	%	No. of viable plantlets	%
0	40	100	38	95
20	37	92.5	37	92.5
40	37	92.5	37	92.5
60	38	95	34	85
80	37	92.5	27	67.5
160	38	95	32	80
320	33	82.5	27	67.5
640	30	75	19	47.5
1280	7	17.5	1	2.5

Note: The number of replicates per dose were 40

Mites culturing and rearing

Work on multiplying adult mites in large number has been carried out. It was observed that the complete life cycle of mites (from adults to adults) under Malaysian weather was approximately 9 weeks. They favour warm to hot dry conditions to multiply. Under normal environment, a female mite would produce approximately 5 eggs per day. In this study, of the five mites used to inoculate an *in vitro* orchid plant, two were male mites. Male mites were morphologically smaller than female mites.

***In vitro* screening for mite tolerance**

In vitro screening on ion beam irradiated *Den. mirbellianum* plantlets, has shown that after one week of inoculation with mites, majority of the tested plants from all doses of irradiation, showed a low level of infestation. Plants irradiated at doses above 1 Gy in particular, only showed less than 5% infestation (Table 2). In week 2 and 3, more infestation on the plants'

leaves was observed, especially those irradiated at doses below 0.8 Gy (Table 3 and Table 4). However, there was no significant difference in the number of surviving adult mites, the number of eggs and the number of larvae produced after 3 weeks of inoculation on *in vitro* plants. This suggested that the irradiated plants did not produce toxin that can kill mites, but rather develop internal resistance to mite infestation. Table 5, 6 and 7 show detailed results on the number of surviving adult mites, eggs and larvae produced on the irradiated plants tested, respectively.

Table 2. Severity of infestation after 1 week of inoculation with mites

Dose (Gy)	# of plants	No. of plants at different infestation scale (%)				
		1	2	3	4	5
0	52	43 (82.69)	4 (7.69)	5 (9.61)	0	0
0.4	50	44 (88.0)	5 (10.0)	1 (2.0)	0	0
0.8	57	51 (89.47)	4 (7.02)	2 (3.51)	0	0
1.0	26	26 (100)	0	0	0	0
2.0	10	9 (90.0)	1 (10.0)	0	0	0

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves, 4 : 11-25% infested leaves
5 : > 25% infested leaves

Table 3. Severity of infestation after 2 weeks of inoculation with mites

Dose (Gy)	# of plants	No. of plants at different infestation scale (%)				
		1	2	3	4	5
0	52	33 (63.46)	6 (11.54)	7 (13.46)	5 (9.62)	1 (1.92)
0.4	50	38 (76.0)	1 (2.0)	7 (14.0)	4 (8.0)	0
0.8	57	47 (82.46)	3 (5.26)	2 (3.51)	5 (8.77)	0
1.0	26	21 (80.77)	4 (15.38)	1 (3.85)	0	0
2.0	10	7 (70.0)	2 (20.0)	1 (10.0)	0	0

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves, 4 : 11-25% infested leaves,
5 : > 25% infested leaves

Table 4. Severity of infestation after 3 weeks of inoculation with mites

Dose (Gy)	# of plants	No. of plants at different infestation scale (%)				
		1	2	3	4	5
0	52	33 (63.46)	4 (7.69)	5 (9.62)	8 (15.38)	2 (3.85)
0.4	50	37 (74.0)	2 (4.0)	3 (6.0)	3 (6.0)	5 (10.0)
0.8	57	45 (78.95)	3 (5.26)	3 (5.26)	4 (7.02)	2 (3.51)
1.0	26	21 (80.77)	0	4 (15.38)	1 (3.85)	0
2.0	10	7 (70.0)	1 (10.0)	2 (20.0)	0	0

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves 4 : 11-25% infested leaves, 5 : > 25% infested leaves

Table 5. Number of surviving adult mites after inoculation on *in vitro Den. mirbellianum* orchids

Dose (Gy)	# of inoculated mites (initial)	# of Adult Mites (%)		
		Week 1	Week 2	Week 3
0	260	166 (63.85)	162 (62.31)	165 (63.46)
0.4	250	147 (58.8)	146 (58.4)	141 (56.4)
0.8	285	184 (64.56)	182 (63.86)	176 (61.75)
1	130	70 (53.85)	68 (52.31)	69 (53.08)
2	50	32 (64.0)	32 (64.0)	32 (64.0)

Table 6. Number of eggs after inoculation on *in vitro Den. mirbellianum* orchids

Dose (Gy)	# of inoculated mites (initial)	# of Eggs (%)		
		Week 1	Week 2	Week 3
0	260	93 (35.77)	116 (44.62)	122 (46.92)
0.4	250	87 (34.8)	92 (36.8)	102 (40.8)
0.8	285	54 (18.95)	124 (43.51)	114 (40.0)
1	130	61 (46.92)	68 (52.30)	63 (48.46)
2	50	31 (62.0)	34 (68.0)	34 (68.0)

Table 7. Number of larvae after inoculation on *in vitro Den. mirbellianum* orchids

Dose (Gy)	# of inoculated mites (initial)	# of Larvae (%)		
		Week 1	Week 2	Week 3
0	260	1 (0.38)	13 (5.0)	2 (0.77)
0.4	250	0	6 (2.4)	3 (1.2)
0.8	285	0	2 (0.7)	8 (2.81)
1	130	0	2 (1.54)	4 (3.08)
2	50	0	0	2 (4.0)

The severity of infestation on the infested *Den. mirbellianum* plantlets was monitored for 9 weeks, or until a complete life cycle of mites (Table 8). At this stage, it was also observed that all non-irradiated plants tested showed a minimum 5 % of the infestation symptom on their leaves. Some of these control plantlets were also severely damaged and died. Irradiated plantlets did also exhibit infestation symptoms at various scales, ranging from very mild to heavily infested, but there were also plantlets that showed no sign of infestation and completely healthy. The percentages of non-infested plantlets were gradually increased with the increase in treated irradiation doses. The percentages of non-infested plantlets were 34 %, 24.56 %, 50 % and 60 % for those irradiated at 0.4, 0.8, 1.0 and 2.0 Gy, respectively. These results showed an increase in resistance towards mite infestation on irradiated seedlings.

Table 8. Severity of infestation after 9 weeks of infestation with mites

Dose (Gy)	# of plants	No. of plants at different infestation scale (%)				
		1	2	3	4	5
0	52	0 (0)	0 (0)	0 (0)	9 (17.31)	43 (82.69)
0.4	50	17 (34.0)	5 (10.0)	2 (4.0)	1 (2.0))	25 (50.0)
0.8	57	14 (24.56)	2 (3.51)	1 (1.75)	6 (10.53)	34 (59.65)
1.0	26	13 (50.0)	2 (7.69)	2 (7.69)	2 (7.69)	7 (26.92)
2.0	10	6 (60.0)	1 (10.0)	1 (10.0)	1 (10.0)	1 (10.0)

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves 4 : 11-25% infested leaves,
5 : > 25% infested leaves

Overall, 50 potential *Den. mirbellianum* mutants were identified as tolerant to mite infestation and successfully planted in the glasshouse. Of these, 11 plants were from 0.4 Gy, 31 from 0.8 Gy and 8 from 1.0 Gy. These plants were further propagated in the glasshouse for subsequent screening at flowering stage. Figure 3 shows the chronology for producing mite tolerant *Den. mirbellianum.*

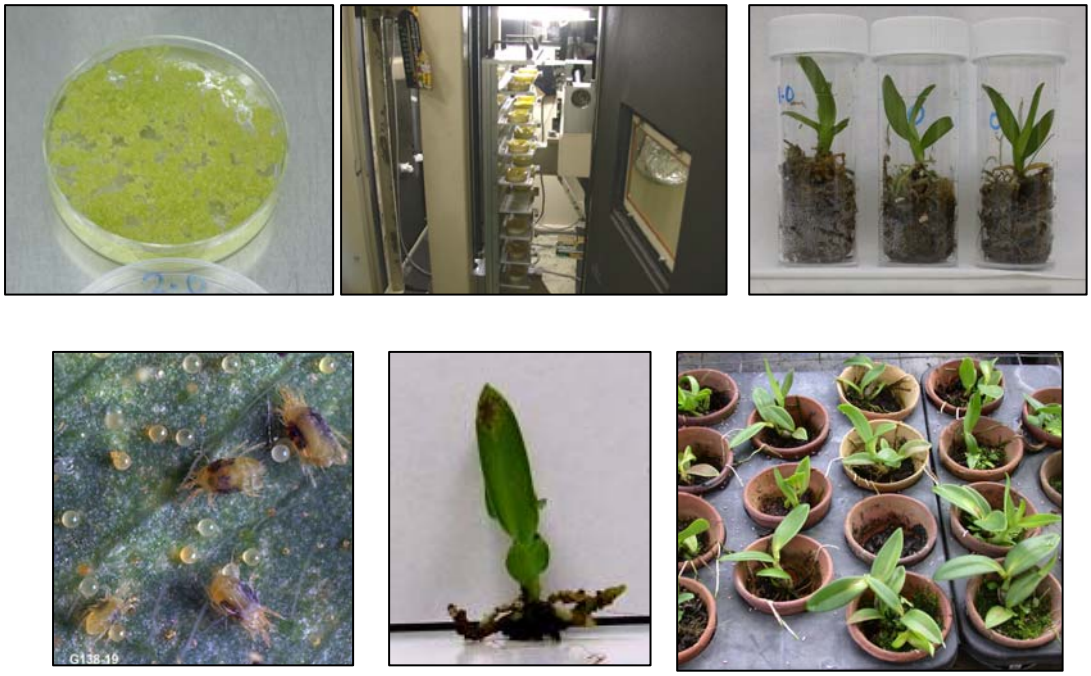

Figure 3. Development of potential mite resistant *Den. mirbellianum* mutants

A: Orchids PLBs used for irradiation B: Ion beam irradiation facility, JAEA, Japan

C: Individual plantlets in vials for in vitro challenge-infestation

D: Infesting pest (mites) E: Symptom of infestation on orchid leaf

F: Potential mite resistant orchid mutants in glasshouse

***In vitro* thrips infestation**

Thrips have been known to cause injury to leaves and buds in infested orchid plants. Among the symptoms observed are browning or blotching of the leaves and bud destruction (http:www.ct.gov/caes/cwp/view.asp). Another symptom is severe flower break as a result of virus infestation transmitted by thrips (Pataky 1990).

An experiment was carried out to screen *in vitro* irradiated plantlets against thrips. Due to a limited number of thrips, only a small number of irradiated plants were used in this experiment. Table 9 and Table 10 show detailed results on the number of infested plants and the severity of infestation with thrips, after week one and two, respectively.

Based on this work, thrips were seen to cause rapid and severe damages on the infested plants as compared to mites. After only 2 weeks of infestation, all control or non-irradiated plants showed symptoms of infestation. Of these, 100 % of *Den. mirbellianum* and 83.3 % of *Den. jayakarta*, exhibited severe damage on more than 25 % of their leaf areas. A number of irradiated *Den. jayakarta* and *Den. mirbellianum* plantlets displayed early tolerance to these infestations. Overall from the *in vitro* screening, 2 *Den. mirbellianum* and 5 *Den. jayakarta* plants were selected as potential tolerant to thrips. Figure 4 shows some infestation symptoms on *in vitro* plantlets infested with thrips.

Table 9. Severity of infestation after one week of infestation with thrips

Orchid species **Dose (Gy)**	**# of plants**	**No. of Infested plants**				
		1	**2**	**3**	**4**	**5**
Den. mirbellianum (gamma irradiated)						
0	5	3	2	0	0	0
0.4	5	5	0	0	0	0
0.8	5	3	2	0	0	0
1.0	5	5	0	0	0	0
Den. jayakarta (gamma irradiated)						
0	6	1	1	4	0	0
10	5	5	0	0	0	0
30	5	4	1	0	0	0
Den. jayakarta (ion beam)						
1.0	5	3	1	1	0	0
2.0	5	2	3	0	0	0

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves 4 : 11-25% infested leaves,
5 : > 25% infested leaves

Table 10. Severity of infestation after 2 weeks of infestation with thrips

Orchid species Dose (Gy)	# of plants	No. of Infested plants				
		1	2	3	4	5
Den. mirbellianum (ion beams)						
0	5	0	0	0	0	5
0.4	5	2	2	1	0	0
0.8	5	0	2	0	3	0
1.0	5	0	3	0	2	0
Den. jayakarta (gamma irradiated)						
0	6	0	1	0	0	5
10	5	2	0	1	0	2
30	5	1	1	1	0	2
Den. jayakarta (ion beam)						
1.0	5	1	1	0	1	2
2.0	5	1	0	1	1	2

Note; 1 : no infestation, 2 : < 5% infested leaves, 3 : 5-10% infested leaves 4 : 11-25% infested leaves,
5 : > 25% infested leaves

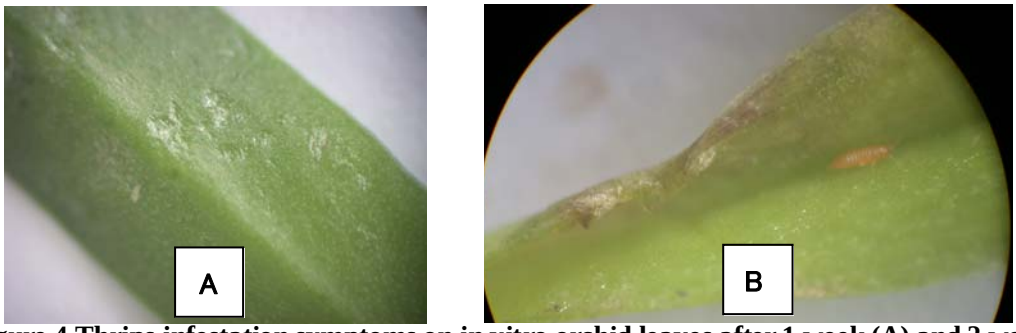

Figure 4.Thrips infestation symptoms on in vitro orchid leaves after 1 week (A) and 2 weeks (B)

<u>Potential mutant lines from *in vitro* screening</u>

Den. mirbellianum

Based on *in vitro* mite screening tests, 50 *Den. mirbelliannum* lines, which were irradiated with ion beams, have been identified as potential mite tolerant mutants. These lines were already planted in the glasshouse. Of these, 11 plants are from 0.4 Gy, 31 from 0.8 Gy and 8 from 1.0 Gy. From *in vitro* thrips screening, 2 plants were found tolerant.

Den. jayakarta

Based on *in vitro* thrips screening, 3 *Den. jayakarta* plants irradiated with gamma rays were identified as potential thrip tolerant, whereas 2 plants irradiated with ion beams were tolerant.

<u>*In vivo* thrips screening</u>

After 3 months of infestation, healthy and surviving plants were transferred into small pots for hardening. Subsequently they were allowed to grow under shade in the greenhouse. The plants were regularly sprayed with fertilizers for growth but were not sprayed with any pesticides. In nature, expressions of disease or infestation symptoms in plants are influenced by a combination of factors such as susceptibility of the host, infective pathogen and favourable environmental conditions (Singh & Singh, 2005). Therefore, these potential mutant plants were also screened at flowering stage by challenge-infestation with the target insects, since insects like thrips especially feed on flowers. It was also a way to confirm the stability of the trait in natural growing conditions.

To date, the tests were carried out on irradiated *Den. jayakarta* plants and potential mite resistant mutants of *Den. mirbellianum* only, since *Den.* Sonia 'BOM 17 Red' have not flowered yet. Infestation severity index was developed based on the level of damages caused by thrips to these flowers. The scale was between 1 and 5, where 1 was resistant and 5 was totally susceptible (Figure 5).

Based on the *in vivo* thrips screening, all samples of *Den. jayakarta* plants from the control and a fragmented dose (gamma, 3 x 10 Gy) populations, were found to be susceptible to insects. Two lines from a single dose (gamma, 30 Gy) population exhibited minor damages (scale 2) on their flowers, and thus could be considered potential resistant lines. These plants have been tagged and maintained in the glasshouse.

For mite tolerant *Den. mirbellianum* plants, the same index was applied and one plant was found tolerant to thrips. All the thrips and mite tolerant mutants are now being propagated in the nursery, to achieve large number of clones.

Genetic transformation

The objective of this genetic transformation work was to study the possibility of generating insect resistant plants through the insertion (transformation) of chitinase gene. From the experiments on *Den. mirbellianum* using *A. tumefaciens* carrying a chitinase gene, 5.59% of the putatively transformed PLBs were obtained after the first selection on hygromycin selection media. These PLBs were transferred to fresh selection media to let them to proliferate. The PLB-to plant conversion was also carried on the same selection media. A total of 13 hygromycin resistant plants have been successfully achieved. At present, the plants are being maintained, and further screening and evaluation will be carried out in the transgenic facility, which is currently under construction.

Conclusion

Some observations and findings of this project were;

1. The optimum ion beam irradiation dose for PLBs was 0.8 - 1.0 Gy.
2. The method for *in vitro* rearing of mites was established and from this study, the complete life cycle of mites in Malaysian conditions was found to be 9 weeks.
3. An *in vitro* insect infestation procedure was developed for preliminary selection of insect tolerant mutants at tissue culture stage.
4. *In vitro* mite infestation study on regenerated *Den. mirbellianum* orchids has showed an increase in resistance towards mite infestation on ion beam irradiated plantlets. The percentages of mite resistant mutant plantlets gradually increased with increasing irradiation doses.
5. Through *in vitro* mite infestation study, a total of 50 potential mite tolerant plantlets

were identified. Of these 50 plants, one plant was found tolerant to thrips when secondary screening was carried out at flowering stage (*in vivo*).

6. Through *in vitro* infestation with thrips, 2 *Den. mirbellianum* plantlets and 5 *Den. jayakarta* plantlets were tolerant. These plants are being propagated in the glasshouse, and secondary screening for thrip tolerance will be carried out once the plants are flowered.
7. *In vivo* thrip screening at flowering stage have identified 2 clones of *Den. jayakarta* which were tolerant to this insect.
8. All identified mutants are currently being propagated to achieve large numbers of clones, and will be transferred to a private collaborator for pre-commercialization studies.
9. Genetic transformation study on *Den. mirbellianum* has generated a number of putative transgenic plants which will be screened and evaluated in the transgenic facility.

Acknowledgement

The authors would like to thank Mr. Shuhaimi Shamsudin, Mr. Suhaimi Musa and Ms. Zainab Harun for technical assistance, JAEA counterparts; Dr. Atsushi Tanaka, Dr. N. Shikazono, Dr. Yutaka Oono, and Dr. N. Hase, for ion beam irradiation and technical advice, Assoc. Prof. Dr. Chitrapan Piluek of Kasetsart University for supplying irradiated *Den.* Sonia 'BOM 17 Red' PLBs and Ms. Ismiyati Sutarto of BATAN for supplying *Den. jayakarta* PLBs. We are also grateful to Research Management Centre, Malaysian Nuclear Agency for providing financial support.

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3. Thailand

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'**

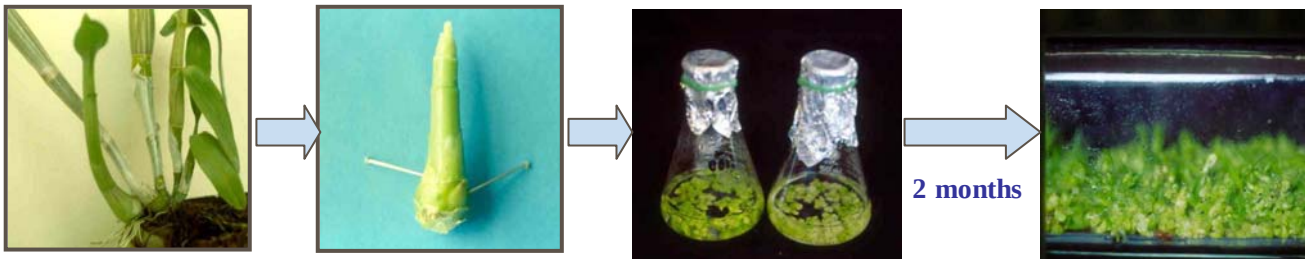
***Den*. Pinky Sem 'Rinnapa'**

***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.

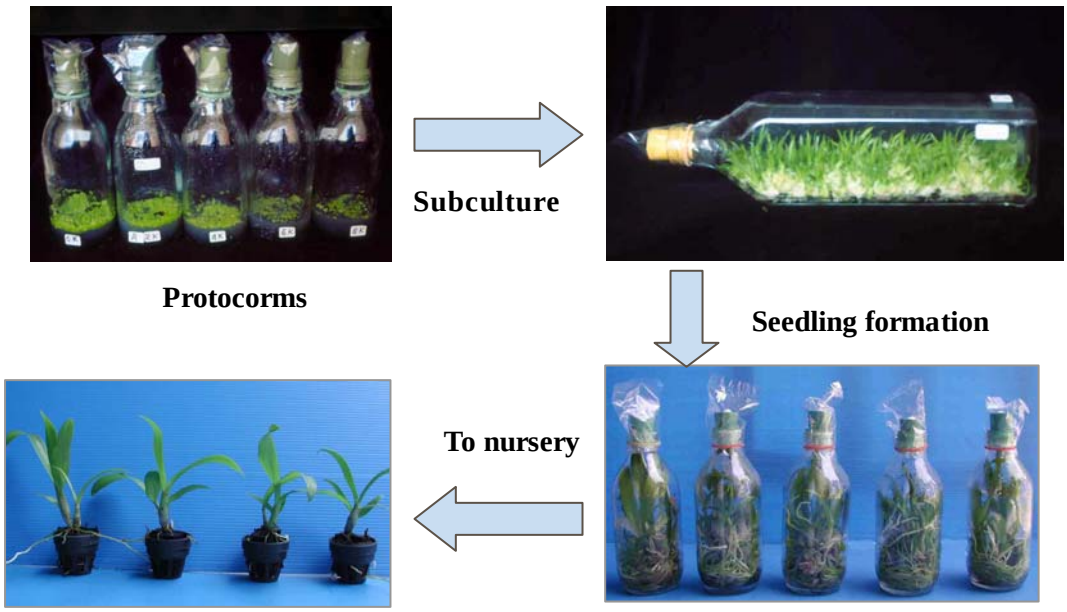


Experiment 3 Exchange of material clones

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)

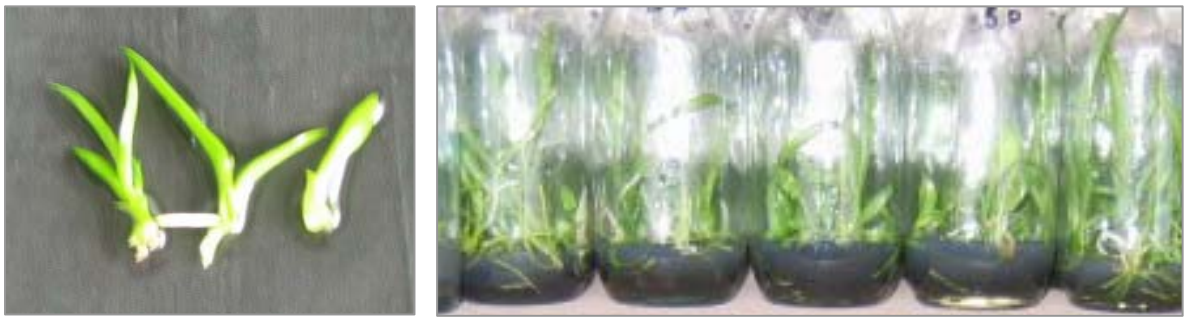
Two replications of PLBs were irradiated. For the first replication, irradiated PLBs were subcultured to the new medium, 20 pieces of PLBs were transferred to each bottle whereas only 10 pieces/bottle was used in the second replication.

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).

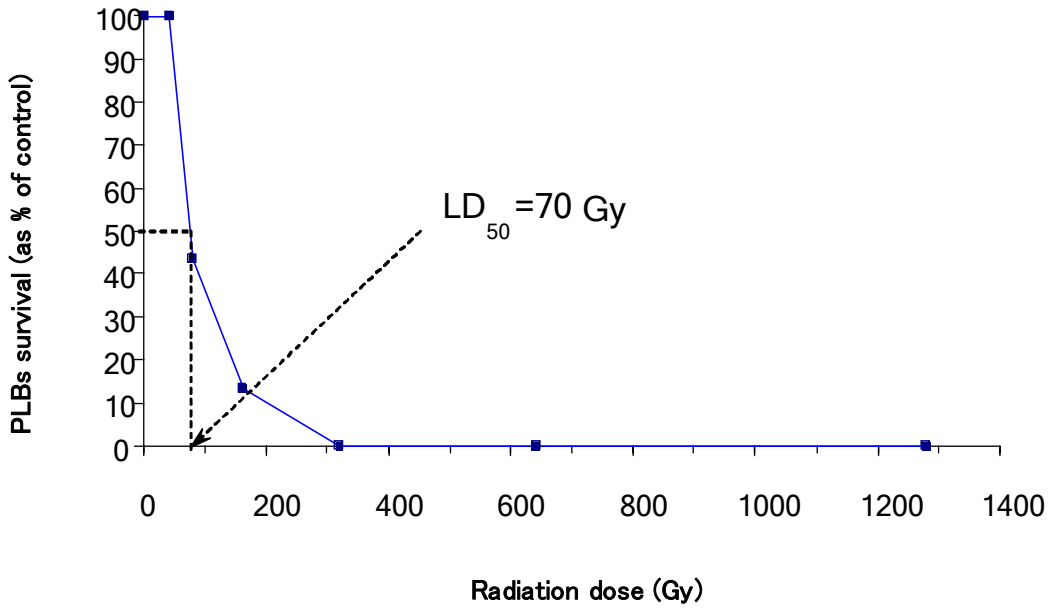


Figure 1. Relationship between radiation dose and survival of protocorm like bodies (PLBs) 2 months after irradiation

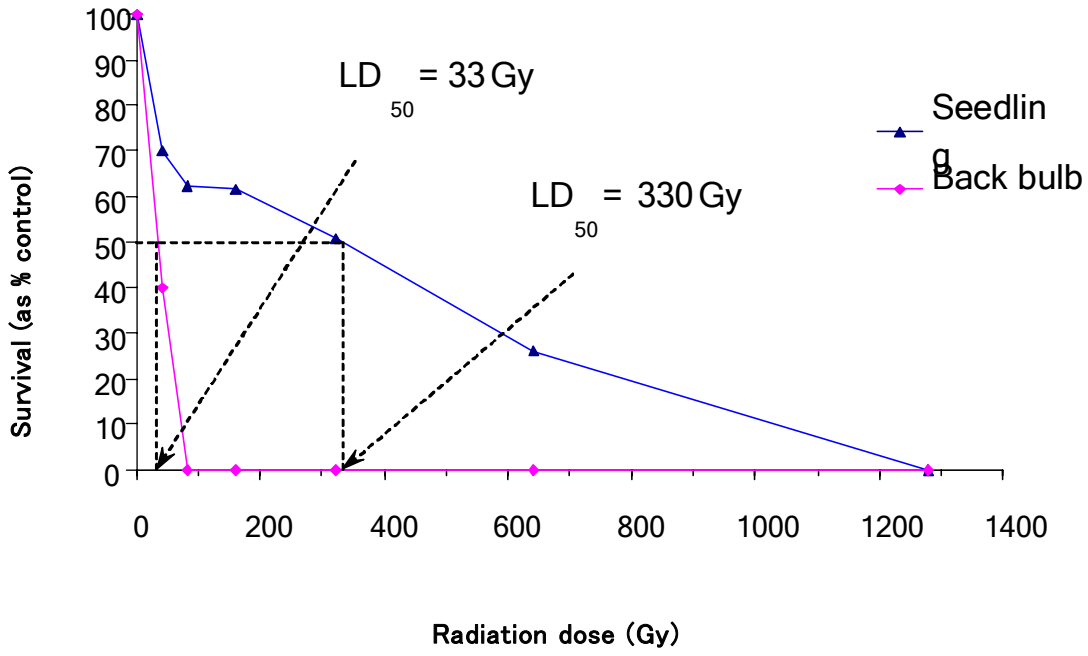


Figure 2. Relationship between radiation dose and survival of plantlet and back bulb 4 and 2 months after irradiation, respectively.

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.

Acknowledgement

The authors appreciate the research grant from Kasetsart University Research and Development Institute (KURDI) and the expert service from Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University.

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This report was prepared by the Nuclear Safety Research Association (NSRA) as a part of activities on Forum for Nuclear Cooperation in Asia (FNCA), organized by MEXT.