A REVIEW ON GAMMA GREENHOUSE AS A CHRONIC GAMMA IRRADIATION FACILITY FOR PLANT BREEDING AND IMPROVEMENT PROGRAM

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ABSTRACT

The research on radiation induced mutation has been conducted as one of the promising method of plant breeding in Malaysia since 1980s. Nuclear Malaysia is leading research institute in Malaysia conducting plant mutation breeding research. Gamma Greenhouse facility located in Nuclear Malaysia is one of the irradiation facilities to serve as a chronic irradiation facility for inducing mutation in various organisms including plants, fungi and microbes. Chronic irradiation refers to the exposure of materials at a lower dose rate over a long period of time. Previous studies have shown that this type of irradiation can minimize radiation damages to living materials and produces a wider mutation spectrum, therefore is very useful for trait improvements in irradiated organisms. Experiments on induce mutation using Gamma Greenhouse facility for crop improvement program have been conducted since its first operation in 2009. Various plant species including ornamental and herbal plants, food crops and industrial crops have been irradiated to improve their traits such as higher yield and biomass, pest and disease tolerance, higher bioactive compounds, longer bloom time and many others. Most of these crop improvement programs were done through collaborations with other agencies in Malaysia such as universities, research institutes and government departments. A number of publications on crop improvement using Gamma Greenhouse have been published in local and international journals as well as seminar presentations at national and international levels. The outputs from induced mutation via chronic radiation using Gamma Greenhouse could be of great interest for plant breeders dealing with improvement and development of new cultivars. This paper discusses the activities and achievement in plant breeding and improvement using Gamma Greenhouse Facility in Malaysia.

Keywords: Chronic gamma irradiation, mutation breeding, mutation spectrum, plant breeding and improvement, Gamma Greenhouse facility

INTRODUCTION

Research in plant mutagenesis for crop improvement has been conducted in Malaysia since 1980s. Mutagenesis is a process by which the genetic information of an organism is changed in a stable manner, resulting in mutation occurring at molecular levels. This technique is used nowadays by plant breeders to develop new varieties by generating and utilizing genetic variability through both chemical and physical mutagenesis (Shu et al., 2012). Gamma radiation is one of the physical mutagens for plant mutagenesis in which alteration at DNA levels can produce new traits towards crops improvement. There are two types of gamma irradiation for crop improvement which are acute and chronic gamma irradiation. Acute gamma radiation is the exposure at high dose in short
period of time, whilst chronic gamma irradiation is the exposure that is continued over long period of time (IAEA, 1977).

Malaysian Nuclear Agency (Nuclear Malaysia) is the leading research institute for mutation breeding using both acute and chronic gamma rays in Malaysia. At present, more than 30 new mutant varieties have been successfully developed by Nuclear Malaysia, either alone or through collaboration with other agencies such as Malaysian Agricultural Research and Development Institute (MARDI), National Landscape Department, United Plantation, University Putra Malaysia (UPM), International Atomic Energy Agency (IAEA), Japan Atomic Energy Agency (JAEA) and others (Zaiton et al., 2012). One facility for chronic gamma radiation called Gamma Greenhouse was built in 2005 to cater for mutagenesis research using low dose radiation (Azhar and Ahsanulkhaliqin, 2014). Radiation breeding is characterized by its three merits which are developing new mutant varieties with desired traits, addition of a few traits without eliminating existing desirable traits, and improvement of vegetative propagated and sterile plants. Even though conventional plant breeding is available for improvement and selection for superior genotype from the selected population, in some cases mutagenesis is preferred as the alternative method to speed up the improvement process (IAEA, 2005).

Activities on chronic gamma irradiation for plant improvement using Gamma Greenhouse were started for trials in 2009 and in full force since January 2010. Among the plants used in the initial stage were ornamental landscaping plants such as hibiscus, canna, turnera, cucurma and sanseviera with the main objectives were to develop database for optimum chronic irradiation dose and suitable experimental design for each species. Since then, various other plant species have been irradiated with multiple aims such as to improve yield, resistance to pest and diseases, unique flower colour, longer bloom period and others (Shuhaimi et al., 2011).

Nowadays, chronic gamma irradiation of living plant becomes a major interest for plant breeder to induce useful mutants in plant breeding and improvement (Kang et al., 2010). Chronic gamma irradiation produced wider mutation spectrum with minimal effect of radiation damage for developing a new mutant variety with superior genotype (Azhar and Ahsanulkhaliqin, 2014). Thus, Gamma Greenhouse facility in Nuclear Malaysia has the potential to become not only an irradiation center, but also a training and knowledge center for research in chronic gamma irradiation in this region. This paper reviews the activities of Gamma Greenhouse Facility and the achievement in plant breeding and improvement using this facility in Malaysia.

**GAMMA GREENHOUSE FACILITY**

Gamma Greenhouse is a circular greenhouse of 15 meter radius with a $^{137}$Cs source at the center. Gamma Greenhouse facility consists of Gamma Greenhouse Dome with Main Control Room outside the concrete shielding. The buffer zone area is 150 meters radius from the source and guarded with concrete based of 3 meters height barbed wire fencing. Figure 1 shows the overall view of Gamma Greenhouse and Figure 2 shows the inside view (at the center). Plants to be irradiated were arranged in 15 rings in which every ring has different dose rate per hour (Figure 3). Generally plants that are more sensitive to radiation need to be placed in a far distance from the $^{137}$Cs source and those that are less sensitive could be placed closer to the center. However, radio-sensitivity tests need to be carried out for all new plant samples in order to find the most suitable dose and dose rate for generating mutation.
Figure 1: Bird’s-eye view of Gamma Greenhouse

Figure 2: View at the center of Gamma Greenhouse

Figure 3: Dose mapping for Gamma Greenhouse

Dose-rate
R1: 2.67 Gy/h
R2: 0.66 Gy/h
R3: 0.30 Gy/h
R4: 0.17 Gy/h
R5: 0.11 Gy/h
R6: 0.07 Gy/h
R7: 0.05 Gy/h
R8: 0.04 Gy/h
R9: 0.03 Gy/h
R10: 0.03 Gy/h
R11: 0.02 Gy/h
R12: 0.02 Gy/h
R13: 0.02 Gy/h
R14: 0.01 Gy/h
R15: 0.01 Gy/h
Plants Irradiated at Gamma Greenhouse Facility for Plant Breeding and Improvement Research

Since its first operation in 2009, many experiments regarding plant breeding and improvement have been conducted in Gamma Greenhouse. Many plant species including food crop, ornamentals, industrial crop, herbal and medicinal plants has been irradiated in this facility (Table 1). Most of these crop improvement researches were done through collaborations with other agencies in Malaysia such as universities, research institutes and government departments.

In its early operation, a training course on mutation breeding using chronic gamma irradiation using Gamma Greenhouse was conducted with the help of foreign experts on chronic gamma irradiation namely Dr Hitoshi Nakagawa from Institute of Radiation Breeding, Japan and Prof. Dr Siranut Lamseejan from Kasetsart University, Thailand. This training course was carried out from 3 to 7 August 2009 with the objectives to provide knowledge and skill to mutation breeding scientists in Malaysia (Figure 4). The participants were from Nuclear Malaysia and other research institutes and universities such as UPM, UKM and MARDI. In addition, Prof. Siranut Lamseejan (Figure 5) was once again invited to Nuclear Malaysia in late 2010 under MOSTI’s Brain Gain Malaysia Program to help Malaysian scientists in setting up the right experimental designs, screening and selection procedures for low dose-irradiated plants.

<table>
<thead>
<tr>
<th>No</th>
<th>Plant Species</th>
<th>Common Name</th>
<th>Traits to be Improved</th>
<th>Year</th>
<th>Collaborator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oryza sativa</td>
<td>Rice</td>
<td>Yield and early maturity</td>
<td>2015</td>
<td>UPM, UiTM</td>
</tr>
<tr>
<td>2</td>
<td>Hibiscus cannabinus L.</td>
<td>Kenaf</td>
<td>High biomass</td>
<td>2016</td>
<td>LKTN</td>
</tr>
<tr>
<td>3</td>
<td>Stevia rebaudiana Bertoni</td>
<td>Stevia</td>
<td>High biomass and high bioactive compound</td>
<td>2012</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Capsicum sp.</td>
<td>Chili</td>
<td>Yield and disease resistance</td>
<td>2016</td>
<td>UPM</td>
</tr>
<tr>
<td>5</td>
<td>Hibiscus rosa sinensis</td>
<td>Hibiscus</td>
<td>Flower colour and longevity</td>
<td>2010</td>
<td>JLN</td>
</tr>
<tr>
<td>6</td>
<td>Arachis sp.</td>
<td>Groundnut</td>
<td>Yield</td>
<td>2016</td>
<td>UPM</td>
</tr>
<tr>
<td>7</td>
<td>Cocos sp.</td>
<td>Coconut</td>
<td>Yield and early fruiting</td>
<td>2014</td>
<td>MARDI</td>
</tr>
<tr>
<td>8</td>
<td>Dendrobium Sonia</td>
<td>Orchid</td>
<td>Flower Colour</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Dendrobium jayakarta</td>
<td>Orchid</td>
<td>Flower colour and insect resistance</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
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<td>Lily</td>
<td>Flower colour</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Alpinia luteocarpa</td>
<td>Bamboo ginger</td>
<td>Plant morphology</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Chrysanthemum sp.</td>
<td>Chrysanthemum</td>
<td>Flower colour and form</td>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Musa sp.</td>
<td>Banana</td>
<td>Disease resistance</td>
<td>2014</td>
<td>MARDI</td>
</tr>
<tr>
<td>14</td>
<td>Ananas sp.</td>
<td>Pineapple</td>
<td>Disease resistance</td>
<td>2014</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Canna sp.</td>
<td>Canna</td>
<td>Flower colour and form</td>
<td>2010</td>
<td>JLN</td>
</tr>
<tr>
<td>16</td>
<td>Turnera sp.</td>
<td>Turnera</td>
<td>Longer bloom time</td>
<td>2010</td>
<td>JLN</td>
</tr>
<tr>
<td>17</td>
<td>Jathropa sp.</td>
<td>Barbados nut</td>
<td>Yield</td>
<td>2013</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>Curcuma alismatifolia</td>
<td>Summer tulip</td>
<td>Flower colour and form</td>
<td>2014</td>
<td>UPM</td>
</tr>
<tr>
<td>19</td>
<td>Piper nigrum</td>
<td>Black pepper</td>
<td>Yield</td>
<td>2014</td>
<td>LADA</td>
</tr>
</tbody>
</table>
Figure 4: Training on plant mutation breeding using chronic gamma irradiation was conducted for plant breeders and potential users of Gamma Greenhouse.

Figure 5: Prof. Dr Siranut Lamseejan (right) with a Malaysian scientist at Gamma Greenhouse

Creating New Characters of Plant Using Chronic Gamma Irradiation

Gamma irradiation can create the new characters in the irradiated plants such as higher yield and biomass, pest and disease resistance, higher bioactive compound and many more through the right screening and selection process. Selecting the optimum dose for induce mutation for each cultivar is very important in order to get higher mutant frequency in the mutant population. Several variations have been observed in irradiated plants through chronic gamma irradiation at Gamma Greenhouse facility. Some of these variations are stable whilst others are lost in the subsequent generations. The variations on the irradiated plants were observed and recorded to determine the efficiency of irradiation. Some of the variations observed in the first vegetative generation of irradiated plants ($M_1V_1$) are shown in Figure 6.
In rice, dwarfism induced mutation were observed when the plant was put closest to the $^{137}$Cs source. The rice plants were shorter in rings nearer to the source as compared to those that were put in rings further away from the source (Figure 7). Besides, chronic gamma irradiation has significantly affected the agro-morphological traits such as number of tiller per plant and number of grain per plant (Aziliana et al., 2015).

Changes of the leaf shape were detected in *Stevia rebaudiana* Bertoni when exposed to the chronic gamma irradiation (Figure 8). The effective doses for *Stevia rebaudiana* Bertoni in chronic irradiation were 10, 20, 30, and 40 Gy (Norazlina et al., 2014). Besides, some irradiated plants were observed to have more branches and these traits were promising in stevia cultivation especially for producing higher biomass.
In kenaf (*Hibiscus cannabinus* L.), variation in flower shape was observed in cultivar at the ring 3 with total accumulated dose 204 Gy. Additionally, the highest plant height, stem girth and high biomass were also observed in selected irradiated plants using chronic gamma irradiation (Zulmadi et al., 2016). These findings showed the effectiveness of the chronic gamma irradiation to create superior genotypes of *Hibiscus cannabinus* with desirable traits (Figure 9).
Figure 9: Chronic gamma irradiation of kenaf (*Hibiscus cannabinus* L.) with an objective to generate mutants with higher biomass.

**Significant Output from Plant Breeding and Improvement Research at Gamma Greenhouse Facility**

Numerous publications on plant breeding and improvement research using Gamma Greenhouse irradiation facility have been published in local and international journals as well as seminar presentations at national and international levels (Aziliana et al., 2015; Norazlina et al., 2014; Taheri et al., 2014; Taheri et al., 2016; Zaiton et al., 2011; Zulmadi et al., 2016). These publications are very important to disseminate research findings in plant mutation breeding disciplines. Thus, researchers around the world can get access to this information and subsequently create great interest in improvement and development of new cultivars via chronic gamma irradiation.

Besides, this facility was recently recognized as IAEA Collaborating Center for plant mutagenesis via chronic gamma irradiation. This facility is also one of the most visited irradiation facilities in Nuclear Malaysia. Among the high ranking officials from international agencies that have visited Gamma Greenhouse were; former Chargé d'Affaires, U. S. Mission to the United Nations, Ms. Laura E. Kennedy in 2014 and IAEA Director General, Mr. Yukiya Amano and his team in 2015 (Figure 10). With this acknowledgement, Gamma Greenhouse can become a center of excellence for plant mutagenesis research via chronic gamma irradiation.
Figure 10: Visit by Mr. Yukiya Amano, Director General of IAEA (top) and Chargé d'Affaires, U. S. Mission to the United Nations, Ms. Laura E. Kennedy (bottom) to Gamma Greenhouse Irradiation Facility

CONCLUSIONS

Gamma Greenhouse facility has played a significant role in plant breeding discipline for developing new variety with improvement of selected traits via chronic gamma irradiation. A number of mutant varieties from various plant species were generated from the chronic gamma irradiation using this facility since its operation in 2009. Additionally, this facility can become one of the centers of excellent and training for plant mutagenesis research via chronic gamma irradiation in this region. Collaborations with local and international researchers also highly recommended for giving the high impact and value added in research conducted. In the future, we hope more mutant varieties with useful traits could be developed via chronic gamma irradiation and transferred to relevant end-users.
ACKNOWLEDGEMENTS

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REFERENCES


