Glowing of Transgenic *Phalaenopsis Amabilis* Leaf Inserted by Firefly Luciferase Gene Using Biolistic Method

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**Abstract**-- *Phalaenopsis amabilis* orchid plb was used as the target for shooting in biolistic method by using the firefly luciferase gene. When the putative transformants were checked by PCR, 21 transgenic *P. amabilis* showed positive bands. Glowing leaf of transgenic *P. amabilis* was observed by DNA illuminator.

**Index Term**-- *Phalaenopsis amabilis*, luciferase gene, glowing leaf

I. **INTRODUCTION**

*Phalaenopsis amabilis* is one of the most important orchids grown for commercial production cut flowers and potted plant, and is becoming an important ornamental plant in the world. The orchid free flowering with inflorescences bearing to more than 20 flowers, flowering 2 times a year and has a long-lasting vase-life make it ideal for global cut flower markets [1].

For further improvement of orchid species, genetic transformation is now expected to be a tool for introducing traits such as disease resistance, herbicide resistance, flower colour and shape, which has been difficult through conventional breeding technique based on sexual crossing [2]. In this study, for increasing aestethics of the orchid, we inserted luciferase gene into the genome of Phalaenopsis amabilis. The purpose is to enable the plant glowing in the dark.

II. **MATERIAL AND METHODS**

1. **Establishment of embryo culture**

   Seeds of *P. amabilis* were surface sterilized using 10% chlorox for 10 min. Then the sterilized seeds were washed with distilled water three times. Sterile seeds were cultured in ½ MS liquid medium with 10% coconut water and shaked at 120 rpm for 3 weeks.

2. **Shooting of embryos**

   The tungtens were coated with DNA (plasmid containing wasabi and bar gene and co-plasmid containing luc gene). The co-plasmid containing luc gene was shown in fig. 1. Subsequently, the coated DNA was shot onto embryos. The embryos were then dropped by ½ MS liquid medium. Thereafter, the embryos were put in the dark for 2 weeks for recovery.

   ![Fig. 1. Plasmid of luc gene][3]

   3. **Shot embryos in selection medium**

   The shot embryos were subcultured into 3 µM MSO for 2 weeks. The green and yellow plbs were subcultured into 3 µM MSO every 2 weeks until the green plbs were stable. The stable green plbs were subcultured into 4 µM MSO. The green and big plbs were subcultured into plantlet media (1.2 MS with 10% banana) containing 4 µM MSO. The plantlets were subcultured every 3 weeks.

4. **Polymerase chain reaction (PCR)**

   DNA analysis was performed on genomic DNA of young leaf of transgenic Phalaenopsis amabilis plantlet as described by Dellaporte et al. [5].
DNA was subjected to the PCR. The primer pair were design to amplify the entire 1.87 kb luciferase gene; LUC 5: 5’-CAATTACCAACAACAACA and LUC 3:3’-TTTTTAGTTACCTATACC. Amplification condition was 2 min at 94°C, 35 cycles of 30 second at 94°C, 30 second at 50.9°C, 2 min at 72°C and 10 min at 72°C using a DNA thermal cycler.

5. Glowing of leaf
The putative transgenic leaf was put in Petri dish and dropped by luciferin. After 10 min, the leaf was put into DNA illuminator and picture of the glowing of leaf was taken.

III. RESULT AND DISCUSSION
Eventhough the shooting of co-plasmid luc gene was concurrently shot with DNA containing wasabi and bar gene, the transgenic P. amabilis could show the luc gene bands when checked by PCR. Fig. 2 shows electroforegram of luc gene. The transgenic plantlets of P. amabilis no 2-22 showed positive bands of luc gene.

The glowing of transgenic P. amabilis leaf was shown in fig. 3. The part of the leaf without luciferin could not glow. If the luciferin was poured onto the leaf, LUC gene will produce luciferase. After several minutes, ATP will be formed and produced energy and light. One of the transgenic P. amabilis propagated by itself [4].

Figure 4. shows the plantlet of transgenic Phalaenopsis amabilis. The plantlet could developed and survived on the medium containing MSO. MSO is a herbicide and used in selection medium. Another paper about Phalaenopsis amabilis with resistance to soft rot disease and herbicide (by using plasmid containing wasabi and bar genes) has been published [4].

Transgenic cells, tissues, or plants can be readily identified by the luminescence emitted upon addition of a minute amount of luciferin. Successful transformation of plant cells depends critically on two steps: an efficient method for delivering DNA into target cells and the identification of stably transformed cells [3]. In this study, we were successful in performing transformation and identifying the transformed cells or tissues.

Figure 2 shows result of PCR screen of DNA sample of transgenic plants of Phalaenopsis amabilis. Expected product size 600bp

![Fig. 2. PCR of transgenic Phalaenopsis amabilis](image-url)
Summary and Conclusions
In summary, we have demonstrated successful Phalaenopsis amabilis transformation through phenotypic and molecular characterization of plants regenerated from particle-gun mediated gene introduction (biolistic). This result signals the availability of genetic transformation as a tool for genetic improvement in Phalaenopsis orchids. The leaf of transgenic P. amabilis was glowing. We obtained 21 transgenic P. amabilis after checking by PCR. Genetic programs for the manipulation of important commercial traits like insect and disease resistance, novel flower form and color, free flowering, and extended vase life can now be implemented. Commercial companies recognizing this potential have already embarked on such projects. The increasingly concerted effort between government agencies and private industries targeting on joint economic benefits, will broaden this spectrum of research and development. Currently, we are maintaining the transgenic orchids for flowering.

REFERENCES