LAPORAN AKHIR RISET
Program Hibah Kerjasama Luar Negri dan Publikasi Internasional

Production of Red and White Variegated Leaf of Aglaonema for Domestic and Export Purpose

Periset Utama:
Dr. Totik Sri Mariani M.Agr.

Nama KK/P/PP:
Sains dan Bioteknologi Tumbuhan

Lembaga Penelitian dan Pengabdian Pada Masyarakat
INSTITUT TEKNOLOGI BANDUNG
Desember 2010
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I. HALAMAN IDENTITAS

1. Judul
Production of Red and White Variegated Leaf of Aglaonema for Domestic and Export Purpose

2. Program Riset
Kerjasama Luar Negeri dan Publikasi Internasional

3. Waktu Pelaksanaan
Juni 2010-November 2010

4. Tim Riset

a. Nama Lengkap Ketua Tim : Dr. Totik Sri Mariani M.Agr.
b. NIP : 131944837
c. Pangkat/Golongan : Penata/III C
d. Jabatan : Lektor
e. Fakultas/Sekolah & Prodi : Sekolah Ilmu dan Teknologi Hayati & Biologi
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5.1 Anggota Tim Riset:

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<tbody>
<tr>
<td>1</td>
<td>Dr. Any Fitriani</td>
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<td>UPI Bandung</td>
<td>20 6</td>
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<tr>
<td>2</td>
<td>Dr. Tet Fatt Chia</td>
<td>Genetika Molekuler Tumbuhan</td>
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<td>5 6</td>
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5.2 Asisten Peneliti/Mahasiswa:

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5.3 Biaya yang disetujui oleh ITB : Rp 125.000.000,-

Mengetahui,
Ketua Kelompok Keahlian
Sains dan Bioteknologi Tumbuhan

(Dr. Robert Manurung)
NIP: 130704301

Bandung, 9 Desember 2010
Ketua Tim Riset,

(Dr. Totik Sri Mariani M.Agr)
NIP: 131944837

Dekan Sekolah Ilmu dan Teknologi Hayati, ITB

(Prof. Dr. Inian Ahmad Musmeinan)
NIP: 131572753
II. EXECUTIVE SUMMARY <Ditulis dalam Bahasa Inggris >

1. TITLE OF RESEARCH : Production of Red and White Variegated Leaf of Aglaonema for Domestic and Export Purpose

2. HEAD OF RESEARCH TEAM : Dr. Totik Sri Mariani M.Agr.

3. TEAM MEMBERS : 1. Dr. Any Fitriani, 2. Dr. Tet Fatt Chia

4. OFFICIAL ADDRESS :
   a. Laboratory of Plant Physiology, School of Life Sciences and Technology, Bandung Institute of Technology
   b. Laboratory of Plant Molecular Genetics, National Institute of Education, Nanyang Technological University, Singapore.
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5. EXTENDED ABSTRACT :

   Ornamental plants have significant meaning along the history of human civilization. Since long time ago, ornamental plant were mostly used to express feeling as well as for increasing the beauty of environment. Various ethnic groups in Asia, Africa and Latin America continue the tradition of using ornamental plants to brighten ceremonies and national day celebrations. Currently, the most rapidly expanding domestic crops are foliage plants.

   Aglaonema is one of the beautiful foliage plants. It has a good combination of leaves color, such as green and red, green and white, pink and green, red, and so on. This Aglaonema were produced by crossing/breeding method. The problem is it take a long time (about 2 years) and the result is unpredictable. By doing mutagenesis followed by tissue culture, it will be faster (4 months). Through mutagenesis technique, new combination of colour, red and white, will be obtained. Therefore, the purpose of this research is to create red and white colour combination of Aglaonema by mutagenesis through particle bombardment.

   Firstly, tissue culture of Aglaonema was performed to produce about 1000 shoots of Aglaonema. The shoots were then shot by particle bombardment method. Thereafter the shot shoots were cultured and maintained until mutated shoot was observed.

   About 1000 shoots were obtained from tissue culture experiment. Initially, small protruding shoot was growing from stem region near root and it was used as the explant. The shoots were growing and small shoots proliferated on the bulb region on the MS medium supplemented with 1.5 ppm thidiazuron. The small shoots were then subcultured on proliferation medium containing 1.5 ppm thidiazuron and 3 ppm BAP. The shoots
proliferated on proliferated medium and the shoots were continuously subcultured until 1000 shoots were obtained.

Multiplication rate of Aglaonema was observed in this research. The multiplication rate curve followed a sigmoid curve. It consists of lag phase at the first up to third subculture (2 to 6 weeks), exponential phase at the forth subculture (8 weeks) and begin to enter stationary phase at fifth subculture (10 weeks). At exponential phase, the multiplication rate was very high, up to 17 shoots. After 5th subculture, 1000 shoots was obtained from 2 axillary shoot explants. Therefore, 1000 shoots could be obtained in 10 weeks period.

Thidiazuron is a cytokinin and could promote shoot proliferation. In Aglaonema application of thidiazuron alone also induced callus formation. Therefore BAP was also added and could avoid the callus formation.

According to Akasaka et al. (2000) thidiazuron is the most efficient cytokinin compared to BAP, zeatin and kinetin. Yeh et al. (2007) also used thidiazuron combined with dicamba in tissue culture of Aglaonema using inflorescence explants.

The 1000 shoots were then shot by particle bombardment method. The concentration of NMU (Nitroso methyl Urea) used were 1;2,5;5;10;25;50;100;250;500;1000;2500;5000 μM. There were 3 colour of shoots after 3 weeks of shooting, green, pale green and white. Dead shoots were also observed. The percentage of green, pale green, white and dead shoots varied according to the concentration of NMU applied. Up to now, one red and white leaf of Aglaonema was observed on 2500 μM NMU. Observation of the mutated shoots are still underway.

When non mutated shoots were elongated on medium containing 3 ppm of BAP and were rooted on 3 ppm of IBA, plantlets of Aglaonema were developed. The plantlet was then acclimatized on moss and transferred to the soil later on.

It was concluded that 1000 shoots of Aglaonema could be obtained through tissue culture method. One red and white leaf of Aglaonema was obtained through particle bombardment method using 2500 μM NMU.

6. LIST OF RESEARCH OUTPUT

b. Photo of prototype:

III. EVALUASI DIRI <1 halaman>

1. CAPAIAN:
   a. TUJUAN YANG TERTULIS DI PROPOSAL:
      1. To produce 1000 shoots of Aglaonema
      2. To create red and white colour combination of Aglaonema by mutagenesis through particle bombardment
   b. TUJUAN YANG TELAH DICAPAI:
      1. 1000 shoots of Aglaonema has been produced.
      2. One red and white leaf of Aglaonema has been obtained
   c. TUJUAN YANG BELUM DICAPAI: ---

2. PRODUK RISET

<table>
<thead>
<tr>
<th>Yang Dijanjikan Pada Proposal</th>
<th>Yang Dihasilkan Pada Penelitian</th>
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<tbody>
<tr>
<td>Propototipe Plantlet of Aglaonema Red and white Aglaonema</td>
<td>Propototipe Plantlet of Aglaonema Red and White Aglaonema</td>
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<td>HAKI Belum, (Di Tahun ke 2)</td>
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3. ALAMAT HOMEPAGE KK YANG BERISI HASIL PENELITIAN:

4. KEGIATAN DISEMINASI HASIL RISET: Belum
5. SINERGI DENGAN KEGIATAN DAN PROYEK RISET LAIN: Tidak ada

6. KEMANFAATAN PROYEK RISET:
   TA 1 buah

7. PERMASALAHAN YANG DIHADAPI DAN SARAN PERBAIKAN: Dana turunnya terlambat dan kita harus menalangi dana untuk belanja barang dan jasa terlebih dahulu baru direimburse. Mohon agar ITB dapat menyediakan dana talangan. Terima kasih.

8. RENCANA KELANJUTAN PENELITIAN:
   a. To multiply the red and white Aglaonema obtained by tissue culture method
   b. To acclimatize red and white Aglaonema plantlet by aeroponic system
   c. To commercialize red and white Aglaonema in Indonesia as well as export them to Singapore

IV. LAMPIRAN OUTPUT PENELITIAN

A. DRAFT PUBLIKASI

Mariani et al. (2010)-Micropropagation of Aglaonema using axillary shoot explants
Micropropagation of Aglaonema using axillary shoot explants
Totik Sri Mariani¹, Any Fitriani², Tita Puspita¹, Widaningsih¹, Adhityo Wicaksono¹, Tet Fatt Chia³
¹School of Life Sciences and Technology, Bandung Institute of Technology, Ganesha 10, Bandung 40132, Indonesia.
²Program Study on Biology, Indonesia University of Education, Dr. Setiabudhi 229, Bandung 40154, Indonesia
³National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616
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Abstract : Micropropagation of Aglaonema was performed using axillary shoot as the explants. Shoots could be induced on the bulb region on MS medium supplemented by 1.5 ppm thidiazuron. For shoot proliferation, the small shoots multiplied on MS medium supplemented by 1.5 ppm thidiazuron and 3 ppm BAP. Addition of BAP was important to avoid callus growth. The highest multiplication rate of Aglaonema shoot was achieved at 5th subculture. After 5th subculture (10 weeks) 1000 shoots was obtained from 2 axillary shoot explants. Plantlet was developed on MS medium containing 3 ppm IBA. Acclimatization was performed successfully with 100% survival rate on moss followed on soil.

Key words: Aglaonema, axillary shoot, multiplication rate, plantlet, thidiazuron
Ornamental plants have significant meaning along the history of human civilization. Since long time ago, ornamental plant were mostly used to express feeling as well as for increasing the beauty of environment. Various ethnic groups in Asia, Africa and Latin America continue the tradition of using ornamental plants to brighten ceremonies and national day celebrations. It has also been noted that with the establishment and growing affluence of a society, the use of ornamental plants increases in popularity. Currently, the most rapidly expanding domestic crops are foliage plants for patio or indoor use, bedding and garden plants.

Aglaonema is one of the beautiful foliage plants. It has a good combination of leaves color, such as green and red, green and white, pink and green, red, and so on. In this study, we performed tissue culture of Aglaonema up to acclimatization. Yeh et al. (2007) used inflorescence of Aglaonema as an explants. We used axillary shoot of Aglaonema as an explants. In this study, we also calculate multiplication rate of Aglaonema. To our knowledge, there has been no report in the micropropagation of Aglaonema using axillary shoot explants.

**Material and Method**

**Material**

Plant of Aglaonema var. Cochin.

**Method**

1. **Hormonal Injection**

The lower stem of Aglaonema plant was injected by 30 ppm of BAP to induce axillary shoot. The axillary shoot was then used as an explants.

2. **Sterilization of Explant**

Explant was washed in running water for 1 hr. The explants was then soaked in fungicide for 30 min. Thereafter, the explants was dried in petri dish layered by sterilized filter paper. Inside laminar air flow, the explant was soaked in 70% alcohol for 2 min. The explants was then washed by sterile aquadest. Subsequently, the explants was sterilized by 50% chlorox plus 2 drops of tween (10 min) for the explants near root and 20% chlorox plus 2 drops of tween for the explants near shoot (10 min). Thereafter, the explants was washed 3 times by sterile aquadest. The explants was then dried in petri dish layered by sterile filter paper.

3. **Tissue Culture of Aglaonema**

The sterilized explant was cut on the node and sowed onto the M1 medium (Table 1). After 1 week, the sterile explant was subcultured onto M2 medium (Table 1). In this medium, the shoot will grow and several small shoots will be developed. After 4 weeks of culture, the small shoots were subcultured onto M3 medium (Table 1). The shoots were proliferated in M3 medium.
For shoot elongation, the proliferated shoots were subcultured onto M4 medium (Table 1). For rooting, the elongated shoots were subcultured onto M5 medium (Table 1).

4. Acclimatization

The plantlet of Aglaonema was acclimatized on moss and the pot was covered by holed plastic for 1 month. The plastic was then opened and the plant was transferred to soil media in pot.

Result and Discussion

1. Hormonal Injection

Stem of Aglaonema plant was injected with 30 ppm benzylaminopurine (BAP). After two weeks, shoots were growing from the root region (Fig.1). The shoots growing indicated that BAP as a cytokinin could induce shoots formation. Big shoot (Fig 1, arrow) could not be used as an explant but the small protruding shoot (Fig. 1, arrow head) could be used as an explants.

2. Tissue culture of Aglaonema

Stem of Aglaonema containing small protruding shoot was used as an explants. The explant was cultured on M1 medium (Table 1). After one week of culture on M1 medium, the steril explants was transferred onto M2 medium containing 1.5 ppm thidiazuron (Table 1). In M2 medium, the shoots were growing and small shoots proliferated on the bulb region (Fig. 2). The small shoots were subcultured on proliferation medium (M3) containing 1.5 ppm thidiazuron and 3 ppm BAP (Table 1). The shoots proliferated on M3 media after 2 weeks of culture (Fig. 3) and 4 weeks of culture (Fig. 4).

Multiplication rate of Aglaonema could be seen in figure 7. The multiplication rate curve followed sigmoid curve. It consists of lag phase at the first up to third subculture (2 to 6 weeks), exponential phase at the forth subculture (8 weeks) and begin to enter stationary phase at fifth subculture (10 weeks). At exponential phase, the multiplication rate was very high, up to 17 shoots. After 5th subculture, 1000 shoots was obtained from 2 axillary shoot explants. Therefore, 1000 shoots could be obtained in 10 weeks period.

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After the shoots were elongated on medium containing 3 ppm of BAP and were rooted on 3 ppm of IBA, plantlets of Aglaonema were developed (Fig.5). The plantlet was then acclimatized on moss and transferred to the soil later on (Fig. 6).

The basal medium used in this study is Murashige and Skoog (MS) medium. According to Sagawa and Kunisaki (1990), MS is a high-salt medium. For further considerations and for economical purpose, the concentration of medium could be lower such as half- or third-strength MS (Ma and Wang, 1977).

Plantlets from axillary shoots usually retain the chimera tissue arrangement and resemble the mother plant (Sagawa and Kunisaki, 1990). For future prospect, extension of micropropagation techniques to foliage and bedding plants has already contributed toward the rapid growth of the foliage and nursery industries (Ammirato et al., 1990). The reproducible method of Aglaonema propagation in this study could give a contribution in foliage and nursery industries. There are three considerations in tissue culture propagation of ornamentals: revenues, expenses and investments in the business (Jones, 1990).

In conclusion, we found that micropropagation of Aglaonema could be performed from axillary primordial shoot. We could obtain 1000 shoots of Aglaonema in 10 weeks period.

Acknowledgements
Hibah Kerjasama Luar Negeri dan Publikasi Internasional Dikti greatly facilitated this study and are gratefully acknowledged.

References
Explanation of Table:
Table 1. Medium composition for micropropagation of Aglaonema

Explanation of Figures:
Figure 1. Shoots of Aglaonema (→) were growing 2 weeks after BAP injection. Small protruding shoot (⇒) to be used as an explant. Figure 2. Proliferated shoots on M2 medium containing 1.5 ppm thidiazuron. Figure 3. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after two weeks of culture. Figure 4. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after four weeks of culture. Figure 5. Plantlet of Aglaonema 4 weeks of culture on media containing 3 ppm IBA. Figure 6 Aglaonema plant 2 months after acclimatization. Figure 7. Multiplication rate of Aglaonema shoot

Table 1. Medium composition for micropropagation of Aglaonema

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<td>M1 (MS Medium)</td>
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<td>M2 (MS Medium)</td>
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<td>M3 (MS Medium)</td>
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<td>M4 (MS Medium)</td>
<td>3 ppm BAP</td>
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<tr>
<td>M5 (MS Medium)</td>
<td>3 ppm IBA</td>
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Figure 1. Shoots of Aglaonema (→) were growing 2 weeks after BAP injection. Small protruding shoot (□) to be used as an explant. Figure 2. Proliferated shoots on M2 medium containing 1.5 ppm thidiazuron. Figure 3. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after two weeks of culture. Figure 4. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after four weeks of culture. Fig. 5. Plantlet of Aglaonema 4 weeks of culture on media containing 3 ppm IBA. Fig. 6. Aglaonema plant 2 months after acclimatization.
B. FOTO PROTOTYPE

1. Plantlet Aglaonema

2. Red and white Aglaonema

The red colour is not clear in this picture