

## ABSTRACT

### **MEDIA OPTIMATION FOR *IN VITRO* SEED GERMINATION AND SEEDLING GROWTH, AND EFFECTS OF MEDIA AND BENZYLADENINE ON HYBRID *Phalaenopsis* PLANTLET ACCLIMATIZATION**

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The worldwide popularity of *Phalaenopsis* led to the creation new hybrids that is continuously needed to meet the consumers taste change. However, the technical expertise and high cost for *in vitro* seed germination, seedling growth and plantlet acclimatization often limit the *Phalaenopsis* breeding among orchid growers in Indonesia. The objectives of this research were to obtain optimized and less costly media formulations and methods for *in vitro* seed germination and seedling growth, as well as plantlet acclimatization. This research consisted of several steps of orchid breeding, starting with collection of parent plants, dialel crossing of two parents of hybrid *Phalaenopsis* (pink with dark purple dotted stripes flower x yellow petal with dark purple *labellum*), plant maintenance during seedpod development, *in vitro* seed germination and seedling growth, and plantlet acclimatization. All activities were conducted in the Plant Science Laboratory, Lampung University since April 2014 to June 2015.

Three experiments were conducted, each of which used a completely randomized design with three replicates, namely : (1) Effects of basal medium (1/2 MS vs 3 g/l Growmore Compound Fertilizer, NPK 32:10:10) and tryptone concentrations (0, 2 and 4 g/l) on *Phalaneopsis* seed germination; (2) Effects of basal medium (1/2 MS vs 3 g/l Growmore Compound Fertilizer, NPK 32:10:10) and tryptone concentrations (0, 2 and 4 g/l) on *in vitro* seedling growth; and (3) Effects of media (*sphagnum moss* vs shredded fern stem) and *benzyladenine* (BA) concentrations (0, 20 and 40 mg/l) on survival and growth of *Phalaenopsis* plantlets.

Data of experiment I were collected at 2 months after seed sowing, including the score of seed germination, percent of protocorms with leave primordia, and fresh weight of 100 protocorms. Data of experiment II were collected at 3 months after seedling culture in the media, including length and width of leaves, number of leaves, number of roots, length of longest roots and seedling fresh weight. Data

of experiment III were collected at 4 months after the plantlets were acclimatized in the *ex vitro* condition, including the plantlet survival, number of leaves, length and width of leaves, number and length of roots and plantlet fresh weights. All data were subjected to analysis of variance and if necessary was followed by mean separation using least significant difference (LSD) at  $\alpha=0.05$ .

Results of experiment I showed that after two months of culture, *Phalaenopsis* seed germination and the percentage of protocorm with leaf primordia were significantly affected by basal media, tryptone concentrations and interaction between the two factors. Without addition of tryptone, ½ MS produced better seed germination and higher percentage of protocorms with leaf primordia compared to Growmore basal medium. However, addition of 2 g/l or 4 g/l tryptone into Growmore medium caused better seed germination as well as higher percentage of protocorms with leaf primordia than those in ½ MS medium, either without or with the addition of tryptone. The best medium for the hybrid *Phalaenopsis* seed germination and protocorm growth was 3 g/l Growmore supplemented with 2 or 4 g/l tryptone, followed by ½ MS (without tryptone). Higher percentage of protocorms with leaf primordia indicated faster growth of protocorms compared with the lower one, in which the higher proportion consisted of protocorms in globular shape. In addition, the highest fresh weight of 100 protocorms was obtained in ½ MS without tryptone.

Results of experiment II showed that after three months of culture, the number and length of leaves as well as number and length of roots of *Phalaenopsis* seedlings were not influenced by basal media, addition of tryptone and interaction between those two factors. However, basal media significantly affected leaf width, number of shoots, and fresh weight of seedlings, while addition of tryptone and interaction between the two factors only significantly affected leaf width. Growmore medium resulted in higher number of shoots and seedling fresh weight, whereas the highest leaf width was obtained in Growmore medium without tryptone.

Results of the experiment III showed that after 4 months in the *ex vitro* condition, plantlet survival as well as their growth were neither affected by the potting media, application of *benzyladenine* (BA), nor interaction between those two factors, with the exception that *sphagnum moss* resulted in more number of leaves and spraying plantlets with 40 mg/l BA decreased their fresh weights. All treatments assigned, i.e., *sphagnum moss* or shredded fern stem, without or with application of 20 or 40 mg/l BA, resulted in 100 % *Phalaenopsis* plantlet survival with almost the same growth, which indicated that both *sphagnum moss* and shredded fern stem were suitable media for *Phalaenopsis* plantlet acclimatization. Spraying of *Phalaenopsis* plantlets with 20 mg/l or 40 mg/l BA solution during acclimatization in general did not affect their growth.

Keywords: *Phalaenopsis*, seed germination, seedling growth, *in vitro*, basal media, tryptone, acclimatization, *sphagnum moss*, shredded fern stem.

## ABSTRAK

### OPTIMASI MEDIA UNTUK PERKECAMBAHAN BIJI DAN PERTUMBUHAN *SEEDLING IN VITRO* SERTA PENGARUH MEDIA DAN *BENZILADENIN* TERHADAP KEBERHASILAN AKLIMATISASI PLANLET *Phalaenopsis* HIBRIDA

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Popularitas anggrek *Phalaenopsis* di Indonesia maupun di seluruh dunia memerlukan pemuliaan tanaman yang terus menerus untuk mengantisipasi perubahan selera konsumen. Akan tetapi, aktivitas pemuliaan *Phalaenopsis* oleh para penganggrek di Indonesia seringkali terkendala oleh sulitnya perkecambahan biji dan pertumbuhan *seedling in vitro* serta aklimatisasi planlet. Penelitian ini bertujuan untuk mendapatkan formulasi media yang optimal dan murah untuk perkecambahan biji dan pertumbuhan *seedling Phalaenopsis* hibrida *in vitro*, serta mempelajari pengaruh media dan *benziladenin* (BA) terhadap keberhasilan aklimatisasi serta pertumbuhan planlet *Phalaenopsis* hibrida. Penelitian ini terdiri dari serangkaian aktivitas yang dimulai dengan pemilihan tanaman tetua, persilangan dialel lengkap dua tetua *Phalaenopsis* (*Phalaenopsis* berbunga pink dengan garis-garis total berwarna ungu tua dengan *labellum* ungu tua x *Phalaenopsis* berbunga kuning dengan *labellum* ungu tua), pemeliharaan tanaman selama perkembangan polong buah, perkecambahan biji dan pertumbuhan *seedling in vitro*, serta aklimatisasi planlet. Semua aktivitas penelitian tersebut dilaksanakan di Laboratorium IlmuTanaman mulai dari April 2014 hingga Juni 2015.

Dalam penelitian ini dilakukan tiga percobaan, masing-masing menggunakan rancangan acak lengkap dengan tiga ulangan. Perlakuan pada masing-masing percobaan disusun secara faktorial (2 x 3), yaitu: (1) Pengaruh media dasar (1/2 MS vs 3 g/l pupuk lengkap Growmore, NPK 32:10:10) dan konsentrasi tripton (0, 2 and 4 g/l) terhadap perkecambahan biji *Phalaenopsis*; (2) Pengaruh media dasar (1/2 MS vs 3 g/l pupuk lengkap Growmore, NPK 32:10:10) dan konsentrasi tripton (0, 2 and 4 g/l) terhadap pertumbuhan *seedling Phalaenopsis in vitro*; dan (3) Pengaruh media (*sphagnum moss* vs cacahan pakis) dan konsentrasi *benziladenin* (BA) (0, 20 and 40 mg/l) terhadap keberhasilan aklimatisasi dan pertumbuhan *seedling*. Pengamatan percobaan I dilakukan pada umur 2 bulan

setelah penyemaian biji, meliputi skor banyaknya biji yang berkecambah, persentase protokorm yang sudah membentuk primordial daun, dan bobot 100 protokorm. Pengamatan percobaan II dilakukan pada umur 3 bulan setelah penanaman *seedling*, meliputi variable panjang dan lebar daun, jumlah daun, jumlah akar, panjang akar terpanjang, dan bobot basah *seedling*. Pengamatan percobaan III dilakukan setelah 4 bulan pada kondisi *ex vitro* untuk variable persentase keberhasilan aklimatisasi, jumlah dan panjang daun, jumlah dan panjang akar serta bobot segar planlet. Semua data dianalisis ragamnya dan jika terdapat perbedaan nyata antar perlakuan dilanjutkan dengan analisis pemisahan nilai tengah dengan uji beda nyata terkecil (BNT) pada taraf nyata 0.05.

Hasil percobaan I menunjukkan bahwa banyaknya biji *Phalaenopsis* yang berkecambah dan persentase protokorm yang sudah membentuk primordial daun dipengaruhi oleh media dasar, konsentrasi tripton maupun interaksi antar keduanya. Tanpa penambahan tripton, media  $\frac{1}{2}$  MS lebih baik dari pada media Growmore. Namun demikian, penambahan 2 g/l atau 4 g/l tripton kedalam media dasar Growmore menghasilkan perkecambahan biji *Phalaenopsis* dan persentase protokorm berprimordia daun lebih tinggi dari pada media  $\frac{1}{2}$  MS tanpa tripton maupun  $\frac{1}{2}$  MS + 2 atau 4 g/l tripton. Media terbaik untuk perkecambahan biji *Phalaenopsis* hibrida adalah 3 g/l Growmore yang ditambah dengan 2 or 4 g/l tripton, diikuti oleh media  $\frac{1}{2}$  MS tanpa tripton. Tingginya persentase protokorm yang sudah membentuk primordial daun mengindikasikan bahwa pertumbuhan protokorm lebih cepat dari pada yang mayoritas masih berbentuk globular. Bobot 100 protokorm tertinggi didapatkan pada perlakuan media  $\frac{1}{2}$  MS tanpa tripton.

Hasil percobaan II menunjukkan bahwa setelah tiga bulan dalam kultur *in vitro*, pertumbuhan *seedling Phalaenopsis* hibrida tidak dipengaruhi oleh media dasar, konsentrasi tripton dan interaksi antar keduanya. Hal ini ditunjukkan oleh rata-rata jumlah dan panjang daun, jumlah dan panjang akar yang tidak berbeda satu sama lain. Namun demikian, media dasar berpengaruh nyata terhadap lebar daun, jumlah tunas, dan bobot segar *seedling*. Media dasar Growmore menghasilkan jumlah tunas dan bobot *seedling* yang lebih tinggi dari pada media  $\frac{1}{2}$  MS. Pemberian tripton dan interaksi antara tripton dengan media dasar hanya berpengaruh terhadap lebar daun, dimana media dasar Growmore tanpa tripton menghasilkan lebardaun tertinggi.

Hasil percobaan III menunjukkan bahwa setelah berumur 4 bulan keberhasilan aklimatisasi dan pertumbuhan planlet secara umum tidak dipengaruhi oleh media, BA maupun interaksi antar keduanya, kecuali bahwa media *sphagnum moss* menghasilkan jumlah daun lebih banyak dan aplikasi 40 mg/l BA pada planlet justru menurunkan bobot segar planlet. Semua perlakuan yang dicobakan yaitu jenis media baik tanpa maupun dengan aplikasi BA menghasilkan 100 % planlet *Phalaenopsis* hidup dengan pertumbuhan yang hamper sama.

Kata Kunci: *Phalaenopsis*, perkecambahan biji, pertumbuhan *seedling*, *in vitro*, media dasar, tripton, aklimatisasi, *sphagnum moss*, cacahan pakis.