

**PENGARUH PHOTOAUTOTROPHIC MICROPROPAGATION SYSTEM  
TERHADAP AKTIVITAS GEN HSP70 PADA "ANGGREK MACAN"  
(*Grammatophyllum scriptum* (Lindl.) Bl.) SECARA IN VITRO**

EKA FITRIANA CANDRA NINGRUM  
14/364879/BI/09239

**INTISARI**

"Anggrek Macan" (*Grammatophyllum scriptum*) merupakan anggrek alam asli Papua yang populasinya mulai mengalami penurunan, sehingga diperlukan metode yang efektif dan efisien dalam memperbanyak anggrek, salah satunya adalah dengan *Photoautotrophic Micropropagation System* (PMS). PMS merupakan suatu teknik kultur *in vitro* yang dapat meningkatkan kemampuan fotosintesis tanaman dengan memanfaatkan karbondioksida di udara melalui penggantian tutup botol kultur dengan selang. Pengaruh penerapan PMS pada penelitian ini dilihat dari aktifitas *Heat Shock Protein 70* (HSP70) yang berperan sebagai protein *chaperon* pada *plantlet*. Penelitian ini bertujuan untuk mengetahui pengaruh PMS terhadap pertumbuhan *plantlet* anggrek *G. scriptum* secara *in vitro* dengan indikator amplifikasi pada cDNA HSP70 pada *plantlet* dan profil total protein yang dihasilkan. Penelitian ini menggunakan bibit *in vitro* anggrek *G. scriptum* umur 1 tahun yang dikultur dengan perlakuan botol tertutup dan PMS serta variasi sukrosa (0, 5, 10, 20 gr/L). Pengukuran dilakukan terhadap panjang dan jumlah daun serta akar *plantlet* selama pertumbuhan. Total RNA tanaman diisolasi dari daun, digunakan untuk sintesis cDNA. Total cDNA diamplifikasi dengan oligonukleotida primer gen HSP70: HSP70-F1R1, F3R3 dan F1R3. Analisis profil protein terhadap total protein yang diisolasi dari daun anggrek dilakukan dengan SDS-PAGE dan diukur konsentrasi total proteinnya menggunakan metode Bradford. Hasil penelitian menunjukkan bahwa penerapan PMS dapat meningkatkan pertumbuhan morfologi akar dan daun *plantlet*. Hasil amplifikasi cDNA dengan primer HSP70 menunjukkan terbentuknya pita DNA dengan ukuran  $\pm 680$  bp pada semua perlakuan, namun diukur dari intensitasnya, penerapan PMS dapat menurunkan aktivitas gen HSP70 di dalam sel. Analisis profil protein menunjukkan penerapan PMS dapat meningkatkan konsentrasi total protein dan macam protein yang terbentuk dengan berat molekul protein yang bervariasi yaitu 8 macam protein setelah perlakuan PMS selama 1 bulan menjadi 13 macam protein setelah perlakuan PMS selama 2 bulan, masing-masing dengan berat molekul (13,8-48,7) kDa.

Kata kunci : *Grammatophyllum scriptum*, *Photoautotrophic Micropropagation System*, cDNA, Protein, SDS-PAGE

**EFFECT OF PHOTOAUTOTROPHIC MICROPROPAGATION SYSTEM  
ON THE ACTIVITY OF HSP70 GENE IN "ANGGREK MACAN"  
(*Grammatophyllum scriptum* (Lindl.) Bl.) BY IN VITRO CULTURE**

EKA FITRIANA CANDRA NINGRUM  
14/364879/BI/09239

**ABSTRACT**

*Grammatophyllum scriptum* is a natural orchid native to Papua whose population has begun to decline, an effective and efficient method is needed in orchid propagation, one of which is the Photoautotrophic Micropropagation System (PMS). PMS was a culture technique that can improve the photosynthesis ability of plants by utilizing carbon dioxide in the air through the replacement of culture bottle caps with a hose. The effect of PMS application in this study was determined from the activity of Heat Shock Protein 70 (HSP70) which acts as a *chaperone* protein on the *plantlet*. This study was aimed to determine the effect of PMS on the activity of HSP70 gene in "Anggrek Macan" *plantlets* development *in vitro*. As the method, 1-year-old *in vitro plantlet* of "Anggrek Macan" were cultured with closed bottle and PMS treatments with addition of sucrose variations (0, 5, 10, 20 gr / L). The total RNA was isolated from the leaves and then used for cDNA synthesis. The cDNA that has been obtained was amplified with oligonucleotide primers of HSP70 gene : HSP70-F1R1, F3R3 and F1R3 primers. Protein profile analysis was carried out using the total protein isolated from orchid leaves and then analyzed with SDS-PAGE. Protein concentration was measured using the Bradford method. Measurements were made on the length and number of leaves and *plantlet* roots during growth. The results showed that the application of PMS could increase the growth of leaves and roots in *plantlets*. The results of cDNA amplification with HSP70 primer showed the formation of DNA bands with a size of  $\pm 680$  bp in all treatments although the intensity indicated that the PMS treatment reduced the expression of HSP70 gene in cells. Protein profile analysis showed that the application of PMS increased the concentration of total protein and formed proteins with various molecular weights from 13.8 to 48.7 kDa, that 8 kinds and 13 kinds of proteins produced after treated by using PMS in 1 and 2 months, respectively.

Keywords : *Grammatophyllum scriptum*, Photoautotrophic Micropropagation System, cDNA, Protein, SDS-PAGE