

学位論文要旨 Dissertation Abstract

氏名 : MD MANIRUL ALAM
Name

学位論文題目 : Studies on organogenesis in protocorm-like bodies
Title of Dissertation (PLBs) of *Dendrobium kingianum* cultured *in vitro* (*In vitro*におけるデンドロビウム・キングアナムのプロトコーム様球体(PLBs)の器官形成に関する研究)

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The orchids are one of the most beautiful flowering plants in the world. There are many factors like fragrance, color, pattern of blooms are responsible for the popularity of any flower. The propagation of orchids is almost difficult. Thus, organogenesis of orchids *in vitro* is a more controllable and reliable process. Studies on organogenesis of PLBs on two *Dendrobium* cultivar describe some new organogenesis methods of *Dendrobium* tissue culture.

Chapter I. The effect of bio polysaccharides of different molecular weights hyaluronic acid (HA9, HA12 and HA20) and sodium alginate added to modified Murashige and Skoog (MS) media on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* ‘Hallelujah’. We found the highest average number of PLB (16.9/explant) 0.01 mg/L in HA9. In HA12 we found highest number of PLBs (10.1/explant) in 0.1 mg/L but when we use HA20 we found highest number of PLBs (10.9/explants) in 1 mg/L. When we found the best result in 0.01 mg/L of HA9 to compare with HA12 and HA20 and that’s why we use this same concentration under different LED to find out the best combination of LED and HA on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* ‘Hallelujah’. Addition of HA9 (0.01 mg/L) under different LEDs we found the highest number of PLBs (10.33/ explant) under white LED. The highest roots and shoots (0.013/explant) are found in under blue LED light. The effect of sodium alginate we using at different concentration for six weeks. The highest number of PLBs (17.2/explant) we found in 0.01 mg/L in alginate to compared with other concentrations.

Chapter II. Effect of environmental factors and carbohydrate sources on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium* cultivar. In this experiment we use two *Dendrobium* cultivar under different LED with sucrose and trehalose. We also use different temperature effect of *Dendrobium kingianum* ‘hallelujah’. The *in vitro* culture of protocorm-like bodies (PLBs) of *D. kingianum* ‘Hallelujah’ varied significantly under the different LEDs. The green LED light produced the highest number of PLBs in both the

sucrose and trehalose treatments (13.8 and 11.1/ explant, respectively). The white fluorescent lamps (control) resulted in the lowest number of PLBs per explant for both the sucrose and trehalose treatments and the with lowest formation rate (93%). The sucrose supplemented culture medium produced the maximum average number of PLBs (8.0/explant) and the highest number of shoots (2.6/explant) under green LED light for *D. kingianum* Jonathan's Glory 'Dark Joy'. On the other hand, the trehalose supplemented medium under white LED lights increased the average number of PLBs with 100% PLBs formation rate. Green LEDs produced the lowest number of PLBs (4.1/explant) and the lowest PLBs formation (87%), which were lower than for the white fluorescent lamps (control). In this experiment the growth and development of PLBs in *Dendrobium kingianum* 'Hallelujah' were significantly affected by different temperature treatment *in vitro*. The highest average number of PLBs (15.5/explant) we found in 25 °C for 24 hours. The fresh weight (.206 g) recorded also highest in 25 °C for 24 hours. When we set it under 35 °C we see that all the explants are burned.

Chapter III. Effect of elicitors 5-aminolevulinic acid (5-ALA) and N-acetylglucosamine (NAG) on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. In this experiment, In this experiment, the most effective concentration in terms of average number of PLBs in 1 mg/L to compare with control. The maximum number of PLBs (10.3/ explant) was found in 1 mg/L of 5-ALA. The effect of NAG at low concentration enhanced the growth of PLBs formation to compare with control. The highest number of PLBs (10.06/explant) we found in 0.1 mg/L on NAG.

Chapter IV. Effect of plant hormones 6-benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. In this treatment, result has shown that, the highest number of PLBs (15.7/explant) on 10 mg/L of BAP to compare with control (0). In low concentration of BAP (0.01 mg/L) found little shoots also but in high concentration of BAP haven't any shoots and roots in this treatment. The Effect of NAA on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah' with different concentration. The highest number of PLBs (14.9/explant) we found in 1 mg/L of NAA and results showed significantly different with control and high concentration of NAA treatment. The maximum number of roots and shoots are also found in same concentration 1 mg/L of NAA. The maximization of orchid production is leading to higher people demand for technical assistance and information in order to develop orchid growth, development, production and quality with quantity. The culture media are supplemented with phytohormones for controlling the efficient proliferation of PLBs, shoots and roots in orchidaceae plants *in vitro*. The different sources of LEDs are used in this study and all are widely available and generally viewed as well safe materials for environment and also for human.