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In-Vitro Rooting Induction On The Embryo Somatic Of Dendrobium Species From Riau Province Indonesia

Pebra Heriansyah, Hasan Basri Jumin, Maizar

Faculty of Agriculture Islamic University of Kuantan Singingi, Riau, Indonesia

hpebra92@gmail.com

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ABSTRACT

Rooting induction is a very important stage in in-vitro for the anticipation of the extinction, In this paper, the rooting induction of embryo somatic dendrobium species from Riau Province Indonesia is studied using different sucrose concentrations (0 g L⁻¹; 25 g L⁻¹; 50 g L⁻¹; 75 g L⁻¹), and Kinetin (0 mg L⁻¹; 0,1 mg L⁻¹; 1,0 mg L⁻¹; 10 mg L⁻¹), in a modified MS medium containing half the regular concentration of macronutrients at pH 5.6, with 50 g L⁻¹ sucrose and 1,0 mg L⁻¹ kinetin was optimal for a percentage of explant producing root, a number of roots, root high, root and shoot ratio (T/R Ratio).

Keywords: Dendrobium species, In-vitro, Sucrose, Kinetin

ABSTRAK

Suhu rendah dan aplikasi giberelin berpengaruh terhadap pematangan dormansi. Pengaruh lama penyimpanan suhu induksi perakaran secara in-vitro merupakan tahap yang sangat penting dalam kultur in-vitro untuk mengantisipasi kepunahan, induksi perakaran dari embrio somatis Dendrobium spesies dari Provinsi Riau Indonesia dipelajari dengan menggunakan konsentrasi sukrosa yang berbeda (0 g l⁻¹; 25 g l⁻¹; 50 g l⁻¹; 75 g l⁻¹), dan kinetin (0 mg l⁻¹; 0, 1 mg l⁻¹; 1,0 mg l⁻¹; 10 mg l⁻¹), dalam media Murashige and Skoog yang dimodifikasi setengah konsentrasi dari makronutrien pada pH 5,6, dengan 50 g l⁻¹ Sukrosa dan 1,0 mg l⁻¹ kinetin adalah konsen-trasi optimal untuk persentase eksplan memproduksi akar, jumlah akar, tinggi akar, rasio akar dan tunas (Rasio T/R).

Kata Kunci : Dendrobium spesies, In-vitro, Sukrosa, Kinetin

INTRODUCTION

Orchid species is an orchid plant that grows on an area and has not undergone a genetic engineering process, This plant generally grows and develops with a compact color, and does not have many floral patterns, such as white, or red. One orchid species is the Dendrobium orchid, which is the most species in the order Orchidaceae (Heriansyah et al. 2014). The Dendrobium orchid species is already locally endan-gered, due to various

activities such as logging, land burning, and looting.

The planting of Dendrobium orchid plants in-vitro cultures also has problems due to the induction of rooting which requires a relatively long time, After the establishment of the callus and multiplication process so it is necessary to provide induction of rooting treatment.

Rooting induction is the most important part of plants regenerations that play a role in the plant to survival strategies (Du and Scheres 2018), including in-vitro conditions, plants need roots to absorb nutrients and other materials in the medium, generally, there are two types source of rooting, a root that comes from the embryonic root and roots derived from somatic cells (Atkinson et al. 2014), to induce rooting, explant requires energy from the culture medium and sucrose is a source of energy that can be added into the medium as a source of carbon and energy, the addition of sucrose at concentrations of 60 g L⁻¹ needs to be increased to induce rooting explant (Faria et al. 2004), Furthermore to induce rooting also influenced by phytohormones (Yang et al. 2017), One of these hormones is cytokinin, and it serves to control the growth of roots explant (Zhang et al. 2013).

Earlier research on the induction of rooting especially in the *Dendrobium* orchid plant species has not been found, but the research on the use of kinetin on embryo growth that examines kinetin on the growth of embryonic shoots have been done by Heriansyah, 2019. Explaining that the administration of kinetin affects the establishment of a *dendrobium* of explants species (Heriansyah 2019).

This study aims to study the induction techniques of rooting on the embryo somatic orchid plant species, For the conservation of locally endangered orchids due to various activities that interfere with the preservation of habitat from the *Dendrobium* orchids.

The benefit of this study is to prepare the method of conservation on *Dendrobium* orchids, and preventing local extinction. In addition, it can also be used as a reference in the process of plant tissue culture of the type *dendrobium* orchids especially for the-induction of rooting.

METHOD

This research was conducted in the laboratory of tissues culture in Balai Benih Induk Riau Province main seed Hall, Indonesia, in April to June 2015. Embryo somatic of *dendrobium* species were collected from laboratory of tissues culture in Balai Benih Induk Riau Province, Indonesia and that was 6 months after planting, explant in isolation from the shoots and planted in the MS (Murashige and Skoog 1962) medium with 30 g l⁻¹ sucrose and 7.0 g l⁻¹ agar in jar bottles, without the addition of plant hormones

Culture media and culture conditions

The MS (Murashige and Skoog 1962) medium containing 100 mg l⁻¹ myo-inositol was used and solidified by agar at 7.0 g l⁻¹. This studied using different sucrose concentrations (0 g L⁻¹; 25 g L⁻¹; 50 g L⁻¹; 75 g L⁻¹), and Kinetin (0 mg L⁻¹; 0,1 mg L⁻¹; 1,0 mg L⁻¹; 10 mg L⁻¹), The pH of a medium was adjusted to 5.8 prior to autoclaving at 121 OC at 1 kg cm⁻² for 20 min. All cultures were incubated at 25 ± 2 OC under a 16/8-h (day/night) photoperiod provided with white-fluorescent tubes at an intensity of 30 l mol m⁻² s⁻¹.

The experiments were carried out in a completely randomized design with two factors using different sucrose concentrations (0 g L⁻¹; 25 g L⁻¹; 50 g L⁻¹; 75 g L⁻¹), and Kinetin (0 mg L⁻¹; 0,1 mg L⁻¹; 1,0 mg L⁻¹; 10 mg L⁻¹) and and three replications, with fourth degrees of treatment (48 cultures). Were raised for each treatment and experiments were repeated at least three times. Visual observations were made every day. The percentage of explants forming roots, the number of the root, the length of the roots, and the ratio of buds – root (B/R Ratio) was counted after 12 weeks of root induction. The data were analyzed statistically using one-way analysis of variance (ANOVA), using honestly significant different (HSD) multiple range test with the level of significance set at 5%.

RESULTS AND DISCUSSIONS

The Percentage Explants forming Roots

As shown in **Figure 1** The treatment Sucrose 50 g/l combined with Kinetin 0.1 ppm and 50 g/l Sucrose and 1.0 ppm Kinetin, Can be growth it's rooted very well, due to the concentration of sucrose does not exceed 50 g/l kinetin and the concentration of not more than 1.0 ppm, if both of these factors given at high concentration then explant will experience a decrease in the percentage of explants forming roots, granting preferential treatment as in 75 g/l sucrose and Kinetin a root in the amount of 10 ppm were 0.0%.

Plant growth especially on the formation of rooting in in-vitro conditions influenced by

2 factors, namely internal and external the factor (Smeekens et al. 2010), one part of the external factor is the availability of sucrose on MS medium sucrose acts as a source of energy and carbon medium, serves as the modulation of growth and development, as well as a stress response (Rolland et al. 2006). Each plant a different sucrose concentrations want to meet the needs of the plant metabolism, as well as requiring different concentrations at different growth phases, Especially in the induction phase of rooting on somatic embryos dendrobium species want the concentration sucrose 50 g L⁻¹.

Figure 1.Diagram of Percentage Grows Roots

Figure 2. Diagram of The Number Of Root

The Number Of Root

Figure 2 Based on the results of the Statistical analysis, observations of the data parameters the number of roots, grant of sucrose and kinetin interaction did not give significant effects against the number of roots. The influence of Sucrose concentration, the main effect of the real against the growing number of roots and the influence of the main concentration of Kinetin are also influential real against the number of roots.

Sucrose has the role of singly as a source of energy for the explant and synergize with growth hormone in stimulating the formation of plant roots, and kinetin has functions singly interacts with hormone auxin the

formation of the root of explant, in these conditions the concentration hormone auxin a concentration greater than kinetin so stimulate the formation of roots, however the most influential in the synergy is the hormone auxin and its concentration in the medium (Schaller et al. 2015).

Formation of rooting in embryo somatic is also influenced by environmental factors associated with the availability of nutrient density and concentration in the medium, in addition to this factor of light also has an important role in the distribution of hormone plant growth medium of cells of plants, temperature, and humidity also has an important role, dealing with the distribution of water for plants (Duclercq et al. 2009).

Figure 3. Diagram of The length of the root

Lengthening of the root is the result of a combination of various internal and external factors on the explant culture medium on the plant, these factors are, the availability of this type of growth hormone challenged and some other hormone, in addition also influenced by the activity of enzymes that speed up the metabolism of various reactions on explant plant, the existence of the energy obtained from sucrose is used as a source of energy in

the metabolic reactions (Parthibhan et al. 2015).

Hormone gibberellin function in the process of lengthening the cell on rooting explant, the role of the hormone works on plant cell with some other hormone, hormone challenged on the cell plant will stimulate the transport nutrition cell, and will cause of occurrence of the processes of cell enlargement in plants (Nelson et al. 2018).

Figure 4. Diagram of The ratio of buds – root (T/R Ratio)

Figure 5. Source of orchid explant

Figure 6. Orchid Tissue Culture Results

Ratio Of Buds – Root (T/R Ratio)

Table IV. Based on the results of the Statistical analysis, observations of the data parameters the ratio of buds – root (T/R Ratio), grant of sucrose and kinetin interaction did not give significant effects against the ratio of buds – root (T/R Ratio),. The influence of Sucrose concentration, to give a significant effect against the growing the length of the roots and the influence of the main concentration of Kinetin is also influential significant effect against the ratio of buds – root (T/R Ratio).

Formation of the root on the explant can be compared with the number of buds are formed, the ratio of buds – this root is determined by growth hormone concentration in the medium, when the concentration of

auxin in medium higher compared to the concentration of kinetin on the medium it will be formed in the excess amount of rooting than the formation of buds, but when a larger the concentration of kinetin in the medium then the number of shoots that form will be more and more (Bhattacharyya et al. 2016).

CONCLUSION

Sucrose and kinetin Interaction effect significantly to, the percentage of explant to form roots on Dendrobium Orchid plant species. With best treatment 50 g Sucrose/l and 1.0 ppm Kinetin. The awarding of the Sucrose affect significantly to the percentage of explant to form root, the number of roots, root length, root-shoot ratio (T/R ratio) on the Orchid plant Dendrobium SP. With the best

concentration of 50 g/l Kinetin Granting the significant effect. Against the percentage of explant to form root, the number of roots, root length, root-shoot ratio (T/R ratio) on the Orchid plant *Dendrobium* SP. With the best concentration of 1.0 ppm.

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