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IN VITRO CONSERVATION OF MEDICINAL PLANT ST. JOHN'S WORT (*Hypericum perforatum* L.) THROUGH DILUTION OF BASIC MEDIUM

KONSERVASI IN VITRO TANAMAN OBAT ST. JOHN'S WORT (*Hypericum perforatum* L.) MELALUI PENGENCERAN MEDIA DASAR

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ABSTRACT

In vitro techniques could be applied for plant conservation through minimal growth. Growth reduction is generally attained by modifying the culture medium and/or the environmental condition. Conservation of St. John's wort (*Hypericum perforatum* L.) *in vitro* through dilution of basic medium was conducted at the Tissue Culture Laboratory of Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) in 2018. Sterile shoots were cultured into Murashige and Skoog (MS) medium by reducing macronutrients. The treatments used were full-MS + 0.1 mg L^{-1} N^6 - benzyladenine (BA) as control; ¾ MS + 0.1 mg L^{-1} BA; ½ MS + 0.1 mgL^{-1} BA and ¼ MS + 0.1 mg L^{-1} BA. Observation of the culture growth was conducted three months after the treatments. The experiment was arranged in Completely Randomized Design with ten replications. The result showed that the use of a dilution of basic medium affected the growth of St. John's wort. The use of ¼ MS + 0.1 mg L^{-1} BA composition could suppress culture growth (number of shoots, shoots length and number of leaves), without showing necrotic symptom until three months of conservation. This treatment could be used as an alternative to minimize the culture of St. John's wort for *in vitro* conservation.

Keywords: *Hypericum perforatum* L., *dilution of basic medium, in vitro conservation.*

ABSTRACT

Kultur *in vitro* dapat diaplikasikan untuk konservasi tanaman melalui pertumbuhan minimal. Upaya memperlambat pertumbuhan kultur dapat dilakukan melalui modifikasi media atau kondisi lingkungan. Konservasi St. John's wort (*Hypericum perforatum* L.) *in vitro* melalui pengenceran mdia dasar dilakukan di Laboratorium Kultur Jaringan Balai Penelitian Tanaman Rempah dan Obat pada tahun 2018. Eksplan yang digunakan adalah tunas steril yang dikulturkan pada media Murashige dan Skoog (MS) yang telah dikurangi hara makronya. Perlakuan yang digunakan adalah MS + BA 0.1 mg /l sebagai pembanding; ¾ MS + BA 0.1 mg/l; 1/2MS + BA 0.1 mg/l dan ¼ MS + BA 0.1 mg/l. Observasi pertumbuhan biakan dilakukan selama tiga bulan setelah perlakuan. Rancangan yang digunakan dalam penelitian ini adalah Acak lengkap dengan sepuluh ulangan. Hasil penelitian menunjukkan bahwa pengenceran media dasar berpengaruh terhadap pertumbuhan kultur. Penggunaan ¼ MS + BA 0.1 mg/l dapat menekan pertumbuhan kultur (jumlah tunas, panjang tunas dan jumlah daun), tanpa menunjukkan gejala penurunan daya tumbuh sampai umur tiga bulan. Perlakuan ini dapat diguanakan untuk konservasi St. John's wort *in vitro.*

Kata Kunci: *Hypericum perforatum* L., konservasi *in vitro*, pengenceran media dasar.

INTRODUCTION

St. John's wort (*Hypericum perforatum* L.) is one of the potential medicinal herbs with a long history of traditional use. This plant belongs to the Hypericaceae/Clusiaceae family. St. John's wort is the most important species of the genus Hypericum. The main uses in medicine includes treatment of mild and moderate depression, skin wounds and burns (Barnes et al., 2001). Documented pharmacological activities, including antidepressant, antiviral, and antibacterial effects, provide supporting evidence for several of the traditional uses of stated for St. John's wort. This plant contains a vast array of secondary metabolites such as hypericin and pseudohypericin, hypeforin and adhyperforin, and essential oil can be mentioned (Morshedloo et al., 2012; Ebadi, 2002). Recently, hypericin and pseudohypericin have received more attention due to their antitumor (Penjweini et al., 2013) and antiviral (Arumugam et al., 2013).

Considering the potency of St. John's wort which has many benefits as medicine, an attempt at plants conservation should be conducted. To support the conservation of St. John's wort in the ISMCRI, propagation is done through tissue culture technique. Plantlet multiplication is conducted by using Murashige and Skoog (MS) media, supplemented with 0.1 mg L^{-1} BA and has been conserved for over ten years (Syahid and Wahyuni, 2019). *In vitro* conservation of genetic resources has advanced considerably during this decade. One alternative for preventing irreparable loss of biodiversity is the use of *in vitro* conservation of plant germplasm through using slow growth culture.

There are three techniques for *in vitro* conservation which are short-term conservation, medium-term conservation, and long-term conservation. Conservation techniques for short-term storage are also called optimal growth techniques. In this technique, the plant material is stored in the medium and optimal physical condition so that explant grows with optimum speed. Medium-term conservation techniques are called minimal growth techniques, plant material is stored in medium and physical conditions outside the optimal range, whereas long-term conservation uses freezing or non-growth techniques, plant material is stored in frozen conditions in liquid nitrogen with temperatures well below freezing (-196^{o}C) (Engelmann and Engels, 2002; Dube et al., 2011). Among the three kinds of techniques most widely practiced in Indonesia are the minimal

growth techniques because they are efficient, the risk of contamination and mutation is lower, and the resources available in Indonesia are adequate.

The principle of minimum growth technique is to provide a condition for explants (plant material stored) to metabolise and grow in low velocity by regulating the composition of the medium and the physical environment of the culture, i.e. lowering the levels of nutrients, adding osmoregulatory, inhibitors and storing the culture in temperature, intensity of light, and the duration of irradiation below the optimal point. Due to the slow metabolism, they do not need frequent sub-culture that leads to waste of materials, time, and energy (Engelman, 2011; Lata et al., 2010).

In vitro conservation of St. John's wort has been conducted using cryopreservation, and they used shots tips as an explant (Skyba et al., 2011). Conversely, freezing techniques or cryopreservation called requires high technology and skills that in Indonesia has not many institutions that have adequate tools and expertise to perform the technique.

This research's objective was to access *in vitro* storage of St. John's wort under slow growth conditions by dilution of basic medium to develop an efficient protocol for the conservation of species genetic diversity.

MATERIAL AND METHOD

The experiment was conducted at the Tissue Culture Laboratory of Indonesian Spice and Medicinal Crops Research Institute, Bogor from January to July 2018.

Plant materials and culture media

The plant materials used in the study were a sterile shoot of St. John's wort *in vitro.* Murashige and Skoog (MS) medium was used as basal medium enriched with vitamins from B group. The medium was augmented with 3% sucrose and 0.8% agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121^{o}C for 15 min. The cultures were kept inside the culture room at 22 ± 2^{o}C, with 16 h photoperiod of 50 µmol m^{-2} s^{-1} photon flux density provided with cool white fluorescent tubes.

Statictical analysis

The experiment was arranged in a Completely Randomized Design with ten replications. Data were subjected to analyses of variance (ANOVA). The differences of mean value were analyzed using Duncan Multiple Range Test (DMRT) at 5 % level.

RESULT AND DISCUSSION

Number of shoots

The principle of minimal growth conservation is based on minimizing culture conditions or culture media to allow the culture to remain viable slow-growth rate. The use of reducing macronutrients applied significantly affected the culture growth. All reductions of macronutrient treatments showed significantly different results with control.

One month after treatment, there was no significant different on the number of shoots produced. Observation on two months' age of the culture, the number of shoots/projections performed on the reducing macronutrient began to decrease. Three months after treatments, conserved culture on ¾ MS + 0.1 mg L^{-1} BA, ½ MS + 0.1 mg L^{-1} BA, and ¼ MS + 0.1 mg L^{-1} BA performed the lowest shoots production than the other treatments (Table 1).

Table 1. Effect of reducing macronutrients on the growth of St. John's wort during *in vitro* conservation

Treatments (mg L^{-1})	Number of shoots
Full MS + 0.1 BA	58.14 ± 5.76[a]
¾ MS + 0.1 BA	41.86 ± 4.67[b]
½ MS + 0.1 BA	24.29 ± 2.49[c]
¼ MS + 0.1 BA	10.57 ± 1.72[d]
CV (%)	11.88

Note: Number followed by the same letter on the each coloum are not different at 5 % DMRT.

Media using full of macronutrients composition (MS + 0.1 mg L^{-1} BA) showed the faster growth compared to that have a smaller macronutrients content (3/4MS + 0.1 mg L^{-1} BA, ½ MS + 0.1 mg L^{-1} BA and ¼ MS + 0.1 mg L^{-1} BA. Media containing ¼ MS + 0.1 mg L^{-1} BA showed the lowest shoots and shortest shoots length (Figure 1). This study observed that ¼ MS + 0.1 mg L^{-1} BA showed the alternative for reducing growth.

Shoots lenghth

The use of ¼ MS + 0.1 mg L^{-1} BA affected the St. John's wort growth rate *in vitro*. The petiole was shorter, and the leaf size was smaller than the other treatments (Table 2).

Table 2. Effect of reducing macronutrients on the shoot lenghth of St. John's wort during *in vitro* conservation

Treatments (mg L^{-1})	Shoot length (cm)
Full MS + 0.1 BA	3.03± 0.15 a
¾ MS + 0.1 BA	2.94± 0.19 a
½ MS + 0.1 BA	2.59± 0.30 b
¼ MS + 0.1 BA	1.43 ± 0.28 c
CV (%)	9.58

Note: Number followed by the same letter on the each coloum are not different at 5 % DMRT.

Number of leaves and roots

Application of ¼ MS + 0.1 mg L^{-1} BA also reduced the number of leaves of St. John's wort during *in vitro* conservation. The number of leaves produced in this treatment is not significantly different with ½ MS + 0.1 mg L^{-1} BA (Table 3). During *in vitro* conservation, no roots found on all of the treatments.

Table 3. Effect of reducing macronutrients on the number of leaves of St. John's wort during *in vitro* conservation

Treatments (mg L^{-1})	Number of leaves
Full MS + 0.1 BA	11.14 ± 1.07[a]
¾ MS + 0.1 BA	8. 86 ± 1.07[b]
½ MS + 0.1 BA	7.71 ± 0.76[c]
¼ MS + 0.1 BA	7.43 ± 0.98[c]
CV (%)	11.11

Note: Number followed by the same letter on the each coloum are not different at 5 % DMRT.

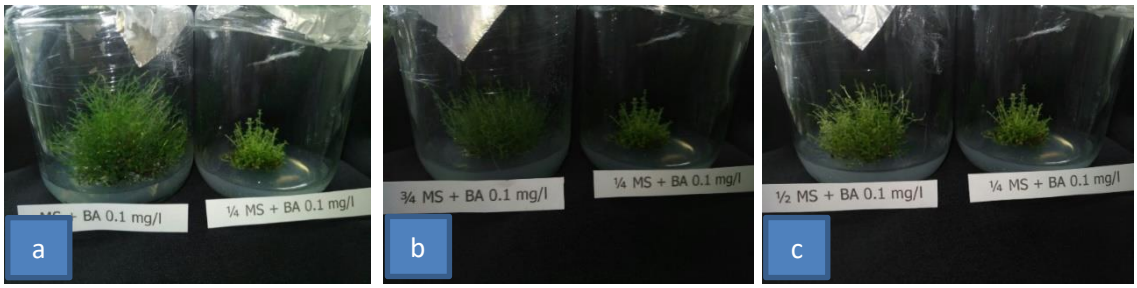

Figure 1. Culture performance during in vitro conservation of St. John's wort *in vitro:* a) Full-MS + 0.1 mg L^{-1} BA and ¼ MS + 0.1 mg L^{-1} BA, b) ¾ MS +0.1 mg L^{-1} BA and ¼ MS + 0.1 mg L^{-1} BA, and c) ½ MS + 0.1 mg L^{-1} BA and ¼ MS + 0.1 mg L^{-1} BA.

Macronutrients are essential substances of the plants for their growth, and the deficiency of them can affect a decrease the growth rate even death. Decreasing the salt concentration into the medium affected reducing of the absorption of the nutrients responsible to the plant growth and development and this condition make the activity of the culture slow. Based on the slow-growth conservation, the use of ¼ MS+0.1 mg L^{-1} BA could be recommended as a technique for conserving the St. John's wort *in vitro*. This treatment could suppress the number of shoots without showing necrotic symptoms. Cultures in this treatment are still alive during three months of conservation.

In this condition, the visual cultures on ¼ MS + 0.1 mg L^{-1} BA were still optimal with vigor stem and fresh leaves. Based on the slow-growth response, the subculture period can be minimized. Reducing macronutrient is one technique to reduce requirement for subculture without causing any damage to the tissue (Rajasekharan and Sahijram, 2015). Reducing salt to 50% on *Epidendrum*

clorocorymbos culture provided better slow-growth storage, and plants could be conserved for four months (Lopez-Puc, 2013). Reducing sucrose and ½ MS salt was also influential in conserving the tips of the shoots of *Prunus malaleb* L. *in vitro* and ginger/*Zingiber officinale* Rosc. (Sota and Kongjika, 2014; Syahid and Bermawie, 2000). Cell growth and morphogenesis of some species may even be promoted by increasing the level of macronutrients. Nitrogen is a very vital component of the basal medium to support plant growth (Chawala, 2002).

According to the nutrient and to the level of deficiency, the deficiency of mineral in plants can cause biochemical, physiological and morphological changes (Monteiro et al., 2000). Macronutrients are essential substances the plants needed for their growth and their deficiencies can result into the decreasing of the growth rate, and even death to the plant.

In the post-storage viability test, 100% recovery was observed regardless of storage duration, provided the culture contained reduced macronutrients. The plant responded to regeneration by developing axillary shoots and nodal zones in MS regeneration medium supplemented with 0.1 mg L^{-1} BA.

CONCLUSSION

Reduced macronutrient concentration can slow the culture growth of St. John's wort *in vitro*. Therefore, it is appropriate for St. John's wort germplasm storage *in vitro*. The use of ¼ MS + 0.1 mg L^{-1} BAP could minimize plant growth (number of shoots, shoots length and number of leaves), during *in vitro* conservation and reduce cost. For further research, another method to prolong the sub-cultured period is needed.

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