In vitro conservation of *Valeriana officinalis* L. through minimal growth

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Abstract. Minimal growth storage could be utilized for in vitro plant conservation. Modifying the culture medium or the environmental condition can reduce the plant growth. The aim of the experiment is to observe the effect of macronutrients on the reduction of valerian culture This research was conducted at Tissue Culture Laboratory at the Indonesian Crops and Medicinal Research Institute (ISMCRI). Single axillary buds with leaves of valerian were used as explants. The treatment applied were :1) full-MS medium + 0.1 mg l^{-1} BAP, 2) ¾ MS + 0.1 mg l^{-1} BAP, 3) ½ MS + 0.1 mg l^{-1} BAP and, 4) ¼ MS + 0.1 mg l^{-1} BAP. After 12 weeks of conservation, the cultures were transferred into a plant growth regulator free-MS medium to observe their regeneration ability after the conservation. Then, the cultures were acclimatized at the greenhouse to observe their further growth. The result showed that using ¼ MS + 0.1 mg l^{-1} BAP was the best treatment to reduce the growth during 12 weeks of conservation. After in vitro storage, the cultures can grow normally in full strength plant growth regulator free-MS medium, which was characterized by the formation of new shoots. Valerian also produced roots in the same medium. All acclimatized plantlets were successfully established at the greenhouse.

1 Introduction

Valeriana officinalis L. is called valerian, a perennial herb that belongs to Valerianaceae. Valerian is endemic to many parts of Europe, Asia, and North America and is widely used in various traditional medicines. Pharmacological studies indicated that this plant possesses multiple biological activities, especially in the area of antioxidant, anti-inflammatory, anti-cancer, anti-convulsive, anti-Parkinson's, anti Alzheimer's diseases, etc. [1]. In addition, Valerian root is a popular herbal supplement used to treat insomnia and anxiety [2]. The roots and rhizome of valerian are the main part of the plant that are medically used. Now valerian extract is considered a dietary supplement primarily comprised of dried root or root extracts formulated into tablets or soft gelatin capsules [3]. Roots and rhizomes of valerian contain valepotriates and valerenic acid (with putative pharmacological activities. Valerenic acids and valepotriates have a role in display tranquilizing effects [4].

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In vitro propagation of valerian using rhizome as an explant was conducted at Indonesian Spices and Medicinal Crops Research Institutes (ISMCRI) laboratory in 2016-2018 [5], using MS medium enriched with 0.1 mg l^{-1} BAP. Subculture was conducted every 8-10 weeks to maintain the culture in good condition cause of high withered leaves, but this technique wastes materials, time, and energy. Therefore, in vitro conservation of valerian is needed to maintain plant germplasm. The fundamental objectives of in vitro conservation technology are to retain and exchange germplasm in a disease-free and genetically stable state through tissue culture. In vitro conservation programs reduce frequent demand for subcultures, which can be accomplished in two ways: by maintaining culture under normal growth or subjecting them to growth-limiting strategies [6].

There are three techniques of in vitro conservations; 1) short-term, 2) medium- term and 3) long-term conservations. 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. The medium-term conservation technique is called the minimal growth technique. The plant material is stored outside the optimal range in medium and physical conditions, whereas long-term conservation uses a freezing or non-growth technique. The plant material is stored in frozen conditions in liquid nitrogen with a temperature well below the freezing point (-196 ºC) [7].

The principle of minimal growth is to maintain a condition for the culture to metabolize and grow at 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, the intensity of light, and the duration of irradiation below the optimal point. Due to the slow metabolism, they do not need frequent sub-culture, leading to waste of materials, time, and energy [8]. In the present study, we observed the effect of macronutrient reduction on the growth of valerian during *in vitro* conservation.

2 Materials and methods

2.1 Study area

In vitro conservation of *V. officinalis* through minimal growth was conducted at Tissue Culture Laboratory at the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI).

2.2 Plant materials

Single axillary buds with leaves of valerian in vitro were used as explants.

2.3 Conservation through minimal growth

Single axillary buds with leaves were cultured in: 1) full-MS medium + 0.1 mg l^{-1} BAP, 2) ¾ MS + 0.1 mg l^{-1} BAP, 3) ½ MS + 0.1 mg l^{-1} BAP and, 4) ¼ MS + 0.1 mg l^{-1} BAP. The Murashige and Skoog (MS) medium contain 3% sucrose as the carbohydrate source, and 8 g/l agar is used to solidify the medium. Before adding the agar, the pH of the medium was adjusted to 5.8 using 0.1 HCL or NaOH. The cultures were incubated in a culture room at 24 ± 2 ºC under 16 fluorescent tube lights with 16/8 hours light/dark. The parameters observed were the number of shoots, roots, shoot lengths, and visual culture during treatment and after the conservation period.

2.4 Culture regeneration after conservation and acclimatization

After 12 weeks of conservation, the culture was transferred into plant growth regulator free-MS medium to observe their regeneration ability after conservation and whether they grow normally and produce new shoots and roots after the conservation period. Then, valerian was acclimatized in the greenhouse. First, Valerian plantlets were removed from culture bottles and washed to remove the agar. The plantlets were then transferred into a small plastic cup containing sterilized planting media (soil, compost, and husk), covered by a polyethylene bag to maintain the humidity. The polyethylene bag was opened gradually for two weeks after transplantation, and in one month, it was removed entirely. The plants were maintained under greenhouse conditions for two months.

2.5 Statistical analysis

The experiment undertaken was by a Completely Randomized Design with ten replications. The data obtained were analyzed using the SAS program. Further testing was carried out by using Duncan at a probability level of 1% (p=0.01).

3 Results and discussion

3.1 Conservation through minimal growth

The reduction in the use of macronutrients significantly affects the growth of valerian. All removal of macronutrient treatments showed quite different results compared to control. Application of ¼ MS + 0.1 mg l^{-1} BAP was the best treatment to reduce the growth during 12 weeks of conservation. The culture showed the slowest growth response compared to other treatments in the number of shoots and shoots length in twelve weeks (Table 1 and 2). The use of full-MS medium produced the highest and longest shoots.

Table 1. Effect of minimal growth on the number of shoots of valerian on twelve weeks of conservation.

Treatments (mg l^{-1})	Number of shoots
Full MS + 0.1 BAP	2.9 a
¾ MS + 0.1 BAP	2.2 b
½ MS + 0 1 BAP	1.4 c
¼ MS + 0.1 BAP	1.2 c

Note: The number followed by the same letter on each column are not significantly different at 1% DMRT.

Table 2. Effect of minimal growth on the shoot's length of valerian, on twelve weeks conservation.

Treatments (mg l^{-1})	Shoots length (cm)
Full MS + 0.1 BAP	3.28 a
¾ MS + 0.1 BAP	2.91 b
½ MS + 0.1 BAP	2.37 c
¼ MS + 0.1 BAP	1.96 d

Note: The number followed by the same letter on each column are not significantly different at 1% DMRT.

Applying macronutrients ¼ MS + 0.1 mg l^{-1} BAP reduced the number of shoots and growth rates, such as shoot lengths and leaf sizes. The petiole was shorter and the leaf size was smaller than the other treatments. The application on full-MS + 0.1 mg l^{-1} BAP medium cannot support the plant growth until 12 weeks and culture growth tended to decrease (Figure 1). During the conservation period, less withered leaves found ¼ MS + 0.1 mg l^{-1} BAP compared to the other treatments (Data not shown). In this condition, the visual culture on ¼ MS + 0.1 mg l^{-1} BAP was still optimal with vigor stem and fresh leaves. Based on slow growth response, the subculture period can be minimized. Valerian can be subcultured after 12 weeks. So, this treatment can be applied for valerian conservation. Reducing macronutrients is one of the techniques to minimize the requirement for subculture without causing any damage to the tissue. Reducing salt to 50% on *Epidendrum clorocorymbos* culture provided better slow growth storage, and plants could be conserved for four months [9]. Reducing sucrose and ½ MS salt also effectively conserves the shoot tips of *Prunus mahaleb* L. *in vitro* [10]. Nitrogen is the most important component of the basal medium [11]. The deficiency of minerals in plants can cause biochemical, physiological, and morphological changes according to the nutrient and the level of deficiency [12, 13]. Macronutrients are essential substances the plants need for their growth and their deficiencies can decrease the growth rate and even cause the death of the plant. The reduction of macronutrients significantly affected the number of roots. Application of ¼ MS + 0.1 mg l^{-1} BA performed the greatest roots (Table 3).

Table 3. Effect of minimal growth on the number of roots of valerian on twelve weeks of conservation.

Treatments (mg l^{-1})	Number of roots
Full MS + 0.1 BAP	0.20 c
¾ MS + 0.1 BAP	1.20 b
½ MS + 0.1 BAP	2.60 a
¼ MS + 0.1 BAP	2.40 a

Note: The number followed by the same letter on each column are not significantly different at 1% DMRT.

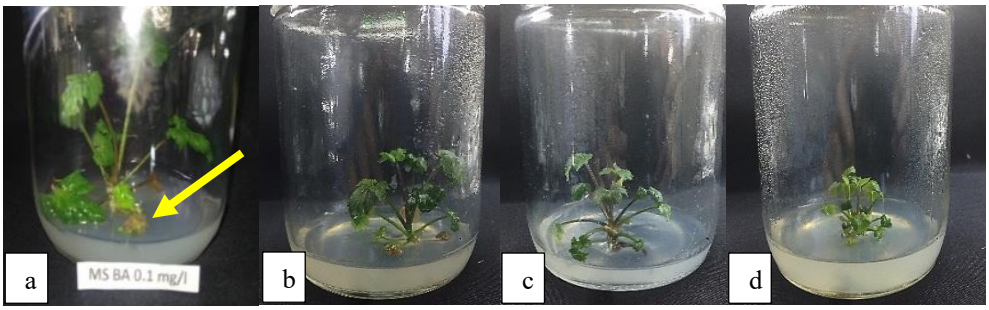

Figure 1. Culture performance of *V. officinalis* during *in vitro* conservation with different concentrations of MS macronutrient supplement with 0.1 mg l^{-1} BA with (a) Full-MS, (b) ¾ MS, (c) ½ MS, (d) ¼ MS, on 12 weeks after culture.

The result obtained for number of roots on ¼ MS + 0.1 mg l^{-1} BAP treatments were significantly different with full MS + 0.1 mg l^{-1} BAP and ¾ MS + 0.1 l^{-1} BAP. When MS strength decrease, the number of roots increases. Application of different strength of MS media in solid and liquid media for *Typonium flagelliforme* propagation indicated that using quarter-strength MS (¼ MS) increased the number of roots on solid media [14].

3.2 Regeneration after conservation and acclimatization

After 12 weeks of storage treatments, the cultures were transferred to a growth regulator free-MS medium to observe their growing ability. The observations showed that the culture could grow normally in MS medium characterized by the formation of a new shoot. There is no effect of reducing macronutrient for further plant growth. The roots can grow when the shoots are transferred into the MS-free medium. Reduced salt could support the formation of roots in vitro. It is interesting as root induction is quite difficult. Valerian roots can be induced using Indol Butyric Acid (IBA) but are limited. Reducing a macronutrient is often used for rooting induction in vitro. Acclimatization is the last step of in vitro conservation to observe the ability of plantlets after treatments. It is important for the survival and successful establishment of plantlets in ex vitro. Acclimatization is conducted by removing plantlets from the culture bottle to an acclimatization medium with high humidity [15]. Many factors influence the success of in vitro plantlet in acclimatization process. Steps of acclimatization, plantlets size and handling, and root differentiation must be considered for transferring the plants from in vitro culture [16, 17]. All acclimatized valerian plantlets were successfully established under greenhouse conditions and showed normal growth.

4 Conclusion

In conclusion, using ¼ MS + 0.1 mg l^{-1} BAP was the best treatment to reduce the growth during 12 weeks of conservation. After in vitro storage, the cultures can grow normally in full strength plant growth regulator free-MS medium characterized by the formation of new shoots. Valerian also produced roots in the same medium. All acclimatized plantlets were successfully established at the greenhouse and showed normal growth.

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