SHOOTS MULTIPLICATION AND ROOTS INDUCTION OF VALERIAN (*Valeriana officinalis* L.) IN VITRO THROUGH BENZYL AMINO PURIN AND INDOL BUTYRIC ACID APPLICATION

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ABSTRACT

Valerian (*Valeriana officinalis* L.) is one of introduction medicinal plants from Europe and Asia, which is potential to be developed.The plant rhizome is used for sedation, hypertension, sleep induction and improve sleep quality.Based on plant usage, Indonesian Spices and Medicinal Crops Research Institute (ISMCRI) had collected valerian since 1983 at Manoko Research Station and conserved them through ex situ conservation. *In vitro* culture is one of the alternatives to maintain dan conserve germplasm. *In vitro* propagation of valerian is needed before plant conserved.A research was conducted on Tissue Culture Laboratory of the Indonesian Spices and Medicinal Research Institute (ISMRI) from September 2016 to March 2017.Murashige and Skoog (MS) medium was used as a basic medium.The research contains of two steps i.e. shoots multiplication and roots induction. For shoot multiplication, a treatment applied was several concentration of Benzyl Amino Purine (BAP): 0.1, 0.3, 0.5, 0.7 dan 0.9 mg l^{-1}. For root induction, a treatment was several concentration of Indol Butyric Acid (IBA): 0.5, 1.0, 1.5 and 2.0 mg l^{-1}. The parameter observed were number of shoots, roots and leaves, shoots and root length and visual culture. The experiment was arranged in completely randomized design with ten replications. Result showed that application of 0.7 mg l^{-1} BAP produced the highest shoots. Roots could be obtained on all of the IBA concentration and also the hair roots. Acclimatization at the green house was successfully established using growth medium containing soil : husk : compost (1 : 1 : 1) with 70% growth percentage. The availability of valerian *in vitro* could be utilized for genetic improvement through biotechnology and germplasm conservation.

Keywords: acclimatization, in vitro, roots induction, shoots multiplication

INTRODUCTION

Valerian (*Valeriana officinalis* L.) is one of the medicinal plants from Europe and Asia. Prospects of plant usage are a quite promising cause of much benefits as medicinal materials such as tranquilizers (sedatives), hypnotic, lowering blood pressure, and improve sleep quality. Traditionally, valerian used as a cure for insomnia, migraine, arthritis and anti-hysterical. The current indications for valerian are restlessness, insomnia, nervousness, and tension (Raal *et al*., 2007; Tariq & Pulisetty, 2008).

Valerian belongs to a family of Valereinaceae (Wang *et al*., 2010). Traditionally, valerian is propagated by seeds and tiller separation. The seed of valerian have low viability and tiller separation is faced on the infection of the disease (Janke, 2004). An alternative obtaining the healthy planting material is through plant tissue culture technique. So that*, in vitro* methods for large scale multiplication would be a viable options and considered a powerful tool to multiply difficult to propagate, rare or endangered and useful species for commercial cultivation as well conservation (Nandi *et al*., 2002). Nowadays, *in vitro* techniques a being used as a powerful tool for plant propagation, particularly in clonal propagating of high-value varieties (Tansaz *et al*., 2014).

Valerian collection at Manoko Research Station, Indonesian Spices and Medicinal Crops Research Institute was conserved since 1983 through *ex situ*. Until now, only one accessions of valerian could survive, so it's needed to conserve using *in vitro* technique. Therefore, establishment of an efficient propagation systems is necessary for the large scale production.

To increase the shoots multiplication and roots induction, growth regulators such as cytokinin and auxin must be applied. For the shoot multiplication, a concentration of Benzyl Amino Purine was applied on *in vitro* culture is different for each plant. Rooting *in vitro* determines the successful of plant acclimatization at the green house. To induce root formation *in vitro*, several auxins like IBA, IAA, and NAA are applied (George *et al*., 2008). For certain plants auxin IBA is often used because it produces the better root.

In vitro propagation of *Valeriana officinalis* L. using crown of seedling has obtained by Tansaz *et al.* (2014). The best medium for regeneration of *Valeriana officinalis.* L. *in vitro* using a novel combined Murashige and Skoog and Gamborg (MB) medium enriched with 0.5 mg l^{-1} BAP + 0.25 mg l^{-1} NAA.

Thus, the present examined the effect of different Benzyl Amino Purine (BAP) and Indol Butyric Acid (IBA) on shoot multiplication and rooting of *Valeriana officinalis* L. *in vitro*

MATERIALS AND METHODS

The study was conducted at Tissue Culture Laboratory of the Indonesian Spices and Medicinal Crops Research Institute (ISMCRI), from September 2016 to April 2017.

Source of Explants

Shoots of valerian were supplied from Manoko Research Station of Indonesian Spices and Medicinal Crops Research Institute on 2015. To provide a sterile planting material, shoots of valerian must be sterilized by soaking it in 70% alcohol for 5 minutes and then followed by several rinses with sterile distilled water. Then shoots were soaked in 0.2% $HgCl_2$ for 5 minutes followed by several rinses in sterile distilled water. Then, shoots were soaked with 20% NaClO during 10 minutes followed by several rinses with sterile distilled water to remove excess of the disinfectants. Shoots were cultured on plant growth regulator free Murashige and Skoog (MS) medium.

The study consisted of two steps i.e.: shoots multiplication and root induction.

1. Shoots multiplication

The sterile shoots was transferred into MS medium solidified with 0.8% agar. As a source energy used sucrose by 30 g l^{-1}. The medium contained various concentration of growth regulators Benzyl Amino Purine (0.1 , 0.3 , 0.5, 0.7 and 0.9 mg l^{-1}). The variables observed were the number of shoots, shoots length and visual of shoots.

2. Root induction

For root induction, the treatment being tested was various concentration of Indol Butyric Acid (0.5 , 1.0 , 1.5 and 2.0 mg l^{-1}). The variables observed were the number of roots, the length of roots, and visual of roots. Culture bottles were stored in shelves in the incubation room culture with the light intensity of 1000 lux for 16 hours a day. Room temperature ranged was 22-25 ^{o}C.

Acclimatization

The *in vitro* grown plantlets with well-developed root systems were acclimatized at the green house using growth medium containing mixture of soil : rice husk : compost (1: 1: 1). And then covered with polyethylene bag to maintain the humidity. The plantlets were acclimated under controlled greenhouse condition at 24ºC and irrigated regularly. The experiment was arranged as a complete randomized design with ten replications.

Data Analysis

Data were statistically analysed, using SAS Statistical Software package (Ver. 9.1). Data obtained was analysed with the interval of 5%. If there are differences among the treatments, then conducted a further test with DMRT.

RESULTS

Shoots Multiplication

Applications of Benzyl Amino Purine (BAP) at all concentrations tested gave a positive response on valerian shoot multiplication *in vitro* (Figure 1). One week after treatment, shoots started to initiate on all of the treatments with a different number. Two months after culturing, the ability of culture to produce shoot was also increased. Application of BAP on 0.1-0.5 mg l^{-1} produced the different significantly number of shoots with BAP 0.7 mg l^{-1}. The use of 0.7 mg l^{-1} BAP performed the highest number of shoots, leaves and shoot length (Table 1). Increasing the BAP concentration to 0.9 mg l^{-1} tended to decrease the number of shoots.

Table 1. Effect of different concentration of Benzyl Amino Purine (BAP) on the number of shoots, shoot length, a number of leaves of *Valeriana officinalis* L., two months after planting

Treatment (mg l^{-1})	Number of shoots	Number of leaves	Shoots length (cm)
BAP 0.1	1.22 b	3.33 b	1.00 d
BAP 0.3	1.67 b	3.78 ab	1.17 cd
BAP 0.5	2.33 b	4.22 ab	2.22 ab
BAP 0.7	4.33 a	4.89 a	2.67 a
BAP 0.9	2.11 b	3.56 ab	1.78 bc

Values followed by the same letters are not significantly different at 5% level of DMRT

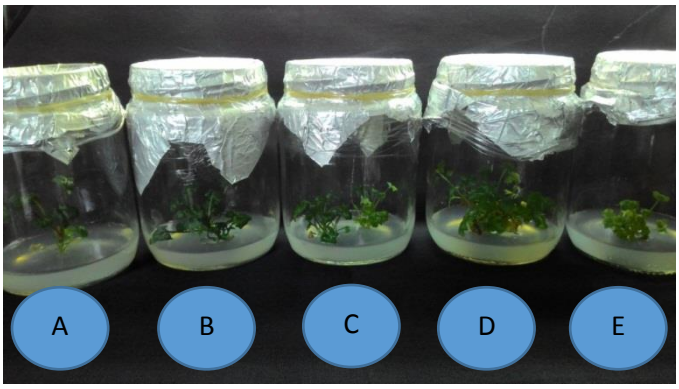

Figure 1. Effect of several concentration of Banzil amino purin on shoots multiplication of *Valeriana officinalis* L. *in vitro*, two months after culturing. (A= 0,1 mg l^{-1} B=0,3 mg l^{-1} C=0,5 mg l^{-1} D=0,7 mg l^{-1} E= 0,9 mg l^{-1})

Root Induction

Root of *Valeriana officinalis L. in vitro* could be produced on all of IBA concentrations. The process of roots formation started from thickening of the stem, on the third weeks. Then, the rooting had begun. Root was rather thick and black (Figure 2). Although the roots obtained very little, but it tended to produce hairy roots that came out from the stem. Number of the roots obtained were not significantly different among all the treatments. Increasing the IBA concentration affected the root length (Table 2).

Table 2. Effect of several concentration of IBA on root growth of *Valeriana officinalis* L. in vitro, two months after culturing

Treatments (mg l^{-1})	Number of root	Root length (cm)	Visual of culture
IBA 0.5	1.2 a	0.20 b	Stem base was thickened, roots was short and hairy roots come out from the stem
IBA 1.0	1.8 a	0.22 b	Stem base was thickened, roots was short and hairy roots come out from the stem
IBA 1.5	1.8 a	0.36 a	Stem base was thickened, roots was short and hairy roots come out from the stem
IBA 2.0	2.0 a	0.40 a	Stem base was thickened, roots was short and hairy roots come out from the stem

Values followed by the same letters are not significantly different at 5% level 0f DMRT.

Acclimatization

Acclimatization of *Valeriana officinalis* L. was conducted using a planting medium mixture of soil : rice husk : compost (1: 1: 1) resulting up to 70% being successful (Figure 3).

Figure 3. The acclimatization process of *Valeriana officinalis* L.
(A) : Acclimatization on one week (B): Valerian planting material on three weeks
(C) Valerian is on 3 months (D) Valerian is on 4 months at the greenhouse

DISCUSSION

Generally, BAP is used on low concentration for plant propagation *in vitro*. The content of cytokinin in valerian tissue was high enough so that the addition of concentration up to 0.7 mg l^{-1} has been able to produce the optimal number of shoots. Application of BAP on 0.7 mg l^{-1} also produced the greatest number of leaves and shoot length.

The response of tissue to the growth regulator given was different for each plant. On micro propagation of Artemisia (*Artemisia chamaemelifolia* Vill.), application of BAP at concentrations of 0.7 mg l^{-1} produced the highest number of shoots within four weeks after culture and increased of BAP concentrations tended to decrease the number of shoots produced. In addition, the increase in BAP concentration also decreased the length of the shoots obtained (Hristova *et al.*, 2013). High concentration of growth regulator usually inhibits the cell division. Abdi and Khosh-Khui (2007) found the shoots regeneration of valerian via direct organogenesis from leaf using a combination of BAP + NAA.

Limited of roots which can be induced on valerian indicated that the application of IBA as exogenous auxin has not been able to optimize the process of root formation *in vitro*. The use of IBA also could induce adventitious roots from leaf explants of *Valeriana jatamansi* Jones (Chen *et al.*, 2014). Tansaz *et al.*, (2014) reported that rooting of Valeriana *officinalis L. In vitro* could be obtained through combination of 0.5 mg l^{-1} Benzyl Amino Purine and 0.25 mg l^{-1}

Naphthalene Acetic Acid. All micro-shoots were successfully rooted in the same regeneration medium and developed long, numerous roots after 2 - 4 weeks and so it was not needed to transfer the shoots into the rooting medium.

According to Abdi and Khosh-Khui (2007), rooting of *Valeriana officinalis* L. obtained after transferring the shoots into rhizogenic medium. Application of NAA 1.0 mg l^{-1} produced the greatest number of roots and plantlet could be acclimatized and transferred at the greenhouse.

Acclimatization is one important aspect of plant propagation through *in vitro* culture. Acclimatization is the process of moving the culture from the heterotroph environment to autotroph, and the plant form i*n vitro* culture will adapt to environmental conditions that were previously controlled humidity, temperature and light intensity. Acclimatization is conducted by conditioning the plants roots can be function immediately, but evaporation from the leaves kept to a minimum condition (Lestari, 2008).

Tansaz *et al*., (2014) also found that all acclimatized plantlet of Valerian (*Valeriana officinalis* L.) were successful in a greenhouse and showed normal growth. Purohit *et al*., (2015) reported that acclimatized of *Valeriana jatamansi* Jonses using a mixture of sterile soil : vermiculite : perlite (1:1:1, v/v/v) was successful and about 90% survival was recorded. On Rue (*Ruta graveolens* L.) Acclimatization, the planting medium used was soil : rice husk : compost (1:1:1), gave the successful growth reached 73.3% at the greenhouse (Syahid & Kristina, 2012).

CONCLUSION

The use of MS + BAP 0.7 mg l^{-1} produced the highest number of shoots (4.33 shoots) within two months. Increasing concentrations of BAP to 0.9 mg l^{-1} tended to decrease the number of shoots. Application of IBA at all concentrations tested produced in small amounts of roots but hairy root formed on all treatments. Acclimatization of valerian in a greenhouse using a planting medium mixture of soil : rice husk : compost (1: 1: 1) was satisfactory successful. The availability of valerian *in vitro* could be utilized for genetic improvement through biotechnology and germplasm conservation.

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Supriadi for discussion, Suryatna, Totong Sugandi and Ramdan Arismaya for their contribution and help in plant conservation at the greenhouse.

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