

Clonal Propagation of *Vetiveria zizanioides* L. through Tissue Culture Technique

Wahyu Widoretno*¹, Arbaul Fauziah², Serafinah Indriyani³, Edi Priyo Utomo⁴

^{1,2,3}Biology Department, Faculty of Mathematics and Natural Sciences,
Brawijaya University, Malang

⁴Chemistry Department, Faculty of Mathematics and Natural Sciences,
Brawijaya University, Malang

e-mail: *¹widoretno@ub.ac.id

Abstrak

Metode perbanyakan *in vitro* tanaman akar wangi (*Vetiveria zizanioides*) secara efektif telah dikembangkan pada penelitian ini. Beberapa variasi media digunakan untuk inisiasi dan multiplikasi tunas. Pembentukan tunas dapat diinisiasi dari eksplan crown yang dikulturkan pada medium MS dengan penambahan zat pengatur tumbuh Benzyladenine (BA) 2 mgL⁻¹. Sedangkan untuk multiplikasi tunas, konsentrasi BA yang efektif dalam media adalah 3 mgL⁻¹, dengan rata-rata jumlah tunas yang terbentuk sebesar 126 tunas/eksplan. Penggunaan BA konsentrasi tinggi (3-5 mgL⁻¹) pada media multiplikasi dapat memacu terbentuknya lebih banyak tunas, namun tunas yang dihasilkan lebih pendek. Sebaliknya, penggunaan BA dengan konsentrasi rendah (1-2 mgL⁻¹) pada media multiplikasi menghasilkan lebih sedikit tunas, tetapi tunas yang dihasilkan lebih panjang. Regenerasi plantlet diperoleh dengan menginduksi akar pada tunas pada media MS yang ditambah dengan zat pengatur tumbuh NAA 1 mgL⁻¹. Tanaman *in vitro* akar wangi telah berhasil ditumbuhkan pada media tanah di bawah kondisi rumah kaca. Dengan menggunakan metode di atas, maka dimungkinkan perbanyakan bibit akar wangi secara massal melalui teknik kultur jaringan.

Kata kunci— *Vetiveria zizanioides*, *in vitro*, inisiasi dan multiplikasi tunas, regenerasi plantlet, Benzyladenin (BA)

Abstract

In vitro propagation method for vetiver (*Vetiveria zizanioides* L.) had been effectively developed in this study. Several variations of media were used for shoot initiation and multiplication. Shoot formation was initiated from crown explant cultured on MS media with the addition of 2 mgL⁻¹ of growth regulator Benzyladenine (BA). Whereas for shoot multiplication, 3 mgL⁻¹ of BA was evidently effective with the average shoot number was 126 shoots per explant. The application of high BA concentration (3-5 mgL⁻¹) in multiplication media was capable of inducing more shoots, but the shoots resulted were shorter. In contrast, multiplication media supplemented with low BA concentration (1-2 mgL⁻¹) yielded less shoots, but the shoots were longer. Plantlet regeneration was accomplished by inducing roots in the shoots yielded on MS media containing 1 mgL⁻¹ growth regulator NAA. *In vitro* plants of vetiver had been successfully grown on soil media under greenhouse condition. By using foregoing method, it is possible to conduct mass propagation of vetiver through tissue culture technique.

Keywords— *Vetiveria zizanioides*, *in vitro*, shoot initiation and multiplication, plantlet regeneration, Benzyladenin (BA)

1. INTRODUCTION

Vetiver (*Vetiveria zizanioides* L.) is perennial grass in which the root contains essential oil consisting of more than 150 sesquiterpenoids. There are many benefits of vetiver essential oil e.g. as fixative in perfume industry, as mixture component in soap and cosmetics industry, as well as for aromatherapy [1].

The need of vetiver oil is increasing along with the advanced development of perfume, cosmetics, and aromatherapy industry. Recently, vetiver oil has become export commodity in international market. However, vetiver oil production in Indonesia is still incapable of fulfilling the need of industry and international trade. It is due to the low production of vetiver plants as the source of vetiver oil and the vetiver oil quality resulted is still unable to meet the market requirements (low uniformity and low quality) [2]

According to Statistic data of Indonesia Plantation in 2012-2014, the vetiver production from 2010 to 2013 were 377, 107, 80, and 71 ton respectively, while the productivity in 2012 and 2013 were 34 and 30 kg/ha respectively. In addition to production, another vetiver oil problem in Indonesia is that the quality of vetiver oil is so low that frequently it is not in accordance with the market demand [3]. According to Mulyono et al. [4], the low quality of Indonesian vetiver oil resulted from the accumulation of low quality and low uniformity of vetiver plants as raw material, as well as less optimum distillation.

With the increasing need of vetiver seeds, the improvement of efficient and effective vetiver propagation technology is needed in order to yield high quality and productive vetiver seeds. One of the methods to increase plant quality is to provide high quality and uniform seeds within short period through tissue culture technique.

Furthermore, not all vetiver plants are able to bear flowers and the germination speed of the seed is so low that it is difficult to yield variants through conventional sexual hybridization. Therefore, in vitro propagation method obtained gives opportunity to yield variants through tissue culture technique. This study was aimed to develop efficient in vitro clonal propagation of vetiver and to determine optimal condition for initiation and multiplication of vetiver by using growth regulator BA.

2. MATERIALS AND METHODS

2.1 Plants Material

Plants material used was vetiver plants derived from Sengklek, Pamalayan Village, Bayongbong District, Garut Regency, West Java.

2.2 Shoots induction and Multiplication

In vitro shoot was induced from crown explant. Vetiver plant was washed with running tap water, then it was trimmed 4 cm long, and the root was removed. The trimmed part of vetiver plant was sterilized using 96% alcohol within one minute, commercial bleaching agent (containing 5.25% NaClO as active material) 50% for 20 minutes, and then rinsed twice with sterile aquadest each for 5 minutes. The sterile trimmed part was cleft then the crown part was cultured on MS media containing BA (1, 2, 3 mgL⁻¹). The culture was incubated at room temperature (25-26°C) with the light intensity of 600 lux for 8 weeks. The observation parameters were the time of the first shoot formation and the number of shoots. The shoots formed were then multiplied on media supplemented with several concentrations of growth regulator BA (1, 2, 3, 4 mgL⁻¹). For every treatment, 6 clumps of shoots were cultured, and each clumps consisted of 5-6 small shoots. The culture was maintained with light intensity of 600 lux at temperature of 25-26°C. After 8 weeks, the number of shoots and the length of each shoot formed were observed.

2.3 Plantlet Regeneration and Acclimatization

Shoot was transferred to MS media containing 1 mgL⁻¹ growth regulator NAA for plantlet regeneration. The shoots forming roots (plantlet) were acclimatized on soil media for plant growth (compos: rice husk charcoal =1:1). After 4 weeks, the success of acclimatization was determined by observing the survival plants.

3. RESULT AND DISCUSSION

Crown explant cultured on media MS supplemented with growth regulator 6-Benzyladenin (BA) was capable of forming shoots. The formation of shoots started 3 weeks after culture and took place on all three media, but the best shoot formation was on media containing 2 mgL⁻¹ of BA, which yield most shoots. Besides, the shoots formed were longer compared to media containing 1 mgL⁻¹ of BA or 3 mgL⁻¹ of BA (Figure 1).

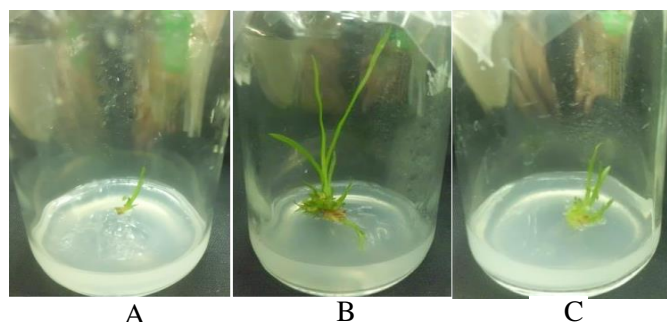


Figure 1. Shoot formation from crown explant on MS media containing several concentrations of BA. A. 1 mgL⁻¹, B. 2 mgL⁻¹, dan C. 3 mgL⁻¹.

The addition of 1 mgL⁻¹ growth regulator BA in media was less able to drive shoot formation, by yielding only 4 shoots per explant. The number of shoots produced on MS media containing 2 mgL⁻¹ BA was significantly higher compared to other two media (Figure 2).

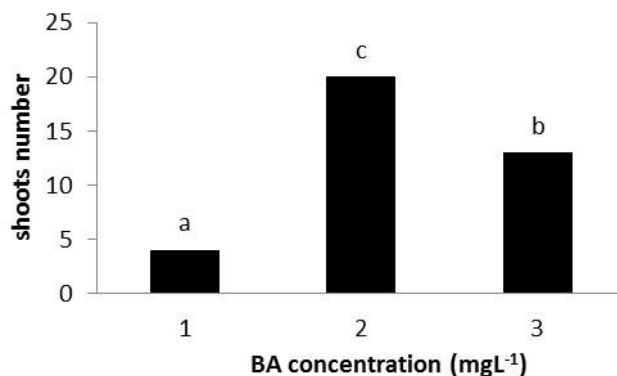


Figure 2. The number of shoots produced on MS media supplemented with several concentrations of BA (note: histogram with bars indicated means. Different letters indicated significant differences at $\alpha = 5\%$, according to one-way ANOVA and Duncan’s test)

Besides affecting shoot formation from explant, concentration of growth regulator affected shoot multiplication as well (Figure 3 and 4). In vitro shoots of vetiver could be multiplied rapidly on MS media with higher concentration addition of BA. Increasing BA concentration in media enhanced in vitro shoot multiplication of vetiver, but it turned out that higher concentration (above 3 mgL⁻¹) of BA application tended to cause the declining of shoots multiplication, although it was not significant. Highest shoot multiplication was obtained from media containing 3 mgL⁻¹ BA, averaging 126 shoots per explant and significantly different

from media supplemented with 1 mgL⁻¹ and 2 mgL⁻¹ BA, which only produced average shoots number of less than 70 shoots per explant (Figure 3).

Besides affecting shoots number, concentration of growth regulator BA in MS media also gave effect to shoots length. Media supplemented with lower BA concentration yielded less but longer shoots, while higher BA concentration resulted in more but shorter shoots (Figure 4). The addition of higher BA concentration to media tended to result in smaller percentage of shoots longer than 5 cm. Shoot percentage of shoots longer than 5 cm on MS media containing 1 mgL⁻¹ BA was almost 10%, which was significantly different from the same percentage of shoots cultured on media supplemented with 3-5 mgL⁻¹ BA. More than 60% of the shoots formed on media with the addition of 3-5 mgL⁻¹ BA were less than 0.5 cm (Figure 3).

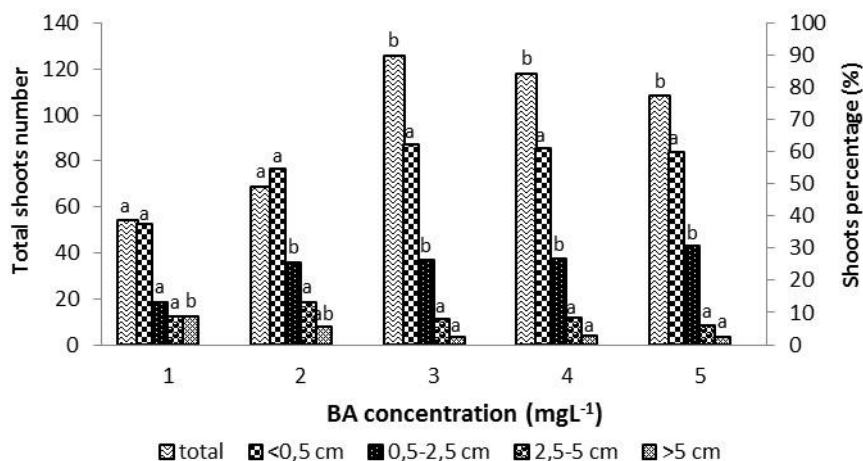


Figure 3. The effect of BA in multiplication media on total shoot number and percentage of shoots based on their length (note: histogram with bars indicated means. Different letters indicated significant differences at $\alpha = 5\%$, according to one-way ANOVA and Duncan's test)

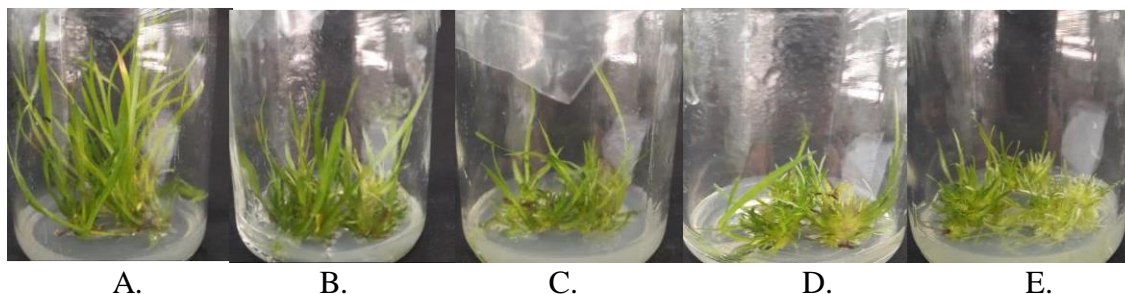


Figure 4. Shoot multiplication on MS media containing growth regulator BA with several concentrations. A. 1 mgL⁻¹, B. 2 mgL⁻¹, C. 3 mgL⁻¹, D. 4 mgL⁻¹ dan E. 5 mgL⁻¹

Shoots were then transferred to MS media + 1 mgL⁻¹ NAA for plantlet regeneration. Generally, roots started to emerged from the shoots 2 weeks after culture. Four weeks after culture, acclimatization was accomplished on soil media (compos: rice husk charcoal =1:1) with relatively high success rate, i.e. more than 90%.

Nowadays, clonal propagation has been considered as regular technology on plant propagation. Steps in clonal propagation include: culture initiation, shoot multiplication, rooting of shoots formed, and plantlet acclimatization on soil media. Clonal propagation has benefits for plant seed production, i.e. high quality and healthy plants [5]. Culture media consisting of mineral constituents (mineral salts), carbohydrate, and growth regulator play important role in clonal propagation [6].

The success of in vitro plant propagation is affected by growth regulator in culture media. The role of growth regulator in determining development and development path of the

cell and tissue cultured is due to the accumulation of specific biochemistry content within cell or tissue [7]. Ikram-ul-Haq & Dahot [8] revealed that different growth regulator, used individually or in combination in media, causes particular and balance maintenance of organic and inorganic compound within growing tissue and in turn it causes the cells or tissues develop into shoots/roots or even subject to death.

Generally, cytokinins is necessary for in vitro shoot induction and shoots multiplication. The study conducted by Sajid et al. [9] showed that the presence of cytokinins contained in media not only determines the regeneration response of banana meristem culture, but it also affected regeneration mode. Initial response of shoot formation is caused by the addition of cytokinins accompanied by elevated level of cytosolic calcium triggered by high uptake from the media. This condition affects cytoskeleton and regulates exocytosis [10].

The speed of in vitro shoot multiplication and shoot elongation is affected by cytokinins type and concentration. For plants clonal propagation, it was proved that benzyladenin (BA) was more effective than kinetin (K), N⁶-(2-isopentenyl), adenine (2iP) and Zeatin (Z) [11, 12, 13]. BA as one of cytokinins types is generally used in plant tissue culture at the range concentration of 0,1-10 mgL⁻¹. Cytokinins stimulates the activity of Cdk-A at transition phase of G1-S and G2-M, which regulate cell cycle progression partly by transcription induction of CycD3/1[14].

The effect of growth regulator BA on shoot formation and multiplication had been reported in several plants. The addition of 10 mgL⁻¹ BA was able to promote adventive shoot formation at initiation and multiplication step of all *Gerbera L.* [6], and it was better compared to kinetin. BA use was also very effective for shoot multiplication of *Hypericum retusum* Aucher [15]. The increasing concentration of BA yielded more banana shoots, and the optimum BA concentration used was 6 mgL⁻¹ [7]. MS media containing 3 mgL⁻¹ BA was also suitable for propagation of *Ficus benjamina* var. Natasja and Starlight [16]. Clonal propagation of *Saintpaulia ionantha* Wendl. was also successfully carried out by culturing leaf explant on MS media containing 3 mgL⁻¹ BA alone or in combination with 1 mgL⁻¹ IAA [17].

In addition to affect the shoots formed, the concentration of BA in media also affected the length shoots of banana [18] and tea [19]. The longest shoot of in vitro tea was obtained by supplementing media with 3 mgL⁻¹ BA, while the shortest shoot was yielded from MS basal media.

Root induction is a significant procedure in plantlet regeneration. In general, shoots regenerated in vitro produce no roots, therefore root induction is necessary by culturing shoots on MS basal media or MS media supplemented with auxins growth regulator NAA. Plantlets with good shoots and roots system had high success rate in acclimatization and transfer process to soil media.

4. CONCLUSION

- Clonal propagation of in vitro vetiver seeds had been successfully conducted through shoot induction from crown explants, shoot multiplication on MS media + 3 mgL⁻¹ BA, and plantlet regeneration on MS media containing 1 mgL⁻¹ NAA.
- By applying foregoing method, it is possible to carry out mass clonal propagation of vetiver through tissue culture technique.

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