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In vitro Plant Regeneration of Java Vetiver (*Vetiveria zizanioides*)

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Abstract*—In vitro* plant regeneration has been successfully obtained from basal shoot explant of *Vetiveria zizanioides* through indirect organogenesis. The explant was cultured in Murashige & Skoog's (MS) media supplemented with 2,4-D, IAA, and kinetin in various concentrations. Callus was well induced in media supplemented with 2ppm 2,4-D, 1ppm IAA, and 1ppm kinetin. This callus was then transferred to MS media supplemented with 1 - 5 ppm of BAP for shoot regeneration. The media supplemented with 3 ppm BAP was a suitable medium for shoot induction, as well as for shoot multiplication. Rooting was well developed in shoot following transferred to half MS media containing 0.2ppm IBA. Plantlet was then transferred to husk charcoal for acclimatization, and almost all (90%) of plantlets were survived during acclimatization.

***Keywords*—**Callus, plantlet regeneration, shoot induction, *Vetiveria zizanioides.*

I. Introduction

JAVA vetiver (*Vetiveria zizanioides* L. Nash) is a plant belongs to Poaceae that can produce an essential oil, known as vetiver oil. This essential oil has been used as a perfume, cosmetics, herbal medicine [1], as well as for aromatherapy [2]. Ecologically, this plant has an important role for preventing soil erosion [3], and for phytoremediation [4].

Indonesian vetiver oil, known also as *Java vetiver oil*, is one of potential export commodity [5]. High market demand of java vetiver, unfortunately, is not concurrently followed by the avaibility of raw materials in fields. Hence, plant tissue culture can be used as an alternative and potential method for mass propagation of this plant. Micropropagation is one of method in plant tissue culture, which can be used to produce a high number of small plant or plantlet in short period. Success in plant micropropagation is highly affected by the ratio of plant growth regulators used in culture media, especially auxin and cytokinin [6].

Plant micropropagation can be directly occurred through organogenesis, or indirectly through callus culture. Indirect regeneration has frequently been occurred in recalcitrant plants, including Poaceae, woody plants and gymnosperm [7], [8]. *In vitro* plant regeneration of these plants, therefore, needs a higher concentration of plant growth regulators or by using high affinity of certain growth regulators.

In Poaceae, plant regeneration through *in vitro* culture has been achieved in rice (*Oryza sativa* L.), when the explant is culture in media supplemented with thidiazuron (TDZ), 2-4-D dan NAA [7]. Plant regeneration has also been obtained from *in vitro* leaf explant of wheat [9]. Plantlet regeneration of *Vetiveria zizanioides* has been reported by using leaf crown [1], leaves [10] and young inflorescence [11].

This study aimed to develop a protocol for enhancing plantlet regeneration in java vetiver using shoot base explant.

II. Procedures

A. Explant Preparation

Plant of *V. zizanioides* was collected from Kamojang area, Garut, West Java. The tillers of this plant were washed carefully by using running tap water. The tillers were then separated from the root, and the outer leaf sheath of each tillers were removed. The clean tillers were then trimmed about 3 cm from the base of shoot. This explant was then soaked in 10% detergent solution for 10min, followed by rinsing in distilled water. The explant was disinfected using 70% alcohol and subsequently surface sterilized using 0.5% natrium hypochlorite, rinsed using distilled water, soaked again 1.0% natrium hypochlorite, and finally rinsed twice by using sterile distilled water.

B. Callus Induction

Sterile explants were grown in callus induction media, which was composed of MS basal media [12] supplemented with several concentrations of plant growth regulators 2,4-D (1, 2, and 4ppm), IAA (1, 2, and 4ppm) and kinetin (1, 2, and 4ppm). The culture was incubated in a culture room at room temperature ($\pm$ 25°C) with 16 hours of light fotoperiodism.

C. Shoots Induction and Multiplication

Callus from callus induction medium was transferred to shoot induction medium, which was consisted of MS basal media supplemented with several concentrations of Benzyl amino purine/BAP (1, 2, 3, 4, and 5ppm). These cultures were incubated at room temperature of 25°C with 16 hours of light fotoperiodism. Regenerated shoots were then transferred to shoot multiplication media, containing MS basal with the optimum concentration of BAP for shoot induction.

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D. Root Induction and Plantlet Acclimatization

Roots were induced in shoot by transferring the shoots into MS or half MS media supplemented with 0, 0.2 or 0.4ppm IBA. Rooted shoots were then transferred into sterile husk charcoal for acclimatization.

III. Results

Basal shoot of *Vetiveria zizanioides* started to form callus in the first week of culture. This callus firstly developed at both cutting edge of explant. Three weeks later, callus had covered whole explant's surface. In this study, there are two types of callus formed, friable and compact callus. Friable callus grew faster than the compact callus. The medium containing 2ppm 2,4-D, 1ppm IAA and 1ppm kinetin was the optimum medium for callus formation, around 46.67% of basal shoot explants was able to develop white or yellowish ehite friable callus (Table I).

TABLE I
The Effect of Plant Growth Regulators on Callus Formation

Growth regulators (ppm)			Number of explants forming callus (%)
2,4-D	IAA	Kinetin	
1	1	1	26.67
1	2	2	30
2	1	1	46.67
2	2	2	6.67
1	4	4	16.67
2	4	4	6.67
4	1	1	10
4	2	2	10
4	4	4	10

From these results, it can be shown that the addition of exogenous growth regulators (2,4-D, IAA, kinetin) at appropriate concentrations, can probably interact with endogenous growth regulators to stimulate cell division. In the present study, three types of growth regulators, namely 2,4-D, IAA and kinetin were used to induce callus. The use of 2,4-D alone could not induce callus formation [1]. Some of monocots are recalcitrant plants, therefore it usually requires a higher concentration or used several types of growth regulators for callus induction [13], [14]. In *Hordeum vulgare*, callus can be induced when the explant is cultured in media containing 2,4-D and dicamba [15].

Four weeks old callus that induced on media containing 2 ppm 2,4-D, 1ppm IAA and 1ppm kinetin was transferred into the shoot induction media. One week following transfer into these media, the morphogenesis response observed in callus. A green spot was developed in the callus (Fig. 1 (a)). In the second week, callus began to regenerate shoots in the medium containing 3ppm BAP (Fig. 1 (b)). The medium containing 3 ppm BAP was the optimum media to induce shoots and also for shoot multiplication. The other media showed no response to shoot regeneration, although the green spot was also observed on callus surface. This green callus, however, was unable to develop shoots.

The use of BAP for shoot regeneration has also been conducted in some Poaceae, i.e. *Triticum aestivum* [16]. BAP seemed to induce shoot regeneration stronger than the other cytokinin, because this growth regulator has higher affinity to react with cytokinin-binding protein [17], [18].

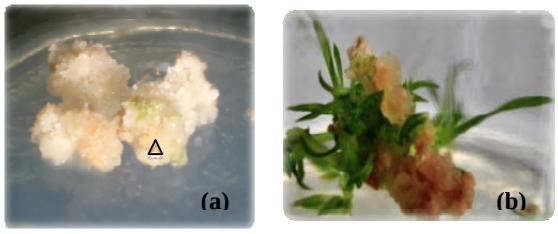

Fig. 1 Shoots induction from vetiver callus on MS medium supplemented with 3 ppm BAP (a) Green spots developed on callus (arrowhead), (b) Callus began to form shoots

Root formation was successfully obtained when java vetiver shoots were transferred into root induction media, containing half-strength MS and 0.2ppm IBA (Fig. 2). Root did not develop in the other media. As stated by Bozwani and Razdan [19], half-strength media is suitable for root induction if full strength media used for shoot regeneration. The use of IBA is also important for root induction in shoot of java vetiver. This auxin is probably responsible for gene regulation to synthesize protein needed for root development [20].

Almost all (90%) of plantlets were survived during acclimatization in husk charcoal media (Fig. 3). Husk charcoal is suitable for plant acclimatization media, because it can absorb and maintain water availability in the media, inhibit the growth of pathogenic organisms, show good aeration and drainage and fix toxic substance [21].

Fig. 2 Root induction in the *in vitro* shoots of java vetiver

Fig. 3 Acclimatization of plantlet in husk charcoal media

IV. Conclusions

In the present study, we found that the MS media containing 2ppm 2,4-D, 1ppm IAA and 1 ppm kinetin was the optimum media for callus induction of java vetiver. The callus could induce shoot and conduct shoot multiplication in MS medium containing 3 ppm BAP. Root formation was obtained in half-strength MS media containing 0.2ppm IBA. Plantlet could survive during acclimatization in husk charcoal media.

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