The Effect of Explant Types and Kinetin Concentration on In Vitro Callus Induction in *Vetiveria zizanioides* (L.) Nash

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Abstract

The aims of this research were to evaluate the effect of explant types and several kinetin concentrations on in vitro induction and growth of callus vetiver (*Vetiveria zizanioides* (L.) Nash). Crown and tiller of vetiver were cultured on Murashige and Skoog's (MS) media supplemented with combination of 2,4-D 0.75 ppm and several kinetin concentrations (0, 0.3, 0.5, 0.75, and 1) ppm. The induction and growth of callus were influenced by type of explant and concentration of kinetin. Formation and growth of callus on tiller explant were faster than crown explant. Callus on tiller explant were formed one week after culture, while callus from crown explant were formed at four weeks after culture. Callus growth on tiller explant also was better than crown explant. Eight weeks after culture, callus fresh weight from tiller explant was 0.35 ± 0.09 g, while callus fresh weight from crown explant was only 0.16 ± 0.08 g. The addition of kinetin in the medium combined with 2,4-D was able to increase callus growth and the optimum concentration of kinetin used was 0.5 ppm. The addition of kinetin more than 0.5 ppm in the medium decreased the callus fresh weight.

Keywords: callus, crown, in vitro culture, tiller, *Vetiveria zizanioides*.

INTRODUCTION

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) is a perennial grass from the family Graminae (Poaceae). It has been utilized as various fields such as industries, economies, and environments [1]. Utilization of vetiver in the various fields is caused by the essential oil contents. The need for essential oils increases along with the development of the medicines, perfumes, cosmetics, and aromatherapy industries. The needs of essential oils in the world, especially vetiver oil is 300 tons per year. However, Indonesia is only able to supply about 28% with the price of essential oil production of approximately 25-30 tons per year [2]. One of the attempts which can be used to increase the essential oil is cell culture. Cell culture is a technique which can be used to increase the production of bioactive compounds in plants. Through tissue culture, bioactive compounds can be produced continuously, controlled and manipulated environment to obtain optimum results [3].

Increasing bioactive compound through cell culture has been carried out by some methods, one of them is callus culture. Callus culture has increased quinone compound in *Peritassa campestris* about 2.5 folds [4] and isopenoid in *Artemisia annua* L. 2.5 folds higher than compounds produced *in vivo* [5]. Thus, the attempt to obtain a callus culture method in vetiver is necessary.

Plant growth regulator is one of the factors which can influence the success of callus cell culture. One of the growth regulator has been used to promote callus formation is kinetin. The use of kinetin in callus induction has been done in *Andropogon gerardii* [6]. In addition, the success of cell culture or callus culture growth is also affected by explant type used. Callus induction has been performed from shoot base explant in *Vetiveria zizanioides* [7] and tiller explant in *Pennisetum purpureum* Schum [8]. However, the used of tiller explant in vetiver plants for callus induction has never been done. Therefore, in this research, the callus induction will be performed by tiller explant in vetiver on media supplemented with several kinetin concentrations. The objectives of the current study were to determine the effect of explant type and kinetin concentration on formation and growth of callus in vetiver.

MATERIALS AND METHODS

Plant material

Plants of *Vetiveria zizanioides* (L.) Nash. were collected from Sengklek, Pamalayan Village, Bayongbong District, Garut, West Java. These plants were washed by using running tap water, then trimmed about 4 cm from the base of shoot and separated from the root. The trimmed plants were disinfected using 96% alcohol for a minute,

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sterilized using 80% commercial whitening agent (containing 5.25% NaClO) for 25 minutes, and finally rinsed by using sterile aquadest twice each for five minutes. Sterile trimmed of plant was cleft, then the crown (part of plant intercourse between tiller and root system) and tiller were used as explant.

Callus Induction

The crown and tiller explant measured ± 0.2 cm from the base were cultured on MS basal medium containing 2,4-D 0.75 ppm and kinetin (0, 0.3, 0.5, 0.75, and 1) ppm. The cultures were incubated at room temperature 25-26^{0}C with 600 lux light intensity for 8 weeks. Each treatment was repeated three times (three bottles). Each bottle consists of one explant for crown and three explants for tiller. The observation parameters were formation and growth of callus including the time of first callus formation, morphology, and callus fresh weight.

RESULT AND DISCUSSION

Callus were induced from crown and tiller explant cultured on MS basal media supplemented with 2,4-D alone or combination of 2,4-D and kinetin. Callus started to form from the edge of explant and then followed by the entire of surface explant. These calli tend to be yellow to whitish yellow and transparent in color. Induction and growth of callus in vetiver is affected by explant types and concentration of kinetin in the medium. However, there are not interaction between explant type and kinetin concentration on formation and growth of callus.

The type of explant had significant effect on formation and growth of callus in vetiver. Callus formation from tiller explant were faster than callus formation from crown explant. Callus from tiller explant started to form a week after culture, while callus from crown explant were formed four weeks after culture. In addition of the faster callus formation, the callus growth from tiller explant was also better than crown explant (Fig. 1).

At 8 weeks after culture, the callus fresh weight from tiller explant was 0.35 ± 0.09 g, while the fresh weight callus from crown explant was only 0.16 ± 0.08 g (Fig. 2). It was shown by the distinct difference in callus growth from both of two type of explants, biomass of callus from tiller explant was higher twice than biomass of callus from crown explant.

Addition of 0.3-1 ppm kinetin in combination with 0.75 ppm 2,4-D did not significantly influence the formation and growth of callus. However, the addition of kinetin in the medium in combination with 0.75 ppm 2,4-D tended to increase callus growth. On the other hand, when the concentration of kinetin was higher than 0.5 ppm (0.75-1 ppm) callus growth began to decrease. Callus fresh weight on medium containing 2,4-D alone was only 0.17 ± 0.07 g, while on medium containing 0.75 ppm 2,4-D in combination with 0.3-1 ppm kinetin was 0.23 ± 0.13 – 0.28 ± 0.15 g. The addition of 0.5 ppm kinetin in the medium produced the highest callus fresh weight than the other treatments, it was 0.32 ± 0.12 g (Fig. 3).

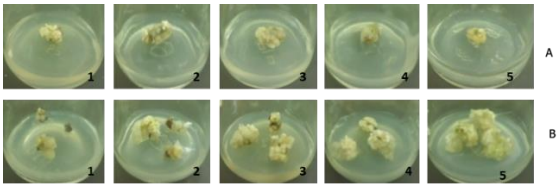

Figure 1. Formation and growth of callus from crown explant (above, A) and tiller explant (bottom, B) on MS basal medium supplemented with combination of 2,4-D 0.75 ppm and several kinetin concentrations.
Description: A1 and B1 without kinetin (control), A2 and B2 kinetin 0.3 ppm, A3 and B3 kinetin 0.5 ppm, A4 and B4 kinetin 0.75 ppm, A5 and B5 kinetin 1 ppm.

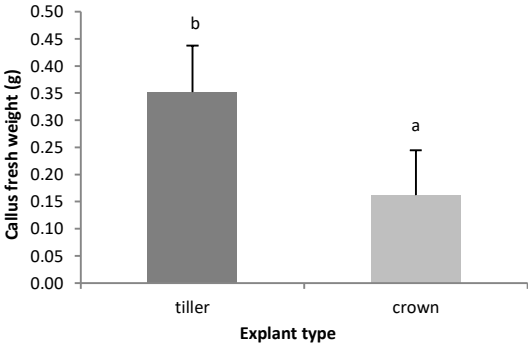

Figure 2. Effect of explant types on vetiver callus fresh weight at 8 weeks after culture.
Note: the same letter of each bar showed no significant on BNT test (α=0.05)

Increased fresh weight in medium supplemented with kinetin was quite large, however there was no significant difference in statistical analysis on biomass of callus on medium only containing 2,4-D alone. This is probably due to the very high variation of callus fresh weight on each treatment.

Some factors such as genotype of plant, explant source, basal medium, and growth regulator in the medium affect the formation and growth of callus [9]. Explant has important role on the success of callus initiation. Explant of

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young tissue generally more responsive than the oldest tissue [10]. Explant from meristematic tissue forms the callus faster than dorman tissue [11].

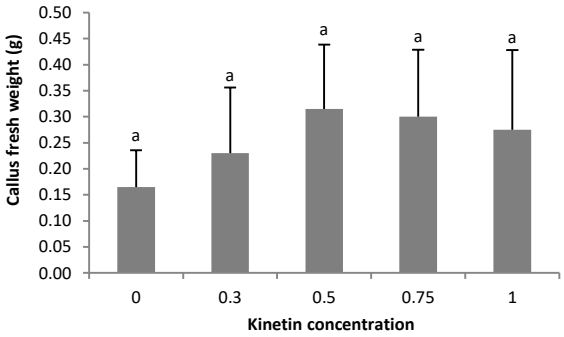

Figure 3. Effect of kinetin concentration in combination with 2,4-D 0.75 ppm on callus fresh weight at 8 weeks after culture.
Note: the same letter of each bar showed no significant on BNT test (α=0.05)

In *Trigonella foenum* Graecum L., the percentage of callus formation from hypocotyl explant was higher than cotyledon explant [12]. In the study [13], the highest percentage of callus formation in *Sphenostylis stenocarpa* Hochst. was formed from leaf and stem compared with root explant. Nodal explant showed better response on callus induction in *Citrus jambhiri* that better than leaf and root explant [14]. While the results of research [15], showed that callus growth in *Ricinus communis* from cotyledon explant was better than root explant.

The difference response of callus formation on each explant were caused by the different physiology condition of each explant. The success of callus formation is increasing with using young tissue as explant source. The tissue that growth actively on earlier of growth period is an excellent explant source, because the ability of the organ division process on the older tissue decreased [16]. According to previous study, the young tissue is generally proliferate easier than old tissue [17].

The success of the callus induction depends on the type and concentration of growth regulator. Callus formation was affected by auxin or combination between auxin and cytokinin. Kinetin is one of growth regulator which often combined with auxin to promote the callus formation [18]. Kinetin promotes the development of cell and affects the development of cell physiology [19]. The addition of kinetin could induce the callus from leaf explant in *Talinum paniculatum* [20] and shoot tip explant in *Shorgum bicolor* [21].

Some research results also showed that the callus formation was affected by explant type used and growth regulator in the medium. In *Vetiveria zizanioides*, the addition of 1 ppm kinetin combination in 2,4-D dan IAA produced callus from shoot base explant 47% [22], whereas 1 ppm kinetin promoted optimum callus growth 85% from axillar bud explant with combination in NAA [23]. The result of other research showed that the addition of 0.5 ppm kinetin in the medium in combination with 2,4-D produced callus 32.78% from leaf disc and 20% hypocotyl explant in tomato [24]. In addition to the type of explant, growth regulator also as important factor that affected the success of callus induction. On leaf explant in *Sauropus androgynous*, the addition of 1 ppm kinetin in combination with NAA in the medium resulted callus formation 20%, while the addition of 0.5 ppm kinetin promotes callus formation only 13% [25]. The addition of kinetin in combination with NAA promotes the callus formation from root explant in *Sphenostylis stenocarpa* Hochst. On root explant in *Sphenostylis stenocarpa* Hochst., the addition of 3 ppm kinetin in the medium produce the percentage of callus higher than 1.5 ppm kinetin, respectively 50% dan 16% [13].

CONCLUSION

Formation and growth of callus in vetiver were affected by explant type and kinetin concentration supplemented in the medium. The formation and growth of callus from tiller explant were faster and better than crown explant. In addition, the formation and growth of callus were also affected by kinetin in the medium. The addition of 0.3-1 ppm kinetin in combination with 0.75 ppm 2,4-D in the medium increased callus growth. However, higher concentration of kinetin, which were 0.75-1 ppm, the callus growth started to decrease.

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