Effect of Plant Growth Regulator and Explant Types on *in vitro* Callus Induction of *Gynura procumbens* (Lour.) Merr

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Abstract
Gynura procumbens (Lour.) Merr is a medicinal plant of Asteraceae. The plant is a potent compound as pharmaceutical raw material which can be increased through plant tissue culture. This study aimed to determine the effect of various combinations of plant growth regulator and explant types on induction callus. The explants used were leaf, stem node, stem internode and petiole while the plant growth regulator used were 0.1 mg/L 2,4-D and 0.1 mg/L BAP, 0.5 mg/L 2,4-D and 1.0 mg/L Kinetin, 0.5 mg/L NAA and 0.5 mg/L BAP, 5.0 mg/L 2,4 D and 0.5 mg/L BAP, 0.1 mg/L 2,4-D and 0.1 mg/L IAA. The explants were cultured in MS medium supplemented with 30% sucrose and 8% agar for 28 days.

The results of this study indicated that the treatment of 0.5 mg/L NAA and 0.5 mg/L BAP on the petiole explants was the best combination of plant growth regulators to produce the highest callus fresh and dry weights (1478.1 mg and 40.0 mg respectively). Callus derived from leaf, petiole and internode explants was friable and compact in texture while node explant-derived callus was compact in texture.

Keywords: *Gynura procumbens,* callus induction, plant growth regulator, explants type.

Introduction

One of the potential plants as pharmaceutical raw materials in Indonesia is *Gynura procumbens*. *G. procumbens* is an important plant of Asteraceae and widely used in Southeast Asia especially in Indonesia, Malaysia and Thailand[1]. The plant is used as a medication for fever, rash, kidney disease, migraine, constipation, hypertension, diabetes mellitus and cancer[2]. *G. procumbens* has several bioactive compounds such as flavonoids, saponins, alkaloids, tannins, terpenoids and glycoside sterols[3]. Flavonoids have many benefits, especially in health such as antioxidants, antiaging, anticancer, anti-inflammatory, cardiovascular, stroke and asthma[4-9].

Plant growth regulators such as auxin and cytokinin are the most important supplements to regulate the growth and development in plant tissue and organ culture[10,11]. Modification of plant growth regulator can increase biomass and secondary metabolite accumulation[12]. Callus cultured from leaf explants of *G. procumbens* in MS medium supplemented with sucrose, erythrosa-4-phosphate and phenylalanine contained flavonoids[13] but callus biomass produced from leaf explants was still low, so in this study we want to optimize plant growth regulators and explant types to induce callus of *G. procumbens* on *in vitro* culture.

Material and Methods

Material: *G. procumbens* was obtained from Purwodadi Botanical Gardens, East Java, Indonesia. The plant has been identified and confirmed by Purwodadi Botanical Garden, Indonesian Institute of Sciences, Pasuruan, East Java, Indonesia.

Callus induction: Leaf, internode, node and petiole of *G. procumbens* were washed with detergent solution and rinsed in running water, then sterilized with 10% v/v chlorox for leaf and petiole and 20% chlorox v/v for internode and node during 5 minutes and rinsed with sterile distilled water three times. Leaf explant was cut ± 2 cm^2 while internode, stem nodes and petiole explants were cut 0.5-1 cm and inoculated in MS medium[14] supplemented with 30 g/L sucrose, 8 g/L agar and various combinations of plant growth regulator: 0.1 mg/L 2.4-dichlorophenoxy acetic acid (2.4-D) and 0.1 mg/L 6-benzyl amino purine (BAP), 0.5 mg/L, 2.4-D and 0.1 mg/L kinetin (Kn), 0.5 mg/L of naphthalene acetic acid (NAA) and 0.5 mg/L BAP, 5.0 mg/L 2.4-D and 0.5 mg/L BAP, 0.1 mg/L 2.4-D and 0.1mg / L indole-3-aceticacid (IAA). Cultures were incubated at 25±3°C under light of 320 lux for 28 days. Fresh weight, dry weight and morphology of callus were observed at the end of cultivation.

Results and Discussion

The addition of various combinations of plant growth regulators on leaf explants showed that the addition of 0.1 mg/L 2, 4-D and 0.1 mg/L BAP was the best treatment compared to the others. The treatment could induce 144.6 mg fresh weight and 5.0 mg dry weight of callus. All various combinations of plant growth regulators on leaf explant formed callus, but the treatment of 0.1 mg/L 2, 4-D and 0.1 mg/L IAA cannot induced callus (Table 1).

Combination of 0.1 mg/L 2.4-D and 0.1 mg/L BAP could produce high callus fresh weight and all explants formed callus. This result was also show at *Centella asiatica* (L.)[15], *Achyranthus aspera* L.[16] and *Glinus lotoides* (L)[17]. The effects of combination of 2.4-D and BAP play an important role as growth regulators in plant and have a remarkable

effect on the percentage of callus induction compared to the other combinations. The interaction effect of 2.4-D and BAP on callus induction of *Tridax procumbens* has also been reported[18].

The addition of 0.5 mg/L NAA and 0.5 mg/L BAP on *G. procumbens* internode explants obtained the highest callus biomass. The biomass reached 581.5 mg fresh weight and 15.3 mg dry weight, but the lowest callus biomass (dry weight) was obtained on treatment of 0.5 mg/L 2.4-D and 0.1 mg/L kinetin (Table 2).

Internode explant-derived callus with a combination of 0.5 mg/L NAA and 0.5 mg/L BAP was the best combination to produce fresh and dry weight. Similar result was shown in *Cucumis sativus* (L.)[19] in which the treatment of 1.0 mg/L NAA and 0.5 mg/L BAP induced 89% callus from stem explants. The best result of callus induction from *Rauvolfia serpentine* stem explants with the addition of various concentrations of NAA and BAP was 80% while in *Catharanthus roseus* stem explant was 85% [20]. Kumlay and Ercisli[21] reported that combination of NAA and BAP produces callus induction 87.5% from node and leaf explants of *Solanum tuberosum* L. The combination of NAA and BAP could also produce 95% callus induction with 4.75 g fresh weight and 2,45 g dry weight from *Tinospora formanii* node explants[22].

The same result was reported by Jan et al.[23] The addition of NAA and BAP on MS media produced 100% callus induction from internode explants of *Ajuga bracteosa*. Malayaman et al[24] reported that the addition of NAA and BAP resulted in 60% callus induction of *Phyllanthus debilis* from internode explants. The application of balanced combination of auxin and cytokinin on the media is a factor in controlling cell division in tissue culture.

The five different treatments of plant growth regulator combinations on callus induction from *G. procumbens* node explants obtained various results. The best result of callus induction was on the combination of 0.5 mg/L 2.4-D and 1 mg/L kinetin reached 415.8 mg fresh weight and 18.3 mg dry weight, but lowest callus biomass was shown in treatment of 5.0 mg/L 2.4-D and 0.5 mg/L that produced 91.0 mg fresh weight and 4.9 mg dry weight (Table 3).

Treatment of 0.5 mg/L 2.4-D and 1.0 mg/L Kn from node explants obtained the highest fresh weight and dry weight. The same response was shown at *Crescentia alata*[25], *Parkia biglobosaceae* stem explants[26], endosperm explants of *Barringtonia racemosa* L.[27] and node explants of *Rauvolfia serpentina*[28]. Callus induction from petiole explants produced highest fresh weigth and dry weight on medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP (Table 4).

Callus induction from petiole explants with various concentrations of plant growth regulators obtained the best results on fresh weight, dry weight and percentage of callus. Similar result was shown in *O. tamineus* petiole explants[29,30], *Rhodiola imbricate* leaf explants[31], leaf explants of *Glinus lotoides*[17], leaves and stems explants of *Artemisia annua* L.[32] However, the addition of NAA and BAP could only produce 91.6% callus induction in shoot explants of *Ipomoea obscura* L.; this result was lower than leaf explants that reached 96.9%[33]. The different results indicated that the different types of plant tissue had different responses in callus induction[34]. Based on the results of this study, the petiole is very potential as an explant source of *in vitro* propagation of *G. procumbens.*

Based on the best result from each treatment on different explants it was shown that the highest production of callus biomass was obtained on petiole explants in medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP which produced callus 1478.1 mg fresh weight and 40.0 mg dry weight. Leaf explants showed the lowest production of callus biomass (Figure 1).

Morphology of the callus from different explants type can be seen in figure 2. Callus from leaf explants was white color and green color; white callus has friable texture while green callus has compact texture (Figure 2A-B). White and green color with friable and compact texture were also shown in internode explants (Figure 2C-D) while in stem node explants callus was yellowish color with compact texture (Figure 2E-F). Petiole explant-derived callus on MS medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP had white in color and friable (Figure 2G) while the addition of 0.5 mg/L 2.4-D and 1.0 mg/L gave green and yellowish compact calli (Figure 2H).

The effect of combination of plant growth regulators on callus morphology varied. The addition of 2.4-D and BAP in *G. procumbens* leaf explants resulted in white friable and green compact calli. This was similar with the study of Mungole et al[33] that the combination of 2.4-D and BAP obtained white friable callus in leaf explants of *Ipomoea obscura* (L.) and brown compact callus in the treatment of 2.4–D in various concentrations. The combination of NAA and BAP obtained green compact callus in *G. procumbens* leaf explants. Similar result was also reported by Elangomathavan et al[29] that the addition of a balanced concentration of NAA and BAP produced green compact callus of *O. stamineus*. However, the addition of high concentration of NAA and BAP produced yellowish white compact callus in leaf explants of *Dianthus caryophyllus* L.[35]

The addition of 0.5 mg/L NAA and 0.5 mg/L BAP in *G. procumbens* internode explants produced white friable and green compact calli. The addition of the same plant growth regulator and explant type in different plant produced green compact callus, but when the concentration of auxin was enhanced, the light green and greenish white friable calli were formed[29]. Besides, the combination of 2.4-D and Kn

could induce brownish white compact callus in *G. procumbens* internodus.

However, the addition of 2.4-D and Kn in node explants of *Ipomoea obscura* (L.) produced greenish white friable callus[28], reported in *Thymus persicus* callus induction as well[36]. It indicated that different plant growth regulators and tissue types led to different responses in callus morphology.

Callus induction of *G. procumbens* petiole explants on MS medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP produced white friable callus. However, the addition of NAA and BAP in petiole explant produced greenish white friable callus whereas different concentrations between NAA and BAP produced light green friable callus[36]. The addition of a balanced combination of NAA and BAP produced light green compact callus[37]. Color of callus can change because chlorophyll is altered due to reaction among endogenous and exogenous hormones, explant sources and environmental culture conditions such as temperature and light exposure.[38]

Table 1
Effect of plant growth regulators on callus induction of *G. procumbens* from leaf explants

Plant growth regulator (mg/L)					Leaf explants		
2.4-D	**IAA**	**NAA**	**BAP**	**Kn**	**Fresh weight (mg)**	**Dry weight (mg)**	**% Explants formed callus**
0.1	-	-	0.1	-	144.6±0.00^{c}	5.0±0.00^{c}	100%
0.5	-	-		1.0	59.8±0.00^{b}	3.0±0.00^{b}	100%
-	-	0.5	0.5	-	64.8±0.03^{b}	4.4±0.00^{bc}	100%
5.0	-	-	0.5	-	61.5±0.02^{b}	3.3±0.00^{b}	100%
0.1	0.1	-	-	-	0.0±0.00^{a}	0.0±0.00^{a}	0%

Mean values within a column followed by the same letters are not significantly different at p=0.05 according to Duncan's Multiple Range Test

Table 2
Effect of plant growth regulators on callus induction of *G. procumbens* from internode explants

Plant growth regulator (mg/L)					Internode explants		
2.4-D	**IAA**	**NAA**	**BAP**	**Kn**	**Fresh weight (mg)**	**Dry weight(mg)**	**% Explants formed callus**
0.1	-	-	0.1	-	125.3±0.03^{a}	9.3±0.00^{c}	100%
0.5	-	-		1.0	137.3±0.04^{a}	5.4±0.00^{a}	100%
-	-	0.5	0.5	-	581.5±0.10^{c}	15.3±0.00^{e}	100%
5.0	-	-	0.5	-	153.4±0.14 ^{a}	7.6±0.00^{b}	100%
0.1	0.1	-	-	-	376.9±0.10^{b}	12.7±0.01^{d}	100%

Mean values within a column followed by the same letters are not significantly different at p=0.05 according to Duncan's Multiple Range Test

Table 3
Effect of plant growth regulators on callus induction of *G. procumbens* from stem node explants

Plant growth regulator (mg/L)					Stem node explants		
2.4-D	**IAA**	**NAA**	**BAP**	**Kn**	**Fresh weight (mg)**	**Dry weight (mg)**	**% Explants formed callus**
0.1	-	-	0.1	-	93.8±0.01^{a}	4.8±0.00^{a}	100%
0.5	-	-		1.0	415.8±0.04^{c}	18.3±0.00^{c}	100%
-	-	0.5	0.5	-	287.9±0.07^{b}	8.2±0.00^{b}	100%
5.0	-	-	0.5	-	91.0±0.03^{a}	4.9±0.00^{a}	100%
0.1	0.1	-	-	-	304.9±0.03^{b}	8.3±0.00^{b}	100%

Mean values within a column followed by the same letters are not significantly different at p=0.05 according to Duncan's Multiple Range Test

Table 4
Effect of plant growth regulators on callus induction of *G. procumbens* from petiole explants

Plant growth regulator (mg/L)					Petiole explants		
2,4-D	**IAA**	**NAA**	**BAP**	**Kn**	**Fresh weight (mg)**	**Dry weight (mg)**	**% Explants formed callus**
0.1	-	-	0.1	-	52.2±0.03[a]	2.8±0.00[b]	100%
0.5	-	-		1.0	196.5±0.11[a]	8.1±0.00[b]	100%
-	-	0.5	0.5	-	1478.1±0.41[b]	40.0±0.00[c]	100%
5.0	-	-	0.5	-	164.4±0.01[a]	6.8±0.00[b]	100%
0.1	0.1	-	-	-	113.2±0.06[a]	6.9±0.00[a]	100%

Mean values within a column followed by the same letters are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

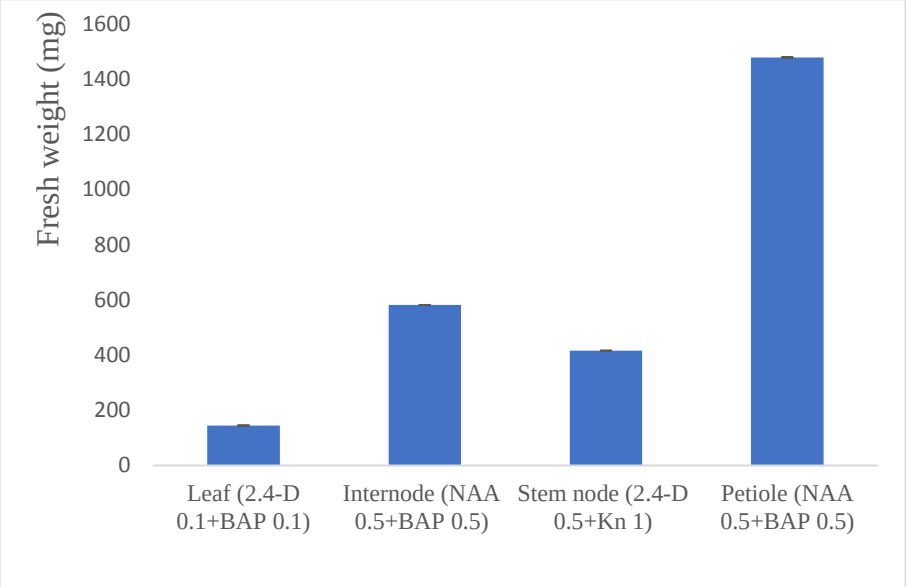

Figure 1: Comparison of the best result (fresh weight) from each treatments (combination of growth regulators) on different explants

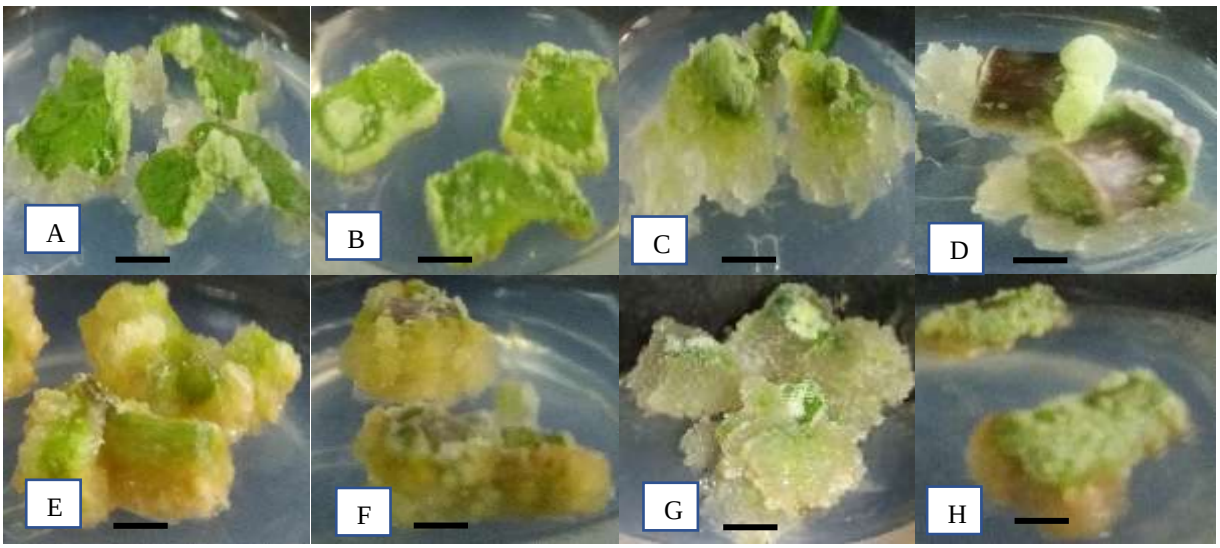

Figure 2: Morphology of *G. procumbens* calli in different growth regulator and explants type after 28 days culture periode; (A-B) leaf explants, (C-D) internode explants, (E-F) stem node explants and (G-H) petiole exlants. Bar = 3 mm

Conclusion

Various concentrations of plant growth regulators (2,4-D, IAA, NAA, BAP and kinetin) affected callus induction in leaf, internode, stem node and petiole explants of *Gynura procumbens*; of the four combinations of plant growth regulators and different types of explants, petiole explants that were cultured in MS medium supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP produced highest biomass. Callus derived from leaf, internode, stem node and petiole explants were friable and compact while node explant-derived callus was compact.

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(Received 18th December 2018, accepted 11th June 2019)