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Efficient Callus and Shoot Induction Protocol from Leaf and Node Explants of Javanese Ginseng (*Talinum paniculatum* (Jacq.) Gaertn.)

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Abstract **Original Research Article**

This present study was carried out to determine an efficient and reproducible callus and shoot induction protocol of *Talinum paniculatum* by using leaf and node explants. Explants were cultured in MS medium supplemented with a combination and concentration 2,4-D and kinetin (0, 1, 2, 3 mgL^{-1}) in single or combination treatment for 14 days. The best response of callus and shoot induction (100%) and the fastest callus (6,0 ± 2,31 days) and shoot induction (5,0 ± 1,15 days) from leaf explants were obtained from MS medium supplemented with (1 mgL^{-1} 2,4-D and 1 mgL^{-1} kinetin) and (3 mgL^{-1} 2,4-D and 1 mgL^{-1} kinetin) respectively. In the case of node explants, none of calli were produced, whereas the best response of shoot induction (100%) and the fastest shoot induction (4,0 ± 0 days) were obtained from MS medium supplemented with 3 mgL^{-1} kinetin. Callus produced from leaf explant has a bright yellow color and friable texture. The optimized protocol of callus and shoot induction for *Talinum paniculatum* plant could be used effectively for mass propagation to fulfill the demand of the pharmaceutical industry as well as for its cultivation.

Keywords: Callus, 2,4-D, explants, kinetin shoot, *Talinum paniculatum*.

INTRODUCTION

Plant tissue culture is one of the powerful tools in plant biotechnology approach for clonal mass propagation using relatively small pieces of plant (explant), multiplication of disease-free plantlets throughout the years without seasonal dependence, production of plantlets with a somaclonal variation that have better stress tolerance, and production of certain secondary metabolites which qualitatively and quantitatively sustainable under aseptic and controllable condition (Kumar & Reddy, 2011; Kumlay & Ercisli, 2015). Callus and shoot induction from explant is an efficient way to accelerate the multiplication and regeneration process of plantlets through micropropagation (Dalila *et al.*, 2013; Kumlay & Ercisli, 2015)

The type of explant and plant growth regulators are the two most factors that influenced efficient callus and shoot induction from explants. Young leaf and node explants are the type of explants frequently used in callus and shoot induction. Young leaf explant consists of competent cells in parenchymatous tissue which actively undergoes cell division, while node explant also contains axillary meristematic tissue, thus it will be more responsive towards callus and shoot induction (Dalila *et al.*, 2013; Kumar & Reddy, 2011; Kumlay & Ercisli, 2015). 2,4-Dichlorophenoxyacetic acid (2,4-D) is a common synthetic auxin used in plant tissue culture to induce callus growth from explant due to its function to dedifferentiate cells which stimulate actively cell division and proliferation. Kinetin is one of the cytokinin groups mostly used to stimulate cell division, cell differentiation, and shoot induction (Dalila *et al.*, 2013; Habibah *et al.*, 2017; Handayani *et al.*, 2021; Kumlay & Ercisli, 2015). A combination of 1,5 mgL^{-1} 2,4-D and 0,5 mgL^{-1} in MS culture medium using *Barringtonia racemose* endosperm explant resulted in 100% callus induction percentage, whereas 93,33% of callus induction percentage resulted from leaf explant in MS medium supplemented with 2,0 mgL^{-1} 2,4-D (Dalila *et al.*, 2013). Sakpere, *et al.*, (2014) also reported a combination of 5,0 mgL^{-1} 2,4-D and 0,1 mgL^{-1}kinetin produced 60% callus induction on *Telfairia occidentalis* leaf explants, whereas nodal explants produce less percentage callus induction (20%). Another research also reported the combination

of kinetin 2,0 mgL^{-1}kinetin and 0,25 mgL^{-1} NAA resulted in 67,50% shoot regeneration percentage from *Solanum tuberosum* nodal-derived callus (Kumlay & Ercisli, 2015). These results suggested that optimum callus and shoot induction is strongly influenced by the combination of PGR at certain concentrations and different type of explants.

Recent developments in plant tissue culture have clearly shown its contribution to the improvement of propagation and secondary metabolites production from various medicinal important plants, including *Talinum paniculatum*. In Indonesia, *Talinum paniculatum* (Jacq.) Gaertn. or commonly known as Ginseng Jawa is an important herbaceous perennial plant which also often consumed as a leafy vegetable. This plant belongs to the Talinaceae family (Lakitan *et al.*, 2021; Silalahi, 2022). It contains various bioactivity such as antioxidant, antibacterial, antifungal, and aphrodisiac. This plant is also rich in secondary metabolites such as flavonoids, saponin, tannin, and steroids (Cerdeira *et al*., 2020; Gamage *et al*., 2017; Lestario *et al*., 2009; Menezes *et al*., 2021; Silalahi, 2022; Yeni *et al*., 2022). Various bioactive compounds contained in all parts of *T.paniculatum* brings many health benefits to cultivating this plant.

Generally, *T.paniculatum* was propagated by stem cutting and seed. However, these propagation techniques were less efficient due to the slow growth of the root from a stem cutting, limited seed availability, seed dormancy, and low germination (A. Yachya & Y.S.W. Manuhara, 2015; Seswita, 2010). Considering the growing demand for *T.paniculatum* plant extract in the pharmaceutical industry and the substitution of expensive importing Korean ginseng, there is an urgent need to develop an efficient protocol for *T.paniculatum* micropropagation through callus and shoot induction. Previous studies focused on *T.paniculatum* in vitro culture have been carried out. Manuhara *et al*., (2015) established adventitious root culture from leaf explant and increased saponin content using 2,0 mgL^{-1} IBA in a liquid MS medium. (Solim & Manuhara, 2017) also reported the effect of 2 ppm IBA in the liquid and solid medium could induce root and shoot induction percentage (100%) from stem explant. Restiani *et al*., (2022) also studied the effect of methyl jasmonate elicitation on saponin accumulation from callus culture. However, the optimization of PGR and type of explants on efficient callus and shoot induction protocol of *Talinum paniculatum* is still limited. Therefore, the aim of this present study was to determine an effective callus and shoot induction protocol from leaf and nodal explants in MS medium supplemented with different combinations and concentrations of 2,4-D and kinetin (0, 1, 2, 3 mg L^{-1}) in single and combination treatments. This established protocol will benefit the effective cultivation as well as the supply of bioactive compounds for the pharmaceutical industry.

MATERIALS AND METHODS

Preparation of plant material

Talinum paniculatum was obtained from the laboratory of Biotechnology, Duta Wacana Christian University. Young, healthy, green, and disease-free of *T.paniculatum* mother plant was used as a source of explant. Plant specimen determination was conducted at Plant Systematic Laboratory, Biology Faculty, Gadjah Mada University, Yogyakarta.

Preparation of culture medium

Medium culture used in this study was Murashige and Skoog (MS) with modification (Murashige & Skoog, 1962). In order to determine the protocol of an efficient callus and shoot induction from different explants (leaf and node), MS medium was supplemented with different combinations and concentrations of plant growth regulators 2,4-D (0, 1, 2, 3 mgL^{-1}) and kinetin (0, 1, 2, 3 mgL^{-1}) was supplemented in MS medium (Table 1). Into the MS medium, added 0,1 g Myo-inositol, 30 g/L sucrose then adjusted the pH of the medium at 5,7 – 5,8 using HCl or NaOH 0,1M. Subsequently, added 0,48 g gelling agent into the medium, homogenized, and pour into a culture bottle. A solid MS medium was sterilized using an autoclave at 121^{o}C for 30 minutes (Natasha & Restiani, 2019; Restiani *et al*., 2022).

Table 1: Various combinations and concentrations of plant growth regulators

Plant growth regulators (mg L^{-1})		Treatment Code
2,4-D	Kinetin	
0	0	T0
1	0	T1
2	0	T2
3	0	T3
0	1	T4
0	2	T5
0	3	T6
1	1	T7
1	2	T8
1	3	T9
2	1	T10
2	2	T11
2	3	T12
3	1	T13
3	2	T14
3	3	T15

Culture establishment

Explants used in this study were leaves and nodes. Young leaves and nodes explants used in this study were the second and third leaves and nodes from shoot tip. Subsequently, explants were pra-sterilized by washing under the running tap water and rinsed using liquid detergent (3%) (v/v) and 3 drops of tween 80 for 45 s, then washed using sterilized aquadest thrice until the explant surface was clean. Further, clean explants were sterilized inside the Laminar Air Flow by

immersing them with 70% alcohol (v/v) for 3 min, then washed 3 times until no remaining alcohol was on the surface of the explant. Sterilized leaves (0,5 - 1 cm) and nodes (2 -3 cm) explants were inoculated into the MS medium supplemented with various combinations and concentrations of 2,4-D and kinetin. The culture was maintained at an incubation room with a relatively stable temperature of 25^{o}C ± 2 ^{o}C, 24 h light, and light intensity 3000 lux (Natasha & Restiani, 2019; Restiani *et al*., 2022). At the end of 14 days of culture, callus induction percentage (Equation 1) and shoot induction percentage (Equation 2) were calculated as follow:

$$\text{Callus Induction \%} = \frac{\text{Number of explants callusing}}{\text{Total number of explants in the culture}} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{Shoot Induction \%} = \frac{\text{Number of shoot regenerated from explants}}{\text{Total number of explants in the culture}} \times 100 \quad \ldots\ldots (2)$$

(Hemmati *et al*., 2020)

Data analysis

All experiments were carried out in Completely Randomized Design (CRD) (Table 1). Data were collected from three replicates and presented as mean ± standard deviation. All data were analyzed descriptively.

RESULTS AND DISCUSSION

Callus and shoot induction stages of leaf and nodes explants

Callus and shoot growth stages from leaf and node explants were initiated within 4 to 14 days and 6 to 14 days after culture, respectively. Callus growth is initiated by swelling of the explant and followed by callus growth at the cut surface of the leaf explants after 10 days of culture. Callus is fully formed at the leaf surface after 14 days of culture (Fig. 1a-d). It is suggesting that wounding of explant stimulates cell dedifferentiation and proliferation to form callus. According to Ikeuchi *et al*., (2013) and Iwase *et al*., (2011), wounding of explant during excision acts as an external signal that triggered the activation of transcription factors WIND1 (Wound Induced Dedifferentiation 1) which in turn upregulated genes encoding cytokinin to induce callus formation. Callus was formed as a mechanism to heal the wound site of explants.

In the beginning, callus has light green in color and friable in texture. After 14 days of culture, the color of callus turned yellowish-green with a friable texture. According to Sari *et al*., (2018), variation in callus color is influenced by pigmentation, type of explant, and influence of light. Green color of callus is caused by the development of chlorophyll pigment inside the cells, while the yellowish-green callus indicates the degradation of chlorophyll pigment in callus. Kinetin is one of the cytokinin hormones that promote chlorophyll formation. This result is in line with results reported by Sari *et al*., (2018) that showed green callus formed from *Myrmecodia tuberosa* cotyledon explant after 2 weeks of culture.

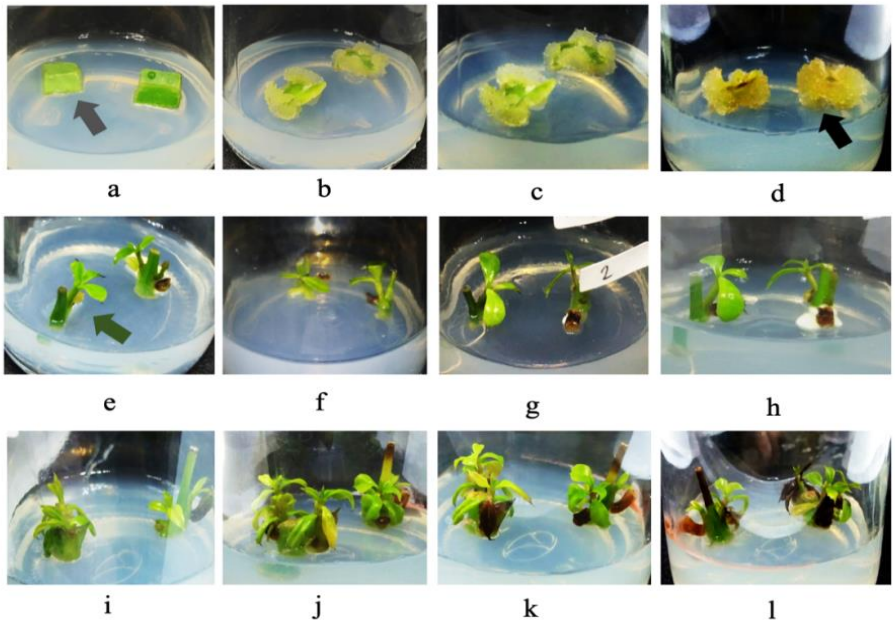

Figure 1: Callus induction stages of *Talinum paniculatum* leaf explant on MS medium containing 2 mgL^{-1} kinetin and 2 mgL^{-1} 2,4-D at 4, 10, 12 and 14 days of culture (a-d), shoot induction stages of *Talinum paniculatum* stem node explant on PGR- free media (e-h) and, MS media containing 3 mg L^{-1} kinetin at 6, 10, 12 and 14 days of culture (i-l). Notes: Grey arrow: leaf explant, black arrow: bright yellow friable callus, and dark green arrow: nodes-derived shoot

Callus begin to form after explant swelling and continued with the formation of callus at the edge of explant. The increasing size of the explant after swelling was presumably caused by the absorption of nutrients from the medium. Callus of Javanese ginseng leaf explants has a friable texture marked by the loose cell interaction thus callus cells could easily be separated. The addition of 2,4-D either in single or combination treatment with kinetin produced friable callus. The addition of auxin 2,4-D stimulates cell elongation by increasing cell wall plasticity which caused the cell wall to become loose and increases the water adsorption into the inner cell through osmotic pressure. Thus, friable callus also contained much water and doesn't have lignified cell wall yet. This result is also in line with Sari *et al*., (2018), which reported the addition of 2,4-D and kinetin in MS medium affected the production of friable callus texture of ant nest (*M.tuberosa*) after one month of culture. According to Purnamaningsih (2006), callus texture usually determines the regeneration capacity to induce shoot and root formation. The friable callus has a higher regeneration capacity to form a shoot than the compact callus. These results indicated that the addition of 2,4-D and kinetin into MS medium influenced the color and texture of the callus.

Shoot induced from nodes explant within 6 days after inoculation in PGR-free medium and medium supplemented with 3 mg L^{-1} kinetin (Fig 1e-l). Shoot induced at the excision site in Javanese ginseng of nodes explant. The same result was also reported by Yusniwati *et al*., (2021) that shoot emerged from axillary meristem located in excised nodes explants in 9,3 days in MS medium supplemented with 0,3 ppm BA. These results indicated that wounding during the excision of explants also stimulates the regeneration of tissue or organ including shoot and root (Ikeuchi *et al*., 2013). In addition, the interaction of exogenous cytokinin added in the medium with the activity of axillary meristem already contained in nodes explant could stimulate cell proliferation and regeneration into new shoots more quickly (Yusniwati *et al*., 2021).

Effects of plant growth regulators and type of explant on callus formation

Leaf explant showed a positive response in callus induction from all medium supplemented with PGRs, except for the PGR-free medium did not show any callus induction (Table 2). Callus induction time from leaf explant ranged from 5,3 ± 2,31 - 10,0 ± 0 days. The fastest callus induction time (5,3 ± 2,31 days) from leaf explant resulted from MS medium supplemented with 3 mgL^{-1} 2,4-D and 3 mgL^{-1} kinetin, whereas the slowest (10,0 ± 0 days) callus induction time resulted from MS medium supplemented with 1 mg L^{-1} kinetin. Furthermore, node explant also showed a positive response in callus induction from all medium supplemented with PGRs, including in PGR-free medium (Table 2). Callus induction from node explant ranged from 4,0 ± 0 - 8,0 ± 2,83 days. Node explant needs minimum time for callus induction within 4,0 ± 0 days in PGR free-MS medium (control) and MS medium supplemented with 2 mg L^{-1} 2,4-D and 1 mg L^{-1} kinetin, while the slowest callus induction (8,0 ± 2,83 days) resulted from MS medium supplemented with 1 mg L^{-1} 2,4-D and 2 mg L^{-1} kinetin. The addition of only 2,4-D (1 – 2 mgL^{-1}) in medium without kinetin and 3 mgL^{-1} kinetin in medium without 2,4-D showed a negative response as none of the calli were induced from node explants. In contrast, the addition of only 2,4-D in a medium without kinetin showed faster callus induction time in leaf explants. Callus induction of *T.paniculatum* leaf and nodes explants in MS medium supplemented with a combination of 2,4-D and kinetin relatively faster than other types of explants in the same MS medium containing a combination of PGRs. Shirsat *et al*., (2021) reported that callus induction of *Caesalpinia bonducella* stem internode ranged from 6 – 9 days after culture in MS medium supplemented with (1 – 3 mgL^{-1}) 2,4-D, while in medium supplemented with (2 - 3 mgL^{-1}) kinetin showed longer callus induction (12 days) from the same explant type. Sari *et al*., (2018) also reported callus induction time from different types of explants (cotyledon, stem, tuber, and root) at 14 days of culture (two weeks) in MS medium supplemented with 0,5 – 2 mg L^{-1} 2,4-D and 0 – 2 mg L^{-1} kinetin. In addition, leaf sheath explant of *Saccharum officinarum* L. cultured in MS medium containing (1, 2,5 and 5 gL^{-1}) 2,4-D showed slower callus induction time within 11,60 ± 0,24 – 21,20 ± 0,20 days (Inderiati *et al*., 2021). Furthermore, Habibah *et al*., (2021) reported faster callus induction time (16 days) from *Dioscorea esculenta* tubers occurred in MS medium supplemented with 0.5 ppm 2,4-D and 0.5 ppm kinetin. These results suggested that callus induction time is strongly influenced by a different type of species, type of explant, and the interaction of endogenous hormones and exogenous PGRs added in the medium. Based on the results, 2,4-D and kinetin are the best combination of PGR used in callus induction due to its higher callogenic activity than others (Carsono *et al*., 2021; Dalila *et al*., 2013; Sari *et al*., 2018).

Data in Table 2 and Figure 2A showed the highest callus induction percentage (100%) from leaf explant resulted in MS medium supplemented with (1 – 2 mgL^{-1}) 2,4-D , 1 mgL^{-1} 2,4-D + 1 mgL^{-1} kinetin, and (2 - 3 mgL^{-1}) 2,4-D + (1 - 2 mgL^{-1}) kinetin, while the lowest callus induction (25%) resulted in medium containing (1 – 2 mgL^{-1}) kinetin without 2,4-D. Meanwhile, the highest callus induction percentage (100%) from nodes explants resulted in MS medium supplemented with 1 mgL^{-1} 2,4-D + 1 mgL^{-1} kinetin, 2 mgL^{-1} 2,4-D + 2 mgL^{-1} kinetin, 2 mgL^{-1} 2,4-D + 3 mgL^{-1} kinetin, and 3 mgL^{-1} 2,4-D + 1 mgL^{-1} kinetin, while nodes explants did not show any callus induction in MS

medium supplemented with 3 mgL^{-1} kinetin without 2,4-D. This study showed similar results with callus induction percentage from *Barringtonia racemosa* leaf explant cultured in MS medium fortified with (0,5 – 2 mgL^{-1}) 2,4-D without kinetin resulted in the highest callus induction percentage (83 – 93%) (Dalila *et al*., 2013). Furthermore, Sakpere *et al*., (2014) also reported that leaf and node explants of *Telfairia occidentalis* showed different responses on callus induction percentage. Leaf explants of *T.occidentalis* showed the highest callus induction percentage (60%) in MS medium supplemented with 2 mgL-1 2,4-D + 5 mgL^{-1} kinetin and 5 mgL^{-1} 2,4-D + 0,1 mgL^{-1} kinetin, whereas nodal explants of *T.occidentalis* showed the highest callus induction percentage (60%) in MS medium supplemented with 5 mgL^{-1} 2,4-D + 3,3 mgL^{-1} kinetin. Based on results suggested that the addition of 2,4-D and kinetin at the lower concentration on the medium gave the best response of callogenesis in leaf explants. It might be due to the effect of 2,4-D (auxin) to increase cell division, causing auxin-sensitive cells that are not dividing to enter S phase and undergoes mitosis, whereas kinetin (cytokinin) stimulates cell division and proliferation (Bong *et al*., 2021). Among all treatments, leaf explants produced more callus induction with the highest percentage (100%) from explants (7 of 15 treatments) than nodes explants (4 of 15 treatments). This result was similar to the previous study by Sánchez-Ramos *et al*., (2022) who reported that leaf explants of *Artemisia ludiviciana* produced more callus induction than nodal explants. These results suggested that leaf explants were more callogenic than node explants.

Table 2: Effects of plant growth regulators on callus induction time from leaf and nodes explants of *T.paniculatum* (Culture medium: MS, culture period: 14 days)

Type of Explant	Plant Growth Regulators (mgL^{-1})		Callus Induction Time (Day)
	2,4-D	KIN	
Leaf	0	0	-
	1	0	7,0 ± 1,15
	2	0	7,0 ± 1,15
	3	0	6,0 ± 0
	0	1	10,0 ± 0
	0	2	8,0 ± 0
	0	3	6,0 ± 0
	1	1	6,0 ± 2,31
	1	2	6,0 ± 0
	1	3	8,7 ± 3,06
	2	1	9,5 ± 1,00
	2	2	7,0 ± 1,15
	2	3	9,3 ± 1,15
	3	1	7,5 ± 3,00
	3	2	7,0 ± 1,00
	3	3	5,3 ± 2,31*
Nodes	0	0	4,0 ± 0*
	1	0	-
	2	0	-
	3	0	6,0 ± 0
	0	1	6,0 ± 0
	0	2	8,0 ± 0
	0	3	-
	1	1	6,0 ± 0
	1	2	8,0 ± 2,83
	1	3	6,0 ± 0
	2	1	4,0 ± 0*
	2	2	7,5 ± 1,91
	2	3	7,5 ± 1,00
	3	1	5,0 ± 1,15
	3	2	6,0 ± 0
	3	3	6,0 ± 0

Notes: Results are presented as means ± standard deviation (SD) (Number of replication of each treatment: 4) (-) none of callus induced (*): the fastest callus induction time

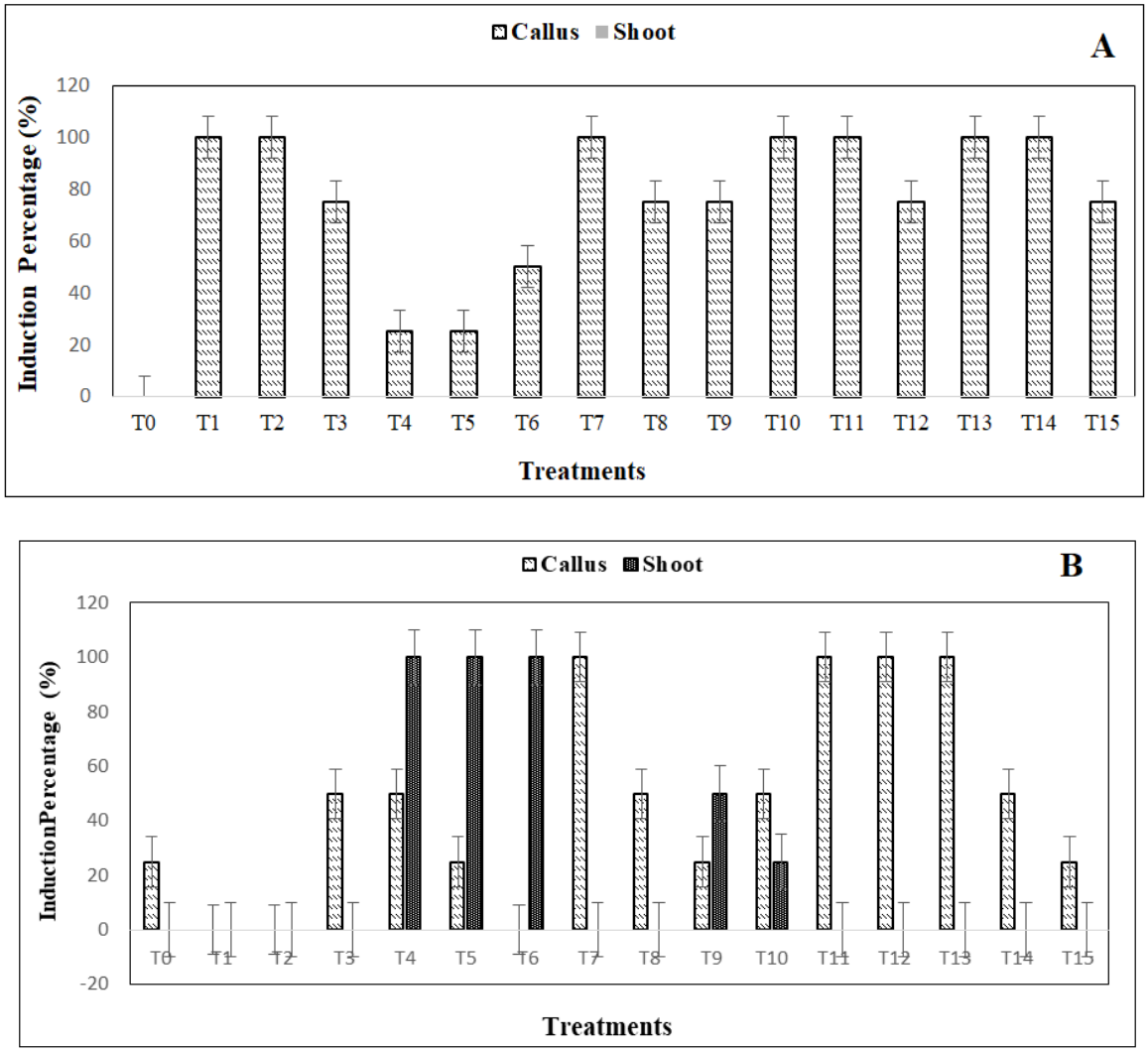

Figure 2: Effects of various treatments on callus and shoot induction percentage from (A) leaf explants (B) nodes explants of *T.paniculatum* (Notes: description of T0-T15 presented in Table 1)

Effect of plant growth regulators and type of explant on shoot formation

The best conditions for explants cultivated on MS medium to regenerate shoots were determined in the current study using different types and concentrations of plant growth regulators and various types of explants. Table 3 and Figure 2B showed that node explants have better growth response in shoot induction than leaf explants (none of the shoots were induced from leaf explants in all treatments). Shoots were induced from node explants within 4,0 ± 0 - 8,0 ± 1,15 days after culture. The addition of a lower concentration of kinetin (1 mgL^{-1}) in MS medium without the addition of 2,4-D showed faster shoot induction from node explants. This result was similar to Yusniwati *et al*., (2021) who reported a minimum time of shoot induction (9,3 days) from patchouli (*Pogostemon cablin* Benth) node explants in MS medium containing 0,3 ppm BA, while the increasing concentration of BA (0,4 – 0,5 ppm) showed longer of shoot induction time ranged from 12,2 – 12,9 days. Furthermore, Ling *et al*., (2013) also reported the increasing concentration of kinetin added to MS medium (1 – 7 mg L^{-1}) significantly affects longer shoot induction time from stem explants of *Labisia pumila* var. alata ranged from (17,5 – 29 days after culture). Shoots induced faster from node explants in a lower concentration of kinetin might be due to the balance ratio of endogenous auxin and cytokinin hormone contained in node explants and exogenous PGR added to the medium (Ling *et al.*, 2013).

As seen in Figure 2B, leaf explants did not show any shoot induction in all treatments, while node explants showed a positive response in shoot induction percentage ranging from (25 - 100%), including in PGR-free medium (control). The best treatment that resulted in the highest shoot induction percentage (100%) was MS medium supplemented with (1 – 3 mgL^{-1}) kinetin without the addition of 2,4-D. Meanwhile, the addition of a higher concentration of 2,4-D in MS medium containing a lower concentration of kinetin reduced shoot induction percentage (25%). This result was linear with Fejér *et al*., (2018) that

reported an increase in kinetin concentration (0,5 – 1 mgL^{-1}) increased shoot induction percentage (100%). In contrast with these results, Premkumar *et al*., (2011) reported reduced shoot induction from stem explants of *Scoparia dulcis* L. in MS medium supplemented with the increasing concentration of kinetin and BAP (2,32 – 9,29 μM). Node explants showed a positive response in terms of shoot induction than leaf explants due to the presence of buds at the node's area. According to Fejér *et al*., (2018), the addition of cytokinin into the media could overcome the apical dominancy and thus could enhance the growth of lateral buds and stimulate shoot formation from node explants. Furthermore, the different shoot growth responses in node explants might be influenced by the interaction of concentration level of endogenous hormone contained in the explant and the concentration level of exogenous PGR added to the culture medium.

Table 3: Effect of plant growth regulators on days for shoot induction and shoot induction percentage from leaf and nodes explants of *T.paniculatum* (Culture media: MS, culture period: 14 days)

Type of Explant	Plant Growth Regulators (mg/L)		Shoot Induction Time (Day)
	2,4-D	KIN	
Leaf	0	0	-
	1	0	-
	2	0	-
	3	0	-
	0	1	-
	0	2	-
	0	3	-
	1	1	-
	1	2	-
	1	3	-
	2	1	-
	2	2	-
	2	3	-
	3	1	-
	3	2	-
	3	3	-
Nodes	0	0	4,0 ± 0*
	1	0	-
	2	0	-
	3	0	-
	0	1	4,0 ± 0
	0	2	8,0 ± 1,15
	0	3	4,50 ± 4,50
	1	1	-
	1	2	-
	1	3	6,0 ± 0
	2	1	4,0 ± 0*
	2	2	-
	2	3	-
	3	1	-
	3	2	-
	3	3	-

Notes: Results are presented as means ± standard deviation (SD). (Replication of each treatment: 4)
(-) none of callus induced (*: the fastest shoot induction time)

CONCLUSION

The present study has successfully established efficient callus and shoot induction protocols from leaf and node explants of *Talinum paniculatum* in solid MS medium supplemented with a combination of 2,4-D and kinetin. The best response of callus and shoot induction (100%) and the fastest callus (6,0 ± 2,31 days) and shoot induction (5,0 ± 1,15 days) from leaf explants were obtained from MS medium supplemented with (1 mgL^{-1} 2,4-D and 1 mgL^{-1} kinetin) and (3 mgL^{-1} 2,4-D and 1 mgL^{-1} kinetin) respectively. In the case of node explants, none of calli were produced, whereas the best response of shoot induction (100%) and the fastest shoot induction (4,0 ± 0 days) were obtained from MS medium supplemented with 3 mgL^{-1} kinetin. Callus produced from leaf explants has a bright yellow color and friable texture. The optimized protocol of callus and shoot induction for *Talinum paniculatum* plant

could be used effectively for mass propagation to fulfill the demand of the pharmaceutical industry as well as for its cultivation.

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