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Research Article

Curcuma Explant Multiplication in MS Medium Supplemented with Various Kinds of Cytokinine and Auxin Concentration

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Abstract

Background and Objectives: Establishing new protocol for *in vitro* propagation of turmeric from vegetative tissue is needed to be studied more due to the demand of turmeric increased every year and also for producing a faster rhizome propagation and free-disease rhizome. The MS solid medium mostly used in *in vitro* culture. To make a faster explant multiplication, cytokinine must be supplemented to MS medium. The objective of this study was to study the effect of various cytokinins and concentration of Naphthalene Acetic Acid on the explant multiplication of turmeric. **Materials and Methods:** This study was conducted in the Laboratory of Tissue Culture, Faculty of Agriculture, Universitas Padjadjaran. Young shoot of sprout rhizome was cultured in MS basal medium supplemented with different kinds of cytokinins (BAP, TDZ and Zeatin) were combined with 0.01 and 1.0 mg L⁻¹ of NAA. All parameters were tested by analysis of t-student test at the 5% significance level. **Results:** Three kinds of cytokinins (BAP, TDZ and Zeatin) were combined with 0.01 and 1.0 mg L⁻¹ of NAA and supplemented to MS solid medium. The result showed that TDZ was more effective than BAP and Zeatin for explant multiplication. The supplementation of TDZ combined with NAA resulted the best effect on shoot multiplication resulted in the highest shoot number compared with control but the supplementation of cytokinins affected in the root elongation resulted the short root length. **Conclusion:** It was concluded that supplementation of cytokinine in MS medium significantly effects on the shoot formation but inhibit the root elongation. The TDZ was the best type of cytokinine that affects shoot multiplication.

Key words: Auxin, cytokinin, *in vitro*, multiplication, turmeric

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Turmeric (*Curcuma domestica* Val.) belong to the Zingiberaceae family and it is one of the most potential herbs in tropical countries. Turmeric is mostly used as a traditional herbal medicine and also as an additional food ingredient, food coloring, food additives and textile dyes^{1,2}. Because of its advantages, therefore turmeric is needed in a great demand in the world around 12,000 t per year but it is only fulfilled by India 1,260 t and a small part of China.

Plant propagation is one of the method in providing turmeric sprout rhizome that increase the turmeric production. Conventional turmeric propagation mostly used by the farmer but it needs a long time to product high quantity of turmeric sprout rhizome. Moreover, the quality of rhizome is lower and also it is not free from disease infection, such as from *Phytophthora myriophyllum* and *Pseudomonas solanacearum*³⁻⁵. Conventional turmeric propagation by rhizome takes more than 8 months⁶. Farmers generally prefer the parent rhizome as planting material with 100 g of rhizome weight because it can produce 50% larger than the amount of planting material⁷. Conventional propagation becomes a problem in turmeric production, because it need a long time to provide the turmeric rhizome. Therefore some alternative method is needed to make a short time in propagation process, such as by tissue culture method.

Turmeric propagation through tissue culture has the advantage in providing free-disease turmeric rhizome and also for medicine purposed it can be extracted for secondary metabolites in the large quantities⁸. Plant growth and development *in vitro* is determined by the composition of culture media that consist of macro nutrients, micro nutrients, vitamins, growth regulators, amino acids, carbon sources, organic materials and compacting materials⁹. Two Plant Growth Regulator (PGR), cytokines and auxins are very important in tissue culture, because they regulate shoot and root formation, respectively¹⁰. Several kinds of cytokinine and auxin are used in tissue culture. However the effectivity in plant growth and development is different. Bharalee *et al.*¹¹ reported that the additional of BAP (6-Benzyl amino purine) and NAA (Naphthalene acetic acid) in MS medium (Murashige and Skoog) gave the best response in *Curcuma caesia* and *Curcuma zedoria* explants and also the highest number of shoot multiplication. Another study showed that *Curcuma zedoria* Roscoe grown on MS+sucrose+BAP media produces 100% shoots and green shoots¹². Other kinds of cytokinine can

be used such as Thidiazon and Zeatin but the effectivity in turmeric *in vitro* culture have not been many reported. This study reported the effectivity of three kinds of cytokinine modified with different concentration of auxin in MS medium.

MATERIALS AND METHODS

Plant material preparation: Rhizome turmeric sprouts of *Curcuma domestica* Val. were washed thoroughly in running water and sterilized as following steps. The explants were rinsed and soaked in detergent solution (5%) for 10 min, then rinsed sterilized water. They were soaked in mixed solution of fungicide and bactericide (10%) for 60 min, then rinsed with sterilized water. The next step was soaked in alcohol 70% for 5 min, then soaked in Clorox solution (15%) for 15 min, in Clorox solution (10%) for 10 min, the last soaked with 0.1% HgCl solution for 5 min and then rinsed with sterilized water three times^{13,14}. Under aseptic condition, 0.6-2.0 cm of explant from sterilized sprout rhizome with shoot tip was used for culture.

Tissue culture media preparation for multiplication: Media culture used the basal medium of Murashige and Skoog¹⁵. The media was prepared by weighing of instant MS (Medium with Vitamin (Phytotech Technology) of 3.72 g, sucrose 25.2 g and agar powder 7.56 g. They were diluted in 1 L of aquades and stirred using magnetic stirrer. Different concentration of BAP, TDZ, Zeatin and NAA as desired treatments was added to MS medium solution. The treatments for multiplication were:

- A : MS 0 (control)
- B : MS+9 mg L⁻¹ BAP+0.01 mg L⁻¹ NAA
- C : MS+9 mg L⁻¹ BAP+1 mg L⁻¹ NAA
- D : MS+1.0 mg L⁻¹ TDZ+0.01 mg L⁻¹ NAA
- E : MS+1.0 mg L⁻¹ TDZ+1 mg L⁻¹ NAA
- F : MS+0.1 mg L⁻¹ Zeatin+0.01 mg L⁻¹ NAA
- G : MS+0.1 mg L⁻¹ Zeatin+1 mg L⁻¹ NAA

Homogenous MS media was adjusted the pH to 5.7. Ten milligram of the adjusted modified MS media was poured into glass culture plugged with plastic plugs and sterilized by autoclaving at 121 °C (15 psi) for 30 min. The sterilized explant was inoculated in these media and the cultures were incubated at 25±2°C of temperature, 75±5% of humidity with the additional of fluorescent light (120 µ mol m⁻² sec⁻¹) of 16 h of photoperiod. During incubation periods, to study

the effect of various and concentration of cytokinin and concentration of NAA on the multiplication of turmeric explant. Several parameter were investigated namely shoot number, shoot height, leaves number, root length and root number.

RESULTS

Modification of culture media by combination of various cytokinin and concentration of NAA significantly affect the shoot number and leaves number of explant during multiplication periods. The additional of 9 mg L⁻¹ BAP+0.01 mg L⁻¹ NAA and 1.0 mg L⁻¹ TDZ+0.01 or 1.0 mg L⁻¹ NAA significantly increased the average number of shoots compared the control. Whereas, the additional zeatin in MS medium did not affect in the increasing the shoot number. The highest value of shoot number was resulted from MS media supplemented with 1.0 mg L⁻¹ TDZ+1.0 mg L⁻¹ NAA with the average number of 3 shoots/explant that is 1.88 shoots/explant higher than control (1.12 shoots/explant)

(Table 1). Beside effects on the shoot number, the application of 1.0 mg L⁻¹ TDZ+0.01 or 1.0 mg L⁻¹ NAA significantly reduced the shoot length compared with control with the value of 2.19 and 2.12 cm, respectively. Whereas the shoot length of control was 3.33 cm (Table 1).

The supplementation of MS medium with several kinds of cytokinin (BAP, TDZ and Zeatin) combined with different concentration of NAA did not give a significantly effect on the change of leaves number and root number but it was significantly effect in the root length compared with control at 14 week after panting. In Medium multiplication, the additional auxin has an effect in increasing the root formation. However in our study showed that the supplementation of MS medium with NAA up to 1 mg L⁻¹ has not been able to increase the roots number, even the roots number was less than the control (Fig. 1, Table 1). The supplementation of 9 mg L⁻¹ BAP+0.01 or 1.0 mg L⁻¹ NAA and also 0.1 mg L⁻¹ Zeatin+1 mg L⁻¹ NAA in MS medium was significantly inhibit the root elongation with the root length was 1.03, 1.25 and 1.21 cm (Table 1).

Table 1: Supplementation of various kind of cytokinin and NAA concentration on MS medium on shoot number, shoot length, leaves number, root number and root length of turmeric explants at 14 weeks after planting

Treatments	Shoot number (shoots/explant)	Shoot length (cm)	Leaves number leaves/shoot	Root number (roots/shoot)	Root length (cm)
MS 0 (control)	1.12	3.33	0.87	3.5	1.73
MS+9 mg L ⁻¹ BAP+0.01 mg L ⁻¹ NAA	1.80	2.92	0.5	4.0	1.03*
MS+9 mg L ⁻¹ BAP+1 mg L ⁻¹ NAA	1.14	2.42	0.2	3.4	1.25*
MS+1.0 mg L ⁻¹ TDZ+0.01 mg L ⁻¹ NAA	2*	2.19*	0.6	3.3	1.43
MS+1.0 mg L ⁻¹ TDZ+1 mg L ⁻¹ NAA	3*	2.12*	0.62	3.25	1.44
MS+0.1 mg L ⁻¹ Zeatin+0.01 mg L ⁻¹ NAA	1	3.04	0.72	3.14	1.54
MS+0.1 mg L ⁻¹ Zeatin+1 mg L ⁻¹ NAA	1	2.37	0.5	2.87	1.21*

The values followed by asterisks indicate a significantly different from the control at $p < 0.05$, according to Student's t-test

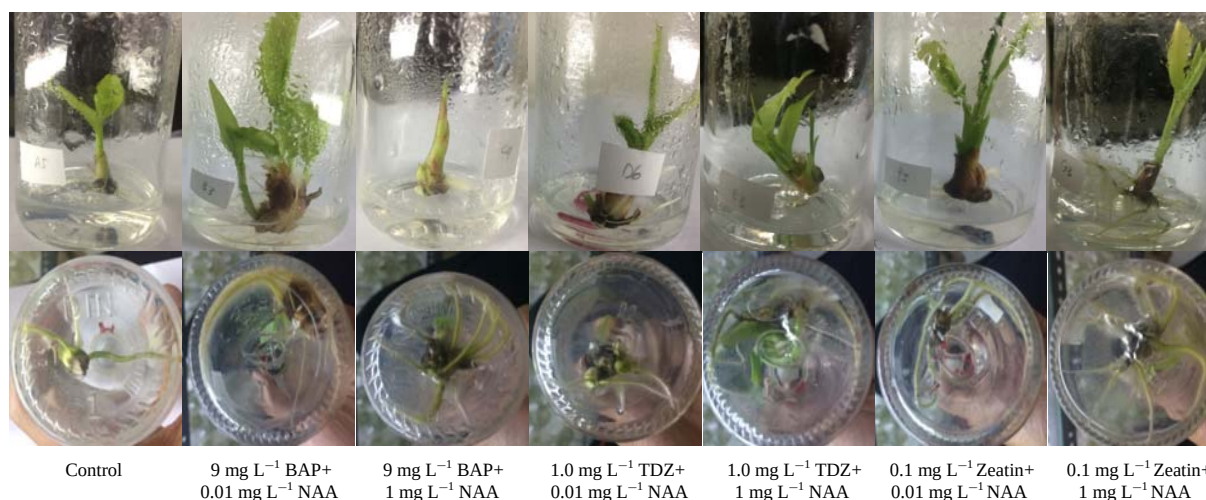


Fig. 1: Shoot and root multiplication of turmeric explant after 14 week after planting (WAP)

DISCUSSION

The success of explant multiplication is characterized by the increasing number of shoots or roots. In this study, it elucidate the effect of various kind of cytokinine and different concentration of NAA on turmeric explants multiplication. Increasing the shoots number and shoot lengths obtained from explants grown in MS medium supplemented with all various kinds of cytokinine (BAP, TDZ and zeatin) with the additional of NAA and TDZ resulted the highest shoots number and shoot lengths compared with control. Lavakumaran and Seran¹⁶ stated that in the early stages of incubation, TDZ had a higher *in vitro* response than BAP. In *Astragalus cariensis*, combination of TDZ and NAA can increase higher shoot regeneration compared with BAP+NAA treated explant¹⁷. Increased the shoots number in TDZ treated explants due to the high concentrations of TDZ associated with isopentenyl (ip) enzymes that promote the cell division and stimulate shoot organogenesis¹⁸.

Explants obtained from MS medium with the additional of TDZ resulted in shorter shoot lengths compared with control (Table 1). That is correspond with the study of Prathanturug *et al.*¹⁹ who stated that cytokinine plays a role in increasing shoot proliferation but inhibits shoot extension.

Kozak and Salata²⁰ suggested that on the medium supplemented with BA ($4.4\text{--}22.2\ \mu\text{mol dm}^{-3}$), the main shoot was characterised by the largest number of leaves (4.1-6.3). However, in this study it can be seen that the supplementation of various kinds of cytokinine and different concentration of auxin up to $1\ \text{mg L}^{-1}$ has no significant effect on increasing the leaves number but it decreased the leaves number (Table 1). The BAP does not effect in increasing the leaves number of ginger culture, because BAP is one type of cytokinine that stimulating shoot formation but inhibits leaf growth²¹. The combination of high concentrations of cytokinine and low concentration of auxin is important in shoot and leaf formation²². This study showed that TDZ and zeatin were less effective in leaf formation compared with control (Fig. 1).

Beside the shoot growth, the successful of explant multiplication is root growth and development. Root formation is associated with endogenous auxin and cytokinine content in plant tissue, followed by the process of cell elongation and enlargement²³. This study investigated that the roots formation of curcuma explant occurred at 2 weeks after planting. However, after 14 weeks, statistically the root number and root length were comparable with control (Table 1). It can see that curcuma explant grown in MS Medium (control) has been able to form the roots. Present

study corresponds with Marlin²⁴ who showed that exogenous auxin is not necessary used for root. Although, as statistically the root number and root length were not significantly different but their values were fewer and shorter compared with control, indicating that cytokinine inhibited root growth of turmeric explants. Gaspar and Coumans²⁵ suggested that high concentrations of cytokinine be able to inhibit the root formation.

Auxin is very important role in the formation of plant roots but it is needed in low concentration. At 14 WAP the NAA $0.01\ \text{mg L}^{-1}$ resulted a higher root number compared with control and $1.0\ \text{mg L}^{-1}$ of NAA in all kinds of cytokinine. The highest root number was observed in MS medium supplemented with BAP and $0.01\ \text{mg L}^{-1}$ of NAA but in the another kinds of cytokinine resulted a lower value (Table 1). Pamungkas *et al.*²⁶ stated that high concentrations of exogenous auxin inhibits root growth, as it inhibits the control of endogenous auxin in plants. Murch and Saxena²⁷ reported that TDZ would increase the accumulation and transport of auxin in the tissues, thus stimulating root formation but in the high concentration of cytokinine will inhibit the roots formation. The role of cytokines in the formation of roots i.e., it is able to induce auxin biosynthesis and increase auxin levels in developing young tissues such as in roots and buds²⁸. The root length in all treatments was lower than control, this suggested that high concentrations of cytokinine inhibit the process of root elongation. Root growth is inhibited by high concentrations of cytokines. In banana, high concentration of BAP and TDZ decrease the shoot height and root length²⁹⁻³¹. Besides cytokinine, high concentration of auxin also can inhibit the root elongation. Alarcon *et al.*³² reported that a high concentration auxin will increase the number of roots but inhibit root elongation.

CONCLUSION

The study concluded that supplementation of cytokinine in MS medium significantly affects on the shoot formation but inhibit the root elongation. About $1.0\ \text{mg L}^{-1}$ TDZ combined with $1\ \text{mg L}^{-1}$ NAA produced the highest shoot number and shoot length compared with control.

SIGNIFICANCE STATEMENT

Turmeric is an important commodity for additional food ingredient and also for traditional medicine. Many problems are found in turmeric propagation such as long period in producing rhizome. *In vitro* technique can be as one methods in turmeric propagation to get a high quantity and

quality of rhizome but it still has a problem in multiplication protocol. In this study we reported that for turmeric multiplication, TDZ combined with NAA is very effective in shoot formation and resulted the highest number of shoot.

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