ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 67 1 Number 1, 2019

COCONUT WATER AND IAA EFFECT ON THE *IN VITRO* GROWTH OF *TRIBULUS TERRESTRIS* L.

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To link to this article: https://doi.org/10.11118/actaun201967010009
Received: 5. 9. 2018, Accepted: 19. 12. 2018

To cite this article: AKHIRIANA ENIK, SAMANHUDI, YUNUS AHMAD. 2019. Coconut Water and IAA Effect on the In Vitro Growth of *Tribulus terrestris* L. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(1): 9–18.

Abstract

Tribulus (*Tribulus terrestris* L.) is a medicinal plant with considerable implementation such as aphrodisiac and anti-inflammation drugs. This research was conducted to study the effectivity of coconut water and IAA (*Indole-3-acetic acid*) application on various concentration as the growth regulator for tribulus growth on the *in vitro* and its effect toward observation variables. The material used were cotyledon from tribulus embryo and MS (Murashige and Skoog) medium. The research method used a Completely Randomized Design with first factor of coconut water concentration of 0 mL L^{-1}, 50 mL L^{-1}, 100 mL L^{-1}, and 150 mL L^{-1} and IAA concentration of 0 ppm, 0.15 ppm, 0.20 ppm, and 0.25 ppm as the second factor. Observed variables were shoots emergence time, number of shoots, shoot height, number of leaves, roots emergence time, number of roots and regression between observation variables. The result showed that the combination of 150 mL L^{-1} coconut water and 0.25 ppm IAA gave the highest shoot height, roots emergence time, and number of roots. Coconut water treatment on 150 mL L^{-1} concentration alone gave the best result on shoots emergence time, number of shoots, and number of leaves, while IAA concentration of 0.25 ppm independently gave the highest number of leaves. Regression analysis result indicate that the number of roots has a positive correlation with shoots height, number of shoots and number of leaves.

Keywords: medicinal plant, effectivity, growth regulator, shoots, leaves, roots, regression

INTRODUCTION

Tribulus (*Tribulus terrestris* L.) is known as a medicinal plant. This plant has been long used by the Chinese and Indians in traditional medicine methods (Kasote *et al*, 2017). Tribulus derived from *Zygophyllaceae* family of annual plant belonging to C4 plants. At first, this plant originated from the Mediterranean region, but then distributed to several countries within Europe, Asia, Africa, and Australia (Qureshi *et al.*, 2014). Tribulus contains a lot of active compounds that are beneficial to human health such as saponins, flavonoids, alkaloids, and other nutrients (Vaidya *et al*, 2018).

The content of the active compound is useful for humans as an aphrodisiac (Ammar *et al*, 2018) reduces blood levels of triglyceride, and cholesterol (Sharma and Kanwar, 2018), diuretic, antibacterial, hepatoprotective, immunomodulatory, antihypertensive, anti-inflammation drugs and anticancer (Sharma, 2017; Zahra, 2018).

On land cultivation, there are some obstacles in the cultivation practices such as the long dormancy period of seed which causing low germination rate for seedlings (Petkov, 2011). In areas with a tropical climate, this plant can only be cultivated during the dry season. Alternative propagation that can be used is tissue culture. Tissue culture gives some advantages of large quantity production of seedlings, short time production, more uniformed explant growth and has a better quality than the seedlings obtained from conventional propagation, while increasing the production of secondary metabolites in the medical herbal plants (Paric *et al*, 2017) and obtaining secondary metabolites of plants in a short time.

The main factor which affect the success of tissue culture is growth regulator substance. PGR (plant growth regulator) mainly used in tissue culture, especially in culture initiation are auxin and cytokinin. PGR requirement in plant tissue culture will vary depending on the type of plant, explant material, and the expected result. Coconut water has a natural content of auxin and cytokinin. Coconut water is widely used in tissue culture such as the culture of banana, dendrobium, jewel orchid, doctorbush, and carnation (Mondal *et al*, 2015; Parthibhan *et al*, 2015; Sherif *et al*, 2016; Raja *et al*, 2018; Khatun *et al*, 2018). The MS (Murashige and Skoog) medium produce the highest number and length of roots on *in vitro* banana growth, but half MS medium added by 100 mL L^{-1} coconut water and 400 ppm AB-mix nutrient can produce the best plant vigor. The use of coconut water can reduce the costs in tissue culture due to cheaper price and easier to obtain than synthetic PGR (Prabowo *et al*, 2018).

The coconut water contain natural PGR such as IAA up to 20.89 mg L^{-1}, 50.09 mg L^{-1} kinetin, and 28.65 mg L^{-1} zeatin (after heated at 121 ºC). In addition, while coconut water contain high cytokines, it also required an additional auxin hormones in order to obtain optimal growth of tissue culture. The optimal growth of tissue culture can be achieved by balancing the two hormones used (Kristina and Syahid, 2012). The WPM medium added by 4 ppm BAP and 1 ppm IAA produce the best growth of shoot and leave of *Sterculia foetida,* but WPM medium added by 4 ppm BAP and 0.5 ppm IAA is able to produce complete plantlet by forming buds, leaves, and roots (Yuniastuti *et al*, 2018). This study aims to determine the effectiveness of coconut water and IAA combination at various concentrations on tissue culture growth of tribulus in MS medium and regression between observation variables.

MATERIALS AND METHODS

The research was conducted during the growth of tissue culture of tribulus from August 2017 until April 2018 and carried out in Plant Physiology and Biotechnology Laboratory of Faculty of Agriculture, Sebelas Maret University (UNS) Surakarta. The average temperature of the tissue culture was 22 – 28 °C and the moisture was 60 – 80 %. Plant was the part of tribulus seed of 1 – 3 months old. The study was initiated by germinating tribulus seed on a petridish in a semi-*in vitro* state. The seed then added into bottle for initial surface sterilization for 1 hour, followed by detergent soaking and rinsed by tap water. Later, it was soaked again with 1 g L^{-1} bactericide and 1 g L^{-1} fungicide for 1 night. Tribulus seeds were then put into a petridish with tissue and stored in dark condition (light intensity 0 cd), on averange temperature of 27.3 °C and 79 % humidity. Explant material was taken from tribulus which had been germination and the cotyledons part which had opened.

The material used for the MS medium preparation was 8 g L^{-1} agarose, 30 g L^{-1} saccharose, aquadest, 50 mL L^{-1} macro stock solution, 10 mL L^{-1} micro stock solution, 50 mL L^{-1} vitamin stock solution, 50 mL L^{-1} FeEDTA, 0.1 g L^{-1} PVP (*Polyvinylpyrrolidone*), coconut water (0 mL L^{-1}, 50 mL L^{-1}, 100 mL L^{-1}, and 150 mL L^{-1}), and IAA (0 ppm, 0.15 ppm, 0.20 ppm, and 0.25 ppm). The pH level required for tribulus culture is 5.8, if the pH is too low, 0.1N NaOH / KOH can be used, if it is too high then addition of 0.1N HCl can be done to obtain the required pH, and once the media is formulated, it was put into the culture bottle to be sterilized. The media sterilization was done by autoclave in 121 °C temperature with 120,658.3 pa pressure for one hour.

The explants used in this study were seeds that had been germinated and the cotyledon part which had opened. The seeds was sterilized using a detergent and rinsed with tap water, which then the sprouts were taken and put into the LAFC (Laminar Air Flow Cabinet). The sprouts were sterilized by 10 g L^{-1} bactericide + 10 g L^{-1} fungicide for 1 hour, 250 mg L^{-1} antibiotics for

30 minutes, 50 mL L^{-1} chlorox for 15 minutes, 2 drops L^{-1} betadine for 2 seconds then rinsed with aquadest for 1 minute, and last was immersion in ascorbic acid for 30 minutes. Sterilized explants were planted in tissue culture media, on the each bottle is planted with one explant.

This research use a Completely Randomized Design. The first factor was coconut water with concentration of 0 mL L^{-1}, 50 mL L^{-1}, 100 mL L^{-1}, and 150 mL L^{-1}, and as second factor was IAA concentration of 0 ppm, 0.15 ppm, 0.20 ppm, and 0.25 ppm, which in total 16 treatments combination were obtained and three replication was done each.

Data obtained were analysed by the analysis of variance (ANOVA) and means were compared using Least Significant Differences were assessed by Duncan's Multiple Range Test at 5 % level. The statistical analyses were performed using the programme of Statistical Package for the Social Sciences (SPSS) ver. 21.

RESULTS AND DISCUSSION

A. Shoots Emergence Time

Based on statistical analysis done on observation data of shoots emergence time (Tab. I), it showed that coconut water treatment independently gave significant result, whereas single IAA treatment gave no significant difference and combination between coconut water and IAA had no interaction.

The control treatment gave very significant different result compared to 50 mL $L^{-1,}$ 100 mL L^{-1}, and 150 mL L^{-1} coconut water concentration treatment, while treatment coconut water of 50 mL L^{-1}, 100 mL L^{-1}, and 150 mL L^{-1} did not gave significantly different result compared with in the treatments. Although there was no significant difference between coconut water treatment concentrations of 50 mL L^{-1}, 100 mL L^{-1}, and 150 mL L^{-1}, but the increase of coconut water concentration was directly

1: *Explant of tribulus tissue culture.*

I: *The effect of coconut water and IAA concentration on shoots emergence time, data are presented as mean ± standard deviation, n = 48*

Coconut water concentration (mL L^{-1})	Shoot emergence time (DAP)
0	42.66 b ± 3.85
50	13.58 a ± 0.39
100	5.16 a ± 1.61
150	3.66 a ± 1.83
IAA concentration (ppm)	**Shoot emergence time (DAP)**
0	12.08 a ± 0.57
0.15	12.33 a ± 0.53
0.20	18.91 a ± 0.42
0.25	21.75 a ± 0.03

Note: Numbers followed by different character in the same column shows significant difference in DMRT at 5% level, DAP: Day After Planting

proportional to the increase in speed of shoots emergence time.

Addition of 150 mL L^{-1} coconut water was able to accelerate shoots appearance to 9.92 days faster than 50 mL L^{-1} coconut water, and 1.5 days faster than the treatment of 100 mL L^{-1} coconut water. In tribulus tissue culture by using tribulus cotyledon explant, the faster result was shown by the treatment of 150 mL L^{-1} coconut water at 3.66 DAP. The initiation of tribulus cotyledon with 150 mL L^{-1} coconut water concentration usage were good enough to grow shoots faster than the initiation of callus which gaves 16 DAP shoot emergence time (Raghu *et al*, 2010; Singh and Goyal, 2016). Shoots in tissue culture can grow well due to the hormones which given in the shoots growth was in easily available, appropriate, and balanced condition for shoots formation.

B. Number of Shoots

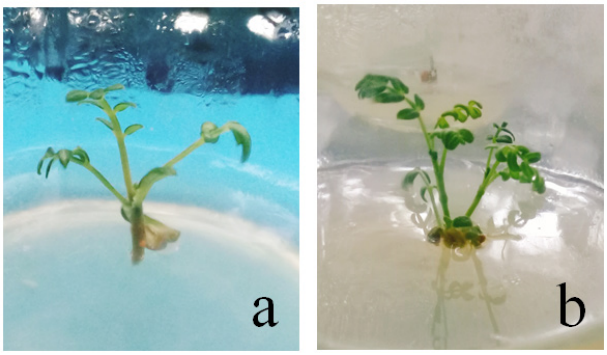
Tissue culture of *Tribulus terretris* L. with concentration combination of coconut water and IAA gave 1 or 2 shoots/explant similarly to tissue culture of *Tribulus terretris* L. with combination of NAA and BAP (Singh and Goyal, 2016). The result of statistical analysis in this study showed that the coconut water treatment was independently gave a significantly different result to the number of shoots. While single IAA treatments did not gave significant results to the number of shoots and combination of coconut water treatment and IAA did not show interaction (Tab. II).

Treatment of 150 mL L^{-1} coconut water gave the best result on shoot emergence time of 1.25 days on tribulus tissue culture which significantly different and increased 150 mL L^{-1} shoot rate compared to 0 mL L^{-1}, 50 mL L^{-1}, and 100 mL L^{-1} coconut water concentration. Number of shoot observation shows the best results on 150 mL L^{-1} coconut water treatment. Identical result was shown in the culture of stevia tissue culture with the addition of 150 mL L^{-1} coconut water concentration which able to grow new shoots in higher rate than the addition of coconut water of

II: *The effect of coconut water and IAA concentration on number of shoot, data are presented as mean ± standard deviation, n = 48*

Coconut water concentration (mL L^{-1})	Number of shoots
0	1.00 a ± 0.01
50	1.00 a ± 0.01
100	1.00 a ± 0.01
150	1.25 b ± 0.03
IAA concentration (ppm)	**Number of shoots**
0	1.00 a ± 0.01
0.15	1.00 a ± 0.01
0.20	1.08 a ± 0.01
0.25	1.16 a ± 0.02

Note: Numbers followed by different character in the same column shows significant difference in DMRT at 5% level.



2: *Formation shoot regeneration at 30 DAP on 150 mL L^{-1} coconut water and 0.15 ppm IAA (a) and 150 mL L^{-1} coconut water and 0.25 ppm IAA (b).*

0 mL L^{-1}, 50 mL L^{-1}, 100 mL L^{-1}, and 200 mL L^{-1} concentrations (Sridhar and Aswath, 2014).

Shoot growth is a response to the PGR used in tissue culture. The growth of shoots will be optimal under appropriate quantity of hormone and nutrition used, otherwise shoot growth will be inhibited and disturbed, especially if the quantity is scarce. Each type of culture has its own properties on the concentration and hormones or nutrients type used, This is happened due to the needs of each explant type which vary. Even the same type of plants could use a different explant material, hormones, and nutrients depends on the purpose of culture and the magnitude of result desired.

C. Shoot Height

Based on the statistical analysis done on the shoot height at 90 DAP, it shows that the coconut water treatment and IAA gave significant effect, while the combination between the two treatments showed an interaction (Tab. III). The treatment which gave the best result and was significantly different to other treatments was the combination of 150 mL L^{-1} coconut water and 0.25 ppm IAA with 5.60 cm shoot height.

The addition of shoot height is caused by the cell proliferation that form an elongation and enlargement of new cells on the apical meristem of shoot indicated by taller buds and larger volume. At 90 days observation of shoot height, the combination of 150 mL L^{-1} coconut water concentration and 0.25 ppm IAA was able to give the best result on shoots height.

Combination of 150 mL L^{-1} coconut water as cytokinin and 0.25 ppm IAA as auxin was able to increase shoot height significantly at 90 day of observations, because in coconut water there is a high cytokines and low quantity of auxin, added with a synthetic auxin ie IAA at lower doses than the cytokines which capable of accelerating cell division. Previous studies on banana tissue culture with higher use of cytokines and lower auxin gave similar results of significant increase on explants height (Qamar *et al*, 2015).

D. Number of Leaves

Based on the statistical analysis done on the number of leaves, the result shows that the coconut water treatment and IAA independently gave a significantly different result while the combination of coconut water and IAA treatment did not interact (Table 4). At this observation, 150 mL L^{-1} coconut water treatment gave the highest number of leaves as much as 3.16 and significantly different compared to the coconut water treatment of 0 mL L^{-1}, 50 mL L^{-1}, and 100 mL L^{-1}. Coconut water of 150 mL L^{-1} concentration could increase the number of leaves 3 × than control (0 mL L^{-1}), 2 × than 100 mL L^{-1} concentration, and increase 1.5 × than 150 mL L^{-1} concentration. IAA treatment independently on concentration of 0.15 ppm gave the best result of leaves quantity as much as 2.41 and significantly different compared to control (0 ppm), 0.20 ppm, and 0.25 ppm.

Growth and development of leaves is influenced by several hormones such as cytokinin, auxin, gibberellin, and other hormones. On the observation of the number of leaves, the best treatment was coconut water concentration of 150 mL L^{-1} and IAA treatment of 0.15 ppm independently, 150 mL L^{-1} coconut water gave the higher number of leaves compared to the treatment of 0 mL L^{-1}, 50 mL L^{-1}, and 100 mL L^{-1} coconut water concentration as those in *Jatropha curcas* culture (Toppo *et al*, 2012).

Coconut water can increase the number of leaves due to its high cytokinin and content nitrogen of 43 g L^{-1} in young coconut water. In the formation of leaf, cytokinin serves at morphogenesis and

III: *The effect of coconut water and IAA combination on shoot height, data are presented as mean ± standard deviation, n = 48*

IAA concentration (ppm)	Shoot height (cm)				Average
	Coconut water concentration (mL L^{-1})				
	0	50	100	150	
0	0.90 a ± 0.19	0.93 a ± 0.22	0.93 a ± 0.22	1.56 ab ± 0.09	1.08
0.15	0.66 a ± 0.29	1.73 ab ± 0.29	1.66 ab ± 0.23	1.70 ab ± 0.19	1.44
0.20	1.16 ab ± 0.21	1.26 ab ± 0.19	1.23 ab ± 0.18	2.93 b ± 0.33	1.65
0.25	0.40 a ± 0.31	1.33 ab ± 0.12	1.46 ab ± 0.10	5.60 c ± 1.17	2.20
Average	0.73	1.31	1.32	2.90	

Note: Numbers followed by different character shows significant difference in DMRT at 5% level.

leaf enlargement while nitrogen serves at protein synthesis and amino acids, which then used for growth and increase the number of leaves (Kristina and Syahid, 2012). In the number of shoots observation, although coconut water and IAA give significantly different results, the combination between the two treatments did not gave an interaction. Tab. II shows that giving 0.20 ppm and 0.25 ppm IAA will decrease the number of leaves lower than 0.15 ppm IAA.

E. Roots Emergence Time

Observations when the roots appear are carried out every day for 90 DAP. Based on the observations, only a few treatments whose plantlets appeared roots. Root emergence in plantlets is very important because the roots function to absorb water, minerals and other materials needed for plant growth and development.

IV: *The effect of coconut water and IAA concentration on number of leaves, data are presented as mean ± standard deviation, n = 48*

Coconut water concentration (mL L^{-1})	Number of leaves
0	1.08 a ± 0.11
50	1.41 ab ± 0.07
100	1.91 b ± 0.01
150	3.16 c ± 0.18
IAA concentration (ppm)	**Number of leaves**
0	1.50 a ± 0.05
0.15	2.41 b ± 0.07
0.20	1.58 ab ± 0.04
0.25	2.08 ab ± 0.02

Note: Numbers followed by different character in the same column shows significant difference in DMRT at 5 % level.

V: *The effect of coconut water and IAA concentration on the roots emergence time*

Treatments	Roots emergence time (DAP)
0 mL L^{-1} Coconut water + 0 ppm IAA	-
0 mL L^{-1} Coconut water + 0.15 ppm IAA	-
0 mL L^{-1} Coconut water + 0.20 ppm IAA	-
0 mL L^{-1} Coconut water + 0.25 ppm IAA	27.00
50 mL L^{-1} Coconut water + 0 ppm IAA	-
50 mL L^{-1} Coconut water + 0.15 ppm IAA	-
50 mL L^{-1} Coconut water + 0.20 ppm IAA	-
50 mL L^{-1} Coconut water + 0.25 ppm IAA	29.00
100 mL L^{-1} Coconut water + 0 ppm IAA	-
100 mL L^{-1} Coconut water + 0.15 ppm IAA	-
100 mL L^{-1} Coconut water + 0.20 ppm IAA	-
100 mL L^{-1} Coconut water + 0.25 ppm IAA	26.00
150 mL L^{-1} Coconut water + 0 ppm IAA	28.33
150 mL L^{-1} Coconut water + 0.15 ppm IAA	25.50
150 mL L^{-1} Coconut water + 0.20 ppm IAA	19.66
150 mL L^{-1} Coconut water + 0.25 ppm IAA	19.33

Note: –: No root emergence.

Early root occurance in tissue culture will produce a greater number roots and the absorption of nutrients, minerals, and materials needed by the plantlets contained in the media will be optimum. Tab. V shows that the treatment that can grow roots is the combination of 0 mL L^{-1} coconut water + 0.25 ppm IAA, 50 mL L^{-1} coconut water + 0.25 ppm IAA, 100 mL L^{-1} coconut water + 0.25 ppm IAA, 150 mL L^{-1} coconut water + 0 ppm IAA, 150 mL L^{-1} coconut water + 0.15 ppm IAA, 150 mL L^{-1} coconut water + 0.20 ppm IAA, and 150 mL L^{-1} coconut water + 0.25 ppm IAA.

The addition of 150 mL L^{-1} coconut water and 0 ppm, 0.15 ppm, 0.20 ppm, and 0.25 ppm IAA were able to stimulate and accelerate when roots emergence time, while 0 mL L^{-1}, 50 mL L^{-1}, and 100 mL L^{-1} coconut water were only combined with 0.25 ppm IAA which is able to stimulate and accelerate roots emergence time. Coconut water at high concentrations can stimulate and accelerate when the roots emergence time because the coconut water contains auxin. If the concentration of coconut water increases in the media, the auxin content will be higher so that the explant needs for auxin to stimulate and accelerate roots emergence time. Even in this study the provision of 150 mL L^{-1} coconut water without the addition of IAA has been able to produce roots, so it can be concluded that the administration of coconut water with a high enough concentration alone can stimulate root formation.

The best treatment that is able to provide the fastest root appearing is the combination of 150 mL L^{-1} coconut water and 0.25 ppm IAA is 19.33 DAP. This study of 150 mL L^{-1} coconut water concentration combined with various IAA concentrations all grow roots. The addition of organic materials such as coconut water in tissue culture can accelerate when the roots appear in tissue culture because there is an auxin content, so that the increase in the addition of coconut water concentration of 150 mL L^{-1} combined with 0.25 ppm IAA will accelerate more when the roots appear and increase the chance of root formation compared to other treatments (Hartati *et al*, 2017).

F. Number of Roots

Based on the results of statistical analysis on the number of roots it showed that the combination of coconut water and IAA had an interaction. The combination of treatments that gave the highest number of roots and significantly different with other treatments was 150 mL L^{-1} coconut water and 0.25 ppm IAA of 10.66 roots.

The growth of root quantity of tribulus tissue culture was influenced by the combination of coconut water and IAA with best combination of 150 mL L^{-1} coconut water and 0.25 ppm IAA which yield root quantity as much as 10.66. Coconut water and IAA were able to give a significant effect on the root growth of tribulus tissue culture due to the activity of auxin hormone in the form of synthetic IAA addition and natural IAA contained in coconut water (Yong *et al*, 2009). In this study it showed that the increase of IAA concentration either synthetic or natural would be directly proportional to the increase in the number of roots. The successful combination of coconut water and IAA in increasing the number of roots also has been reported by Iqbal *et al* (2013) on the micropropagation of banana plants.

G. Regression between Observation Variables

The effect of roots growth observation variables on other observation variables can be known by performing regression analysis. The equation of regression line stated the relation of independent

VI: *The effect of coconut water and IAA concentration on the number of roots, data are presented as mean ± standard deviation, n = 48*

IAA concentration (ppm)	Number of roots				
	Coconut water concentration (mL L^{-1})				Average
	0	50	100	150	
0	0.00 a ± 0.34	0.00 a ± 0.34	0.00 a ± 0.34	2.33 ab ± 0.53	0.58
0.15	0.00 a ± 0.34	0.00 a ± 0.34	0.00 a ± 0.34	3.00 ab ± 0.74	0.75
0.20	0.00 a ± 0.34	0.00 a ± 0.34	0.00 a ± 0.34	3.66 b ± 0.72	0.91
0.25	0.33 a ± 0.28	0.66 ab ± 0.29	1.33 ab ± 0.47	10.66 c ± 2.54	3.24
Average	0.08	0.16	0.33	4.91	

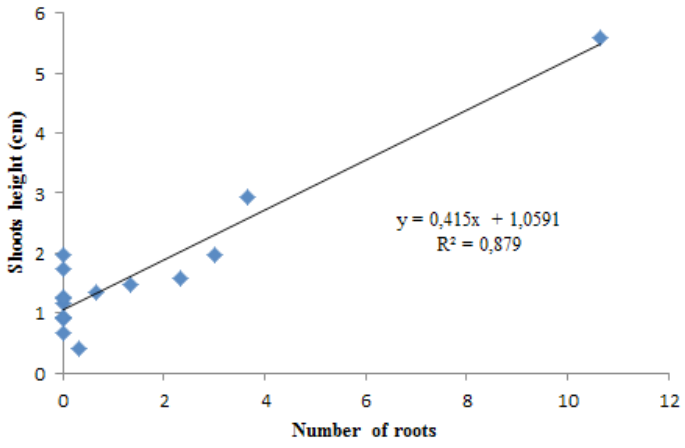
Note: Numbers followed by different character shows significant difference in DMRT at 5 % level.

variables (X axis) which is the number of roots with the dependent variables (Y axis) which is the shoots height, number of shoots, and number of leaves. The effect of the independent variable value on the dependent variable is shown in the correlation coefficient value (r).

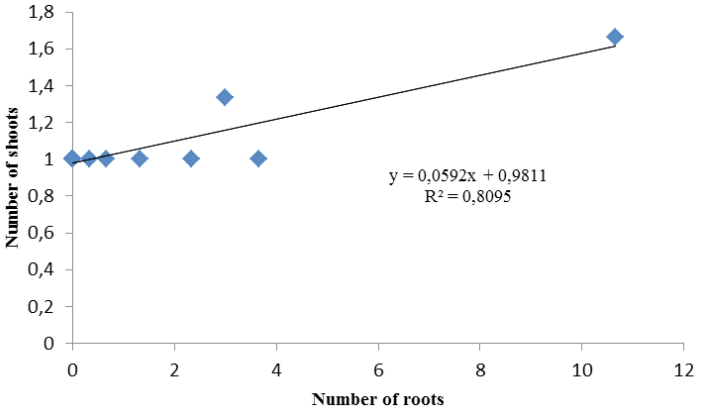
Fig. 3 shows the effect of number of roots towards the shoots height, indicating that the relationship between number of roots and shoot height has a positive correlation which means that the increase in number of roots is in line with the increase in the shoots height. Correlation coefficients number of roots influence on shoot height is 0.879, which average value of 87.9 %. It is indicating that shoot height variation is caused by number of roots while the rest was caused by other factors. This results is in line with research on tissue culture of Dianthus *carthusianorum* and Eulophia *graminea* orchid, the results showed that increase number of roots value was followed by an increase in shoot height (Muszynska and Hanus-Fajerska, 2017; Romeida *et al*, 2018).

Fig. 4 shows the effect of the number of roots towards the number of shoots which have positive correlation. It means that the increase in number of roots is in line with an increase in the number of shoots. Correlation coefficients number of roots influence on the number of shoots is 0.809, which means that 80.9 % of the number of shoots variation was caused by number of roots while the rest was caused by other factors. Roots and shoots are the most important organ for plants. Both of these organs are related, indicating that the explants with large number of roots will have a better shoot growth due to increased process of nutrient absorption and nutrients by roots for shoots growth. In the study of tribulus tissue culture using a combination of coconut water and IAA, the relationship of roots and shoots height gave a positive correlation. The addition of the number of roots will be followed by the height of the shoots reported by Lim *et al* (2018) on tissue culture of oil palm.

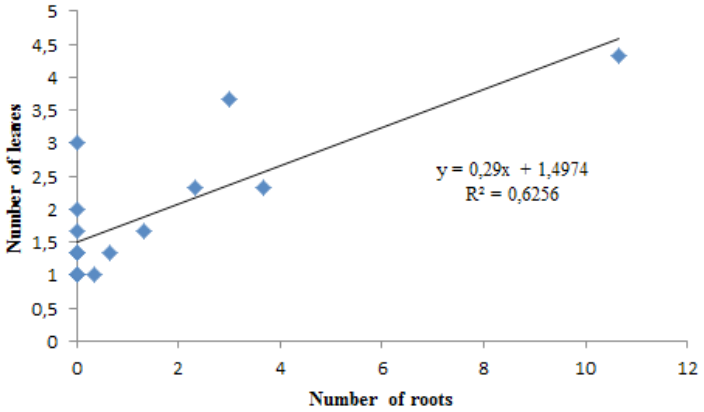
Fig. 5 shows the effect of roots growth towards the number of leaves, indicating that the relationship between number of roots and number of leaves has a positive correlation which means that the increase in number of roots is followed by an increase in the number of leaves. Correlation coefficients of number of roots on the number of leaves is 0.625 which means that 62.5 % of the number of leaves variation in was caused by number of roots while the rest was caused by other factors. This results is in line with research conducted by Waman *et al* (2016) in tissue culture of banana which results showed an increase number of roots was followed by an increase in number of leaves. Number of roots effect towards the shoot height, number of shoots and number of leaves was positively correlated. Root is an important organ for plants that are cultivated in tissue culture because the root is the only organ capable of absorbing nutrients and hormones contained in the culture media. Nutrients and hormones absorbed by the roots are then used for plantlet growth, so that the higher roots growth tend to increase the growth of plantlet, such as increasing the shoot height, number of shoots, and number of leaves.



3: *Regression line between number of roots and shoot height.*



4: *Regression line between number of roots and number of shoots.*



5: *Regression line between number of roots and number of leaves.*

CONCLUSION

Based on the results, it can be concluded that the combinations of coconut water and IAA were able to accelerate shoot height growth, roots emergence, and increase the number of roots. The best treatment was the combination of 150 mL L^{-1} coconut water and 0.25 ppm IAA, but this treatment was not able to increase the number of shoots, number of leaves and shoot emergence time. Treatment that was able to accelerate the emergence of shoots and the number of shoots was the treatment of 150 mL L^{-1} coconut water independently, while the treatment which able to increase the number of leaves 150 mL L^{-1} coconut water or 0.25 ppm IAA independently. From this study, it can be seen that the treatment of 150 mL L^{-1} coconut water concentration has been able to increase the growth of shoots and roots, even though the best result of respons were obtained in the combination of coconut water and IAA. This was shown by the application of 150 mL L^{-1} coconut water in the combination of IAA 0 ppm (without IAA) has grown shoots and roots, so that 150 mL L^{-1} coconut water alone can be used as a potential of plant growth regulator to reduce costs and increase the growth in tribulus tissue culture. Regression analysis result indicate that increase in the number of roots is followed by an increase in the growth of shoot height, number of shoots, and number of leaves, because nutrients and hormones absorbed by the roots are then used for shoots and leaves growth.

Acknowledgement

The authors would like to thank The Ministry of Research, Technology, and Higher Education of Indonesia who has funded this research through the scheme of Insentif Riset SINas (INSINas).

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