

## RUNNER-TIP CULTURE OF STRAWBERRY (*Fragaria x ananassa* Duch) GROWN ON SEVERAL SHOOT-INDUCTION MEDIUM

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### ABSTRACT

A research regarding “Runner-tip culture of strawberry (*Fragaria x ananassa* Duch) Grown on Several Shoot-induction Medium” has been investigated. The objective of the research was to find out the most suitable medium for shoot production from runner-tip culture of strawberry at establishment step of micropropagation. The research was laid out in a Completely Randomized Design, 4 treatments of medium type for shoot induction and 10 replication, each was represented by one (1) bottle with 6-8 explants. The treatments were summarized as follows: T1 = MS ; T2 = MS + 2 ppm BAP + 0.01 ppm NAA; T3 = MS + 1 ppm of TDZ; T4 = WPM + 2 ppm BAP + 0.01 ppm NAA. The parameters observed were days of the bud emergence, the average number of shoots per explant, and the average number of leaves per explant. It can be concluded that among medium used in the current research, the medium of MS added with 1 ppm thidiazuron (TDZ) was the most suitable medium for shoot production of strawberry from the explant of runner-tips. The treatment was resulted in the earliest time of bud emergence, and producing the highest number of shoots and leaves.

*Keywords:* runner-tip, shoot-induction medium, strawberry, thidiazuron

### INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch) is a fruit that originally grown in subtropical area, however, it is also cultivated in tropical country, such as Indonesia. Pancasari is a sub district in Buleleng Regency, Bali, Indonesia with an altitude of  $\geq 1100$ m above sea level, an area where strawberry is cultivated intensively. The farmers obtain the planting materials for beginning plantation from tissue culture-propagated plants which a relatively expensive due to come from outside Bali. They reported that using runners as planted

materials for subsequent plantation lead to a decrease yield due to pathogen attack (Wayan Seria, pers.com. 2018). The condition is in accordance with Dijkstra (1993), Swartz et al. (1981), Nehra et al. (1994) which suggested that using runners as planting materials for strawberry is not suitable because of their sensitivity to pathology agents. Palai et al. (2015) also proposed that several soil pathogens cause vigour declines and ultimately death in strawberry by damaging the root. Moradi et al. (2011) proposed that micropropagated strawberry plants have been introduced to

prevent most of the plant and soil transmissible diseases. So, it is important to produce strawberry plants for field planting material using micropropagation.

There are many methods of plant micropropagation, one of those is direct organogenesis, in which the production of plantlet without following the step of callus production. In this method, micropropagation follows several steps, i.e. culture establishment, shoot multiplication, rooting and hardening off (Dwiyani, 2015). Among those steps, shoot multiplication greatly determines the number of plantlets produced. Shoot multiplication is started with the number of shoot produced at the culture establishment step. The shoot production at the step of culture establishment influences the number of shoot at the step of shoot multiplication and finally greatly affect the plantlet production in micropropagation. The current research reports the shoot production of strawberry (*Fragaria x ananassa* Duch) at the step of culture establishment using explants of runner-tip that grown on several shoot-induction medium. Shoot induction medium is the culture medium that contains plant growth regulator of cytokinin type in greater ratio compared to auxin type. The objective of the research was to find out the most suitable medium for shoot production from runner-tip culture at the step of culture establishment.

## MATERIALS AND METHODS

The research was carried out at The laboratory of Plant Tissue Culture of The Agriculture Faculty, Udayana University, Bali, Indonesia during March to July 2019. Strawberry (*Fragaria x ananassa* Duch) variety of Rosalinda that was taken from an orchard in Pancasari, Buleleng Regency, Bali (Indonesia) was used as source of mother plants.

Undeveloped runners (Figure 1) from the mother plant were taken as source of explants. Those then were surface sterilized following the protocols that has been established for strawberry in our laboratory (The laboratory of Plant Tissue Culture of The Agriculture Faculty, Udayana University) as in the following. The undeveloped runners were washed in the flow tap water, soaked (while shaking) in the 2% detergent solution for 5 minutes, then they were rinsed for three times with sterile water. In the outside laminar, those runners were then soaked and while being shaking in the 10% of sodium hypochlorite solution for 5 minutes. After that, they were rinsed in the sterile water for three times and then were put in the laminar. (The laminar has been UV sterilized for 30 minutes before using). In the laminar, those runners were surface sterilized again by soaking them in the 20% of sodium hypochlorite solution for 2 minutes. They were then rinsed with sterile water for three times, and then were dip

in the 70% alcohol for 5 seconds, then were rinsed again with sterile water for three times and were put in the sterile petri dish. The next step, runners were cut, about 3-5 mm-length of tips were taken, then they were dip in the 50 ppm of filter sterilized-ascorbic acid solution, then were put in the sterile filter paper for desiccation without any rinse. In order to prevent browning, the use of 50 ppm of filter sterilized-ascorbic acid solution has been established in the tissue culture protocol of our laboratory since 2008. The last step, those runner tips were then planted in several shoot-induction medium. The culture was maintained in the culture room under temperature of 24<sup>0</sup>C, room RH of 70%, and light source came from the fluorescent lamps with approximately intensity of 3000 lux.



**Fig. 1.** Undeveloped runner for source of explants; Bar= 1cm; An arrow shows the runner

We used four (4) types of medium culture for shoot induction, i.e. 1) Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) ; 2) MS medium added with 2 ppm of 6-Benzylamino-purine (BAP) + 0.01

ppm of 1-Naphthaleneacetic acid (NAA); 3) MS added with 1 ppm Thidiazuron (TDZ); and 4) Woody Plant Medium (WPM) (Mc. Cown & Lloyd, 1981) added with 2 ppm of BAP + 0.01 NAA + 1 g/L of active charcoal. Two grams of Polyvinylpyrrolidone (PVP) per liter were incorporated in each medium in order to prevent browning. Medium were added with 30g/L sucrose and solidified with 2 g/ L gellan gum, adjusted to pH of 5.8 and autoclaved with 1.16 kg cm<sup>-2</sup> of pressure at 121<sup>o</sup>C for 30 minutes. The treatments were summarized as follows: T1 = MS ; T2 = MS + 2 ppm BAP + 0.01 ppm NAA; T3 = MS + 1 ppm of TDZ; T4 = WPM + 2 ppm BAP + 0.01 ppm NAA;. Those treatments were laid out in Completely Randomized Design and replicated 10 times, each was represented by one (1) bottle with 6-8 explants. The parameters observed were days of the bud emergence, the average number of shoots per explant, and the average number of leaves per explant. Data was processed by analysis of variance (ANOVA). The significant different among the means then was continued with the Least Significant Difference (LSD) test at 1% level of probability.

## RESULTS AND DISCUSSION

The data of bud emergence can be seen at Table 1. Among the medium used, medium MS added with 1 ppm TDZ gave the best

result compared to other type of medium. A hundred percent (100%) of explants produced buds on MS medium added with 1 ppm TDZ (T3), while others were 60%, 50% and 40% for T4, T2 and T1 respectively. Table 2 shows

data for the number of shoot and the number of leaves per-explant. For both tables, it is clearly that TDZ was superior in stimulating of bud emergence, and the number of buds and leaves.

Table 1. Days of bud emergence

Treatments	Rep. 1 (dap)	Rep. 2 (dap)	Rep. 3 (dap)	Rep. 4 (dap)	Rep. 5 (dap)	Rep. 6 (dap)	Rep. 7 (dap)	Rep. 8 (dap)	Rep. 9 (dap)	Rep. 10 (dap)
T1 (MS)	-	11	-	10	9	-	10	-	-	-
T2 (MS +2ppm BAP+0.01ppm NAA)	10	11	-	-	-	-	10	7	9	-
T3 (MS+1 ppm TDZ)	2	2	2	3	1	1	2	2	3	4
T4 (WPM + 2 ppm BAP + 0.01 ppm NAA)	4	5	3	-	-	-	3	4	4	-

Remarks: Rep.=replication, consist of 1 bottle with 6-8 explants each; dap= days after planting; the punctuation mark of “ - “ means no bud was detected from all explants.

Table 2. The effect of medium on the shoot and leaf number

Treatments	The average number of shoots per explant	The average number of leaves per explant
T1 (MS)	0.5 c	0.2 c
T2 (MS +2ppm BAP+0.01ppm NAA)	0.9 c	0.8 c
T3 (MS+1 ppm TDZ)	7.4 a	8.3 a
T4 (WPM + 2 ppm BAP + 0.01 ppm NAA)	2.7 b	2.9 b

Remarks: The same letter behind values of mean of treatment indicates no significant differences among the mean treatment based on The Least Significant Difference (LSD) at 1% level of probability and vice versa for different letter.

This species has morphological characteristics, among others: in exuvia there are five pairs of abdominal compound pores that are shaped like a crown or splinal.

Compound pores with a series of rod-like processes in a ring. One pair on the head and four pairs on the subdorsal part of segments five to eight.

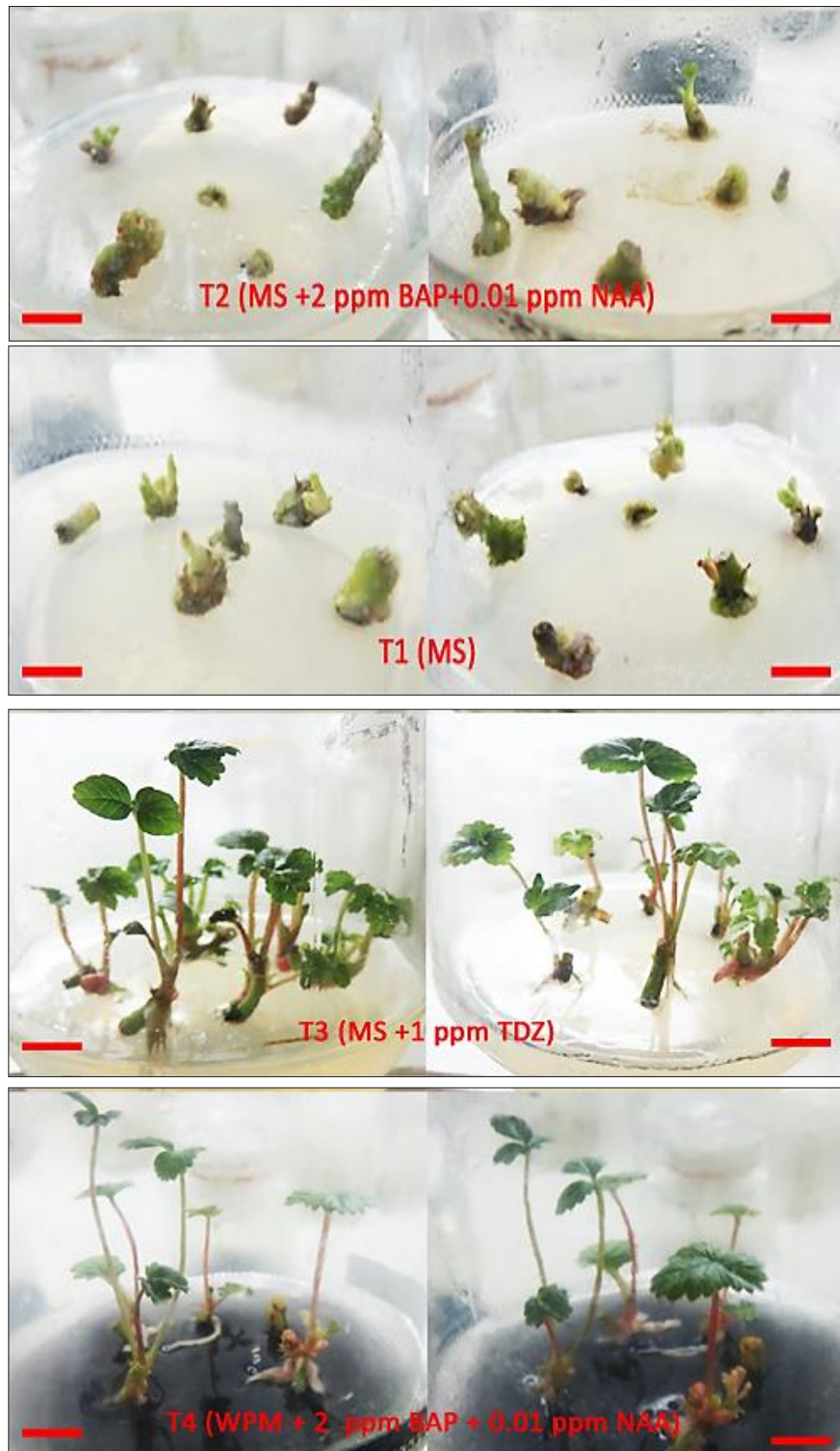
Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) was earlier categorized as cytokinin because of its natural cytokinin-like response (Pai and Desai, 2018). It has cytokinin activity and has been reported to be effective in stimulating adventitious shoot formation and promoting axillary shoot proliferation in micropropagation (Lu, 1993) and also reported as a potent cytokinin for woody and recalcitrant species in tissue culture (Huetteman & Preece, 1993). Jones *et al* (2007) reported that regeneration of *Echinacea purpurea* L. grown in vitro (in solid and liquid medium) was induced with the use of TDZ. Naz *et al.* (2012) also reported the potentiality of TDZ on multiple shoot induction for a woody legume, *Bauhinia tomentosa* L. The use of TDZ in inducing embryogenesis was also reported in *Phalaenopsis* orchid (Mose *et al.*, 2017). Mode of action of TDZ in affecting plant tissue culture was reviewed by Pai and Desai (2018). It was suggested that the presence of TDZ affects metabolism of endogenous plant growth regulators during morphogenesis and regulates endogenous growth. Pai and Desai (2018) also reported that in some experiments, increased levels of endogenous auxin, ethylene, and ABA were recorded in response to TDZ treatment.

In the current research, the number of shoots and the number of leaves of strawberry

were significantly affected by application of TDZ compared to other type of cytokinin (BAP) at 14 days after planting. Many reports were found on micropropagation of strawberry. However, most of the reports used BAP as source of cytokinin for inducing shoot (Biswas, *et al.*, 2010; Mozafari & Gerdakareh, 2012; Tanziman *et al.*, 2013, Harugade *et al.*, 2014) and few reports used TDZ (Passey *et al.*, 2003; Cappelletti *et al.*, 2016). Among those reports, Tanziman *et al.*, (2013) used runner tip as an explant. The current finding used combination of runner tip as an explant and TDZ for the most suitable shoot induction (Table 2 and Figure 2).

Regarding the use of BAP for shoot induction and production, we found that BAP showed its role in inducing shoot if WPM was used as basic medium instead of MS, and when 1 g/L active charcoal (AC) was incorporated. However, it cannot be concluded that this was caused by WPM or AC per se. Although it was not counted, however, roots grew faster on AC-enrich medium (T4) compared to T3. The role of AC for preventing browning in tissue culture was reported elsewhere. However, AC also stimulated root growth in tissue culture (Agrawal *et al.*, 2002; Tyagi and Prakash, 2004; Firoozabady *et al.*, 2006; Joshi and Dhawan, 2007).

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**Fig. 2.** The Growth of explants at 14 days after planting on several medium; bar=0.5cm

We found that the current research produced average number of 7.3 shoots / explant with explant of runner tip and using media of MS + 1 ppm TDZ. Other previous research produced 10.4 shoots, with nodal segment of runner as explant on the medium of MS + 2 ppm BAP + 0.5 ppm NAA (Mir *et al.* 2010), 7 shoots with explant of nodal segment of runner grown on MS medium added with 0.5 ppm BAP (Ashrafuzzaman *et al.*, 2013), and 5.4 shoots/explant using explant of runner segment planted in MS medium supplemented with 1 ppm BA + 0.1 ppm NAA + 1 ppm adenine sulphate + 150 ml coconut water (Jhajhra *et al.*, 2018). The difference between those of previous researches with the current research is in the time of observation. The number of shoots / explant in the previous research was observed after 1-3 subculture, while the current research was in the step of culture establishment, before first subculture was done. However, we did also subculture on the same medium after 14 days of planting, but for T3 only. The number of shoot on T3 treatment was observed and counted at 10 days after first subculture; it was average of 15.2 shoots / explant. We suggest that TDZ greatly affected the number of shoots.

Despite of the effective use of TDZ in shoot induction on some plant species, however, prolonged use of TDZ may induce abnormality such as reported by Naz *et al*

(2012) and Dewir *et al.* (2018). Therefore, to avoid adverse effect of the use of TDZ, the long exposure of explants on TDZ should be avoided.

## CONCLUSION

Among medium used in the current research, it can be concluded that medium of MS added with 1 ppm thidiazuron (TDZ) was the most suitable medium for stimulating shoot induction at the step of culture establishment.

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