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PEMBENTUKAN SCALP DAN TUNAS PADA KULTUR IN VITRO TANAMAN PISANG TANDUK SEBAGAI RESPONS TERHADAP KONSENTRASI THIDIAZURON

SCALP AND SHOOT FORMATION IN RESPONSE TO THIDIAZURON CONCENTRATIONS IN VITRO CULTURE OF PLANTAIN 'TANDUK'

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ABSTRACT

This experiment aimed to study the formation of scalps and banana shoots 'Tanduk' in vitro in response to thidiazuron (TDZ). Shoot explants measuring ± (1 x 1 x 1) cm3 were cultured in precondition media (MS+ 5 mg/l benzyladenine) for 4 weeks, then transferred to treatment media, namely MS + TDZ. This experiment was carried out using a completely randomized design with 3 replications and 7 treatments of TDZ concentrations (0.5; 1.0; 1.5; 2; 2.5; 3; 3.5; 4.0 mg/l). Each experimental unit consisted of 5 bottles, each containing one explant. Observations of scalp number were carried out at 8 weeks of age. The scalps were subcultured on MS+5mg/l BA media to induce shoots. After 8 weeks in the shoot induction medium, the number of shoots was recorded. The results showed that at 8 weeks on the culture all of the explants formed scalps, with the highest number of scalps (6 scalps/explant) was at 1 mg/l TDZ. The scalp number decreased to 4.4 to 3.5/explant as the TDZ concentration increased from 1.5 to 4 mg/l. After 8 weeks on shoot induction media containing 5 mg/l BA, the highest number of shoots (3.8 shoots/explant) was obtained on the scalp from the 0.5 mg/l TDZ treatment. The shoot number decreased to (3.2-1.9/explant) with increasing TDZ concentrations from 1.0 to 4.0 mg/l.

KATA KUNCI:
Pisang tanduk, Thidiazuron, in vitro, Scalp

ABSTRAK

Percobaan ini bertujuan untuk mempelajari pembentukan scalp dan tunas pisang 'Tanduk' in vitro sebagai respons terhadap thidiazuron (TDZ). Eksplan pucuk tunas berukuran ± (1 x 1 x 1) cm3 dikulturkan di media prekondisi (MS+ 5 mg/l benziladenin) selama 4 minggu, lalu ditransfer ke media perlakuan, yaitu MS + TDZ. Percobaan ini dilakukan menggunakan rancangan acak lengkap dengan 3 ulangan dan tujuh perlakuan TDZ (0.5; 1.0; 1.5; 2; 2.5; 3; 3.5; 4.0 mg/l). Setiap satuan percobaan terdiri dari 5 botol, masing-masing berisi satu eksplan. Pengamatan terhadap jumlah scalp yang terbentuk dilakukan pada umur 8 minggu. Selanjutnya, scalp yang terbentuk disubkultur ke media MS +5 mg/l BA untuk menginduksi tunas. Setelah 8 minggu di media induksi tunas, diamati jumlah tunas yang terbentuk. Hasil percobaan menunjukkan bahwa pada 8 MST, semua eksplan yang dikulturkan di media MS+0,5-4 mg/l TDZ dapat membentuk scalp, dengan jumah scalp terbanyak (6 scalp/eksplan) didapat pada perlakuan 1 mg/l TDZ. Jumlah scalp menurun menjadi antara 4,4 dan 3,5 per eksplan seiring dengan makin meningkatnya konsentrasi TDZ dari 1,5 hingga 4 mg/l. Setelah 8 minggu di media induksi tunas yang berisi 5 mg/l BA, jumlah tunas terbanyak (3,8 tunas/eksplan) didapat pada scalp dari perlakuan 0,5 mg/l TDZ. Jumlah tunas menurun menjadi antara 3,2 dan 1,9 per eksplan dengan makin tingginya konsentrasi TDZ dari 1,0 hingga 4,0 mg/l.

1. INTRODUCTION

The needs for bananas and plantains (*Musa* spp.) rises from year to year in Indonesia. Therefore, *Musa* spp. production must be increased, among other things, by expanding areas of plantation which consequently needs planting materials in a large number. To produce them, one way is to apply plant tissue culture techniques, because thousands or even millions of planting materials could be produced in the same size, in genetically identical, and in a relatively short time. A plantain 'Tanduk' needs plant tissue culture technique because it conventionally produces only limited number of suckers per plant (1-5 suckers).

The in vitro propagation of plantain 'Tanduk' via axillary branching has been reported. In this way, multiple shoots were induced from shoot tips as explants, roots were promoted from the shoots, and the plantlets were finally acclimatized.

Another multiplication of Musa spp. in vitro through scalp formatian also reported. The scalp was described as a bulbous structure, looked like cauliflower and contained tiny white tissues (Dhed'a, et al., 1991; Sholi et al., 2009). This scalp contained clumps of meristematic buds leading to shoot tips then resulting in multiple shoots tissues (Dhed'a, et al., 1991; Sholi et al., 2009).

Scalp induction of Musa in vitro was reported by use of media containing cytokinins, such as thidiazuron (Shirani et al., 2010; Sadik et al., 2015; Annisa et al., 2021), benziladenine (Shirani et al., 2010, Manurung et al., 2021; Ramírez-Villalobos and García, 2008) and kinetin (Shirani et al., 2010). The scalps could result in shoots by transfering on the media containing benzyladenine (Shirani et al., 2010; Elhory et al., 2009).

In term of plantain 'Tanduk', to induce scalp was to supplement media with BA 100 µM and IAA 1 µM (Elhory et al., 2009). To use TDZ in order to induce scalp in plantain 'Tanduk', as far as we know, has not been reported. However, in other genotypes of Musa spp, TDZ was effective to induce scalp, in the case of Musa spp. such as Rastali (AAB) using TDZ 7.5 µM, Berangan Intan (AAA) (TDZ 0.5 µM), and Berangan (AAA)(TDZ 0.5 µM) (Shirani et al. 2010). Sadik et al (2015) use TDZ 26 µM to induce scalp effectively. This research aimed to investigate effect of TDZ concentrations on scalp induction in in vitro plantain Tanduk.

2. RESEARCH METHODS

2.1 Plant Materials

Sword suckers of plantain 'Tanduk' approximately 50 cm in length and 12 cm in rhizome diameter were taken from the east Lampung, Indonesia. The suckers were peeled and cut into 12 cm in length. They were soaked in 150 mg/l of ascorbic acid and 2 g/l of mancozeb fungicide for 15 minutes and then were soaked with streptomycin for 15 minutes.

Apical buds along with rhizome ± 4 cm in length were sterilized with solution of NaOCl 2.6%, 1.6%, 0.8%, and 0.3% in series for each 30 minutes. The sterilization was used by shaking 190 rpm, ending up with three rinses with water. The apical buds were then trimmed to 1.5x1.5x 2 cm, soaked in ascorbic acid 150 mg/l, and shaked with NaOCl 1.6% and Tween-20 two drops/100 ml for 10 minutes and followed by three rinses with sterile distilled water. The apical buds were trimmed again to 1x1x1 cm, and then shaked with NaOCl 0.5% for 5 minutes, and followed by three rinses with sterile distilled water. The explants were soaked in NaOCl 0.3% while being vacumed, and then followed by three rinses with sterile distilled water. They were then cultured on precondition media.

2.2 Media Preparation

The precondition media contained MS basal salt (Murashige and Skoog, 1962), sucrose 30 g/, BA 5 mg/l, ascorbic acid 200 mg/l, citric acid 150 mg/l, thiamine-HCl 0.1 mg/l, pyridoxine-HCl 0.5 mg/l, nicotinic acid 0.5 mg/l, and glycine 2 mg/l. The components were solubilized, then the solution was determined at pH 5.8, put in the agar 8 g/l as gelling agents, and heated until boiled. The media were dispensed to 250-ml bottles (25 ml each), and the bottles were covered with heat resistent plastic sheets. The bottles containing the media were autoclaved at 1.2 kg/cm2 for 20 minutes. The treatment media components were the same as those of the preconditioned media, except devoid of BA, but put in TDZ.

2.3 Experimental Design

Explants were cultured on precondition media for 4 weeks and then subcultured to the treatment media containing different concentrations of TDZ (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg/l). The experiment was set up in a completely randomized design with three replications. One replications consisted of 7 bottles containing one explant. The data recorded at 8 weeks in culture were subjected to analysis of variance and separation of the means were done with LSD 5% (least significant difference).

3. RESULTS AND DISCUSSION

3.1 Results

After 4 week-culture on precondition media, the explants were cultured on the media containing different concentrations of TDZ as treatments. The TDZ concentrations significantly affected the scalp number. In the increase of TDZ concentrations from 0.5-4.0 mg/l, the scalp number rised up to 1 mg/l of TDZ and then dropped to 4 mg/l of TDZ. The increase in 1.0 to 1.5 mg/l resulted in significant decrease in scalp number. The highest number was 6.0 scalps per explant at 1.0 mg/l of TDZ (Fig.1).

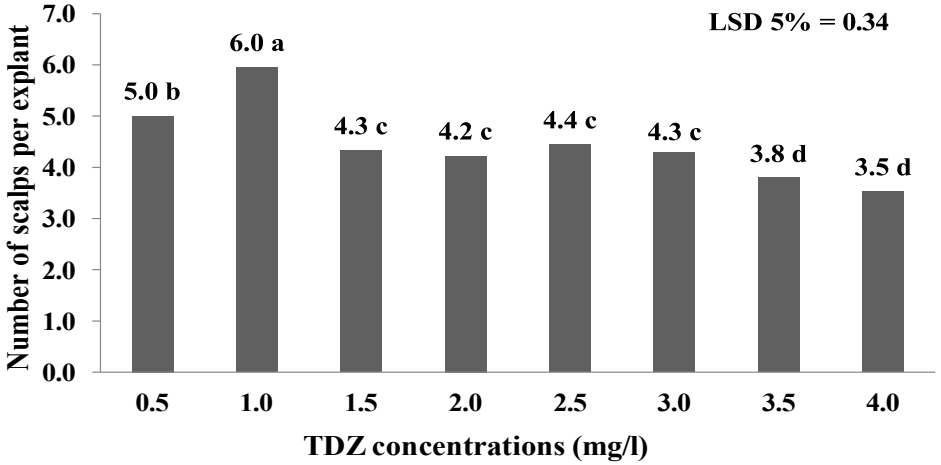

Figure 1. Effects of TDZ concentrations on number of scalps per explant on culture in 8 weeks. Numbers followed by the different letters differ at P ≤ 0.05 according to LSD multiple range test.

In addition to scalps, the shoot buds and shoots were also induced from the explants (Figure 2). Both the highest shoot buds and shoots were obtained at 0.5 mg/l of TDZ (Figure 3), then the both decreased as the TDZ concentrations increased starting from 1.0 mg/l to 4.0 mg/l. Based on

the LSD mentioned, at TDZ 0.5 mg/l the number of shoot buds and shoots were more or less the same (Figure 3).

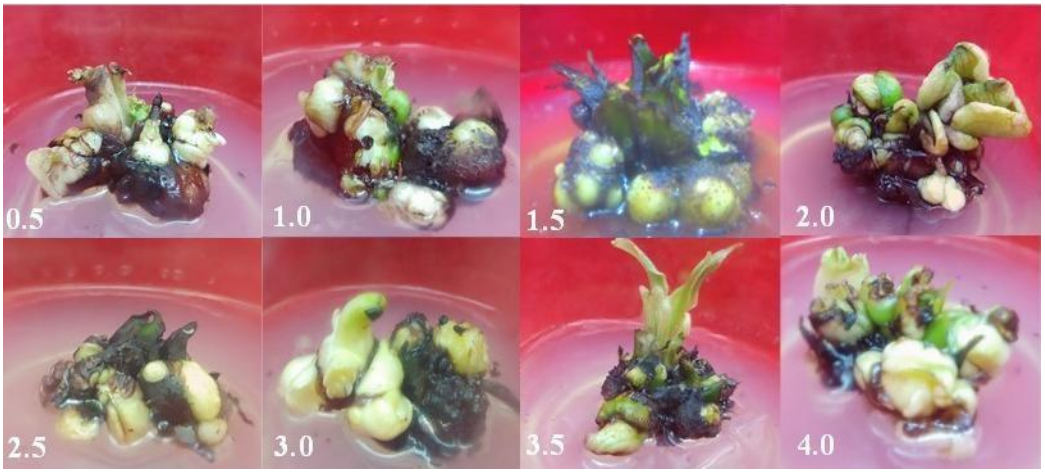

Figure 2. The scalp formation in 8 week-old propagules in media containing different TDZ concentrations. The numbers indicate TDZ concentrations in mg/l.

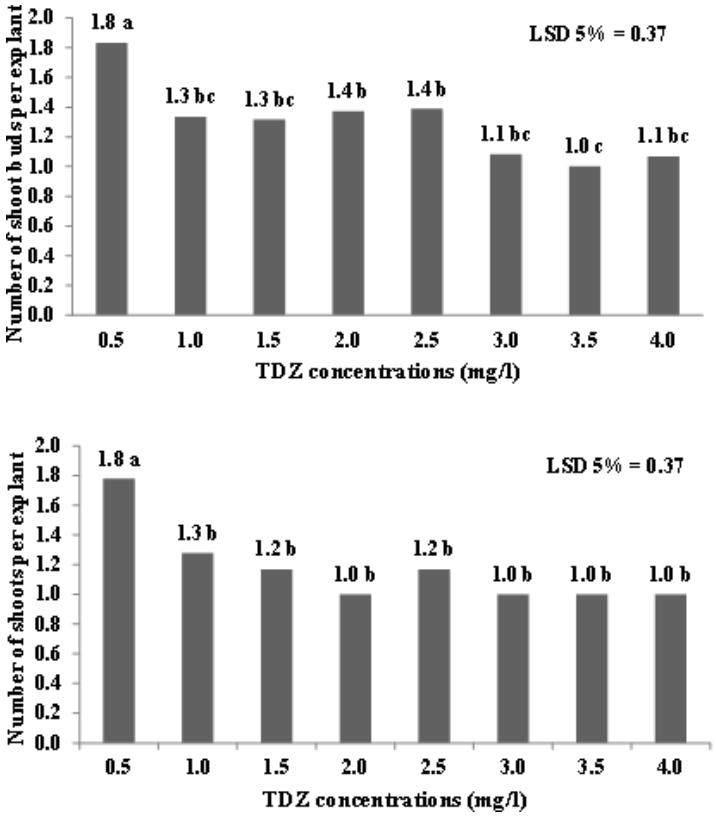

Figure 3. Effects of TDZ concentrations on number of shoot buds (above) and number of shoots (below) per explant on culture in 8 weeks. Numbers followed by the different letters differ at P ≤ 0.05 according to LSD multiple range test.

To induce shoot formation, the scalp was cultured on the media containing 5 mg/l of BA for 8 weeks (Figure 4). In this media, the number of shoots was significantly dependent upon the

TDZ-induced scalps. The highest shoots were produced by the scalps previously induced at 0.5 mg/l of TDZ, then the shoot number decreased at from 1.0-4.0 mg/l of TDZ (Figure 4 and 5).

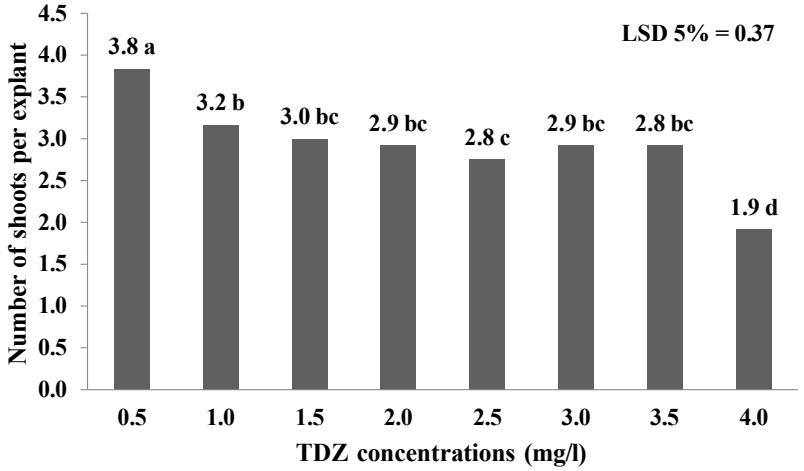

Figure 4. Number of shoots produced by scalps resulted from the induction of different TDZ concentrations. The scalps were cultured on the media containing 5 mg/l of BA for 8 weeks. Numbers followed by the different letters differ at P ≤ 0.05 according to LSD multiple range test.

3.2 Discussion

Some reports showed that cytokinin induced axillary branching in Musa spp. in vitro (Huq et al., 2012; Ahmed et al., 2014; Razani et al., 2020; DRZ et al., 2016; Youmbi et al., 2014; Kiruwa et al., 2024; Agbadje et al., 2021; Uzaribara et al., 2015; J. Lohidas and Sujin, 2015; Waman et al., 2016; Shah et al., 2020; Elyazid et al., 2021; Tamimi and Othman, 2020; Cruz-Rosero et al., 2017). Some other reports showed that cytokinin induced scalps and then they became multiple shoots in tissue culture of Musa spp. (Shirani et al., 2010; Fitramala et al., 2016; Razani et al., 2019). Similar to these reports, this our present study showed that TDZ resulted ini scalps and they were on the media containing benzyladenine turned into multiple shoots (Figure 4 and 5). Concerning the scalp production in tissue culture of Musa spp, Shirani et al. (2010) proposed that since scalps were highly proliferating, they were suitable for a mass clonal propagation, and could be subject to genetic mutation and engineering.

In our present study, all of the TDZ concentrations induced scalp production in tissue culture of plantain Tanduk (Figure 1 and 2), the most effective being 1 mg/l. Other studies reported that TDZ was also effective to induce scalp production in vitro in Musa spp. (Shirani et al., 2010; Sadik et al, 2015; Annisa et al., 2021). Shirani et al. (2010), using Rastali (AAB genome) as their study, reported that the most effective TDZ concentration was 7.5 µM. On the other hand, in our present research, the most effective TDZ was 4.5 µM (1 mg/l) to produce scalps in vitro of Tanduk (AAB genome). Much higher concentration of TDZ (26 µM) was required to be effective for scalp production in tissue culture of banana Nakinyika (Sadik et al., 2015). These different effectiveness of TDZ concentrations might be dependent upon different genotypes related to their difference in indogenous hormonal condition.

In our present study, after the scalps were induced by different concentrations of TDZ, they were moved to media containing 5 mg/l BA. This showed that the increase in TDZ resulted in the decrease in shoot number (Figure 4) even though all the scalps subjected to the same concentration of BA (5 mg/l). This might be attributable to the carry-over effects of TDZ, meaning that the effects were not only the BA but also the combined BA and TDZ which both of them acted as cytokinins. TDZ was considered as a potent cytokinin and could accumulate in cells and act

as endogenous cytokinins (Huetteman and Preece, 1993). Therefore, in Figure 4 was consistent with other reports on the effects of cytokinin consentration on shoot number in tissue culture of Musa spp. In other words, when shoot number is maximum, it decreases as the cytokinin concentration increases.

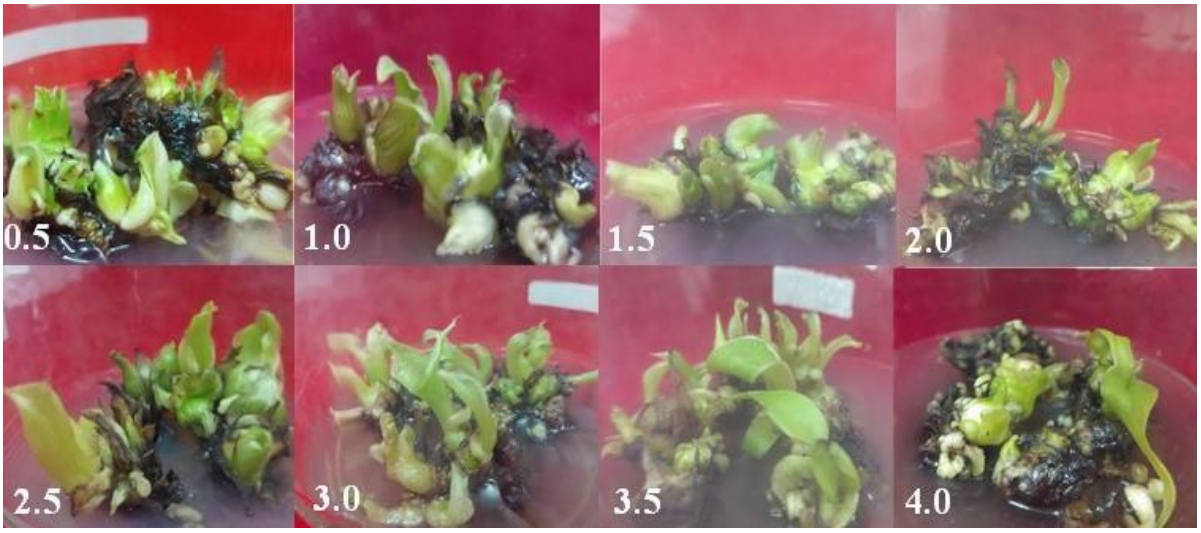

Figure 5. Shoot formation derived from scalps in media containing 5 mg/l of BA for 4 weeks. The scalps were previously induced by different concentrations of TDZ. The numbers indicate TDZ concentrations in mg/l.

4. CONCLUSION

The results showed that thidiazuron (TDZ) 0.5-4.0 mg/l led to scalp formation. TDZ treatment of 1 mg/l resulted in the highest number of scalps (6 scalps/explan), then the scalp decreased (3.5-4.4 scalps per explant) as the concentrations of TDZ increased.

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