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Somatic Embryogenesis and Plant Regeneration from the Apical Meristem of Wrukwona Napiergrass (*Pennisetum purpureum*) Treated with Thidiozuron and Cupric Sulfate

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ABSTRACT

This study focused on the effectiveness of somatic embryogenesis and regenerated plant in Wrukwona napiergrass. Previously, we studied *in vitro* propagation of 4 cultivars of napiergrass (*Pennisetum purpureum*) and showed that only 3.3% of Wrukwona cultivar formed embryogenic callus on day 30 and 21.7% on day 60 of incubation. To improve callus formation performance, it is necessary to develop a special propagation method for Wrukwona cultivar in terms of various growth regulators and additional compounds. This study used several rates of 2.4-dichlorophenoxyacetic acid (2.4-D), benzyl amino purine (BAP), and thidiozuron (TDZ). The result showed that the use of medium Murashige & Skoog (MS) with 2.4-D and BAP at a high ratio of 2.4-D, and TDZ 2 µM formed 78.6% embryogenic callus on day 60th and no albino was found in the regenerated plant. The best combination of growth promotor for embryogenic callus formation was 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 µM TDZ. Callus proliferation with MS media added with 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, 2 µM TDZ, and 5 µM $CuSO_4$ gave the best proliferation results, with regeneration reaching 65%. All regenerants successfully grew in soil. It can be concluded that somatic embryogenesis of *P. purpureum* cv. Wrukwona can be produced from MS culture medium using 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 µM TDZ. Effective multiplication was carried out by adding 5 µM $CuSO_4$ to the same medium as the embryogenic callus formation, and effective regeneration was carried out with MS media containing 2 mg L^{-1} BAP.

Keywords: apical meristem; embryogenic calli; napiergrass; Wrukwona

INTRODUCTION

Biotechnology can be applied to produce and enhance the forage quality by plant breeding methods. One of the ways can be done with tissue culture technique. Breeding efforts have been carried out in various tropical forage grasses, including *Pennisetum purpureum* (Umami *et al.*, 2016), *Brachiaria decumbens* (Suseno *et al.*, 2021), *Cencrus ciliaris* (Kumar *et al.*, 2015), alfalfa (Nurmaningrum *et al.*, 2017), *Imperata cylindrica* (Umami *et al.*, 2012), *Sorghum bicolor* (Saied *et al.*, 2014), *Brachiaria ruziziensis* (Ishigaki *et al.*, 2014), *Urochloa* sp. (Pereira *et al.*, 2016), *Cenchrus ciliaris* (Dwivedi *et al.*, 2016), and *Panicum virgatum* (Lin *et al.*, 2017).

Pennisetum purpureum is one of the superior forage grasses that is widely used in the tropics and subtropics regions. This research used Wrukwona *P. purpureum*, commonly known as Wrukwona napiergrass. Rengsirikul et al. (2013) showed that Wrukwona had higher annual biomass than the other plant varieties (Muaklek, Dwarf, Taiwan A148, Bana, Tifton, Common, and Kampheng SAN). Ishii *et al.* (2016) reported that Wrukwona napiergrass could withstand winter conditions and weed.

Plant transformation may help the breeding system of *P. purpureum* because special traits can be manipulated into the forage species by applying genetic engineering methods. The method offers chances to enhance *P. purpureum*. Moreover, a transformation system and breeding mutation which are combined with this pasture would be essential to increase the gene pool of *P. purpureum*.

Propagation in tissue culture can be done through somatic embryogenesis. The advantages of this technique include the embryos produced are bipolar, resembling zygotic and somatic embryos, planting does not

depend on time/season, can produce large numbers of plants, and have the same characteristics as the parent. In addition, tissue culture techniques produce sterile and disease-free plants (Maulana *et al.,* 2019). The somatic embryogenesis process in tissue culture produces callus as material for genetic transformation. The callus is then regenerated into a complete plant (Suseno *et al.,* 2016). Callus regeneration is very important to ensure the success of callus culture. However, callus regeneration can be influenced by callus age, genotype, explant source, and culture conditions, including the environment and culture media used. Fiah *et al.* (2014) suggested that the regeneration ability of a callus can be decreased, even maybe disappear along with the length of the callus culture period.

In a previous study, based on *in vitro* propagation of 4 cultivars of *P. purpureum*, the results showed that the Wrukwona cultivar formed only 3.3% embryogenic callus on day 30% and 21.7% on day 60 of incubation (Umami *et al.,* 2016). Improved callus formation performance needs to be developed with special propagation methods to Wrukwona napiergrass by comparing various growth regulators and additional compounds. This study used several compositions of 2.4-dichlorophenoxyacetic acid (2.4-D), benzyl amino purine (BAP), and Thidiozuron (TDZ). Sugito *et al.* (2006) stated that one of the aspects that can influence the formation process of somatic embryos is its type and composition of plant growth regulators. Thidiazuron is a very strong cytokinin group for shoot formation, 2.4-D is an auxin group for root formation, and gibberellin is a hormone that triggers dormancy. A research by Nurmaningrum *et al.* (2017) reported that the combination of BAP and TDZ in Murashige Skoog (MS) media could induce shoot formation and propagation. However, there is no published data on the combination of TDZ with auxin cytokinin and cupric sulfate ($CuSO_{4)}$ in the somatic embryogenesis formation of Wrukwona napiergrass. This study focused on the effectiveness of somatic embryogenesis and regenerated plant in Wrukwona napiergrass.

MATERIALS AND METHODS

This research was conducted in Frontier Science Research Center, University of Miyazaki Japan. This research was conducted in 4 stages.

Stage 1

Explant sterilization. Shoot-tillers of Wrukwona napiergrass were used as explants and then washed with water to eliminate strands and small sand particles. The sterilization of the shoot tillers was conducted by absorbing them in 70% (v/v) ethanol for two minutes, preceded by soaking them in a solution of 2% (v/v) sodium hypochlorite (NaOCl). The NaOCl solution with shoot tips was agitated for fifteen minutes, preceded by 3 repetitions washes in purified water for two minutes. After sterilization, the mature leaves were detached from the main explant stem. Explants with the same shape dimension were put in the glass containing MS media (Murashige & Skoog, 1962) added with many ingredients of 2.4-D, BAP, and TDZ.

Embryogenic callus induction. The cultured of initial explants were cultured in MS medium which contains 0.3% phytagel, 3% sucrose, and 0.1% (v/v) as preservation for growth medium of the plant tissue (PPM) which was added with many combinations including 0 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D0B0.5T0); 0 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D0B0T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D2B0.5T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ (D2B0.5T2); 2 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 4 μM TDZ (D2B0.5T4); 2 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D2B0T0); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D3B0.5T0); 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ (D3B0.5T2); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 4 μM TDZ (D3B0.5T4); 3 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D3B0T0). The best combination was used on stages 2.

Stage 2

The best combination on stage 1 was used on stage 2 to find out the proliferous callus, embryogenic callus formed, and % of embryogenic callus formed. Embryogenic callus was proliferated using solidified MS media containing the optimum mixture of 2,4-D, BAP, and TDZ with 0.5 μM $CuSO_4$ (CL) or 50 μM $CuSO_4$ (CH). The best combination was used on stage 3.

Stage 3

Stage 3 was the process of plant regeneration. Embryogenic callus from the media of callus induction was transferred onto regeneration medium that contains MS basal media consisting of 0.3% phytagel and 3% sucrose improved with 2 mg L^{-1} BAP in mixture with 0.01 and 0.1 mg L^{-1} naphthalene acetic acid (NAA), as the control of hormone-free media (Gondo *et al.,* 2017). In 2 weeks, the regeneration of the plant was measured. Elongated green shoots were segregated and moved to MS medium with light situations to induce root growth. Plantlets were rooted from embryogenic callus and then transmitted to soil and sustained in the greenhouse, waiting for its growth. All the plant's media had pH 5.6-5.8 before being sanitized at 121 °C for 15 minutes. The culture plants were nurtured under 3500 lux fluorescent lights for 16 h at 27 °C.

Stage 4

Stage 4 was forage characteristics. Regenerant was planted in pots with a diameter of 30 cm and a height of 40 cm. Treatments of D3B0.5T2CL, D3B0.5T2CH, and control (parental plants) were observed. The yield, physical and chemical properties of plants were observed to determine the diversity of the regenerant results. Harvesting was conducted at 30 days old. Forage characters observed were plant height, biomass production, and chemical composition, including dry

matter (DM), crude protein (CP), crude fiber (CF), and organic matter (OM). The CP and CF contents in plant samples were determined using the method of AOAC (2005).

Statistical Analysis

Data analysis was conducted using a completely randomized design with a significant level of 5%. This analysis was facilitated by software R version 4.0.2. The package Agricolae was selected to support the statistical analysis process. Descriptive test was applied to identify the attributes of data observation. The normality of data was evaluated using Shapiro-Wilk test. Homogeneity of variance among treatments was examined using Bartlett's test. The comparison means of callus formed, embryogenic callus formed, proliferation callus, embryogenic callus formed, regenerated callus, shoot formed, and morphological characters among treatment applications were assessed using Kruskal-Wallis test and followed by Nemenyi test.

RESULTS

Table 1 presents the effect of hormone concentration on embryogenic callus formation in the apical meristems of tiller shoots of Wrukwona napier grass. The use of 2.4-D, BAP, and TDZ showed the effects on callus formed and embryogenic callus formations. The highest values for callus and embryogenic callus formations were observed in the medium D3B0.5T2 with a combination of 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ, which were 47.00 and 25.00, respectively. Calli formed and embryogenic formed are shown in Figures 1 and 2.

Callus growth that did not form embryogenic callus on D2B0.5 media is shown in Figure 3a, and the raised embryogenic callus using D3B0.5T2 media is shown in Figure 3b-e, which indicates the presence of an embryogenic callus on the surface of the callus. The effects of several combinations of TDZ and $CuSO_4$ on the callus proliferation of embryogenic callus of Wrukwona napier grass are shown in Table 2. The proliferation of callus number and percentage of embryogenic callus formation grew on medium D3B0.5T2CL with the combination of 3 mg L^{-1} 2.-D, 0.5 mg L^{-1} BAP, 2 μM TDZ, and 5 μM $CuSO_4$ showed the highest values, which were

Table 1. The different levels of plant growth hormone concentration on the formation of embryogenic callus from shoot apical meristems of *Pennisetum purpureum* cv. Wrukwona on 60 days after culture

Treatments	No. of callus formed (%)	No. of embryogenic callus formed (%)
D0B0.5T0	0.50±0.55^{h}	0.00±0.00^{g}
D0B0T0	0.00±0.00^{i}	0.00±0.00^{g}
D2B0.5T0	4.83±0.75^{f}	0.00±0.00^{g}
D2B0.5T2	10.00±0.89^{d}	4.67±0.52^{d}
D2B0.5T4	18.00±1.10^{c}	10.00±1.55^{c}
D2B0T0	0.50±0.55^{h}	0.17±0.41^{g}
D3B0.5T0	7.17±1.60^{e}	2.33±1.03^{e}
D3B0.5T2	47.00±1.41^{a}	25.00±1.26^{a}
D3B0.5T4	20.00±0.63^{b}	13.00±0.89^{b}
D3B0T0	1.83±1.17^{g}	1.17±0.75^{f}
p-value	<0.001**	<0.001**

Note: Means in the same column with different superscripts differ significantly (p<0.05). **= significant; 2.4-D= 2.4-dichlorophenoxyacetic acid; BAP= benzyl amino purine, TDZ= thidiozuron.
D0B0.5T0= 0 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 0 μM TDZ;
D0B0T0= 0 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ;
D2B0.5T0= 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ;
D2B0.5T2= 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ;
D2B0.5T4= 2 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 4 μM TDZ;
D2B0T0= 2 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ;
D3B0.5T0= 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ;
D3B0.5T2= 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ;
D3B0.5T4= 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 4 μM TDZ;
D3B0T0= 3 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ.

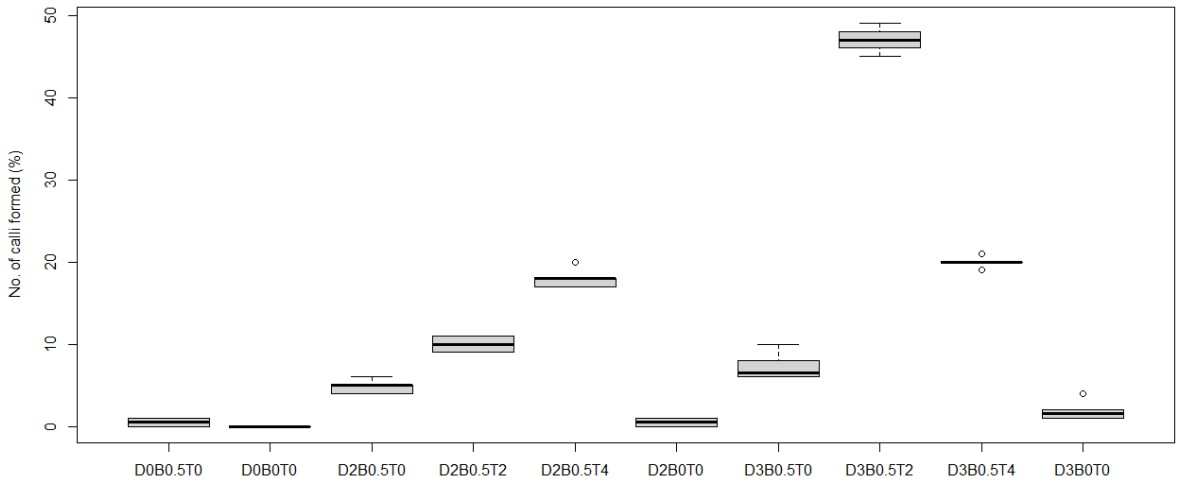

Figure 1. Number of callus formed on media containing 0 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D0B0.5T0); 0 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D0B0T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D2B0.5T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ (D2B0.5T2); 2 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 4 μM TDZ (D2B0.5T4); 2 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D2B0T0); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 μM TDZ (D3B0.5T0); 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ (D3B0.5T2); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 4 μM TDZ (D3B0.5T4); 3 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 μM TDZ (D3B0T0).

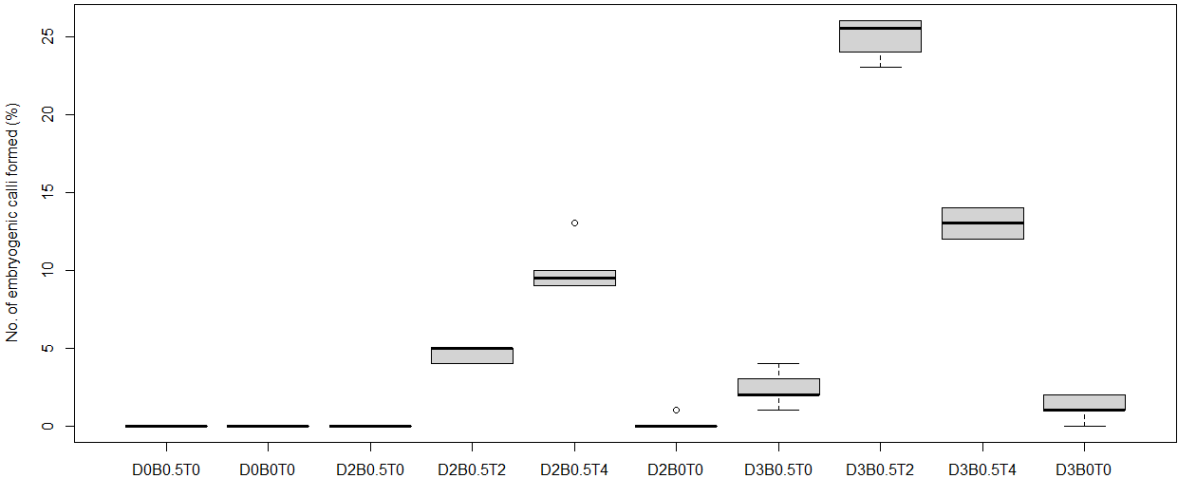

Figure 2. Number of embryogenic callus formed (%) on media containing 0 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 0 µM TDZ (D0B0.5T0); 0 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 µM TDZ (D0B0T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 µM TDZ (D2B0.5T0); 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 µM TDZ (D2B0.5T2); 2 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 4 µM TDZ (D2B0.5T4); 2 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 µM TDZ (D2B0T0); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 0 µM TDZ (D3B0.5T0); 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 µM TDZ (D3B0.5T2); 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 4 µM TDZ (D3B0.5T4); 3 mg L^{-1} 2.4 D, 0 mg L^{-1} BAP, and 0 µM TDZ (D3B0T0).

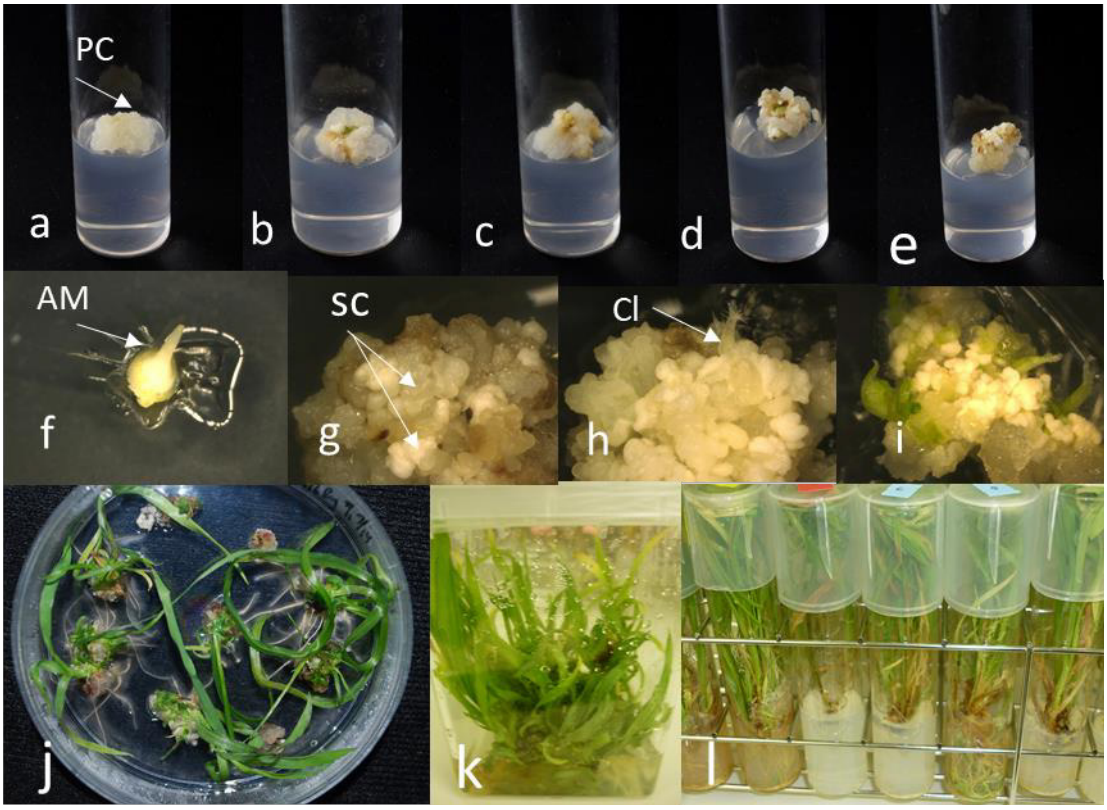

Figure 3. Plant regeneration stage of *Pennisetum purpureum* cv. Wrukwona from embryogenic callus. (a) Callus formed as primary callus from apical meristem after 10 d of culture, under room temperature and light condition on MS media containing 2 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg L^{-1} benzylaminopurin (BAP). (b) Callus formation on MS medium containing 2 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, and 2 µM thidiozuron (TDZ). (c) Callus formation on MS media containing 2 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, and 4 µM TDZ. (d) Callus formation on MS media containing 3 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, and 2 µM TDZ. (e) Callus formation on MS media containing 4 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, and 4 µM TDZ. (f) A swelled apical meristem from shoots apical. (g) Somatic embryo developed into scutellums on MS medium containing 2 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, 2 M TDZ, and 5 µM CUSO4. (h) Plant regeneration on MS medium containing 2 mg L^{-1} BAP. (i) Regenerated plant after 21 days of culture. (j) Regenerated plant after 30 days of culture. (k) Regenerated plant in rooting culture medium containing 2 mg L^{-1} BAP. (l) Plant regeneration ready for acclimatization stage. AM= apical meristem; PC= primary callus; SC= scutellum; Cl= coleoptiles.

Table 2. The callus proliferation of embryogenic callus of *Pennisetum purpureum* cv. Wrukwona treated by hormones thidiozuron and cupric sulfate

Treatments	Callus		
	No. of proliferated callus	No. of embryogenic callus formed	% of embryogenic callus formed
D3B0.5T2C0	25.00±0.89^{c}	8.00±0.63^{c}	32.03±2.69^{b}
D3B0.5T2CH	35.00±0.89^{b}	15.00±0.89^{b}	42.84±1.88^{a}
D3B0.5T2CL	45.00±0.89^{a}	20.00±0.63^{a}	44.44±1.00^{a}
p-value	<0.001**	<0.001**	0.002**

Note: Means in the same column with different superscripts differ significantly (p<0.05). **= significant; 2.4-D= 2.4-dichlorophenoxyacetic acid; BAP= benzyl amino purine, TDZ= thidiozuron. D3B0.5T2C0= 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ (control); D3B0.5T2CL= 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ with 0.5 μM $CuSO_4$; D3B0.5T2CH= 3 mg L^{-1} 2.4 D, 0.5 mg L^{-1} BAP, and 2 μM TDZ with 50 μM $CuSO_4$

45.00 and 20.00, respectively. The proliferation of callus from apical meristem developed into callus and regenerated is shown in Figure 3f-i.

Table 3 represents the consequences of hormones that were concentrated on plant regeneration from the embryogenic callus of *P. purpureum* from D3B0.5T2 CL plants. The highest numbers of regenerated callus (%) and shoots formed were observed in the N0B2 media with a combination of 0 mg L^{-1} NAA and 2 mg L^{-1} BAP, which were 20 and 45, respectively. Figure 3j-l shows the regeneration process of embryogenic callus until root formation.

Table 4 shows the proliferation and regeneration in the medium D3B0.5T2CL (regenerant A) and medium D3B0.5T2CH (regenerant B), and then morphological characteristics of plants were carried out. Table 4 shows the morphological characters and nutrition content of Wrukwona napiergrass harvested at 30 days. Regenerant A had morphological characters and nutritional content that were not significantly different from the parents. Regenerant B was significantly different, but the value was lower than the parent plant and regenerant A. The highest plant height and biomass production were observed in regenerant A. The highest nutrient content was shown in control or parent plants. However, no significant differences were observed among the treatments in DM content and CP contents.

Table 3. Plant growth hormone concentration on plant regeneration from callus of *Pennisetum purpureum* cv. Wrukwona in D3B0.5T2 CL medium

Treatments	Callus	
	No. of regenerated callus (%)	No. of shoots formed
N0.01B2	7.00±0.63^{c}	12.00±0.63^{c}
N0.1B2	9.83±1.33^{b}	19.00±0.89^{b}
N0B0	5.00±0.63^{d}	7.00±0.63^{d}
N0B2	20.00±1.41^{a}	45.00±1.41^{a}
p-value	<0.001**	<0.001**

Note: Means in the same column with different superscripts differ significantly (p<0.05). **= significant; N0.01B2= 0.01 mg L^{-1} naphthalene acetic acid (NAA) and 2 mg L^{-1} benzyl amino purine (BAP); N0.1B2= 0.1 mg L^{-1} NAA and 2 mg L^{-1} BAP; NOB0= combination of 0 mg L^{-1} NAA and 0 mg L^{-1} BAP; N0B2= combination of 0 mg L^{-1} NAA and 2 mg L^{-1} BAP.

DISCUSSION

A primary callus was formed after 10 days of MS media culture (Figure 3a). The best embryogenic callus development was obtained in MS media containing 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ. Subsequently, yellowish embryogenic callus covered most nodular-shaped and compacted callus (Figure 3d). Table 1 shows all treatments using BAP, 2.4-D, and TDZ. This shows its effect on callus formed and embryogenic callus formed. Even though all media with 2.4-D, BAP, and TDZ could produce callus, the occurrence development of embryogenic callus varied among media in a range of 8.3%- 41.6%. A combination of 3 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ had the highest influence on callus formed and embryogenic callus formed. Research by Maulana *et al.* (2019) showed that the inclusion of 2.4-D affected the formation and induction of callus that tended to be faster. Research by Budisantoso *et al.* (2017) also showed that the addition of 2.4-D effectively increased callus formation and the percentage of callus. Rahayu *et al.* (2003) stated that adding 2.4-D

Table 4. Morphological character and nutritional content of *Pennisetum purpureum* cv. Wrukwona at harvest age of 30 days

Variables	Treatment			*p* Value
	Control	Regenerant A	Regenerant B	
Plant height (cm)	142.29±1.33^{a}	142.2±1.56^{a}	122.69±7.20^{b}	<0.001**
Biomass production (kg/pot)	6.51±0.20^{a}	6.46±0.24^{a}	4.68±0.82^{b}	0.001**
DM content (%)	20.58±0.71^{a}	20.76±0.75^{a}	20.42±0.66^{a}	0.140^{ns}
OM content (%)	80.71±0.62^{a}	80.63±0.33^{a}	72.42±11.09^{b}	<0.001**
CF content (%)	35.49±0.83^{a}	35.27±0.68^{a}	35.09±1.22^{b}	<0.001**
CP content (%)	11.23±0.21^{a}	11.27±0.24^{a}	11.32±0.32^{a}	0.611^{ns}

Note: DM= dry matter; OM= organic matter; CF= crude fiber; CP= crude protein; regenerant A= medium D3B0.5T2CL; regenerant B= medium D3B0.5T2CH. **= significant; ns= not significant.

in the media would induce cell division and extension in seeds which can engage callus development and increase natural chemical compounds of flavonoids.

The TDZ and $CuSO_4$ can affect the callus proliferation of the embryogenic callus of Wrukwona napiergrass shown in Table 2. Gondo *et al.* (2005) reported that adding BAP and $CuSO_4$ was useful for the multiplication of highly regenerative embryogenic callus of *Paspalum dilatatum* without the emergence of albino plants during long-term culture. After two-three subcultures, embryogenic callus moved to MS media with the same hormone for proliferation and scutellum development into coleoptiles (Figure h), several green plantlets were regenerated well, as shown in Figure 3 (i-j).

The results of this study indicated that the TDZ concentration at 2 μM showed better results compared to the concentration at 4 μM in callus formation which was in line with the conclusions of Nurmaningrum *et al.* (2017), who stated that low TDZ concentrations stimulated more shoot formation than high concentrations. The TDZ can also increase the ability of shoot multiplication (Lestari, 2011).

Table 3 shows the effects of hormone concentration on plant regeneration from the embryogenic callus of *P. purpureum* from D3B0.5T2CL plants. Arsyam *et al.* (2017) stated that the addition of 2.4-D accelerated cell regeneration to form callus. Ahmed & Anis (2012) reported that suitable TDZ concentrations could sufficiently induce high shoot regeneration, which is possibly beneficial for extensive proliferation and plant preservation.

For morphological character and nutritional content, there was no significant difference in morphological characters and nutritional content between the plants of regenerant A and parents, which is shown in Table 4. This result shows that Wrukwona napiergrass has the same character as its parent, and the method used in the tissue culture process was suitable for breeding material sources. This result shows that Wrukwona napier grass has the same character as its parent and the method used in the process of tissue culture was correct. TDZ concentration used in this research was effective with a concentration of 2 μM. Research by Dewir *et al.* (2018) reported that TDZ (>2.0 μM) caused unaccepted deviations in plant morphology, for example, the abnormal morphology of the leaf, fascinated shoots, and inflated shoot base in several species of plants. Differences in regenerant B indicate the diversity that will become a new source of germplasm. Silva *et al.* (2012) state that plants with different genotypes have different abilities to produce embryogenic callus, differentiation, and regeneration of somatic embryos. The results of this current research indicate that Wrukwona napiergrass can be developed as animal feed. It can be stated to be somatic embryogenesis culture that is stable for the formation of embryogenic callus.

CONCLUSION

It is concluded that somatic embryogenesis of *P. purpureum* cv. Wrukwona can be produced from MS culture medium using 2 mg L^{-1} 2.4-D, 0.5 mg L^{-1} BAP, and 2 μM TDZ. The effective multiplication was carried out by adding 5 μM $CuSO_4$ to the same medium as the embryogenic callus formation, and effective regeneration was carried out with MS media containing 2 mg L^{-1} BAP. There is no difference in the morphology and nutrient content of the regenerant. Somatic embryogenesis culture was stable for the formation of embryogenic callus.

CONFLICT OF INTEREST

Materials discussed in this document is not associated to conflict of interest with any financial, personal, or other relationships with other people or organization.

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