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Plantlets Regeneration from Crown Bud Slicing of Pineapple (*Ananas comosus*)

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2.4-dichlorophenoxy-acetic acid; benzyladenine; *Ananas comosus; in vitro* culture; micropropagation.

Abstract

Pineapple propagation by lateral shoots, suckers or crowns is often confronted with limited number of regenerated seedlings and high diversity in flowering and fruit formation. In order to solve this problem, this study offer an alternative method by using tissue culture techniques. This study aimed to determine the effect of growth regulators on plantlet regeneration from bud slicing of pineapple cv. Tangkit. Four levels of 2.4-D (0.0, 0.001, 0.01 and 0.1 ppm) in combination with BA (0.0, 0.1, 1.0 and 10.0 ppm) were tested on solid MS medium. Cultures were incubated in total darkness for a week followed by transfer to 16-hour photoperiod. Results showed that explants treated with 2,4-D and/or BA succeeded in regenerating adventitious shoots. Average leaf number did not differ significantly among treatments (P = 0.60). Highest leaf number (2.99 ± 0.23) was obtained on medium with 0.01 ppm 2,4-D without BA, followed by 0.1 ppm 2,4-D without BA (2.85 ± 0.33). Meanwhile, roots were only formed on medium with 0.1 ppm 2.4-D without BA (4.2 ± 0.37 per shoot). Thus, complete plantlets were regenerated only on medium supplemented with 0.1 ppm 2,4-D without BA. The growth of plantlets was relatively uniform, and plantlet acclimatization succeeded 100% on Jiffy pots. The finding of optimum concentration of 2.4-D and BA in this study is important to develop standard protocol for *in vitro* propagation of pineapple cv. Tangkit. Thus, the benefit of producing seeds in large quantities and relatively uniform in growth is made possible through tissue culture technique.

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INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is one of important fruit commodities from Bromeliaceae family cultivated in Jambi Province. The cultivation of pineapple in Jambi has become a priority since 1983, with Tangkit Baru Village, Muaro Jambi Regency as the production centre (Zulkarnain, 2017). The Tangkit cultivar has been registered in the Ministry of Agriculture Republic of Indonesia as a local variety of Jambi Province, with registration No. 40/PVL/2009. The fruit has soft aroma, sweet and fine texture with little fibre. The shelf life of this fruit may last up to seven days after harvest. Another advantage of pineapple cv Tangkit is that their ability to grow on peat soils with pH below 5.0.

In general, pineapple fruits are high in fibre, an important component of a healthy diet that improve the digestion system. They also contain a good array of vitamins and minerals including vitamins A and C, folic acid, calcium, and manganese (Hossain *et al.*, 2015). Another benefit of pineapple fruit is to improve skin and hair health, increase energy and reduce body weight. Many studies have suggested that consuming pineapples may relieve osteoarthritis and diarrhea (Pavan *et al.*, 2012), decrease the risk of obesity and its associated cardiovascular dysfunction (Ahmed, 2016), besides it also has a strong antioxidant capacity (Lu *et al.*, 2014). These benefits, along with the sweet taste, fresh aroma and attractive shape make pineapple attracts many people, both for fresh consumption or processing industries such as jam, syrup and canned pineapple.

Pineapples cv Tangkit are routinely propagated vegetatively by means of lateral shoots, basal suckers or crowns as they are parthenocarpy (do not producing seeds). However, vegetative propagation using lateral shoots, suckers and crowns are confronted with the limited number of propagules produced. Crowns are always carried along with the fruit at the time of marketing, while suckers are often available in limited number. The size of the seedling often varies greatly, giving rise to high diversity in flowering time and fruit formation (Sripaoraya *et al.*, 2003). In addition, being vegetatively propagated, conventional hybridization for better pineapple varieties are cumbersome and time consuming (Mhatre, 2007). Therefore, an alternative of pineapple propagation method that can be used is by tissue culture techniques. Many authors have reported successful production of pineapple via tissue culture system during the last few years (Atawia *et al.*, 2016, Danso *et al.*, 2008, Yapo *et al.*, 2011, Zulkarnain & Neliyati, 2017, Zuraida *et al.*, 2011).

In an earlier study of pineapple tissue culture, Mapes (1973) was successfully regenerated plants from shoot culture, while Sita *et al.* (1974) obtained plantlet from meristem culture of Smooth Cayenne cultivar. Regeneration of pineapple plants through somatic embryogenesis has also been reported by a number of researchers (Daquinta *et al.*, 1996, Sripaoraya *et al.*, 2003). Soneji *et al.* (2002) used leaf segments as explant materials. Most protocols, however, used dormant axillary buds from fruit crowns as planting materials (Atawia *et al.*, 2016, Rahman *et al.*, 2001, Soneji *et al.*, 2001, Sripaoraya *et al.*, 2003). These dormant buds, however, need to be stimulated to grow into maturation stage in order to regenerate plants. For this reason, auxin and cytokinin should play an important role in explant organogenic response during the culture. The dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA) are two widely used auxin and cytokinin in tissue culture system of many plants (Kristanti *et al.*, 2013, Sari *et al.*, 2014, Wahyuni *et al.*, 2017).

This paper describes a simple *in vitro* procedure for obtaining pineapple cv Tangkit plants using axillary buds from crown slicing as initial explant materials. This procedure will facilitate clonal propagation techniques of this pineapple cultivar in order to provide uniform quality seeds.

METHODS

Preparation of plant material

Fruit crowns were isolated from pineapple cv Tangkit obtained from farmers' gardens in Tangkit Baru village, Muaro Jambi district. All leaves attached to crowns were removed to obtain a "naked crown" with clearly visible dormant axillary buds. The crowns were then sliced horizontally into three parts to obtain a section of the base, the middle and the tip pieces. The middle pieces were used as the source of the explant material, while the other two parts were discarded. The pieces were then sterilized by immersion in 0.02 % $HgCl_2$ solution for 5 minutes, then rinsed three times with sterile distilled water. After the sterilization process was completed, the pieces were cut into small cube block of about 0.5 × 0.5 × 0.5 cm with a dormant axillary bud. The blocks were then used as planting material and ready to be cultured in the medium within culture flasks.

Medium composition and environmental conditions

Culture medium used was MS composition (Murashige & Skoog, 1962), supplemented

with vitamins (myo-inositol, glycine, nicotinic acid, pyridoxine-HCl and thiamine-HCl) and 3% sucrose (w/v). Plant growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) (0.0; 0.001; 0.01 and 0.1 ppm) and benzyl adenine (BA) (0.0; 0.1; 1.0 and 10 ppm) were added to the medium, producing 16 treatment combinations. The pH of the medium was set to 5.8 ± 0.02 before compacting with 0.8% (w/v) agar (Difco Bacto) and distributed into culture flasks each of 10 mL. The medium was then autoclaved at a pressure of 1.06 kg cm^{-2} and temperature of 121 °C for 15 minutes.

One explant was cultured on medium in culture flask of each treatment combinations. The cultures were then incubated in total darkness for 7 days prior to shifted on shelves within the culture room at 25 ± 1 °C and photoperiod of 16 hours per day under a light intensity of 50 µmol m^{-2} s^{-1} from white TL lamps (Phillips Indonesia).

Acclimatization

The regenerated plantlets (buds with shoots and roots) were removed from culture flasks and transplanted in Jiffy pots (Jiffy-7 ™) which had been soaked in water for 15 minutes. Each pot was planted one plantlet. The plantlets were then maintained in a shade house with light intensity ranging from 100 to 170 µmol m^{-2} s^{-1}, natural photoperiod and day/night temperature of 25/18 °C and relative humidity of approximately 80%.

Design and Data analysis

The experiment was arranged in a simple randomized design with five replications. Parameters suchs as percentage of explants regenerating adventitious shoots, root formation, number of leaves and callus proliferation were observed every week for 16 weeks. Data obtained were calculated using decriptive statictics (Microsoft Corporation, 2016) and standard errors (SEs) were determined.

RESULTS AND DISCUSSION

While explants cultured in medium without growth regulator did not show any progress until the end of the trial period (Table 1), all explants cultured in medium equipped with 2,4-D or BA or combination of both regenerated adventitious shoots (Figure 1). This proves that the regeneration of adventitious shoots from dormant axillary buds of Tangkit pineapple requires the presence of auxin and/or cytokinins in culture medium, with 2,4-D as a critical factor for plantlet growth. Some earlier researchers also reported the need for auxin and cytokinin involvement in inducing morphogenetic responses in a number of Bromeliaceae plants. For example *Dyckia distachya* (Pompelli & Guerra, 2005), *Tillandsia eizii* (Pickens *et al.*, 2006), *Vriesea scalaris* (da Silva *et al.*, 2009), *Vriesea reitzii* (Guerra & Dal Vesco, 2010), *Aechmea blanchetiana* and *A. distichantha* (Santa-Rosa *et al.*, 2013) were successfully regenerated by adventitious shoot formation under *in vitro* system. In all of these successes, the presence of cytokinin (BA) was proven to be crucial in the formation of adventitious shoots.

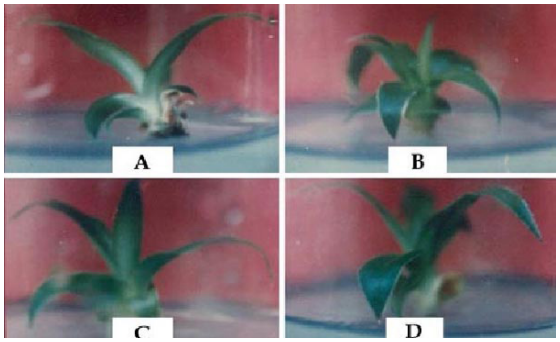

Figure 1. Adventitious shoots formation on crown slicing explants of pineapple cv Tangkit. A = 0.001 ppm 2,4-D + 0.01 ppm BA; B = 0.01 ppm 2,4-D + 10.0 ppm BA; C = 0.001 ppm 2,4-D + 1.0 ppm BA; D = 0.01 ppm 2,4-D + 0.1 ppm BA.

Root formation with an average number of 4.2 ± 0.37 per shoot occured only in the addition of 0.1 ppm 2.4-D without BA, and they are yellowish-white in colour (Figure 2). This is in accordance with report of Sripaoraya *et al.* (2003) regarding tissue culture of pineapple cv Phuket (belongs to Queen group similar to cv Tangkit). Therefore, in our study, complete plantlets regeneration occured only from explants cultured on medium with 2.4-D alone.

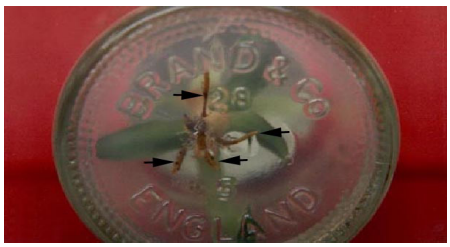

Figure 2. Roots (indicated by arrowheads) formed on adventitious shoot grown from crown bud slicing of pineapple cv Tangkit treated with 0.1 ppm 2,4-D alone.

In general, the use of synthetic auxin in tissue culture system was aimed at accelerating root

Table 1. The effect of 2,4-D and BA on shoots, roots and leaves formation on crown slicing explants of pineapple cv Tangkit.

2,4-D (ppm)	BA (ppm)	Shoot formation (%)	Root formation (%)	Average leaf number
0.0	0.0	0	0	-
	0.1	100	0	2.57 ± 0.50
	1.0	100	0	2.59 ± 0.39
	10.0	100	0	2.83 ± 0.18
0.001	0.0	100	0	2.72 ± 0.21
	0.1	100	0	2.59 ± 0.39
	1.0	100	0	2.59 ± 0.34
	10.0	100	0	2.67 ± 0.31
0.01	0.0	100	0	2.99 ± 0.23
	0.1	100	0	2.85 ± 0.33
	1.0	100	0	2.78 ± 0.26
	10.0	100	0	2.74 ± 0.32
0.1	0.0	100	100	2.98 ± 0.43
	0.1	100	0	2.86 ± 0.22
	1.0	100	0	2.78 ± 0.29
	10.0	100	0	2.61 ± 0.48

± Standard Error. Each treatment consists of 5 replications.

formation and increasing rooting rate. Rovere *et al*. (2013) and Yu *et al*. (2017) suggested that root formation in tissue culture will take place when auxin is present in culture medium. The presence of auxin, either alone or in combination with a low concentration of cytokinin is a critical factor for the proliferation of root primordia on cultured explants (Zulkarnain, 2009). This is probably due to their participation in the regulation of cell cycling, division, and differentiation (Tapingkae *et al.*, 2012, Zulkarnain *et al.*, 2015).

The effect of 2,4-D and BA in the range of concentrations tested did not give any significant difference in leaves formation ($P = 0.60$). The average number of leaves ranged from 2.57 ± 0.50 to 2.99 ± 0.23. These results indicate that plantlet growth does not show high diversity, so, this *in vitro* propagation method is very appropriate effort to obtain pineapple with a uniform growth. This is apparently more advantageous compared to conventional method by using suckers. Sripaoraya *et al*. (2003) claimed that propagation using suckers will result in non-uniform growth and flowering times among progenies due to differences in suckers age at planting time.

All cultured explants did not produce callus, though 2,4-D is known as a strong auxin that frequently used to induce callus formation in a number of plant species such as sweet potato (Oggema *et al.*, 2007), wheat (Rashid *et al.*, 2009), sugar cane (Tahir *et al.*, 2011), gendarussa (Wahyuni *et al.*, 2017) and orchid (Budisantoso *et al.*, 2017). This finding, however, is in accordance with report by (Soneji *et al.*, 2002) where MS medium supplemented with 2,4-D was not conductive to induce callus growth on leaf explants of pineapple. This probably due to the concentration of 2,4-D was still too low to induce callus proliferation. Ikeuchi *et al*. (2013) suggested that in general, callus proliferation will occur when auxin and cytokinin present in intermediate rasio. High rasio of auxin-to-cytokinin or cytokinin-to-auxin will lead to root and shoot regeneration, respectively. In pineapple cv Phuket, Sripaoraya *et al*. (2003) found that callus was formed on leaf explants with the presence of 2,4-D concentrations of 0.5 to 2.0 ppm, while in our experiment the highest concentration of 2.4-D was only 0.1 ppm. This result confimed the report of De Silva *et al*. (2008) who found that 2,4-D application of up to 54.3 ppm failed to induce calli from meristemic globular bodies of pineapple cv Moris

and Josapine. Thus, we believe that the very low concentration of 2,4-D employed in culture medium was the key factor causing the absence of callus formation in this trial.

Figure 3. *In vitro* plantlet of pineapple cv Tangkit transplanted onto Jiffy pot during acclimatization process.

Acclimatization was done after the plantlets were large enough (at 16 weeks after culture initiation). Only plantlets generated on medium supplemented with 0.1 ppm 2,4-D alone were subjected to acclimatization as they have root system. They were transplanted onto Jiffy pots ready-made medium (Figure 3). All explants acclimatized on Jiffy pot showed good growth and development, so that they can be transferred to polybag with soil for further planting in the field. The acclimatization rate of 100% indicated that both environmental conditions and substrate used to support plantlet growth have met their requirements.

The finding of optimum concentration of 2,4-D and BAP in this study is useful for in vitro shoots formation from crown bud slicing of pineapple. The shoots can be subcultured to new medium to develop multiple shoots, or transferred to rooting medium to grow into complete plantlets which can be acclimatisized for field evaluation. Through this study of *in vitro* propagation technique, a standard protocol for mass propagation of pineapple cv. Tangkit can be developed. The research is very important to provide the needs of quality seeds, because normal vegetative propagation of pineapple is hampered by the limited availability of propagation materials.

CONCLUSION

Propagation of pineapple cv Tangkit is made possible through tissue culture techniques using crown bud slicing as explants source. This is a new approach to the effort of multiplying pineapple plants in large quantities. In addition, plants generated through tissue culture were proven to be relatively uniform in growth, making it very profitable in cultivation.

REFERENCES

Ahmed, M. M. (2016). Pineapple Juice Ameliorates the High Fat Diet-induced Alterations in Cardiac Gene Expression Pattern in Male Rats. *International Journal of Biochemistry Research & Review*, *15* (4), 1-11.

Atawia, A. R., F. M. A. EL-Latif, S. F. EL-Gioushy, S. S. Sherif & O. M. Kotb. (2016). Studies on Micropropagation of Pineapple (*Ananas comosus* L.). *Middle East Journal of Agriculture Research*, *5* (2), 224-232.

Budisantoso, I., N. Amalia & K. Kamsinah. (2017). In Vitro Callus Induction from Leaf Explants of *Vanda* sp. Stimulated by 2,4-D. *Biosaintifika: Journal of Biology & Biology Education*, *9* (3), 492-497.

da Silva, A. L. L., E. T. H. Franco, E. B. Dornelles, C. L. R. Bortoli & M. Quoirin. (2009). In Vitro Multiplication of *Vriesea scalaris* E. Morren (Bromeliaceae). *Iheringia Série Botânica*, *64* (2), 151-156.

Danso, K. E., K. O. Ayeh, V. Oduro, S. Amiteye & H. M. Amoatey. (2008). Effect of 6-Benzylaminopurine and Naphthalene Acetic Acid on *In Vitro* Production of MD2 Pineapple Planting Materials. *World Applied Sciences Journal*, *3* (4), 614-619.

Daquinta, M. A., A. Cisneros, Y. Rodríguez, M. Escalona, M. Pérez, I. Luna & C. G. Borroto. (1996). Embríogénesis somática en pina (*Ananas comosus* (L.) Merr.). *Acta Horticulturae*, *425*, 243-246.

De Silva, M. A. Kadir, M. A. Aziz & S. Kadzimin. (2008). Callus Induction in Pineapple (*Ananas comosus* L.) cv. Moris and Josapine. *International Journal of Agricultural Research*, *3* (4), 261-267.

Guerra, M. & L. Dal Vesco. (2010). Strategies for the Micropropagation of Bromeliads. *In* S. M. Jain & S. J. Ochatt [eds.], Protocols for *In Vitro* Propagation of Ornamental Plants, 47-66. Humana Press, New York.

Hossain, M. F., S. Akhtar & M. Anwar. (2015). Nutritional Value and Medicinal Benefits of Pine-

apple. *International Journal of Nutrition and Food Sciences*, *4* (1), 84-88.

Ikeuchi, M., K. Sugimoto & A. Iwase. (2013). Plant Callus: Mechanisms of Induction and Repression. *The Plant Cell*, *25*, 3159-3173.

Kristanti, I., N. A. Habibah & L. Herlina. (2013). Optimization of 2,4-D Concentration, BA, and Long Irradiation to Promote Regeneration Buds from Callus of Soybean. *Biosaintifika: Journal of Biology & Biology Education*, *5* (1), 50-57.

Lu, X.-H., D.-Q. Sun, Q.-S. Wu, S.-H. Liu & G.-M. Sun. (2014). Physico-Chemical Properties, Antioxidant Activity and Mineral Contents of Pineapple Genotypes Grown in China. *Molecules*, *19*, 8518-8532.

Mapes, M. O. (1973). Tissue Culture of Bromeliads. Proceedings of International Plant Propagator Society, 23, 47-55. . Tissue culture of bromeliads. Proceedings of International Plant Propagator Society, 23, 47-55.

Mhatre, M. (2007). Micropropagation of Pineapple, *Ananas comosus* (L.) Merr. *In* S. M. Jain & H. Häggman [eds.], Protocols for Micropropagation of Woody Trees and Fruits, 499–508. Springer, Dordrecht, The Netherland.

Microsoft Corporation. (2016). Microsoft Office 365 ProPlus Version 1708. Microsoft Corporation, New York, USA.

Murashige, T. & F. Skoog. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473-497.

Oggema, J. N., M. G. Kinyua & J. P. Ouma. (2007). Optimum 2,4-D Concentration Suitable for Embryogeneic Callus Induction in Local Kenyan Sweet Potato Cultivars. *Asian Journal of Plant Sciences*, *6* (3), 484-489.

Pavan, R., S. Jain, Shraddha & A. Kumar. (2012). Properties and Therapeutic Application of Bromelain: A Review. *Biotechnology Research International*, 2012, 1-6.

Pickens, K. A., J. Wolf, J. M. Affolter & H. Y. Wetzstein. (2006). Adventitious Bud Development and Regeneration in *Tillandsia eizii*. *In Vitro Cellular & Developmental Biology - Plant*, *42* (4), 348-353.

Pompelli, M. F. & M. P. Guerra. (2005). Micropropagation Enables the Mass Propagation and Conservation of *Dyckia distachya* Hassler. *Crop Breeding and Applied Biotechnology*, *5*, 117-126.

Rahman, K. W., M. N. Amin & M. A. K. Azad. (2001). *In Vitro* Rapid Clonal Propagation of Pineapple, *Ananas comosus* (L.) Merr. *Plant Tissue Culture*, *11*, 47-53.

Rashid, U., S. Ali, G. M. Ali, N. Ayub & M. S. Masood. (2009). Establishment of an Efficient Callus Induction and Plant Regeneration System in Pakistani Wheat (*Triticum aestivum*) Cultivars. *Electronic Journal of Biotechnology*, *12* (3), 1-12.

Rovere, F. D., L. Fattorini, S. D'Angeli, A. Veloccia, G. Falasca & M. M. Altamura. (2013). Auxin and Cytokinin Control Formation of the Quiescent Centre in the Adventitious Root Apex of Arabidopsis. *Annals of Botany*, *112* (7), 1395-1407.

Santa-Rosa, S., F. V. D. Souza, Á. M. Vidal, C. A. da S Ledo & J. R. F. de Santana. (2013). Micropropagation of the Ornamental Vulnerable Bromeliads *Aechmea blanchetiana* and *Aechmea distichantha*. *Horticultura Brasileira*, *31*, 112-118.

Sari, N., E. S. R & Sumadi. (2014). Optimization of Type and Concentration of PGR in Embryogenic Callus Induction and Regeneration into plantlets on *Carica pubescens* (Lenne & K.Koch). *Biosaintifika: Journal of Biology & Biology Education*, *6* (1), 52-59.

Sita, G. L., R. Singh & C. P. A. Layer. (1974). Plantlets through shoot tip culture in pineapple. *Current Science*, *43*, 724.

Soneji, J. R., P. S. Rao & M. Mhatre. (2001). Somaclonal Variation in Micropropagated Dormant Axillary Buds of Pineapple (*Ananas comosus* L., Merr.). *The Journal of Horticultural Science and Biotechnology* 77 (1), 28-32.

Soneji, J. R., P. S. Rao & M. Mhatre. (2002). *In Vitro* Regeneration from Leaf Explants of Pineapple (*Ananas comosus* L., Merr.). *Journal of Plant Biochemistry and Biotechnology*, *11* (2), 117-119.

Sripaoraya, S., R. Marchant, J. B. Power & M. R. Davey. (2003). Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). *In Vitro Cellular and Developmental Biology - Plant*, *39*, 450-454.

Tahir, S. M., K. Victor & S. Abdulkadir. (2011). The Effect of 2, 4-Dichlorophenoxy Acetic Acid (2,4-D) Concentration on Callus Induction in Sugarcane (*Saccharum officinarum*). *Nigerian Journal of Basic and Applied Science*, *19* (2), 213-217.

Tapingkae, T., Z. Zulkarnain, M. Kawaguchi, T. Ikeda & A. Taji. (2012). Somatic (Asexual) Procedures (Haploids, Protoplasts, Cell Selection) and Their Applications. *In* A. Altman & P. M. Hasegawa [eds.], Plant biotechnology and agriculture: Prospects for the 21st century, 139-162. Academic Press, Oxford.

Wahyuni, D. K., P. Andriani, A. N. M. Ansori & E. S. W. Utami. (2017). Callus Induction of Gendarussa (*Justicia gendarussa*) by Various Concentration of 2,4-D, IBA, and BAP. *Biosaintifika: Journal of Biology & Biology Education*, *9* (3), 402-408.

Yapo, E. S., T. H. Kouakou, M. Kone, J. Y. Kouadio, P. Kouame & J.-M. Merillon. (2011). Regeneration of Pineapple (*Ananas comosus* L.) Plant through Somatic Embryogenesis. *Journal of Plant Biochemistry and Biotechnology*, *20* (2), 196-204.

Yu, J., W. Liu, J. Liu, P. Qin & L. Xu. (2017). Auxin Control of Root Organogenesis from Callus in Tissue Culture. *Frontiers in Plant Science*, *8*, 1385.

Zulkarnain, Z. (2009). *Kultur Jaringan Tanaman: Solusi Perbanyakan Tanaman Budidaya*. Jakarta, IDN:

PT Bumi Aksara.

Zulkarnain, Z. (2017). *Budidaya Buah-Buahan Tropis*. Yogyakarta, IDN: Deepublish.

Zulkarnain, Z. & N. Neliyati. (2017). The Effect of NAA and BAP on Tissue Culture of Tangkit Pineapple (*Ananas comosus* (L.) Merr. cv. Tangkit). *Biospesies*, *10* (1), 1-10.

Zulkarnain, Z., T. Tapingkae & A. Taji. (2015). Applications of In Vitro Techniques in Plant Breeding. *In* J. M. Al-Khayri, S. M. Jain & D. V. Johnson [eds.], Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools, 293-328. Springer International Publishing Switzerland, Switzerland.

Zuraida, A. R., A. H. Nurul Shahnadz, A. Harteeni, S. Roowi, C. M. Z. Che Radziah & S. Sreeramanan. (2011). A Novel Approach for Rapid Micropropagation of Maspine Pineapple (*Ananas comosus* L.) Shoots using Liquid Shake Culture System. *African Journal of Biotechnology*, *10* (19), 3859-3866.