Pengaruh NAA dan BAP terhadap Kultur Jaringan Nenas Tangkit (*Ananas comosus* (L.) Merr. cv. Tangkit)

The Effect of NAA and BAP on Tissue Culture of Tangkit Pineapple (*Ananas comosus* (L.) Merr. cv. Tangkit)

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Abstract. This study was aimed at investigating the effect of NAA and BAP on the development of basal slip slices in tissue culture of pineapple cv. Tangkit. The experiment was conducted at the Plant Biotechnology Laboratory Agricultural Faculty University of Jambi from July through to October 2016. Six level of NAA concentrations (0, 1, 2, 3 , 4 and 5 mgL^{-1}) were tested in combination with six levels of BAP concentration (0, 1, 2, 3 , 4 and 5 mgL^{-1}). A Completely Randomized Design with 5 replicates was employed in this trial. Data on the percentage of explants forming shoots, time required for shoot formation, number of shoots growing per explants, and callus proliferation were recorded. Results showed that without the involvement of plant growth regulators there had been no growth on cultured explants. However, shoot growth was found on explants cultured on medium supplemented with 1- 3 mgL^{-1} NAA or 1 - 5 mgL^{-1} BAP. The application of NAA higher than 3 mgL^{-1} was found to suppress shoot growth even in combination with BAP. Shoot formation was found to be fast on medium supplemented with 1 mgL^{-1} NAA + 4 mgL^{-1} BAP, but took longer time on medium with or 1 mgL^{-1} NAA + 5 mgL^{-1} BAP as well as medium with 1 – 2 mgL^{-1} NAA only. The same phenomenon was also found on medium with 1 - 2 mgL^{-1} NAA only. This study also revealed that most of cultured explants regenerated only one shoot, but explants cultured on medium with 1 mgL^{-1} NAA along with 1 mgL^{-1} BAP produced 2.33 shoots on the average. The addition of 1 mgL^{-1} NAA without BAP and 1 mgL^{-1} BAP without NAA could increase average shoot growth by 1.78 and 1.44, respectively.

Keywords: In vitro culture, micropropagation, naphthalene acetic acid, benzyl amino purine.

Abstrak. Penelitian in bertujuan untuk mengetahui pengaruh zat pengatur tumbuh NAA dan BAP terhadap perkembangan eksplan potongan tunas buah (*basal slip*) pada kultur jaringan nenas Tangkit. Penelitian dilaksanakan di Laboratorium Bioteknologi Tanaman Fakultas Pertanian Universitas Jambi dari bulan Juli hingga Oktober 2016. Perlakuan yang diuji adalah zat pengatur tumbuh NAA dengan konsentrasi 0, 1, 2, 3 , 4 dan 5 mgL^{-1} yang dikombinasikan dengan BAP dengan konsentrasi 0, 1, 2, 3, 4 dan 5 mgL^{-1}. Percobaan disusun dalam Rancangan Acak Lengkap dengan 5 ulangan untuk setiap perlakuan. Parameter yang diamati adalah jumlah eksplan yang menumbuhkan pucuk, kecepatan pembentukan pucuk, jumlah pucuk yang berkembang, dan proliferasi kalus. Hasil penelitian menunjukkan bahwa perlakuan tanpa zat pengatur tumbuh sama sekali tidak efektif untuk menginduksi pertumbuhan tunas. Pertumbuhan tunas aterjadi apabila dalam medium kultur diberi NAA 1 - 3 mgL^{-1} atau BAP 1 - 5 mgL^{-1}. Pemberian NAA di atas 3 mgL^{-1} cenderung menghambat pertumbuhan tunas meskipun dikombinasikan dengan BAP. Pembentukan tunas semakin cepat dengan pemberian NAA 1 mgL^{-1} + BAP hingga 4 mgL^{-1}, namun mengalami perlambatan pada pemberian NAA 1 mgL^{-1} + BAP 5 mgL^{-1}. Hal yang sama juga terjadi pada medium dengan 1 - 2 mgL^{-1} NAA tanpa BAP. Sebagian besar perlakuan hanya menumbuhkan satu pucuk, namun perlakuan BAP 1 mgL^{-1} ditambah NAA 1 mgL^{-1} menumbuhkan rata-rata 2,33 pucuk per eksplan. Pemberian NAA 1 mgL^{-1} tanpa BAP dapat meningkatkan pertumbuhan hingga rata-rata 1,78 pucuk per eksplan, dan pemberian BAP 1 mgL^{-1} tanpa NAA menghasilkan pertumbuhan pucuk rata-rata 1,44 pucuk per eksplan.

Kata kunci: Kultur in vitro, mikropropagasi, asam naftalen asetat, benzil amino purin.

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is one of important fruit crops belongs to Bromeliaceae that is grown by farmers in Tangkit Baru Village, Muaro Jambi Regency since the end of 1960s. Pineapple from this region has been registered and released as national elite variety known as pineapple cv. Tangkit (pineapple Tangkit). In general, pineapple Tangkit is vegetatively propagated using suckers or crown since this plant is parthenocarpic (producing no seeds). However, vegetative propagation using suckers or crown resulting in very limited number of propagules, transmission of diseases, and non-uniform growth (Mengesha *et al.*, 2013). Crowns are attached on fruits and always brought to market, meanwhile suckers availability is very limited in field. Besides, there is wide variety in sucker size resulting in great non-uniformity in flowering and fruit harvesting time within progeny (Sripaoraya *et al.*, 2003). The limited number of available plant material for propagation has become a major problem in producing qualified pineapple seedlings. Therefore, the use of plant biotechnology through *in vitro* culture is the choice of method for pineapple mass propagation as it has been proved to be successful with other soft-wood crops such as banana (Anbazhagan *et al.*, 2014; Ngomuo *et al.*, 2014; Kahia *et al.*, 2015) and strawberry (Moradi *et al.*, 2011; Danial *et al.*, 2016; Diengngan *et al.*, 2016).

In vitro propagation of pineapple offers many advantages over conventionally vegetative method. Drew (1980) reported that it was possible to produce 1,250,000 pineapple plantlets within 8 months by using 30 explants as starting material. Further, Almeida *et al.* (2002) reported that 161.080 pineapple plants could be regenerated within eight months from only one explant as starting material. In recent years, there have been reports the success of pineapple seed production through *in vitro* technique (Firoozabady and Gutterson, 2003; Be and Debergh, 2006; Danso *et al.*, 2008; Hamad *et al.*, 2013).

The success of pineapple propagation via *in vitro* culture technique depends on a number of factors that can be found during culture growth. In general, *in vitro* culture of pineapple is done by using dormant axillary shoots from crown (Soneji *et al.*, 2000a; Sripaoraya *et al.*, 2003), and multiple shoot induction from *in vitro* regenerated leaf segments (Soneji *et al.*, 2000b). Almeida *et al.* (1997) reported that the use of 3 mg L^{-1} BAP in and 2 mg L^{-1} IAA was the best combination for the regeneration of pineapple plantlets. Meanwhile Danso *et al.* (2008) claimed that optimal condition for plantlet regeneration in MD2 pineapple tissue culture was with liquid or solid MS medium provided with 7,5 mg L^{-1} BAP in combination with 2 mg L^{-1} NAA. In general, culture growth in liquid medium is better than solid medium (Firoozabady and Gutterson, 2003; Danso *et al.*, 2008). In addition, Danso *et al.* (2008) reported that 5 mg L^{-1} BAP in liquid medium produced more MD2 plantlets than solid medium supplemented with 7,5 mg L^{-1} BAP.

Besides plant growth regulators, explant source also important for the success of *in vitro* culture of pineapple. In their investigation on tissue culture of pineapple cv. Maspine, Zuraida *et al.* (2011) successfully regenerated plantlets from shoot tip culture, while Souza *et al.* (2012) obtained plantlets from meristem culture. Plantlet regeneration through somatic embryogenesis in pineapple have also been reported by a number of authors (Sripaoraya *et al.*, 2003; Yapo *et al.*, 2011; Roostika *et al.*, 2012). Rahman *et al.* (2001) used dormant axillary shoots from crown of mature fruit to regenerate plantlets of Giant Kew and local Khulna of Bangladesh.

This study was aimed to obtain *in vitro* pineapple seedlings using basal slip slices as starting materials. This procedure will facilitate clonal propagation of pineapple Tangkit to produce quality and uniform seedlings.

MATERIALS AND METHODS

The trial was conducted at the Plant Biotechnology Laboratory, Agricultural Faculty University of Jambi, from July through to November 2016. Plant materials used were basal slip isolated from pineapple cv. Tangkit grown by farmer in Tangkit Baru Village, Muaro Jambi Regency, Jambi. All leaves were removed leaving "naked shoot" with clearly identified dormant axillary shoots on leaf axils. The "naked shoots" were then washed thoroughly under tap water plus detergent for five minutes, then rinsed with sterile water before being dipped in Benlox 50WP (50% Benomil) plus Agrept 20WP (20% Streptomycin Sulphate) solution for about an hour. Following from this, the "naked shoots" were left for 15 minutes in 50% Clorox (5.2% $NaOCl_2$), then 20% Clorox for 10 minutes, and finally rinsed with sterile distilled water.

Culture medium used were modified MS medium (Murashige and Skoog, 1962), supplemented with vitamins, myo-inositol and 3% (w/v) sucrose the plant growth regulators were added according to the treatments. Medium pH was adjusted to 5.8 ± 0.02 before being solidified with 0.8% agar (Difco Bacto), and poured into culture flasks each with 10 mL. Then the flasks with medium in it were autoclaved at 1.06 kg cm^{-2} and temperature of 121 ^oC for 15 minutes.

Five levels of BAP (0, 1, 2, 3, 4 and 5 mgL^{-1}) and five levels of NAA (0, 1, 2, 3, 4 dan 5 mgL^{-1}) were tested in this study. The experiment was arranged in a completely randomized design with five replicates, and each replicate consisted of four explants cultured in separated culture flasks.

The "naked shoots" obtained from basal slip were sliced into 4 sections longitudinally. Explants were cultured individually on previously prepared medium supplemented with plant growth regulators according to treatments. Cultures were kept under dark condition for 7 days before being transferred to culture room with 16 hours photoperiod, light intensity of 50 µmol m^{-2} $s^{-1}$and daily temperature of 25 ± 1^oC.

Observation on explant growth and development were conducted on daily basis as well as monitoring on contaminated cultures. Data on the following parameters: 1) percentage of explants growing shoots, 2) time required for shoot formation, 3) number of shoots growing per explants, and 4) callus proliferation, were recorded following one week of culture initiation. Data were presented by ranking of means (Compton, 1994). In addition, qualitative data were also presented visually in the form of picture to support quantitative data.

RESULTS AND DISCUSSION

Results

Percentage of explants growing shoots

Results of the study showed that without the involvement of plant growth regulators there had been no explant growth. However, shoot development was found on explants cultured on medium supplemented with 1 – 2 mgL^{-1} NAA or 3 – 5 mgL^{-1} BAP. The application of NAA more than 3 mgL^{-1} was found to inhibit shoot growth even in combination with BAP at any concentrations. (Table 1). In the observation of NAA as single factor, it can be seen that the use of 1 mgL^{-1} NAA resulted in the highest percentage of explant growing shoots (40%), while in BAP as single factor the highest percentage of culture growing shoots was 1 mgL^{-1} BAP.

Table 1. The effect of various concentrations of NAA and BAP on the percentage of explant growing shoots on in vitro culture of pineapple cv. Tangkit using basal slip slicing as explant source.

NAA (mgL^{-1})	BAP (mgL^{-1})						
	0	1	2	3	4	5	Average
0	-	-	-	20	20	20	20.00
1	20	60	20	20	60	60	40.00
2	40	20	20	40	40	40	33.33
3	-	40	40	40	20	20	32.00
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
Average	30.00	40.00	26.67	30.00	35.00	35.00	-

Speed of shoot growth

Shoot formation was found to be fast on medium supplemented with 1 mgL^{-1} NAA in combination with 4 mgL^{-1} BAP (10 days after culture initiation), On medium without plant growth regulators or medium with only NAA or BAP, shoot formation took longer. In the addition of NAA only, shoot formation was found to be faster with 1 mgL^{-1} NAA (15.56 days after culture initiation on the average). Meanwhile, with BAP only, shoot formation was faster on medium provided with 5 mgL^{-1} BAP (14.29 days after culture initiation on the average) (Table 2).

Table 2. The effect of various concentrations of NAA and BAP on the speed of shoot formation on in vitro culture of pineapple cv. Tangkit using basal slip slicing as explant source.

NAA (mgL^{-1})	BAP (mgL^{-1})						
	0	1	2	3	4	5	Average
0	-	-	-	38.00	13.00	11.00	20.67
1	29.00	13.67	13.00	12.00	10.00	15.67	15.56
2	23.00	24.00	29.00	14.00	20.50	20.50	21.83
3	-	23.00	13.00	15.50	26.00	10.00	17.50
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
Average	26.00	20.22	18.33	19.88	17.38	14.29	-

Shoot number

This study also revealed that most of cultured explants regenerated only one shoot, but explants cultured on medium with 1 mgL^{-1} NAA along with 1 mgL^{-1} BAP produced 2.33 shoots on the average. The addition of 1 mgL^{-1} NAA without BAP could increase shoot growth by 1.78, and 1 mgL^{-1} BAP without NAA could increase shoot growth by 1.44 on the average.

Table 3. The effect of various concentrations of NAA and BAP on the average of shoot number formed on in vitro culture of pineapple cv. Tangkit using basal slip slicing as explant source.

NAA (mgL^{-1})	BAP (mgL^{-1})						
	0	1	2	3	4	5	Average
0	-	-	-	1.00	2.00	1.00	1.33
1	1.00	2.33	2.00	2.00	1.33	2.00	1.78
2	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	-	1.00	1.00	1.00	1.00	1.00	1.00
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
Average	1,00	1,44	1,33	1,25	1,33	1,25	-

Discussion

The success of in vitro technique as a tool for plant propagation much depends upon the characteristics of medium used (Saad and Elshahed, 2012; Arab *et al.*, 2014). In principle, basic requirement of explants cultured in vitro is similar to intact plants. Therefore, George and De Klerk (2008) suggested that culture medium should not only provide macro and micro nutrients, but also carbohydrate in the form of sucrose to replace carbon that normally obtained from atmosphere. Better results will be obtained when vitamins, amino acids and plant growth regulators are also present in culture medium (Kadhimi *et al.*, 2014; Swamy *et al.*, 2014). All of these components are available in the medium used in this study.

Treatment without growth regulator proved to be ineffective to induce shoot growth, but the addition of 1 - 3 mgL^{-1} NAA or 1 - 5 mgL^{-1} BAP into the culture medium was also unable to induce a massive scale of shoot growth during in vitro culture of pineapple cv. Tangkit slips. The application of NAA exceeding 3 mgL^{-1} was found to inhibit shoot growth even in combination with BAP. The finding of this trial was presumably due the ideal composition of growth regulators used was not yet achieved. This result was inconsistent with previous study where the addition of 0,001 mgL^{-1} NAA in combination with 0.5 mgL^{-1} BAP was found to effective in promoting multiple shoot induction on crown slices culture (Khan *et al.*, 2004). Further Al-Saif *et al.* (2011) reported that shoot regeneration on explants of pineapple crown pieces occurred on the addition of 0.2 mgL^{-1} NAA plus 2.0 mgL^{-1} BAP. Though there are differences in concentrations used, the results of this investigation showed a consistency with previous study in term of the ratio of NAA and BAP. We found that the shoot growth took place when the concentration of NAA was lower than BAP in culture medium.

Both NAA and BAP showed the same effect on the growth of shoots, and they effectively increased the number of explants producing shoots at a concentration of 1.0 mgL^{-1}. This is in line with reports of Ibrahim *et al.* (2013) that the growth of axillary shoots from explants of pineapple crown pieces increased when cultured on medium MS supplemented with 1.0 mgL^{-1} BAP.

Shoot formation on basal slip pieces of pineapple plants cultured on medium with 1.0 mgL^{-1} NAA was faster than other NAA concentration, (15.56 days after planting). While the medium with 5 mgL^{-1} BAP induced bud formation within 14.29 days after planting. The result obtained in this study was better than the results of Roy *et al.* (2000) and Ibrahim *et al.* (2013) on the in vitro culture of pineapple crown slices, where it was reported that the axillary shoots did not grow until 4 – 5 months after planting because of bud dormancy. The rapid formation of buds in this trial was presumably as a consequence of the loss of

apical dominance as a result of removal of the basal slip terminal bud, which in turn stimulates the growth of axillary shoots.

This trials used explants from basal slip slicing containing a number of axillary buds. The isolation and slicing of those axillary buds into 4 pieces was presumably caused the lost of apical dominance which previously prevented the bud growth. Pierik (1997) reported that the lost of apical dominance enabled the growth and development of axillary shoots. Growth and development of theses axillary shoots increased when BAP was incorporated to culture medium, in which 1 mgL^{-1} BAP promoted the percentage of explant growing shoots (Table 1 and 2).

Until the end of study period, the number of shoots growing from the basal slip slices explants was still very limited. Most treatments only grow one shoot, but medium with 1.0 mgL^{-1} BAP and medium with 1.0 mgL^{-1} NAA produced up 2.33 shoots per explant. Although the number of regenerated shoots was still limited, this study demonstrates the importance of the presence of NAA and BAP in culture medium to stimulate shoot growth. Similar shoot growth have also been reported by Farahani (2014) in which the maximum shoot formation from sucker pieces explants were obtained on medium supplemented with 2.0 mgL^{-1} NAA plus 5.0 mgL^{-1} BAP. Shoots growing during in vitro culture of pineapple cv. Tangkit are presented in Figure 1.

The 2.33 shoots per explant obtained in this study were much lower than those obtained by Al-Saif *et al.* (2011) who reported 12 shoots from explants cultured on medium with 1.75, 2.25 and 3.5 mgL^{-1} BAP, or 10 shoots per explant (Sripaoraya *et al*., 2003) and 7 shoots per explant obtained in response to BAP at 2.0 mgL^{-1}. The result was also not in agreement with the use of 1.0 mgL^{-1} BAP as suggested by Be and Debergh (2006) who reported 9 shoots, and 10 shoots as reported by Firoozabady and Gutterson (2003) in response to 3.0 mgL^{-1} BAP.

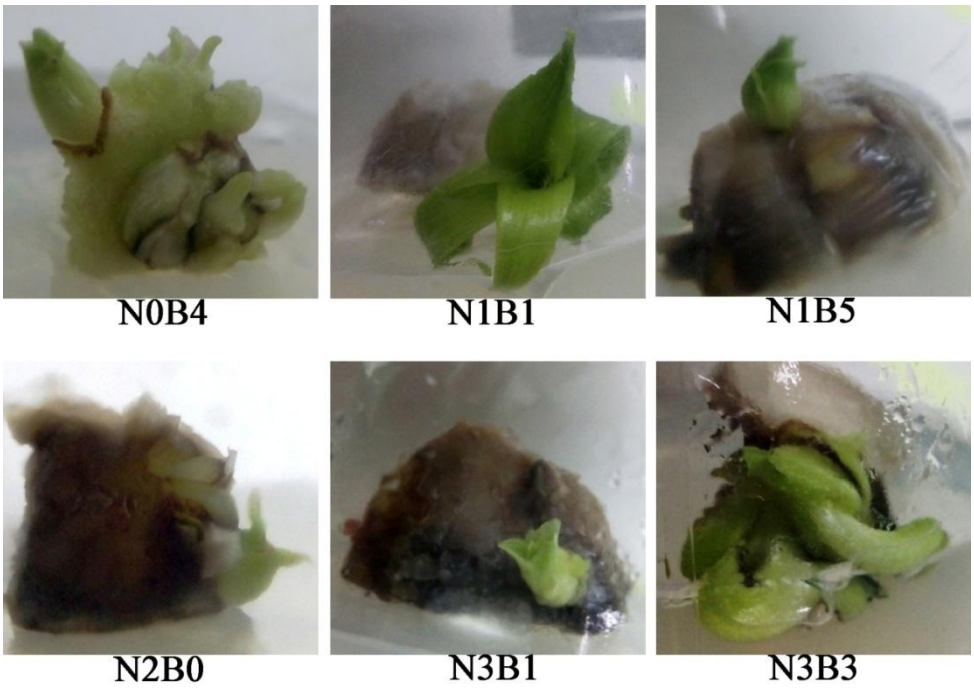

Figure 1. Shoot proliferation and growth on in vitro culture of basal slip slices explants of pineapple cv. Tangkit on MS medium supplemented with NAA and BAP (N0B4 = 4 mgL^{-1} BAP without NAA; N1B1 = 1 mgL^{-1} NAA + 1 mgL^{-1} BAP; N1B5 = 1 mgL^{-1} NAA + 5 mgL^{-1} BAP; N2B0 = 2 mgL^{-1} NAA without BAP; N3B1 = 3 mgL^{-1} NAA + 1 mgL^{-1} BAP; N3B3 = 3 mgL^{-1} NAA + 3 mgL^{-1} BAP).

None of cultured explants showed callus proliferation. This presumably due to concentration ratio of NAA and BAP was out of callus induction zone. Ikeuchi *et al.* (2013) reported that the application of exogenous auxin and cytokinin may induce callus formation in various plant species. However an intermediate ratio of auxin and cytokinin was required for callus induction. When auxin concentration was higher than cytokinin, there will be root formation. On the other hand, when auxin concentration was lower than cytokinin there will be shoot regeneration.

Besides plant growth regulators, Ikeuchi *et al.* (2013) suggested that callus formation on cultured explants could be performed when there was mutation resulting in the loss of function in the process of cell wall formation. As it has been understood that a sequential deposition of cell wall materials, such as cellulose, hemicelluloses and pectin, was an important factor in presser-ving cellular differentiation status. As an example, mutant of Glucuronyltransferase1 (GUT1) which was called *non-organogenic callus with loosely attached cells* (nolac-H18), developed into callus on shoot culture of *Nicotiana plumbaginifolia* (Iwai *et al.*, 2002).

CONCLUSION

It can be concluded that: 1) the presence of NAA and BAP in culture medium can increase the number of explants forming shoots, speeding shoot formation, and increasing shoot growth, and 2) the combination of 1 mgL^{-1} NAA + 1 mgL^{-1} BAP is the best for obtaining shoot growth and development from basal slip slice explants of pineapple cv. Tangkit.

In order to obtain more comprehensive results, further research is suggested with the target of: 1) finding more effective explants sterilization method, 2) testing NAA and BAP in liquid medium, and 3) extending the observation period to get better figure of culture growth and development.

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REFERENCES

Al-Saif, A. M., A. B. M. S. Hossain and R. M. Taha. 2011. Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro*. African Journal of Biotechnology 10: 5291-5295.

Almeida, W. A. B., A. P. de Matos, A. de Sousa and S. da. 1997. Effect of benzylaminopurine (BAP) on *in vitro* proliferation of pineapple (*Ananas comosus* (L) Merr). Acta Horticulturae 425: 242-245.

Almeida, W. A. B., G. S. Santana, A. P. M. Rodriguez and M. A. de Costa. 2002. Optimisation of a protocol for micropropagation of pineapples. Revista Brasileira de Fruticultura 2: 296-300.

Anbazhagan, M., B. Balachandran and K. Arumugam. 2014. In vitro propagation of *Musa* sp. (Banana). International Journal of Current Microbiology and Applied Sciences 3: 399-404.

Arab, M. M., A. Yadollahi, A. Shojaeiyan, S. Shokri and S. M. Ghojah. 2014. Effects of nutrient media, different cytokinin types and their concentrations on in vitro multiplication of G · N15 (hybrid of almond · peach) vegetative rootstock Journal of Genetic Engineering and Biotechnology 12: 81-87.

Be, L. V. and P. C. Debergh. 2006. Potential low-cost micropropagation of pineapple (*Ananas comosus*). South African Journal of Botany 72: 191-194.

Compton, M. 1994. Statistical methods suitable for the analysis of plant tissue culture data. Plant Cell, Tissue and Organ Culture 37.

Danial, G. H., D. A. Ibrahim and M. S. Omer. 2016. Response of running shoot tips of strawberry (*Fragaria* x *ananasa*) for *in vitro* propagation in Kurdistan Region of Iraq. International Journal of Environment, Agriculture and Biotechnology 1: 164-169.

Danso, K. E., K. O. Ayeh, V. Oduro, S. Amiteye and H. M. Amoatey. 2008. Effect of 6-Benzylaminopurine and Naphthalene acetic acid on *in vitro* production of MD2 pineapple planting materials. World Applied Science Journal 3: 614-619.

Diengngan, S., M. Mahadevamma and B. N. S. Murthy. 2016. Efficacy of *in vitro* propagation and crown sizes on the performance of strawberry (*Fragaria×ananassa* Duch) cv. Festival under field condition. Journal of Agricultural Ssience and Technology.

Drew, R. A. 1980. Pineapple tissue culture unequalled for rapid multiplication. Queensland Agriculture Journal 106: 447-451.

Farahani, F. 2014. Micropropagation and growth of *in vitro* pineapple (*Ananas comosus* L. Merr.) in Iran. Plant Archives 14: 337-341.

Firoozabady, E. and N. Gutterson. 2003. Cost effective *in vitro* propagation methods for pineapple. Plant Cell Reports 21: 844-850.

George, E. F. and G.-J. De Klerk. 2008. The Components of Plant Tissue Culture Media I: Macro- and Micro-Nutrients. *In* E. F. George, M. A. Hall and G.-J. De Klerk [eds.], Plant Propagation by Tissue Culture (3rd Edition), 65–113. Springer, Dordrecht, The Netherlands.

Hamad, A. H. M., R. M. Taha and S. Mohajer. 2013. *In vitro* induction and proliferation of adventitious roots in pineapple (*Ananas comosus* L.) cultivars of smooth cayenne and morris. Australian Journal of Crop Science 7: 1038-1045.

Ibrahim, M. A., H. A. Al-Taha and A. A. Seheem. 2013. Effect of cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *in vitro*. Acta Agriculturae Slovenica 101: 15-20.

Ikeuchi, M., K. Sugimoto and A. Iwase. 2013. Plant callus: mechanisms of induction and repression. The Plant Cell 25: 3159-3173.

Iwai, H., N. Masaoka, T. Ishii and S. Satoh. 2002. A pectin glucuronyltransferase gene is essential for intercellular attachment in the plant meristem. Proceedings of National Academi of Science USA 99: 16319-16324.

Kadhimi, A. A., A. N. Alhasnawi, A. Mohamad, W. Y. Wan Mohtar and B. C. M. Z. Che Radziah. 2014. Tissue culture and some of the factors affecting them and the micropropagation of strawberry. Life Science Journal 11: 484-493.

Kahia, J., F. Ndaruhutse, B. Waweru, N. Bonaventure, A. Mutaganda, P. Y. Sallah, N. P. Kariuki and T. Asiimwe. 2015. *In vitro* propagation of two elite cooking banana cultivars- FHIA 17 and INJAGI. International Journal of Biotechnology and Molecular Biology Research 6: 40-47.

Khan, S., A. Nasib and B. A. Saeed. 2004. Employment of in vitro technology for large scale multiplication of pineapples (*Ananas comosos*). Pakistan Journal of Botany 36: 611-615.

Mengesha, A., B. Ayenew and T. Tadesse. 2013. Acclimatization of *in Vitro* Propagated Pineapple (*Ananas comosuss* (L.), var. Smooth cayenne) Plantlets to *ex Vitro* Condition in Ethiopia. American Journal of Plant Sciences 4: 317-323.

Moradi, K., M. Otroshy and M. R. Azimi. 2011. Micropropagation of strawberry by multiple shoots regeneration tissue cultures. Journal of Agricultural Technology 7: 1755-1763.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Ngomuo, M., E. Mneney and P. A. Ndakidemi. 2014. The in Vitro Propagation Techniques for Producing Banana Using Shoot Tip Cultures. American Journal of Plant Sciences 5: 16-14-1622.

Pierik, R. L. M. 1997. *In Vitro* Culture of Higher Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rahman, K. W., M. N. Amin and M. A. K. Azad. 2001. *In vitro* rapid clonal propagation of pineapple, *Ananas comosus* (L.) Merr. Plant Tissue Culture 11: 47-53.

Roostika, I., I. Mariska, N. Khumaida and G. A. Wattimena. 2012. Indirect organogenesis and somatic embryogenesis of pineapple induced by dichlorophenoxyacetic acid. Jurnal Agro*Biogen* 8: 8-18.

Roy, S. K., M. Rhaman and S. Haque. 2000. Mass Propagation of Pineapple through *In Vitro* Culture. *In* C. Kubota and C. Chun [eds.], Transplant Production in the 21st Century: Proceedings of the International Symposium on Transplant Production in Closed System for Solving the Global Issues on Environmental Conservation, Food, Resources and Energy, 279-283. Springer, The Netherlands.

Saad, A. I. M. and A. M. Elshahed. 2012. Plant Tissue Culture Media. *In* A. Leva and L. M. R. Rinaldi [eds.], Recent Advances in Plant In Vitro Culture, 29-40. In Tech, Rijeka, Croatia.

Soneji, J. R., P. S. Rao and M. Mhatre. 2000a. *In vitro* regeneration from leaf explants of pineapple (*Ananas comosus* L., Merr.). Journal of Plant Biochemistry and Biotechnology 11: 117-119.

Soneji, J. R., P. S. Rao and M. Mhatre. 2000b. Somaclonal variation in micropropagated dormant axillary buds of pineapple (*Ananas comosus* L., Merr.). Journal of Horticultural Science and Biotechnology 77: 28-32.

Souza, F. V. D., E. A. Chumbinho, D. T. Junghans, H. L. Carvalho and K. C. dos Santos. 2012. *In vitro* culture of pineapple apical meristems for viral removal. Pineapple News - Newsletter of the Pineapple Working Group, International Society for Horticultural Science 19.

Sripaoraya, S., R. Marchant, J. B. Power and 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.

Swamy, M. K., S. K. Mohanty and M. Anuradha. 2014. The Effect of Plant Growth Regulators and Natural Supplements on *in vitro* Propagation of *Pogostemon cablin* Benth. Journal of Crop Science and Biotechnology 17: 1-7.

Yapo, E. S., T. H. Kouakou, M. Kone, J. Y. Kouadio, P. Kouame and J.-M. Merillon. 2011. Regeneration of pineapple (*Ananas comosus* L.) plant through somatic embryogenesis. Journal of Plant Biochemistry and Biotechnology 20: 196-204.

Zuraida, A. R., A. H. Nurul Shahnadz, A. Harteeni, S. Roowi, C. M. Z. Che Radziah and 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: 3859-3866.