Indonesian Journal of Agronomy

ISSN 2085-2916 | e-ISSN 2337-3652
Indonesian J. Agron., *51*(1), 65-72, April 2023
DOI: https://dx.doi.org/10.24831/ija.v51i1.46196

Research Article

Regeneration of Raja (Musa AAB Group) and Kepok (Musa ABB Group) bananas on various stages of in vitro culture

Shafira Puti Nazihah[1], Megayani Sri Rahayu [2,*], and Ni Made Armini Wiendi [2]

[1] Study Program of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, INDONESIA
[2] Department of Agronomy and Horticulture, Faculty of Agriculture, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, INDONESIA
* Corresponding author (✉ megayani@apps.ipb.ac.id)

ABSTRACT

Banana group can be divided into two groups based on their method of its utilization. Banana group that can be eaten immediately after ripening is called "dessert banana", e.g., Raja, while the group that needs particular processing before consumption is called "plantain banana", e.g., Kepok. This study aimed to compare the growth of Raja banana with Kepok plantain through experiments at three stages of the in vitro culture media: the shoot initiation, shoot multiplication, and plantlet acclimatization, also to compare the growth of Raja bananas with Kepok plantains in response to cytokinins BAP and TDZ at shoot multiplication stage. Planting material was in the form of rhizomes that was prepared with the same size. The experiment was performed using a completely randomized design. Results showed that Kepok plantain could adapt faster to the media condition than Raja banana; the phenomenon was evident in the growing speed of Kepok during shoot initiation stage. The proportion of the B genome did not show a direct effect on shoot induction at the multiplication stage or enlargement and organ formation at the acclimatization stage. Shoot induction at the multiplication stage depended more on the composition of the media used. The combination of BAP 3 mg L^{-1} and TDZ 0.01 mg L^{-1} in MS media produced the best shoot induction rate, and TDZ 0.01 mg L^{-1} in MS media had the highest shoot elongation rate.

Keywords: banana, BAP, genome, PGR, plantain, TDZ

Edited by:
Okti Syah Isyani Permatasari

Received:
8 March 2023
Accepted:
10 April 2023
Published online:
27 April 2023

Citation:
Nazihah, S. P., Rahayu, M. S., & Wiendi, N. M. A. (2023). Regeneration of Raja (Musa AAB Group) and Kepok (Musa ABB Group) bananas on various stages of in vitro culture. *Indonesian Journal of Agronomy*, *51*(1), 65-72.

INTRODUCTION

Banana (*Musa* spp.) is non-woody plant from the *Monocot* class, *Zingiberales* order, *Musaceae* family that is commonly found and cultivated in tropics such as Indonesia (CABI, 2021). Banana is herbaceous perennial plant that grows from rhizomes located underground (Kelta et al., 2018). In Indonesia, banana grows throughout the year causing productivity to be quite high, however, its fruit exports are still far below expectation, i.e., about 0.27% of total production in 2018 (BPS, 2019). The low export volume is due to quality problems.

Banana is propagated vegetatively, therefore, the availability of high-quality banana seedlings is important. Continuous availability of banana seedlings facilitates banana cultivation and the availability of high-quality bananas in the market. Nevertheless, the provision of high-quality banana seedlings on a large scale through in vitro culture techniques is still challenging. In vitro culture is a method of plant multiplication through the regeneration of tissue or cells separated from the parent plant, this technique is the best means of reproducing banana seeds in large quantities and in a short time (Wang et al., 2018).

Cultivated bananas are commonly a result of intra-specific and inter-specific hybridization between diploid wild subspecies of *Musa acuminata* and *Musa balbisiana*. Various types of hybridization show different plant performance and size, including differences in resistance to diseases such as yellow sigatoka, black sigatoka and Panama diseases (Berilli et al., 2018). Genetically, bananas are grouped according to its ploidy level (number of chromosome sets) and its relative proportion of *Musa acuminata* (donor A) and *Musa balbisiana* (donor B) in the genome (Dwivanny et al., 2021).

Fruit of banana group that has a sweet taste and can be eaten directly is called 'table or dessert bananas' e.g. Raja, while the group with hard fruits, not too sweet, starchy, and need further processing before consumption is called 'plantain or cooking bananas', e.g. Kepok (Poerba et al., 2016). Both Raja and Kepok cultivars are widely planted in South Kalimantan (Nisa & Rodinah, 2005).

Fruit of Raja banana is sweet-tasting and fragrant. Raja is a triploid banana with AAB genome, while Kepok is a triploid with ABB genome with fruit having a thick skin and bright yellow color when ripe (Poerba et al*.,* 2016). According to Nisa and Rodinah (2005), as well as Resmi and Nair (2011), the presence of more B genome in ploidy affects the level of phenolic content and polyphenol oxidase content and its activity. The study aimed to compare the growth of Raja bananas (AAB) and Kepok plantain (ABB) in certain planting media compositions and to compare Raja bananas with Kepok plantains in responding to plant growth regulator (PGR) of Benzyl Amino Purin (BAP) and Thidiazuron (TDZ) in the multiplication stage.

MATERIALS AND METHODS

Research site

The research was conducted in the Seedling Orchard and Tissue Culture Laboratory at Lebak Bulus, Jakarta from March 2021 to June 2021. The plant materials used rhizomes of Raja and Kepok varieties obtained from the Jakarta Center for Seed and Plant Protection, DKI Jakarta.

Materials preparation

The research started with sterilizing the banana rhizomes both inside and outside the laminar airflow cabinet. Banana buds sized 4-5 mm in length were planted in Murashige and Skoog (MS0) culture media. The bud was maintained until obtaining sterilized explants ready for research use.

Three experiments were conducted representing one for every stage of in vitro culture, i.e., shoot initiation, shoot multiplication and plantlet acclimatization. The experiment used a completely randomized design (CRD).

Shoot initiation

The initiation of banana shoots was conducted in three subculture stages. The first subculture was used to adjust the explants to grow in a sterile environment, the second subculture aimed to stimulate apical shoot initiation, and the third subculture was the stage of axillary shoot initiation. The media used in the initiation stage was Murashige-Skoog media without adding PGR. The observation was carried out using a CRD one-factor model, namely the banana cultivar. The observation consisted of 2 treatments combination with 8 replications per treatment. Data collection was carried out every week for 3 weeks.

The variables observed were the length and the width of the rhizome (cm). The length of the rhizome was measured from the base of the rhizome to the top of the leaf blade. The width of the rhizome was measured from the right to the farthest left side. The data obtained were analyzed using ANOVA. Data showed a significant difference was further analyzed using Duncan's Multiple Range Test (DMRT) α=5%.

Shoot multiplication

The stage of shoot multiplication was carried out after the initiation of shoots. Shoots were multiplied through the subculture process. Observations of shoot multiplication were carried out on the 6th subculture and using a two-factor CRD model, namely the type of PGR added to media consisting of 3 levels (MS + BAP 3 mg L^{-1}, MS + TDZ 0.01 mg L^{-1}, MS + BAP 3 mg L^{-1} + TDZ 0.01 mg L^{-1}) and banana cultivars consisting of 2 levels (Raja and Kepok varieties). The observations consisted of 6 treatment combinations with 4 replications per treatment. Data collection is carried out every 2 weeks for 8 weeks.

The variables observed were the number of shoots and the height of shoots (cm). The number of shoots was counted from total micro shoots formed in the banana pseudostem. The height of the shoots was measured from the base of the pseudostem to the highest leaf tip. The data was analyzed using F-test. Data showed significant differences and was further analyzed using the Duncan Multiple Range Test (DMRT) α=5%.

Plantlet acclimatization

Shoots grew into plantlets after the multiplication stage, plantlets were then taken out to be planted outside the lab in the acclimatization stage. The media used in the acclimatization stage was a mixture of tricho-compost, rice husk, and soil with a ratio of 1:1:1 (v/v). Plantlets used had the same initial morphology between the two cultivars. The observation of acclimatization plantlets used a one-factor CRD model, which was the banana cultivar. The observation consisted of 2 treatments combination with 7 replications per treatment.

Data were collected every week for 4 weeks. The variables observed were stem length (cm), leaf length (cm), and leaf number. Stem length was measured from the base of the stem to the base of the leaf. Leaf length was measured from the base of the leaf to the furthest end of the longest leaf blade. The number of leaves was counted from fully opened ones. The data was analyzed using an F-test. Data showed a significant difference and was further analyzed using the DMRT α=5%.

RESULTS AND DISCUSSION

Shoot initiation

The length of the rhizome of both cultivars increased (5% for Raja and 13% for Kepok) showing a significant difference, the width of the rhizome also increased (21% for Raja and 26% for Kepok) but the difference was not statistically significant (Table 1). The increase in the size of the rhizome length and width indicates swelling on the rhizome towards the top and towards the side. The difference in the level of swelling between the Raja and Kepok rhizomes may occur due to differences in the activation of endogenous PGR cytokinin hormones synthesized by the two cultivars. Plant cells produce cytokinin endogenously and cytokinin can be stored in a passive state, cytokinin activation can occur due to changes in the environment. Dwivanny et al. (2021) stated that bananas that have a B genome can grow quickly and are more drought-tolerant. Kepok, which has more B genome than Raja, may be able to synthesize more active endogenous cytokinin in the first initiation stage as a response to the change in environment from growing in the field to growing in a controlled sterile environment.

The explant used in the initiation of shoots was rhizomes, consisting of two main parts, i.e., the leaf and the stem. The true stem of the banana that has an apical meristem is located in the inner part of the peduncle. The initiation shoots observed were at stage one on MS0 medium, which aimed to adjust the growth conditions of rhizomes from non-sterile to sterile environments. According to Prabowo et al. (2018), the media treatment without the addition of PGR shows the actual growth ability supported by endogenous hormones. Sakina et al. (2019) reported that the process of adjusting the explant to sterile conditions causes the plant cells to produce PGR cytokinins excessively.

Table 1. Differences in the growth of banana rhizomes on MS0 initiation media.

Cultivars	Rhizome length (cm) at WAP			Rhizome width (cm) at WAP		
	1	2	3	1	2	3
Raja	2.0±0.4	2.1±0.4	2.1±0.4	1.4±0.2	1.5±0.2	1.7±0.3
Kepok	2.5±0.4	2.5±0.4	2.5±0.4	1.5±0.2	1.7±0.2	1.9±0.3
F value	8.7	7.5	7.5	2.3ns	1.2ns	0.9ns
CV (%)	15.8	15.5	15.5	11.6	14.2	16.4

Note: ns=not significant, *=significant at α=5%; CV= coefficient of variation; WAP=week after planting; value ± SD.

Shoot multiplication

Table 2 shows that the treatment of media significantly stimulated the number of shoots of both banana cultivars. The MS media supplemented with 0.01 mg L^{-1} TDZ provided the least response in terms of shoot multiplication among the three levels of media tested. Such responses are likely due to the effect of very low TDZ concentration. The use of TDZ is required in a concentration of 0.20 mg L^{-1} in order to have the same effect as the use of BAP concentration of 3 mg L^{-1} (Lee, 2005). A low concentration for TDZ was used for this experiment because TDZ is a non-degradable enzyme and 0.01 mg L^{-1} is the lowest concentration that was reported to be effective at shoot initiation (Rai et al. 2019).

Table 2. Shoot initiation of two banana cultivars on three different types of multiplication media.

Treatment	Number of shoots at WAP							
	2		4		6		8	
Media								
MS + BAP 3 mg L^{-1}	3.9±1.2	a	5.6±1.6	a	6.4±1.9	a	7.3±1.8	a
MS + TDZ 0.01 mg L^{-1}	2.6±1.2	b	3.9±1.6	b	4.3±1.9	b	4.8±1.8	b
MS + BAP 3 mg L^{-1} + TDZ 0.01 mg L^{-1}	4.6±1.2	a	7.3±1.6	a	8.3±1.9	a	9.0±1.8	a
F value	6.1	**	8.6	**	9.3	**	11.2	**
Cultivars								
Raja	3.8±1.2		5.5±1.6		6.2±1.9		7.1±1.8	
Kepok	3.7±1.2		5.7±1.6		6.4±1.9		6.9±1.8	
F value	0.0	ns	0.1	ns	0.1	ns	0.1	ns
Interaction								
F value	1.6	ns	0.5	ns	0.1	ns	0.4	ns
CV (%)	31.3		29.1		29.4		25.8	

Note: ns=not significant, **= significant at α=1%; CV= coefficient of variation; WAP=week after planting; value ± SD.

MS media added with BAP 3 mg L^{-1} and TDZ 0.01 mg L^{-1} is the best media in inducing shoots of both banana cultivars (Table 2). According to Guo et al. (2011), the synergistic effect of two types of cytokinins is more evident than using single cytokinin. Moreover, combining TDZ with other hormones auxin and cytokinin is more effective to increase shoot initiation. Based on the research conducted by Lisnandar et al. (2015), Kepok (ABB) grown from explant of flower axis and planted in MS media supplemented with BAP and TDZ produces more shoots than Raja (AAB); here Raja cultivar often experiences browning causing shoot growth inhibition.

Table 2 shows that the effect of cultivars on the number of shoots is not significant. The result indicates that the cultivar genome had no effect on the ability of shoot multiplication. It is probable that biosynthesis of polyphenol oxidase causing browning of explant was minimum in the present experiment. However, it needs further evaluation on the polyphenol oxidase analysis to confirm the speculation. It is important to note that cytokinin BAP is the most commonly used PGR in the production of in vitro banana shoot multiplication media, while cytokinin TDZ is also used as an alternative PGR in multiplication media. Therefore, it is not exclusive that a combination of BAP and TDZ supplements in multiplication media increased the number of shoots formed in the present experiment.

MS media with the addition of 0.01 mg L^{-1} TDZ is the media with the highest level of shoot elongation compared to the other two levels of media (Table 3). The differences in media treatment were apparent from 6 to 8 WAP, possibly due to the effect of using TDZ. It is possible that supplement TDZ increases the transportation of endogenous auxin. Metabolism and transport of endogenous auxin are directed towards shoot elongation after hormone activity is directed towards shoot induction (Guo et al., 2011; Pessarakli, 2021). Plants are able to allocate nutrients absorbed based on their environmental conditions to the appropriate metabolic processes to maintain their survival (Magneschi & Perata, 2009).

Table 3. Growth of shoot height of two banana cultivars on three multiplication media.

Treatment	Shoot height (cm) at WAP							
	2		4		6		8	
Media								
MS + BAP 3 mg L^{-1}	2.0±0.2		2.5±0.3		2.7±0.4	b	2.9±0.5	b
MS + TDZ 0.01 mg L^{-1}	1.9±0.2		2.6±0.3		3.3±0.4	a	3.9±0.5	a
MS + BAP 3 mg L^{-1} + TDZ 0.01 mg L^{-1}	1.9±0.2		2.3±0.3		2.4±0.4	b	2.5±0.5	b
F value	0.7	ns	1.8	ns	10.0	**	13.5	**
Cultivars								
Raja	2.1±0.2	a	2.6±0.3	a	3.1±0.4	a	3.3±0.5	
Kepok	1.8±0.2	b	2.2±0.3	b	2.6±0.4	b	2.9±0.5	
F value	5.6	*	9.6	**	9.9	**	2.3	ns
Interaction								
F value	1.8	ns	2.7	ns	4.4	*	3.2	ns
CV (%)	12.0		13.8		14.1		17.6	

Note: ns=not significant, *=significant at α=5%, **= significant at α=1%; CV=coefficient of variation; WAP=week after planting; value ± SD.

MS multiplication media with a combination of BAP + TDZ has the lowest level of shoot elongation compared to the other two levels of media tested (Table 3). It is speculated that a high level of cytokinin in the multiplication media might inhibit shoot growth. Lu (2005) stated that the addition of cytokinin in excessive concentrations inhibits shoot elongation. Thus, the use of TDZ in MS media without other PGR stimulated shoot growth; this is a uniqueness that is only possessed by the cytokinin TDZ. According to Erland et al. (2020), TDZ can be responded to differently by plant cells and can have similar metabolic effects as auxin hormones. Tall shoot growth can be achieved by using auxin hormones, replacing the role of TDZ in planting media.

The application of auxin to in vitro culture media increases shoot length (Pessarakli, 2021). The use of a combination of BAP and auxin or TDZ with auxin at the appropriate concentration can produce a lot and high shoots. BAP 0.5 mg L^{-1} combined with Giberelin GA3 0.3 mg L^{-1} is the best media for producing optimum height of papaya shoots in vitro as reported by Fajri et al. (2021). Table 3 shows that there was a significant to a highly significant effect on shoot height growth among cultivars starting from 4 to 6 WAP. The Raja (AAB) cultivar had higher shoot elongation than Kepok (ABB). Avivi et al. (2013) stated that banana cultivar determines shoot length during multiplication. In the present experiment, the difference in phenotype characters of Raja and Kepok could be due to the effect of more A genomes. According to Megia (2005), banana cultivars with the same genome may express different phenotypes. Therefore, it is possible that other banana cultivars from the ABB group have better elongation than those from the AAB group. However, these speculations need further research.

Plantlet acclimatization

Although plantlets used in this experiment had the same general morphology, both Raja and Kepok plantlets showed variation in stem and leaf blade size, as shown by standard deviation values (Tables 4 and 5). In general, Raja cultivar had a longer stem and leaf as compared to Kepok. At 1 WAP, Raja plantlets sized significantly larger than Kepok (Tables 4 and 5); the bigger plantlets presumably had bigger nutrient absorption resulting in Raja having more superior size than Kepok during observation. Triharyanto et al. (2018) stated that the initial height of the plantlet affects the height of banana shoots in acclimatization media. The proportion of genome A in the ploidy may affect the phenotype expression of Raja and Kepok, but genome is not a determining factor for gene expression because the phenotype of the cultivar depends on many factors. According to research by Ridhawati et al. (2017), gene expression strongly supports growth superiority.

Table 4. Stem length of two banana cultivars on acclimatization media.

Cultivars	Stem length (cm) at WAP							
	1		2		3		4	
Raja	4.5±0.7	a	5.8±0.9	a	7.1±1.1	a	9.0±1.4	a
Kepok	3.0±0.7	b	3.5±0.9	b	4.0±1.1	b	5.1±1.4	b
F value	14.4	*	21.4	*	26.0	*	27.0	*
CV (%)	19.0		19.9		20.5		19.9	

Note: ns=not significant, **= significant at α=1%; CV= coefficient of variation; WAP=week after planting; value ± SD.

Table 5. Leaf blade length of two banana cultivars on acclimatization media.

Cultivars	Leaf blade length (cm) at WAP							
	1		2		3		4	
Raja	6.1±0.6	a	6.9±0.7	a	8.6±1.1	a	9.5±1.2	a
Kepok	4.1±0.6	b	4.3±0.7	b	4.9±1.1	b	5.8±1.2	b
F value	41.1	*	56.1	*	38.5	*	33.6	*
CV (%)	11.7		11.9		16.8		15.8	

Note: ns=not significant, **= significant at α=1%; CV=coefficient of variation; WAP=week after planting; value ± SD.

Cultivars exhibited a very significant effect on the number of leaves (Table 6). Raja bananas can form more leaves than Kepok, this is likely to occur due to the higher level of Raja's endogenous auxin synthesis compared to Kepok. According to Rainiyati et al. (2009), auxin in certain doses stimulates leaf opening of plantlets. However, in this experiment, the planting media used for the acclimatization was not added by any PGR. Therefore, stimulation of PGR on the leaf formation of Raja and Kepok originated from endogenous auxin. It is probable that Raja and Kepok might have different abilities to produce endogenous auxin as a consequence of different genome compositions. In general, the number of functional leaves significantly determines the harvest of banana (Mensah et al., 2012), and determines the yield potential of banana (Sheela & Nair, 2001). Bigger leaves mean more assimilates production for fruit formation (Soares et al., 2012).

Table 6. Number of leaves of two banana cultivars on acclimatization media.

Cultivars	Number of leaves at WAP							
	1		2		3		4	
Raja	3.9 ± 0.7	a	4.1 ± 0.9	a	4.0 ± 0.7	a	4.3 ± 0.8	a
Kepok	2.1 ± 0.7	b	2.0 ± 0.9	b	2.3 ± 0.7	b	2.6 ± 0.8	b
F value	21.6	*	21.8	*	22.7	*	17.3	*
CV (%)	23.0		28.0		21.4		22.5	

Note: ns=no significant effect, *=significant effect at level α=5%, **= significant effect at level α=1% α=1% based on DMRT test; CV=coefficient of variation; WAP=week after planting; value ± SD.

Raja and Kepok experienced decreasing in the number of leaves, the decrease in the number of leaves of Raja occurred from 2 to 3 WAP while the decrease in the number of

leaves of Kepok occurred from 1 WAP to 2 WAP (Table 6). The tendency in decreasing in leaf number of both cultivars is presumably due to the narrow planting distance of the plantlets in the acclimatization stage (Figure 1). Narrow planting distance might increase the light competition among plants. According to Nebangka et al. (2020), narrow planting distance suppresses banana growth. On the other hand, Bhende and Kurien (2015) stated that the horizontal growth of banana is limited although the rhizomes produce offspring. Suppression in leaf growth may also be due to plant responses to environmental conditions such as drought and due to the nature of genome. According to Dwivanny et al. (2021), bananas with genome B are more resistant to drought. It is possible that Kepok undergoes environmental adjustment by shedding its leaves earlier than Raja due to the influence of the genome.

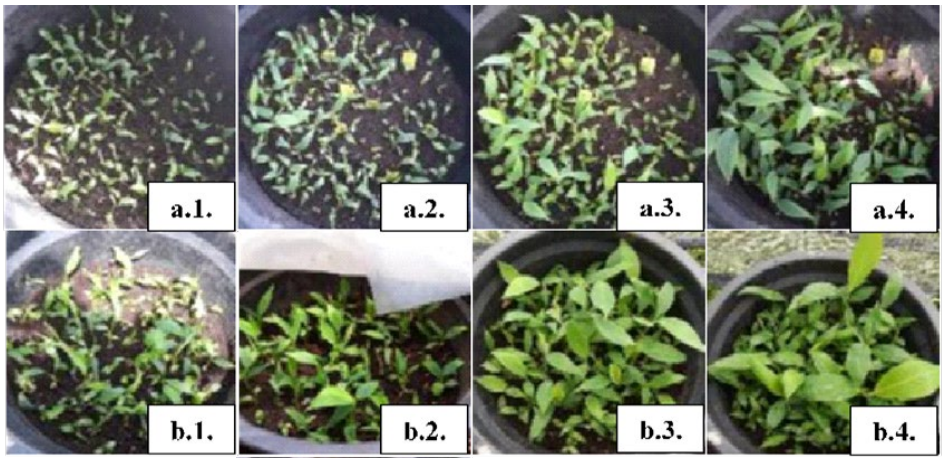

Figure 1. Differences in seed performance of Kepok (a) and Raja (b) banana cultivars during 4 WAP acclimatization. 1 to 4 represent 1 to 4 WAP.

CONCLUSIONS

Kepok banana (ABB) grew faster than Raja banana (AAB) in different growing media, including in media without PGR. However, the proportion of the B genome in the banana ploidy seemed to have no direct effect on the growth ability of Raja and Kepok in vitro culture. Supplement PGR types and their concentrations to in vitro media had a significant effect on the growth of Raja and Kepok during the multiplication stage. A combination of BAP 3 mg L^{-1} and TDZ 0.01 mg L^{-1} in MS media resulted in the best shoot induction, and the concentration of TDZ 0.01 mg L^{-1} in MS media resulted in the highest shoot elongation level of both cultivars. During the acclimatization stage, no direct effect of the proportion of the B genome was observed, but Raja with less B genome had a taller plant than Kepok.

REFERENCES

Avivi, S., Soedarmo, S. H., & Prasetyo, P. A. (2013). Shoot multiplication and acclimatization of three banana's varieties: Raja Nangka, Kepok, and Mas. (In Indonesian.). *Jurnal Hortikultura Indonesia*, *4*, 83-89.

(BPS) Badan Pusat Statistik. (2019). *Annual Statistics of Fruits and Vegetables in Indonesia, 2018*. (In Indonesian.). BPS RI, Jakarta, ID.

Berilli, A. P. C. G., Vigano, M., Sales, R. A. D., Berilli, S. D. S., Fontes, P. S. F., Fontes, A. G., Quartezani, W. Z., Junior, J. D. O. C., Souza, C. M. P. D., & Oliveira, E. V. C. D. (2018). Agronomic performance of different banana cultivars in the Capixaba North Region. *Journal of Experimental Agriculture International, 22*, 1-11.

Bhende, S. S., & Kurien, S. (2015). Sucker production in banana. *Journal of Tropical Agriculture, 53*, 97-106.

[CABI] Centre for Agriculture and Bioscience International. (2021). *Musa (banana).* https://www.cabi.org/isc/datasheet/35124.

Dwivanny, F. M., Wikantika, K., Sutanto, A., Gazali, M. F., Lim, C., & Kamalesha, G. (2021). *Indonesian Banana*. (In Indonesian.). ITB Press, Bandung, ID.

Erland, L. A. E., Giebelhaus, R. T., Victor, J. M. R., Murch, S. J., & Saxena, P.K. (2020). The morpho regulatory role of thidiazuron: metabolomics-guided hypothesis generation for mechanisms of activity. *Biomolecules*, *10*, 1253.

Fajri, R., Efendi, D., & Dinarti, D. (2021). Sterilization and in vitro propagation of axillary shoots of Callina and Caliso papaya cultivars. (In Indonesian.). *Jurnal Agronomi Indonesia*, *49*, 75-81.

Guo, B., Abbasi, B. H., Zeb, A., Xu, L. L., & Wei, Y. H. (2011). Thidiazuron: A multi dimensional plant growth regulator. *African Journal Biotechnology*, *10*, 8984-9000.

Kelta, A., Hajare, S. T., & Banjaw, A. (2018). Studies on in vitro micropropagation in banana. *International Journal of Current Microbiology and Applied Sciences*, *7*, 3366-3375.

Lee, S. W. 2005. Thidiazuron in the improvement of banana micropropagation. *Acta Horticulturae,* 692, 67-74.

Lisnandar, D. S., Fajarudin, A. Efendi, D. & Roostika, I. (2015). Organogenesis of floral axis of AAB and ABB group banana. (in Indonesian.). *Jurnal Hortikultura, 25*, 1-8.

Lu, M. C. (2005). Micropropagation of *Vitis thunbergii Sieb. et Zucc.,* a medicinal herb, through high-frequency shoot tip culture. *Scientia Horticulturae*, *107*, 64-69.

Magneschi, L., & Perata, P. (2009). Rice germination and seedling growth in the absence of oxygen. *Annals of Botany, 103*, 181-196.

Megia, R. (2005). Musa as a genome models. (In Indonesian.). *Hayati*, *12*, 167-170.

Mensah, D. B., Dorcas, Q. M., Kwame, B. R., & Kodjo, D.S. (2012). Comparative study on the field performance of FHIA-01 (hybrid dessert banana) propagated from tissue culture and conventional sucker in Ghana. *Journal of Plant Development, 19*, 41-46.

Nebangka, M., Sumayku, B. R. A. & Pongoh, J. (2020). Potential for abaka banana development (*Musa textilis* Nee) in Karakelang Island. (in Indonesian.). *Cocos, 1*, 1-11.

Nisa, C., & Rodinah. (2005). Tissue culture of several banana cultivars (*Musa paradisiaca L.*) using application combination of NAA and kinetin. (In Indonesian.). *Bioscientiae,* 2, 223-36.

Pessarakli, M. (2021). *Handbook of Plant and Crop Physiology 4th Edition*. CRC Press, Oxford, UK.

Poerba, S., Martanti, D., Handayani, T., Herlina, & Witjaksono. (2016). *Banana Catalog: Koleksi Kebun Plasma Nutfah Pisang Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia.* (In Indonesian.). LIPI Press, Jakarta, ID.

Prabowo, H., Samanhudi, Yuniastuti, E., & Yunus, A. (2018). Effects of media combination with concentration of AB-Mix nutrient on growth of banana shoots on in Vitro. *Bulgarian Journal of Agricultural Sciences*, *24*, 404–410.

Rai, I. N., Sudana, I. M., Astawa, I. N. G., Dwiyani, R., & Fitriani, Y. (2019). Direct organogenesis in vitro propagation of local Balinese banana with thidiazuron. *International Journal of Life Sciences*, *3*, 32-40.

Rainiyati, Lizawati, & Kristiana, M. (2009). Role of IAA and BAP on nodule of Raja Nangka banana (Musa AAB) in vitro. (in Indonesian). *Jurnal Agronomi*, *13*, 51-57.

Resmi, L., & Nair, A. S. (2011). Differential effect of cytokinins in the micropropagation of diploid and triploid Musa cultivars. *Int. J. Integr. Biol, 11*, 35-38.

Ridhawati, A., Anggraeni, T. D. A., & Purwati, R. (2017). The effect of media composition on the induction of shoot and roots and of five agave clones on in vitro culture. (In Indonesian.). *Buletin Tanaman Tembakau, Serat dan Minyak Industri*, *9*, 1-9.

Sakina, S., Anwar, S., & Kusmiyati, F. (2019). Planlet growth of Dendrobium (*Dendrobium sp*.) orchid in vitro on different BAP and NAA concentrations. (In Indonesian.). *Jurnal Pertanian Tropik, 6*, 430-437.

Sheela, V. L., & Nair, S. R. (2001). Growth, flowering and yield potential of tissue culture banana (Musa AAB cv. Nendran). *Journal of Tropical Agriculture, 39*, 1-4.

Soares, J. D., Pasqual, M., Rodrigues, F. A., Lacerda, W. S., Donato, S. L. R., Silva, S. D. O. E., & Paixao, C. A. (2012). Correlation between morphological characters and estimated bunch weight of the tropical banana cultivar. *African Journal of Biotechnology*, *11*, 10682-10687.

Triharyanto, E., Arniputri, R. B., Muliawati, E.S., & Trisnawati, E. (2018). Studies on IAA and BAP of Raja Bulu in vitro multiplication and its acclimation. (In Indonesian.). *Agrotechnology Research Journal*, *2*, 1-5.

Wang, K.H., Uyeda, J., & Sugano, J. (2018). *Banana Pest and Disease Management in the Tropical Pacific: A Guidebook for Banana Growers*. Western IPM Center, Hawaii, USA.