NUSANTARA BIOSCIENCE
Vol. 7, No. 2, pp. 67-72
November 2015

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n070202

Germination and breaking seed dormancy of *Alpinia malaccensis*

REZA RAMDAN RIVAI[1,♥], FITRI FATMA WARDANI[1], MICHA GRACIANNA DEVI[2]

[1]Center for Plant Conservation, Bogor Botanic Gardens, Indonesian Institute of Sciences (LIPI), Jl. Ir. H. Juanda No. 13 Bogor 16003, West Java, Indonesia. Tel./Fax. +62-251-8322187, ♥email: rezaramdanrivai@gmail.com.

[2]Department of Plant Biotechnology, Graduate School of Wageningen University, Droevendaalsesteeg 4, Wageningen, The Netherlands.

Manuscript received: 10 June 2015. Revision accepted: 3 August 2015.

Abstract. *Rivai RR, Wardani FF, Devi MG. 2015. Germination and breaking seed dormancy of* Alpinia malaccensis*. Nusantara Bioscience 7: 67-72. Alpinia malaccensis* belongs to the ginger family, Zingiberaceae, and is generally propagated by vegetative organs. Seed propagation is needed in order to improve the quality of plants through crossbreeding. The hard testa and small endosperm are the main problems in seed germination. The objectives of this research were to determine the best media for germination and method for breaking seed dormancy of *A. malaccensis*. The research was conducted at the Seed Conservation Laboratory, Center for Plant Conservation Bogor Botanic Gardens. A Completely Randomized Design with three replications was used, and the two treatment factors were different germination media and the methods of breaking seed dormancy. Results showed that cocopeat was the best media for increasing *A. malaccensis* germination rate one month after planting, whereas the method of wounding the testa or soaking the seed in water with a temperature of 75 ºC for 5 minutes showed the highest rate of *A. malaccensis* germination after three months of planting.

Keywords: *Alpinia malaccensis*, dormancy, media, germination, Zingiberaceae

INTRODUCTION

Zingiberaceae, the ginger family, contains many high potential medicinal herbal plants known throughout the world (Chudiwal et al. 2010; Sharma and Pegu 2011; Verma et al. 2011; Hanh et al. 2014; Sahoo et al. 2014). *Alpinia malaccensis* is one such species often utilized for medicinal purposes due to its antioxidant content (Sahoo et al. 2012). *A. malaccensis* also contains 3.5% of essential oils, mainly in the form of methyl cinnamate (Setyawan 1999; Ma'mun 2006; Riyanto et al. 2012; Muchtaridi et al. 2014).

Plant species in the genus *Alpinia* are often used as ornamental plants in the form of cut flowers. The increase in consumer demand for fresh-cut *Alpinia* flowers is expected to lead to the development of larger flower size, longer flower stalks and red colored petals (Sunarmani et al. 2011). Given the extensive benefits offered by this species, it is necessary to develop new cultivars through cross or mutation breeding.

A. malaccensis is often bred through vegetative propagation (Yunira 2009). Zingiberaceae produces flower throughout the year, but only about 8% are successful enough to bear fruits (Aswani et al. 2013). One major obstacle that is being tackled by breeders is the limited amount of information about the effectiveness of seed as a propagation material, knowing that multiplication by seeds using crosses is essential in the development of superior types.

The thick testa and small endosperm result in a low germination success rate for *A. malaccensis* seeds (Setyawan 1999; Yunira 2009; Aswani et al. 2013). According to Yunira (2009), only 6.67% of *A. malaccensis* seeds had germinated three months after being planted in rice husk charcoal medium. This study focuses on finding the best type of media and the proper method of breaking *A. malaccensis* seed dormancy.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Seed Conservation, Center for Plant Conservation Bogor Botanic Gardens, Indonesian Institute of Sciences (LIPI), for three months (October 2014 - January 2015). Seeds were extracted from mature *A. malaccensis* fruit in the Bogor Botanic Gardens collection grown in Vak XII.B.III.92. The types of medium used in the experiment were sand, rice husk charcoal, cocopeat, and compost.

The study mainly focused on two factors: germination media and methods for breaking *A. malaccensis* seed dormancy. The effects of these factors were analyzed using a completely randomized factorial experimental design with four levels of germination media as the first factor and three levels of the method used for breaking seed dormancy as the second factor. The germination media were described as follows: (M1) sand, (M2) rice husk charcoal, (M3) cocopeat, and (M4) compost. Tested methods for breaking seed dormancy were: (S1) heating, (S2) without wounding testa (control), and (S3) wounding testa.

There were 12 combinations of treatment with three replications equivalent to 36 experimental units (sowing containers). Each experimental unit consisted of 20 seeds of *A. malaccensis*, in total 720 units of observation. Data were analyzed using analysis of variance by F test at 5% significance level. Significantly different results lead to Duncan's multiple range test (Duncan's Multiple Range Test / DMRT) with 5% level of significance. The software used was Microsoft Excel 2013 and Statistical Tool for Agricultural Research (STAR).

Fruit and seed characteristics were described by direct observation. Fruit characteristics observed included the number of fruits per cluster, ripe fruit skin color, weight and diameter of the fruits. Seed characteristics measured included the number of seeds per fruit, color, weight, length, and width of seeds. The internal structure of seeds was observed using a binocular microscope.

Seed moisture content (MC) was calculated with Constant Oven Temperature methods outlined by Draper et al. (1985) :

$$MC = (M2 - M3) \times \frac{100}{(M2-M1)}$$

where M1: the weight of the sowing container, M2: the seed and the sowing container weight before drying, and M3: the seed and the container weight after drying.

The heating treatment was carried out by soaking the seeds in warm water (75 ^{0}C) for 5 minutes, and the wounding treatment was done by cutting a small portion on the end of hard seed testa without injuring the embryo inside (Kristiaty and Putri 2008; Rodrigo et al. 2012; Yuniarti et al. 2013; Astari et al. 2014). The four different germination media were individually sterilized with hot water before being used in the sowing containers with perforated bottoms. Twenty seeds of *A. malaccensis* were planted in each container after the media had cooled. There were 3 replicate containers for each of the 3 seed treatment in each of the 4 media types. Planting the seeds of *A. malaccensis* in the sand and compost (1: 1) media was also conducted to determine the germination pattern. The entire experiment was kept under paranet shade of 55%.

Observations on the germination rate, germination rate coefficient, germination time (initial and final day), coefficient of uniformity of germination, and seedling growth were carried out every three days. The germination rate was calculated by a formula used by Draper et al. (1985):

$$GR = \frac{n}{N} \times 100$$

Where R= Germination Rate; n= seeds germinated, N= seeds that were treated to germinate. The germination rate coefficient was calculated by using the formula:

$$x = \frac{\sum n}{\sum(t \times n)} \times 100$$

wWhere x: germination rate coefficient, n: seeds germinated, t: day when the seeds germinated. Seedling growth was observed by measuring height with a ruler for every week.

Observations of the microclimate such as pH and relative humidity of the media, light intensity, wind speed, and temperature were made at the beginning of the experiment. Measurements of fresh and dry weight of seedlings, epicotyl and radicle length, quantity and color of leaves and chlorophyll were performed at the end of the experiment (three months after planting). The dry weight measurements were conducted by placing three seedlings from each treatment combination in the oven with a temperature of 60°C for 72 hours. Determination of leaf color was based on the Royal Horticultural Society color chart and the chlorophyll content of seedling leaves was measured using the chlorophyll content meter opti-sciences CCM 200 plus.

RESULTS AND DISCUSSION

Fruit and seed characteristic

A. malaccensis fruit bunches consisted of 13-20 furry golden fruits (Figure 1a). Immature fruits were green and turned yellow when ripe. The fruit broke into three parts when pressed (Figure 1b). Ripe fruit weight ranged from 5.20 to 11.68 g and the diameter ranged from 0.24 to 0.30 cm. Each fruit contained 20-53 black seeds with white arils (Figure 1c); with seed length of 3.00 to 5.61 cm, width of 1.50 to 3.65 cm, and weight of 7.10 to 8.40 g per 100 seeds. The testa was thick and hard. The white perisperm was also thick, and surrounded the endosperm that was attached to the embryo (Figure 1d). According to Setiawan (1999), the base of the seed has a plug that connects the embryo with the aril that will be discharged during the germination.

Seedling growth pattern

Seedling growth of *A. malaccensis* began with the breakdown of the testa and the emergence of the radicle (root candidate) approximately 2-3 weeks after planting. Along with the lengthening of the radicle, the growth of the creamy white epicotyl penetrated the surface of the media around 3-4 weeks after planting. Cotyledons remained under the surface of the media (hypogeal). As the epicotyl grew it changed its color to green. The first leaves appeared about 5-6 weeks after planting (Figure 2).

Germination capacity and seedling height

A. malaccensis seeds germinated under the paranet (55%) with microclimate state at an average daily temperature of 33.8 ° C, 48.9% relative humidity 48.9%, light intensity 11186.67 lux, and wind speed 0.87 m/sec. The seed moisture content was about 35.02% (calculated without extracting water content of aril seed layer). Further investigation is required to determine the viability of the storage system for seeds as plant conservation material in seed banks.

Table 1 shows the significant influence of the media on germination and initial seedling height as well as pH and RH of the media. The method of breaking seed dormancy significantly affected the final germination rate, the initial and final day of germination and initial seedling height. There was an obvious interaction between the media and methods of breaking seed dormancy on the initial seedling height.

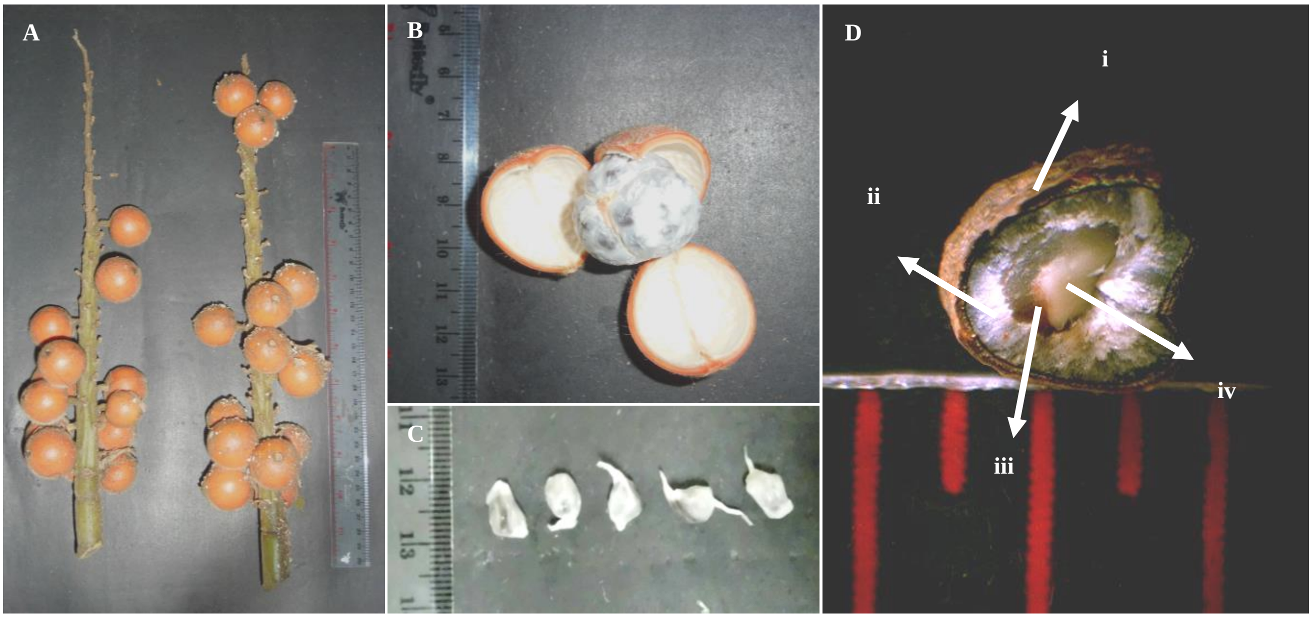

Figure 1. *Alpinia malaccensis* fruit and seed characteristics. A. Fruit bunch; B. Fruit; C. Seed; D. Seed internal structure i. Testa, ii. Perisperm, iii. Endosperm, iv. Embryo.

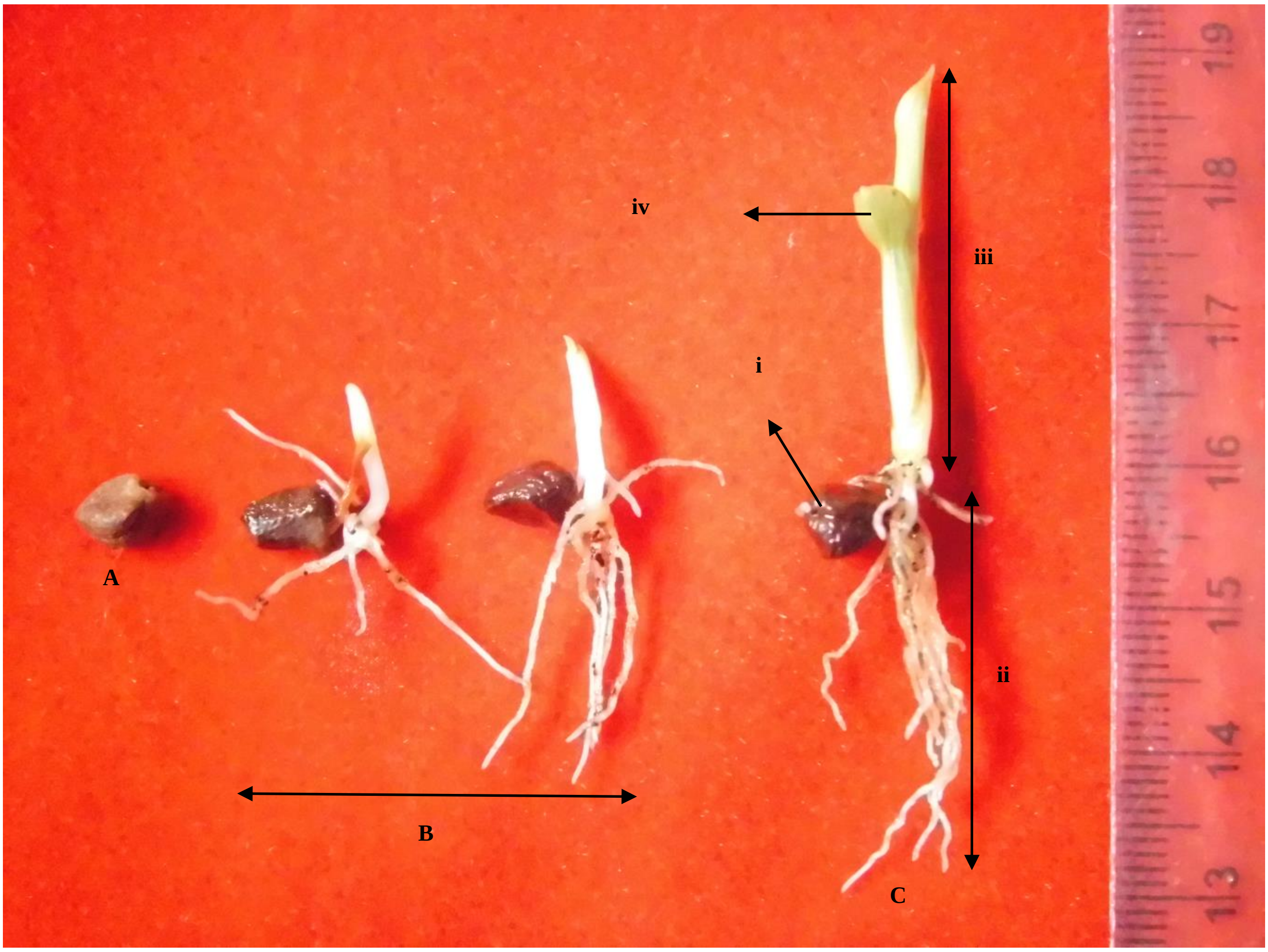

Figure 2. *Alpinia malaccensis* seedling morphology and growth pattern. A. Seed; B. Radicle and epicotyl growth; C. Seedling structure i. Cotyledon, ii. Radicle, iii. Epicotyl, iv. First leaf.

Initial germination was observed one month after sowing the seed. Table 2 shows that the seed germination rate of *A. malaccensis* was best on cocopeat. Almost all media tested had a neutral pH, but cocopeat was more acidic than sand, rice husk charcoal and compost. Cocopeat had moderate moisture content (74.44%). Similar research conducted by Utami et al. (2006) stated that ramin seed (*Gonystylus bancanus*) had the highest percentage of germination in cocopeat media. Yuniarti et al. (2013) also mentioned that using cocopeat was the best for germination of *Acacia crassicarpa*. Cocopeat is obtained by extracting the fiber from coconut shells, and has high porosity, water holding capacity and C/N ratio which make it suitable for germinating tropical plants.

Seeds of *A. malaccensis* are more adaptive to media with medium-high or low moisture content. Sand and rice husk charcoal have good aeration and drainage, but low water holding capacity and therefore seeds do not absorb water optimally for the imbibition process.

According to Copeland and McDonald (2001), imbibition is the process through which water enters the seed. Water that enters through this process then activates gibberellin which activates the germination activity by breaking up the nutrients in the cotyledons. The embryo begins to grow by utilizing the nutrient reserves from the endosperm and perisperm. The radicle will appear and penetrates through the testa due to cell elongation and cell division. This indicates that the radicle has started to grow.

Final germination rate was observed when there were no more seeds germinating three months after planting. Table 3 showed that the method of breaking seed dormancy by soaking the seeds in water temperature of 75°C for 5 minutes and by wounding the testa were significantly better than the control. The initial and final days of germination were faster by injuring the testa because the testa became more permeable to water and air. According to Yunira (2009), water that is absorbed accelerates the imbibition process and speeds the germination process; however, injury to the testa may cause the endosperm to rot.

Breaking seed dormancy by immersion in hot water and wounding the testa have been tested before. Kristiaty and Putri (2008) found that wounding the testa was the best method to break seed dormancy of kedawung (*Parkia javanica*). Soaking the seeds in boiling water (100°C) is not recommended because it resulted in cell death, leading to low germination percentage. Rodrigo et al. (2012) explained that the method of breaking seed dormancy by soaking *Elettaria cardamomum* seeds in water temperature of 50°C for 30 minutes was too long. Yuniarti et al. (2013) and Astari et al. (2014) proved that wounding the testa was the best method of breaking seed dormancy and improving the germination of *Acacia crassicarpa* and *Mucuna bracteata*.

Table 4 shows that there was influence of the media, breaking seed dormancy methods and its interaction with initial seedling height of *A. malaccensis*. *A. malaccensis* seedlings at 4 weeks after planting on charcoaled rice husk soaked water temperature of 75°C for 5 min and wounding the testa had a low initial height than other treatment combinations. Unlike the seedlings grown on rice husk without dormancy breaking treatments had higher initial seedling height than most other treatment combinations.

Table 1. Recapitulation of the influence of media, breaking seed dormancy methods and its interaction with various experimental parameters

Parameter	Factor		
	Media	**Method of breaking seed dormancy**	**Inter-action**
Final germination rate	ns	*	ns
Initial day of germination	ns	*	ns
Final day of germination	ns	*	ns
Germination rate coefficient	ns	ns	ns
Coefficient of uniformity of germination	ns	ns	ns
Initial seedling height	*	*	*
Final seedling height	ns	ns	ns
pH of media	*	ns	ns
RH of media	*	ns	ns

Note: * = significantly different (P <0.05) ns = not significantly different (P> 0.05)

Table 2. *A. malaccensis* initial germination rate of seedling on different media and pH and RH of media

Media	Initial germination rate (%)	pH	RH (%)
Sand	7,22 b	7,00 a	15,00 d
Charcoaled rice husk	5,56 b	7,00 a	28,89 c
Cocopeat	18,33 a	6,14 b	74,44 b
Compost	1,67 b	6,82 a	97,78 a

Note: Figures followed by the same superscript letters in the same column are not significantly different (P> 0.05)

Table 3. Final germination rate, initial day of germination (IDG) and final day of germination (FDG) of *A. malaccensis* using different methods of breaking seed dormancy

Method of breaking seed dormancy	Final germination rate (%)	IDG	FDG
Water immersion in 75 °C, 5 minutes	38,75 a	36,50 a	67,33 a
Without wounding the testa (control)	10,00 b	37,33 a	69,83 a
Wounding the testa	34,58 a	18,42 b	34,08 b

Note: Figures followed by the same superscript letters in the same column are not significantly different (P> 0.05).

Table 4. Initial seedling height of *A. malaccensis* in various media and using different methods of breaking seed dormancy

Breaking seed dormancy method	Germination media			
	Sand	**Charcoaled rice husk**	**Coco-peat**	**Com-post**
	Seedling height (cm)			
Water immersion in 75 °C, 5 minutes	0,11 a	0,08 b	0,71 a	0,11 a
Without wounding the testa (control)	0,30 a	1,15 a	0,54 a	0,12 a
Wounding the testa	0,08 a	0,01 b	0,36 a	0,04 a

Note: Figures followed by the same superscript letters in the same row and column are not significantly different (P> 0.05)

Table 5. Recapitulation of the influence of media, breaking seed dormancy method and its interaction with various experimental parameters

Parameter	Factor		
	Media	**Method of breaking seed dormancy**	**Inter-action**
Seedling fresh weight	ns	ns	ns
Seedling dry weight	ns	ns	ns
Radicle length	*	ns	ns
Epicotyl length	ns	ns	ns
Leaf quantity	ns	ns	ns
Leaf color	*	ns	ns
Amount of chlorophyll	*	ns	ns

Note: * = significantly different (P <0.05) ns = not significantly different (P> 0.05)

Table 6. Radicle length, color and chlorophyll content of leaves of *A. malaccensis* seedling in various media

Media	Radicle length (cm)	Leaf color	Chlorophyll content (cci)
Sand	7,76 b	1,67 c (Light green)	4,83 c
Charcoaled rice husk	13,62 a	3,00 b (Green)	9,63 b
Cocopeat	14,16 a	1,22 c (Light green)	2,93 c
Compost	7,97 b	3,89 a (Dark green)	22,03 a

Note: Figures followed by the same superscript letters in the same column are not significantly different (P> 0.05)

Seedling characteristics

Seedling characteristics were observed 3 months after planting. Table 5 shows that the germination medium significantly affected the radicle length, color, and leaf chlorophyll content.

Figure 4. *Alpinia malaccensis* seedlings after three months planting in different media. A. Sand, B. Rice husk charcoal, C. Cocopeat, D. Compost

Seedling radicles grew longer on rice husk charcoal and cocopeat (Table 6). Both media had porosity and high water-holding capacity, allowing for improved root growth and development.

The type of compost used in this study was Bioposka, which is processed from organic waste from the Center for Plant Conservation Botanic Gardens LIPI. Bioposka is composed of 0.04% P_2O_5, 0.17% K_2O, 9374 ppm Fe, 555 ppm Mn, 21 ppm Cu, Zn 85 ppm and 30 ppm B. The the leaf color of seedlings germinated on the compost media was dark green, darker than others that were grown in sand, rice husk charcoal and cocopeat (Figure 4). This result was supported by the high chlorophyll content of *A. malaccensis* seedling leaf in compost media.

A. malaccensis seed had the highest percentage of germination on cocopeat media one month after planting (18.33%). Breaking seed dormancy by means of wounding the testa or soaking seed in water at 75 ° C for 5 minutes gave the highest percentage of germination three months after planting (34.58% and 38.75%).

Further research is proposed to improve the germination of *A. malaccensis* using various media mixtures and chemical stimulants to break the seed dormancy.

ACKNOWLEDGEMENTS

The research was funded by the Center for Plant Conservation-Bogor Botanic Garden, Indonesia. The authors would like to thank Prof. Dr. Endang Tri Margawati that has guided the writing of this scientific paper.

REFERENCES

Astari RP, Rosmayanti, Bayu ES. 2014. Effect of dormancy breaking physically and chemically with the ability of mucuna seed germination (*Mucuna bracteata* D.C.). Jurnal Online Agroteknologi 2 (2): 803-812. [Indonesian]

Aswani K, Sabu M, Smisha KP. 2013. Reproductive biology of *Etlingera elatior* (Jack.) R. M. SM. ornamental torch ginger. Intl J Plant, Anim Environ Sci 3 (4): 75-80.

Chudiwal AK, Jain DP, Somani RS. 2010. *Alpinia galanga* Wild.- an overview of phytopharmacological properties. Indian J Nat Prod Resour 1: 143-149.

Copeland LO, McDonald MB. 2001. Principles of Seed Science and Technology 4th ed. Kluwer Academic Publishers, Dordrecht.

Draper SR, Bass LN, Bould A, Gouling P, Hutin MC, Rennie WJ, Steiner AM, Tonkin JHB. 1985. Seed science and technology. International Seed Testing Association, Zurich.

Hanh NP, Binh NQ, Adhikara BS. 2014. Distribution of *Alpinia* (Zingiberaceae) and their use pattern in Vietnam. J Biodiv Endanger Sp 2 (2): 1-5.

Kristiaty E. Putri WU. 2008. Seed dormancy in kedawung (*Parkia javanica* (Lam.) Merr.): effect of scarification and chemicals stimulant application on seed germination. Buletin Kebun Raya Indonesia 11 (1): 16-22. [Indonesian]

Ma'mun. 2006. Characteristics of some essential oils of Zingiberaceae family in trade. Bul Litro 17 (2): 91-98. [Indonesian]

Muchtaridi M, Musfiroh I, Subarnas A, Rambia I, Suganda H, Nasrudin ME. 2014. Chemical composition and locomotors activity of essential oils from the rhizome, stem, and leaf of *Alpinia malaccensis* (Burm. F.) Roxb of Indonesian spices. J Appl Pharmaceut Sci 4 (1): 52-56.

Riyanto A, Yunilawati R, Nuraeni C. 2012. Methyl cinnamic isolation of essential oils from laja gowah (*Alpinia malaccensis* (Burm. F.) Roxb). Jurnal Kimia dan Kemasan 34 (2): 237-242. [Indonesian]

Rodrigo WDRJ, Nilanthi D, Senanayake SGJN. 2012. Germination potential of toddy palm (*Caryota urens*), cardamom (*Elettaria cardamomum*) and nutmeg (*Myristica fragrans*) seeds under in vivo conditions. Trop Agric Res Ext 15 (4): 1-4.

Sahoo S, Ghosh G, Nayak S. 2012. Evaluation of *in vitro* antioxidant activity of leaf extract of *Alpinia malaccensis.* J Med Pl Res 6 (23): 4032-4038.

Sahoo S, Parida R, Singh S, Padhy RN, Nayak S. 2014. Evaluation of yield, quality and antioxidant activity of essential oil of *in vitro* propagated *Kaempferia galanga* Linn. J Acute Dis 124-130.

Setyawan AD. 1999. Taxonomic status of the genus *Alpinia* based on characteristics of morphological, anatomical and chemical constituents of essential oils. BioSMART 1 (1): 31-40. [Indonesian]

Sharma UK, Pegu S. 2011. Ethnobotany of religious and supernatural beliefs of the missing tribes of Assam with special reference to the 'Dobur Uie'. J Ethnobiol Ethnomed 7 (16): 1-13.

Sunarmani, Nurmalinda, Amiarsi D. 2011. Consumer preferences of fresh cut flowers of *Alpinia*. J. Hort. 21 (1): 60-67. [Indonesian]

Utami NW, Witjaksono, Hoesen DSH. 2006. Seed germination and seedling growth of ramin (*Gonystylus bancanus* Miq.) on various growing media. Biodiversitas 7 (3): 264-268. [Indonesian]

Verma RK, Mishra G, Singh P, Jha KK, Khosa RL. 2011. *Alpinia galanga* – An important medicinal plant: a review. Der Pharmacia Sinica 2 (1): 142-154.

Yunira I. 2009. Reproductive success of *Alpinia* sp. and *Zingiber* sp.: Natural and Artificial Pollinations. [Hon. Thesis]. Faculty of Agriculture, Institut Pertanian Bogor, Bogor. [Indonesian]

Yuniarti N, Megawati, Leksono B. 2013. Pretreatment techniques and germination methods to maintain the seed viability of *Acacia crassicarpa* breeding. Jurnal Penelitian Kehutanan Wallacea 2 (2): 1-11. [Indonesian]