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Growth performance of *Bruguiera gymnorrhiza* derived from cut-propagule seedling

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Abstract. *Kusmana C, Hidayat T, Istomo, Rusdiana O. 2018. Growth performance of* Bruguiera gymnorrhiza *derived from cut-propagule seedling. Biodiversitas 19: 208-214.* The availability and existence of *Bruguiera gymnorrhiza* seed sources is getting decreased. This causes the generative reproduction of this plant is limited. The present research was carried out to study the growth performance of *B. gymnorrhiza* derived from vegetative cut-propagule seedling by employing different type of cut-propagule, the provision of root promoting hormones, and the provision of NPK fertilizer. The research was conducted at the Greenhouse for 6 months using a completely randomized design (CRD) 3x3x2 factorial with five replications. The results showed that *B. gymnorrhiza* could be propagated by cut-propagule. Seedlings growth which derived from complete propagule apparently had a better performance than those derived from cut-propagule. Roots derived from complete propagule and the bottom part of cut-propagule grow faster than the top part of cut-propagule. Two types of shoot such as shoot existing before planting which was derived from complete propagule and top part of cut-propagule; and shoots existing after planting called as adventitious shoot derived from the bottom part of cut-propagule has been formed. Unfortunately, new complete shoots derived from the bottom part of cut-propagule was not formed. Furthermore, there was no significant effect of root promoting hormone and NPK fertilizer induction on the shoot and root growth of *B. gymnorrhiza*.

Keywords: *Bruguiera gymnorrhiza*, cut-propagule, growth performance, mangrove propagation, seedling

INTRODUCTION

Indonesia is the country with the largest extent of mangrove forests in the world with a coastline of approximately 95,111 km in length and 17,504 islands (Kusmana 2014). Mangrove forest is one of the highly productive coastal ecosystems, which has multiple ecological functions especially for surrounding habitats and for the coastal communities (Woodroffe 1987; Rana et al. 2009; Metcalfe et al. 2011; Jachowski et al. 2013). The main coastal ecosystems in Indonesia are mangroves, coral reefs, seagrass-beds, algal beds, mudflats, estuaries, and beach vegetations as well as small islands with their typical shallow waters. Therefore, the mangrove forest is a very dynamic and highly productive ecosystem. According to Kathiresan (2012), mangrove forests provide an important role in the protection against coastal erosion, and environmental stabilization which also influence the socio-economic aspects of the surrounding communities.

Based on the latest information, the mangrove forest area in Indonesia reaches to 3.2 million hectares (Bakosurtanal 2009). Ministry of Forestry in the year of 2007 reported that potential area to be planted by mangrove (including mangrove forest area) is estimated at 7.8 million hectares (30.7% in good condition, 27.4% moderate-destroyed, 41.9% heavy-destroyed) (Hartini et al. 2010). The destruction of mangrove are caused by many factors, mainly by conversion the land to other uses (Kusmana 2014). Those mangrove depletion results in the diminishing abundance of propagules production, which is important for forest regeneration. In addition, the destruction of coastal area in Indonesia is very vast, therefore the needs of a large number of propagules becomes very important. Some efforts to enlarge the stock of propagules by planting mangrove in degraded area must be done. Furthermore, the management and rehabilitation of mangrove forest ecosystems must be comprehensively conducted for functional sustainability purposes.

Mangrove reforestation using seed or propagule planting is a conventional method to regenerate new tree mangroves individuals such as *Bruguiera gymnorrhiza,* which belongs to Rhizophoraceae family (Tomlinson 1986; Lin 1999; Kusmana et al. 2008). However, the mangrove reforestation using seeds is restricted due to the depletion of growing stock and post dispersal predation of seeds (Smith 1987; Smith et al. 1989; Robertson et al. 1990; McKee 1995; McGuinness 1997; Sousa and Mitchell 1999; Krauss and Allen 2003). Moreover, when mangrove planting does not coincide with the propagule peak season, the availability of seed sources and its range decreases, so that the natural generative reproduction are limited.

One solution to overcome the limited source of propagule is by cutting the propagules into two or three parts used for seedling material. Previous researchers using this method (Tanapeampool 1985; Ohnishi and Komiyama 1998; Mulyani et al. 1999) to grow seedlings from some viviparous mangrove species had been successfully reported. Therefore, the objective of this research was to analyze seedlings growth performance of *B. gymnorrhiza* derived from cut-propagule material.

MATERIALS AND METHODS

Study area and plant material preparation

The research was carried out at the Greenhouse of Faculty of Forestry, Bogor Agricultural University, Bogor, Indonesia for six months (in September 2015–February 2016). Mature propagules of *B. gymnorrhiza* were collected from the mother trees obtained from Pamanukan mangrove forest, Subang, Indonesia (20°18'–20°32' N and 86°41'–86° 48' E). The mixture of soil, compost, and sand with ratio 1:1:1 was used as planting media. Roasting method at 80 °C was used to sterilize culture media. The roasted planting media were incubated in the open area for one day before it was put into a polybag. Propagule were grown under shade treatment with paranet intensity of 70% and covered by a UV-transparent plastic lid.

Experimental design and treatment details

The experiment was arranged in 3x3x2 factorial in a completely randomized design (CRD). There were 3 factors of the treatment, each treatment consisted of 5 (five) replicates of propagules. Those treatments were: types of propagule (A0: complete propagule, A1: top part of cut-propagule, and A2: bottom part of cut-propagule); concentration of root promoting hormones (B0: 0 ppm, B1: 10,000 ppm, and B2: 15,000 ppm); and doses of NPK fertilizers (P0: 0 g and P1: 5 g). Those root promoting hormones contained indole-3-butyric acid (IBA) and napthalene acetic acid (NAA). Furthermore, there were 18 combinations of treatment with 90 cut-propagules as the experiment units.

Three types of propagule (Figure 1) were dipped in each of the root promoting hormones with different concentrations for 5 (five) minutes and planted in each polybag. NPK fertilizers were applied to stimulate the growth of propagules and applied twice during the study period, i.e in the early planting and 4 months after planting. The weeding, watering, and spraying fungicides to control the diseases was performed as part of seedlings maintenance (Dewi et al. 2017).

On the end of the study, each seedling was harvested to determine its biomass. Top biomass was obtained from the total weight of leaves and stems, while root biomass was obtained from the root seedlings. Wet weights were measured to obtained the fresh weight of biomass. Some samples of those seedlings was then dried for 48 hours at 80 °C to obtain constant dry weight or biomass (Wiryono and Siahaan 2013).

Data collection and analysis

Seven growth variables were measured including seedlings height, stem diameter, number of leaves, percentage of seedling root and total root number, total wet weight (TWW) of seedling, total dry weight (TDW) of seedling, and top root ratio (T/R ratio).

Data were analyzed using analysis of variance (ANOVA) with 5% degree to identify the difference among treatments. Duncan's Multiple Range Test (DMRT) was conducted when there was a significant difference among observed parameters (Mattjik and Sumertajaya 2006). All data was analysed using SAS program version 9.1.

Figure 1. Types of propagule cuttings (A0: complete propagule; A1: top part of cut-propagule, and A2: bottom part of cut-propagule)

RESULTS AND DISCUSSION

Results

The recapitulation of the total variance for cut-propagule treated with NPK fertilizer and root promoting hormones (Table 1) showed that the combination of those three factors affected only on the number of leaves. The DMRT results (Table 2) showed that the combination of complete propagule, root promoting hormones 10,000 ppm, and NPK fertilizers 5 g (A0B1P1) produced the highest number of leaves (22 pieces).

The combination of two factors significantly affected the T/R ratio parameters (Table 1). The DMRT results of these combination (Table 3) showed that the best combination for producing the largest T/R ratio (0.18) was combination of A0 and B1.

Single treatment of cut-propagule significantly affected all measured growth parameters (Table 1). The recapitulation of the DMRT results of cut-propagule (Table 4) showed that the seedling growth of *B. gymnorrhiza* treated with complete propagule (A0) apparently was greater than the other treatments. Top part of cut-propagule (A1) treatment resulted in the least number of roots (0.30 pieces), TWW (11.24 g), and TDW (5.39 g). Whereas the cut-propagules treated by bottom part of cut-propagule (A2) not yet success raising up the shoot until the end of study.

Single treatment of NPK fertilizer also significantly influenced on the number of *B. gymnorrhiza* seedling roots (Table 1). The DMRT results of these treatment (Table 5) showed that treatment of P1 produced more root number (3.42 pieces) compared to the treatment of P0 (control)

(2.69 pieces). There were two types of *B. gymnorrhiza* seedlings root (Figure 2) i.e rooting occurred in the uncut propagule (A0 and A2), and rooting growth emerged from a wound tissue after cutting (A1). Furthermore, the root percentage of *B. gymnorrhiza* seedlings (Figure 3) showed that the root percentage of seedlings derived from A0 and A2 was 100, while those originating from A1 varied, where the A1B0P1 treatment produced the highest percentage of root seedlings (60% of root seedlings).

Table 1. The results of ANOVA of *B. gymnorrhiza* seedlings growth derived from cut-propagule treated with root promoting hormones and NPK fertilizer

Parameters	Treatment						
	Types of propagule (A)	Root promoting hormone (B)	Fertilizers (P)	A x B	A x P	B x P	A x B x P
Seedlings height	<0.0001*	0.7865	0.8373	0.9854	0.5966	0.2475	0.2454
Stem diameter	<0.0001*	0.7023	0.2089	0.5638	0.6674	0.5146	0.3518
Number of leaves	<0.0001*	0.1962	0.3473	0.1250	0.6951	0.0439*	0.0258*
Number of roots	<0.0001*	0.3893	0.0317*	0.8626	0.6920	0.1891	0.5505
TWW	<0.0001*	0.6407	0.4556	0.1165	0.7229	0.2753	0.1359
TDW	<0.0001*	0.1868	0.9956	0.6024	0.5102	0.3013	0.2173
T/R ratio	<0.0001*	0.0009*	0.4679	0.0006*	0.8365	0.4110	0.4519

Note: *: significantly different on test-level 5%

Table 2. The DMRT results of the cut-propagule and the application of root promoting hormones and NPK fertilizers combination on the number of *B. gymnorrhiza* seedlings leaves

Types of propagule	Root promoting hormones	NPK fertilizers	
		0 g (P0)	(5 g) (P1)
Complete propagule (A0)	0 ppm (B0)	13.60±2.317 bc	8.20±3.667 c
	10,000 ppm (B1)	11.80±5.237 bc	22.00±1.704 a
	15,000 ppm (B2)	13.80±1.561 bc	14.40±2.715 b
Top part of cut-propagule (A1)	0 ppm (B0)	0.40±0.398 d	1.20±0.801 d
	10,000 ppm (B1)	0.00±0.000 d	1.20±1.199 d
	15,000 ppm (B2)	0.00±0.000 d	0.00±0.000 d
Bottom part of cut-propagule (A2)	0 ppm (B0)	0.00±0.000 d	0.00±0.000 d
	10,000 ppm (B1)	0.00±0.000 d	0.00±0.000 d
	15,000 ppm (B2)	0.00±0.000 d	0.00±0.000 d

Note: Numbers followed by the sama letters and columns are not significantly different

Table 3. The DMRT results of the cut-propagule and root promoting hormones treatment on the T/R ratio of *B. gymnorrhiza* seedlings

Types of Propagule	Root promoting hormones		
	0 ppm (B0)	10,000 ppm (B1)	15,000 (B2)
Complete propagule (A0)	0.16 a	0.18 a	0.08 b
Top part of cut-propagule (A1)	0.01 c	0.01 c	0.00 c
Bottom part of cut-propagule (A2)	0.00 c	0.00 c	0.00 c

Note: Numbers followed by the sama letters and columns are not significantly different

Table 4. The recapitulation of the DMRT results of cut-propagule on all measured growth parameters of *B. gymnorrhiza* seedlings

Types of propagule	Seedling height	Stem diameter	Number of roots	Total wet weight	Total dry weight
Complete propagule (A0)	9.30 a	0.059 a	6.30 a	35.77 a	11.80 a
Top part of cut-propagule (A1)	0.67 b	0.007 b	0.30 c	11.24 c	5.39 c
Bottom part of cut-propagule (A2)	0.00 b	0.000 b	2.57 b	15.85 b	7.39 b

Note: Numbers follewed by the sama letters and columns are not significantly different

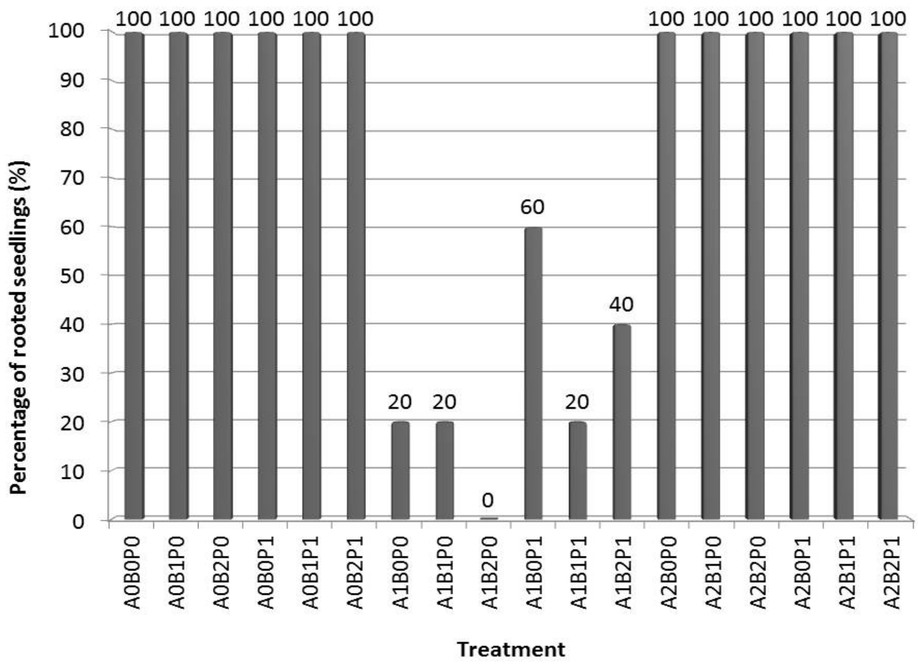

Figure 3. The root percentage of *B. gymnorrhiza* seedlings treated on the combination of three different factors i.e types of propagule (A0: complete propagule, A1: top part of cut-propagule, and A2: bottom part of cut-propagule); concentration of root promoting hormones (B0: 0 ppm, B1: 10,000 ppm, and B2: 15,000 ppm); and doses of NPK fertilizers (P0: 0 g and P1: 5 g)

Table 5. The DMRT results of NPK fertilizer on the number of *B. gymnorrhiza* seedling roots

The concentration of NPK fertilizers	Number of roots
0 g (P0)	2.69 b
5 g (P1)	3.42 a

Note: Numbers followed by the sama letters and columns are not significantly different

A0 **A1** **A2**

Figure 2. Types of *B. gymnorrhiza* seedlings root (A0: complete propagule; A1: top part of cut-propagule, and A2: bottom part of cut-propagule)

Discussion

Cut-propagule system is different from general cutting systems, because the material for propagation was derived from the propagule, therefore the technique can only be carried out when the parent trees are flowering and fruiting. Propagule itself is a food storage organ and other reserve materials on some mangrove trees, one of them is *B. gymnorrhiza* (Tomlinson 1986; Lin 1999; Kusmana et al. 2008).

Height of two months *B. gymnorrhiza* seedlings in Hongkong was ranging from 10–11 cm, and its stem diameter was about 0.03 cm (Zhang et al. 2007), height of four months seedling in Japan was ranging from 12.5 ± 1.8 cm (Takemura et al. 2000). The current study revealed that growth of two and four months seedlings had relatively equal to the height and diameter seedlings derived from complete propagule. In addition, it was revealed that ratio of seedlings height, stem diameter, number of leaves, T/R ratio for six months observation was lower than the previous research done by Krauss and Allen (2003) in Hawaii, USA.

Growth of *B. gymnorrhiza* seedling derived from complete propagule tends to be better than the seedlings derived from cut-propagule. This might happen because the complete propagule has more food reserves than the cut-propagule. The size of propagule of *B. gymnorrhiza* was shorter than propagule of some other viviparous mangrove species (Tomlinson 1986; Lin 1999; Kusmana et al. 2008). According to Ohnishi and Komiyama (1998), the longer size of propagule (e.g. *Rhizophora mucronata*) will affect the higher percentage of successful cutting method. Less food reserves and cut-propagule on top and bottom part, will cause the longer growth of shoots because they must follow several growth stages to be developed.

The shoots of complete propagule and top part of cut-propagule grew earlier before propagule was planted, while bottom part of cut-propagule did not have shoot before planting. According to Ohnishi and Komiyama (1998), on *Kandelia candel* cut-propagule, shoot development on the complete propagule and top part of cut-propagule grow faster than those on bottom part of cut-propagule. On complete and top part of cut-propagule, the shoot might contain the auxin hormone, which stimulate root growth (Sangtiean 1993). Auxin treatment was necessary for direct rooting. When the cuttings were treated with auxin (NAA or IBA), roots usually developed outside resin ducts, in vertical rows along the length of the propagule. Bottom part of cut-propagule until the end of the study produced uncomplete shoot. This was presumably because the stage of shoot development occuring in the bottom part of cut-propagule was still reaching the differentiation stage. In the vegetative propagation through plant cutting, root formation is very important to support the plant growth. In addition, the shoot formation and growth will take place after roots are induced properly (Hartmann et al. 1990). In this study, it was found that roots grow faster than shoots. This result was in line with the study conducted by Ohnishi and Komiyama (1998) in cut-propagule of *K.* candel. In addition, the roots derived from bottom part of cut-propagule and complete propagule grew faster and more nomerous than the top part of cut-propagule. The data are consistent with the study of Ohnishi and Komiyama (1998) which stated that propagule cutting of *K. candel*, the bottom part of cut-propagule roots grow faster. It also occurs in cut-propagule of *R. mucronata* (Mulyani et al. 1999).

Another factor affecting the plant growth is the influence of root promoting hormones. However, the requirement in terms of type and concentration of the root promoting hormones in each type of plant may be different. Plant hormones which includes auxins are endogenous organic compounds in plants. Auxins are synthesized in the stem and root apices and transported through the plant axis. Treatment of exogenous hormone was done when the endogenous hormone levels in plants were low (Hartmann et al. 2002). As presented in Table 1, treatment of root promoting hormones, have no significant effect on root formation. This might be due to the present of endogenous hormones and auxin in seedling *B. gymnorrhiza* was sufficient, thus exogenous hormones are not required. According to Henrique et al. (2006), hormones such as IBA and NAA is more effective than the indole-3-acetic acid (IAA) in stimulating the root formation of plant cutting. Although it does not affect the root growth, treatment of root promoting hormones was effective to improve the T/R ratio. In addition, it have great effects on plant growth and development, especially leave growth (Table 2). In line with Tchinida et al. (2013), the treatment of IBA and NAA on *Ricinodendron heudelotti* cuttings influenced the growth of leaves, but there were no significantly differences in each level of treatment.

The function of root is to collect water and mineral nutrients present in the soil. According to Alongi (2011), the general structure of mangrove roots is similar with other most vascular plants. However, the availability of specific nutrients dictates organismal growth by controlling and limiting activation of cellular and metabolic pathways necessary for their progress. Nitrogen (N), Phosphorus (P), and Potassium (K) availability is always limited to mangrove species. Table 1 shows that the presence of NPK fertilizer influenced root growth. The combination of propagule with root promoting hormones influenced the growth of leaves. This was in line with the study of Alongi (2011) who found that the total number of leaves and roots *B. gymnorrhiza* increased with the application of N and P fertilizers. According to Naidoo (2009), the difference between higher growth rate and productivity of mangrove are due to the nutrient availability is limited. Studies revealed that seedlings treated with NPK fertilizers grew higher and had a bigger stem diameter than the seedlings without NPK fertilizers treatment. According to research in Belize, Central America, N deficiency causes a small diameter and P deficiency causes *R. mangle* plants stunted (Feller 1995; Feller et al. 2002; Lovelock et al. 2006). Moreover K deficiency causes plants susceptible to pests and diseases. N, P, and K is an essential nutrient for plants, so that these three elements must be present in fertilization (Rauf et al. 2000).

Indicator that commonly used to determine the growth of seedlings is biomass. On the other hand, wet weight is used as parameter to determine plant water requirements. According to Putri and Nurhasybi (2010), biomass reflects the accumulation of organic compounds synthesized by plants from inorganic compounds (nutrients, water, and carbohydrates). Based on Table 4, total wet weight was proportional to the total dry weight (biomass) of seedlings. The better or efficient plant physiological processes, the dry weight of the plant will be greater. This means that the plants are able to absorb available nutrients used in the growth process.

The T/R ratio will be obtained from dry weight of shoots and roots of seedlings. Based on the DMRT results, the highest mean value of T/R ratio of seedlings (0.19) were derived from complete propagule combined with root promoting hormones at concentration of 10,000 ppm and NPK fertilizer doses at 5 g. These results indicated that *B. gymnorrhiza* seedlings roots grow better than the shoots of the seedlings. It might occur because *B. gymnorrhiza* is very adaptable to extreme conditions. According to Ball (1988) and Lopez-Hoffman et al. (2007), generally most of mangroves biomass is allocated in the roots than shoots, because of the high salinity. The existence of a larger allocation to the roots is one of the important survival strategy to find water and nutrients in high salinity conditions (Boogaard et al. 1996) for leaf development, photosynthesis, and growth (Lopez-Hoffman et al. 2007). Ye et al. (2003) also reported that, species whose allocated the more biomass in roots indicated that the species is more tolerant in inundation conditions. The T/R ratio itself varies depending on the changes of internal and external conditions of plants. This changes are an adaptation of the character of many plant species (Koler and Kozinka 1992).

As conclusion*, B. gymnorrhiza* can be propagated by cut-propagule. Seedling derived from complete propagule apparently grew better than those derived from cut-propagule. Rooting systems induced by *B. gymnorrhiza* seedlings consists of two type roots i.e roots that grow on the cut-propagule and roots that grow on the propagule without cutting treatment. The shoots of *B. gymnorrhiza* seedlings had two type of shoots which was existed before planted and sprouted after being planted. Until the end of the research, new shoots on the bottom propagule was not been formed, yet. The initiation of primordial shoot was not observed in the research period.

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