

In rice transplanting practiced by local farmers, the seedlings are directly pulled off the growing substrate in clumps. Subsequently, it causes damage to roots of seedling. More damage is commonly observed on seedlings grown on compact and/or relatively dry substrates. Additional damage can occur during separating seedlings to be planted from its clump during transplanting process. More damage is observed in older seedlings since their roots have heavily intertwined between one to the others at the nursery. Therefore, age of seedling at time of transplanting and physical characteristic of substrate should be carefully considered in producing good quality rice seedlings.

Bilderback et al. (2005) noticed that important physical characteristic of growing substrate was having adequate pore spaces for allowing water drainage and creating well-aerated root zone. In addition, Bhat et al. (2014) suggested that a good growing substrate should also contain nutrients to sustain initial plant growth.

Objective of this study is to develop reliable and affordable practices for producing high quality rice seedlings for transplanting to paddy field at riparian wetlands in Indonesia. The study was focused on comparing among different nursery substrates and evaluates appropriate age of the rice seedlings at time of transplanting.

Results and Discussion

Dynamic changes in seedling height

Height of rice seedlings was a very important characteristic for rice cultivation at riparian wetlands. Taller seedling is preferred for overcoming variation in depth of floodwater at time of transplanting to paddy field. Results of this study indicated that significant difference in height amongst seedlings grown on different substrates was observed on day 5, 7, 9, and 21 (Table 1). There was a dynamic weekly change in height amongst seedlings grown on different substrates. Seedlings grown on DBUv substrate exhibited slower growth during the first week but steadily grew to become the tallest seedling at the end of the third-week period. Seedlings sown on mixed substrate of soil and *U. vulgaris* biomass plus biochar (SUv+B substrate) exhibited slower early growth (at the first week) than seedlings grown on the other two substrates. During early second week, seedlings sown on soil amended with biochar (S+B substrate) grew significantly faster as indicated by taller seedlings than those sown on the other two substrates.

The source of nutrients for supporting seedling growth shifted from endosperm to nursery substrate as seedling growth progressed. Aslam et al. (2015) suggested that nutrients contained in the substrate, especially nitrogen, affected rice seedling growth as indicated by increment of seedling height. In case of DBUv substrate, as decomposition of *U. vulgaris* biomass progressed, by which more nutrients were released and became available for supporting seedling growth. Increase in nutrient availability during the third-week period contributed to faster growth of rice seedlings grown on DBUv substrate.

Addition of biochar as amendment to SUv+B or S+B substrate at rate of 1 g/L and 0.25 g/L, respectively, enhanced seedling growth during the first week but did not sustain beyond the second week period (Table 1). Glase et al. (2002) reported that biochar was capable of increasing

germination and vegetative growth. In this study; however, even if biochar has ability to increase solubility of nutrients, rice seedling growth relied more directly on adequate concentration of the essential nutrients. High soil moisture increased solubility of nutrients with or without biochar.

In this study, we realized through direct observation that fine-sized biochar is also soluble in the water. Therefore, significant portion of applied biochar in floating seedbed system was dissolved in water, since the growing substrate was water-saturated and in direct contact with water underneath the raft in this floating system. Amount of applied biochar was gradually depleted at the third week period. Therefore, fine-sized biochar is not suitable as an amendment for nursery substrate in floating seedbed system.

No effect of substrate on number of seedling leaves

In this research, the average number of leaves was not significantly different amongst rice seedlings grown on three types of substrates (Fig 1). It seems that number of leaves at seedling stage in rice is predominantly driven by genetic rather than environmental factors. Seedlings grown on DBUv substrate developed their third and fourth leaves, insignificantly earlier than those grown on the other two substrates. However, this could be an indication that differences in number of leaves might become significant during later stage of vegetative growth.

Lakitan et al. (2018) found that number of leaves at late vegetative growth stage was higher in rice plants grown on biochar-treated soil. Kartika et al. (2018a) also reported that biochar affected leaf area ratio. Moreover, there were significantly higher leaf area index in rice plants at edge rows compared to those at internal rows in 4:1 Jajar-Legowo planting system (Kartika et al., 2018b). These findings justified that non-genetic factors influenced leaf development at later vegetative growth stage.

Less broken roots in porous substrate

The root length was measured after the roots were pulled out of the substrate and washed. Pulling roots of seedling out of the substrate is done for simulating a common practice by local farmers during transplanting process. Root damage was higher in high density substrate (or low substrate porosity). Result of this study indicated that root length of the seedlings was significantly affected by substrates (Fig 2).

During the first week, roots of seedling grown on S+B substrate were longer than those grown on SUv+B and DBUv substrates. However, root length of all seedlings was not significantly different during the second week. Interestingly, during the third week, roots of seedlings grown on DBUv substrate were longer than those grown on SUv+B and S+B substrates. Shorter roots of the seedlings grown on SUv+B and S+B substrates were mainly due to significant damages (broken) of the roots at time they were pulled out of the substrates.

Quality of rice seedlings was not solely evaluated based on physical appearance of above-ground organs but also depended on level of damages to the root at time of the seedlings were pulled out of their substrate during transplantation. The substrate density played a significant

Table 1. Significant differences in height of rice seedling sown on different growing substrates on selected dates during the first 3-week period.

Substrate	Age of seedling (day)			
	5	7	9	21
DBUv	2.81 ± 0.345 a	5.00 ± 0.444 a	8.38 ± 0.997 a	25.07 ± 1.893 a
SUv+B	1.96 ± 0.391 b	4.51 ± 0.656 a	8.48 ± 0.476 a	24.57 ± 2.592 ab
S+B	2.93 ± 0.350 a	6.87 ± 0.335 b	10.64 ± 0.445 b	23.04 ± 1.151 b

Growing substrate did not significantly affect seedling height on other observed days during the first 3-week period. Data are presented as means ± standard deviation. The means followed by similar letter within each column were not significantly different at LSD_{0.05}.

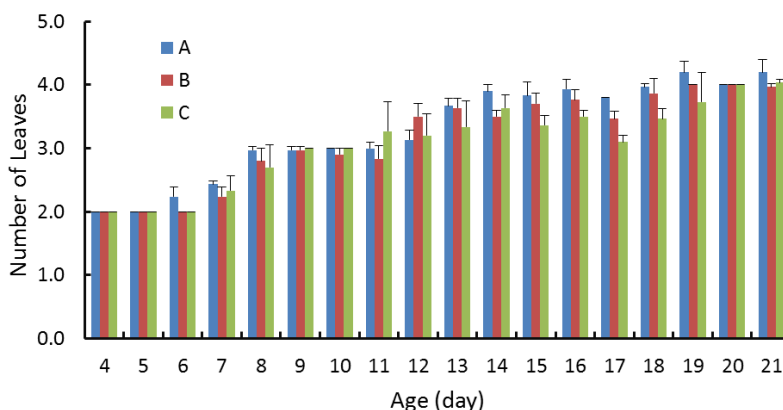


Fig 1. Number of leaves in rice seedlings grown on different growing substrate using floating seedbed during the first 3-week period. A = DBUv substrate; B = SUv+B substrate, and C = S+B substrate.

Table 2. Leaf chlorophyll concentration index (CCI) of rice seedling grown on floating seedbed using different nursery substrates.

Substrate	Age (day)				
	7	14	17	21	24
DBUv	25.66 ± 2.67 a	27.23 ± 2.81 a	22.40 ± 2.53 a	23.83 ± 1.20 a	21.46 ± 1.50 a
SUv+B	24.83 ± 0.83 a	24.76 ± 0.20 ab	22.73 ± 1.60 a	21.73 ± 1.00 a	20.83 ± 0.62 a
S+B	26.80 ± 2.20 a	21.96 ± 1.55 b	20.96 ± 0.76 a	21.16 ± 1.55 a	19.36 ± 1.54 a

Data are presented as means ± standard deviation. The means followed by similar letter within each column were not significantly different at LSD_{0.05}.

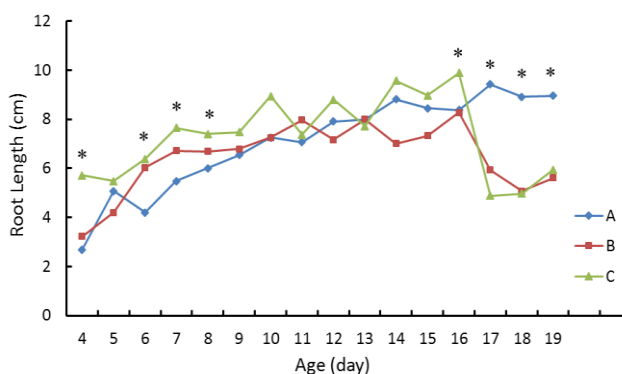


Fig 2. Differences in average length of intact roots after being pulled out of growing substrate. Asterisk indicated that length of intact root was significantly affected by types of substrate used. A = DBUv substrate; B = SUv+B substrate, and C = S+B substrate.

Table 3. Specific substrate weight, seedling density, shoot/root ratio, and total dry matter of rice seedling grown on floating seedbed using different growing substrate.

Substrate	Specific substrate weight (kg.m ⁻²)	Seedling density (cm ⁻²)	Shoot/root ratio	Total dry matter (g)
DBUv	19.36 ± 4.10 a	7.84 ± 3.93 a	0.41 ± 0.06 a	13.61 ± 1.57 a
SUv+B	31.40 ± 1.66 b	7.61 ± 2.33 a	0.37 ± 0.16 a	13.95 ± 0.84 a
S+B	36.16 ± 1.76 c	8.03 ± 1.58 a	0.31 ± 0.04 a	13.81 ± 0.89 a

Data are presented as means ± standard deviation. The means followed by similar letter within each column were not significantly different at LSD_{0.05}.

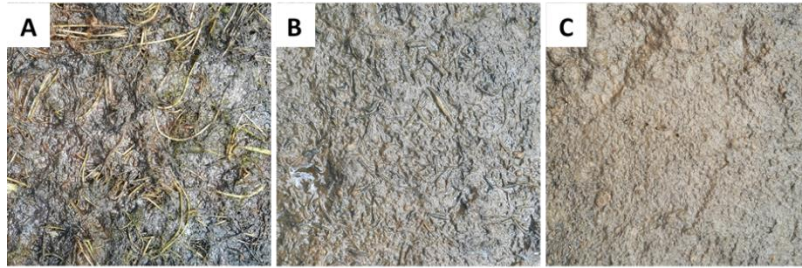


Fig 3. Substrates used in rice seedling preparation on floating seedbed, consisted of (A) decomposing *Utricularia vulgaris* biomass without soil (DBUv substrate); (B) mixed substrate of *U. vulgaris* biomass and soil plus wood biochar powder (SUv+B substrate); and (C) soil plus biochar (S+B substrate).

contribution to root damage in rice seedlings. Length of recovered intact root in rice seedling grown on the most porous substrate of DBUv was significantly higher than those planted on the more compacted SUv+B and S+B substrates. Wilson et al. (2001) reported that composition of growing substrate with different porosity level greatly influenced the quality of seedlings. Porous substrate of DBUv allows root to grow with lower resistance compared to more compact substrates. Addition of biochar into substrate was meant to increase its porosity (Glaser et al., 2002). Bruun et al. (2014) stated that biochar facilitated root development by providing a wider or additional pore space. Furthermore, Abiven et al. (2015) reported that biochar increased root area, root depth, and smooth root development. Similarly, Yang et al. (2015) also reported that biochar applications improved root properties, including length, volume, average diameter, number of root tips and root forks. Biochar-amended substrates (SUv+B and S+B) were successful in allowing roots to protrude through the substrate, yet this substrate still caused significant damage to roots at time of the seedlings were pulled out of the substrate during transplanting process. Since broken part of the roots mostly occurred at younger tissues near root tips, then length of recovered intact roots in SUv+B and S+B substrates were shorter than those in DBUv substrate (Fig 2).

The leaf chlorophyll was not affected by different substrates

Leaf chlorophyll concentration index (CCI) is commonly used as indicator for plant health and leaf nitrogen content. CCI in rice seedlings up to 21 days old was not significantly different regardless of substrates (Table 2). The CCI of seedlings grown on DBUv substrate were consistently higher (but not statistically different) than those grown on SUv+B and S+B substrates, except at 14 DAS, when CCI of seedlings grown on DBUv substrate was significantly higher than CCI of seedlings grown on S+B substrate. Brunetto et al. (2012) found that CCI value declined as plant got older unless nitrogen fertilizer was applied. Although, it was not statistically significant, there was a trend that CCI gradually decreased as rice seedling grew to 3-week-old. The CCI has been used as reliable proxy for leaf nitrogen and chlorophyll content (Monostori et al., 2016; Yang et al., 2018; Huang et al., 2017). Gradual decrease of CCI indicated that nitrogen availability in nursery substrates has slowly depleted as rice seedlings grew approaching end of the third week period.

Substrate effect on seedling morphological characteristics

Porosity of a substrate can be estimated by measuring specific weight of the substrate. Porous substrate has low specific weight and, reversely, compact substrate has high specific weight. DBUv substrate was the most porous substrate among the three substrates used in this study. Measurements on specific substrate weight (Table 3) confirmed that porosity of substrates used in this study was significantly different.

Specific weight of DBUv substrate was significantly lower than those of SUv+B and S+B substrates. Specific weight of SUv+B substrate was also significantly lower than that of S+B substrate. Therefore, amongst the three substrates used, the most porous was DBUv substrate, while the most compact was S+B substrate, and the mixed SUv+B substrate was in between the previous two substrates. Despite of differences in their porosity, nursery substrate did not significantly affect seedling density, shoot/root ratio, and total dry matter (Table 3).

In this study, similarity in seedling density regardless of substrate porosity differences was associated with similar viability rate and sowing density of seeds. Similar shoot/root ratio indicated that during the first 3-week-period, rice seedlings planted on different nursery substrates allocated similar portion of assimilates between shoot and roots. Despite differences observed on seedling height and root length, total dry weight of the seedlings sown on different substrates were not significantly different during the first 3-week period on floating seedbeds.

Materials and Methods

Plant materials, raft construction, and substrate preparation

Ciherang rice variety was used in this experiment. Ciherang is one of popular rice variety grown by local farmers in Indonesia and also commonly grown at riparian wetlands in South Sumatra. Ciherang is not a hybrid variety; therefore, local farmers can reproduce and use seeds from their previous harvest to be planted at the next growing season. The experiment on use of floating seedbed for rice seedling production was conducted in 2017 at an off-campus research facilities in Jakabaring (104°46'44" E; 3°01'35" S), Palembang, Indonesia.

Floating rafts and nursery substrates were prepared 5 days earlier before seed sowing. The rafts were made of biomaterial consisted of leaf blades and spongy base of local

swamp sedge, identified as *Scleria poaeformis* RETZ. Average length of the leaf blade was about 1.5 meters. These long leaves were stitched together to form suitable raft as floating seedbed for rice seedling production. The raft was tested for its stability and carrying capacity (buoyant force) in outdoor experimental pools. Test procedures were conducted according to Siaga et al. (2018). Carrying capacity of the raft was varied, depending on thickness of the raft. In our study, the double-layered raft had ability to withstand loads up to 32.56 kg.m⁻².

Three different substrates were evaluated in this research. The substrates were: (1) made of decomposed biomass of local aquatic plants (*Utricularia vulgaris* L.), denoted as DBUv substrate; (2) mixture of soil and decomposed *U. vulgaris* biomass at 1:1 v/v plus biochar at rate of 0.25 g/L of the mixed media, denoted as SUV+B substrate; and (3) soil amended with biochar at rate of 1 g/L, denoted as S+B substrate (Fig 3). Biochar used was wood biochar, crushed from chunky to fine powder, passed through 1 mm screen.

Procedures of rice seed sowing

Frames made of polyvinyl chloride (PVC) pipes were placed on the raft for separating among the three different substrates and for holding the substrates from falling off the raft. Then, perforated water-penetrable plastic layer was placed within each frame prior to placing the nursery substrates. The most crucial part was the bottom part of the substrate which should have been in direct contact with water surface for keeping the substrate moisture content at optimum level for supporting rice seedling growth. Based on specific weight of each substrate, thickness of the substrate varied from 1.5 to 2.0 cm to set up the required condition, i.e. bottom part of the substrate in direct contact with water surface. Upper surface of growing substrates was leveled before seeds were sown.

Seeds were soaked in water for separating and excluding low quality seeds. Then, the good quality seeds were kept for two days in multi-layer wet clothes as a hydro-priming treatment. At end of the two-day hydro-priming, most of seeds were germinated and their radicles protruded seat coat. The primed seeds were evenly dispersed on leveled nursery substrate. Sowing density was 3 kg rice seeds per square meter. After the seeds were sown, the rafts were covered with white perforated plastic sheets for three days to protect seeds from wild bird attack.

Measurement of chlorophyll concentration index (CCI)

CCI was measured on healthy leaves at 7, 14, 17, 21, and 24 DAS using a chlorophyll meter (Konica Minolta SPAD-502 Plus). Measurements were taken at around 9.00 am when the leaves were at turgid condition and all morning dews on leaf surface had fully evaporated. Measurement was cancelled on rainy days. Data were recorded based on average of three different leaves for representing each treatment in each block.

Experimental design and statistical analysis

Experimental design used was Randomized Block Design. Three outdoor experimental pools were used, each assigned as a block. Treatments consisted of three different nursery

substrates, i.e. DBUv, SUV+B, and S+B substrates. Each of the substrate was randomly placed on one of three rafts within each experimental pool. In total, nine rafts made of swamp sedge *Scleria poaeformis* were used for the three treatments and three blocks. Data on growth parameters and CCI of the rice seedlings were collected daily from 4 to 21 DAS.

The analysis of variance was carried out using the SAS application for testing significant effect of treatments (the nursery substrates) on selected primary and secondary growth parameters of rice seedlings sown on floating seedbeds. If the treatments significantly affected any of growth parameters, further test was performed using the Least Significant Differences test at $p < 0.05$ (LSD_{0.05}) for specifying differences amongst treatments.

Conclusion

Considering quality of seedlings produced and availability of required biomaterials, use of DBUv substrate was the most appropriate practice in floating rice seedling production in riparian wetlands. On the other hand, application of fine sized biochar to water-saturated nursery substrate in floating seedbed system should not be employed, since the biochar was gradually dissolved in water.

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