

Photosynthesis Rate, Sugar and Starch Content of Sago Leaves (*Metroxylon* sp.) at Different Preparation Methods of Sago Seedlings

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Abstract

Photosynthesis rate plays a significant role in plant growth and development. A study was conducted to determine the best methods to grow sago planting materials from sago suckers. Photosynthesis rate, stomatal density, intercellular CO_2 concentration, stomatal conductance, transpiration, sugar and starch content of the sago leaves from different methods of planting was determined. The field experiment was carried out at the Cikabayan Experimental Station from January 2020 to February 2021; the leaf morphology was conducted at the Microtechnical Laboratory and Testing Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia. The treatments for the sago seedlings were immersion of the bare-rooted seedlings in water, immersion in water with media mix in polybags, transplanted to media mix in polybags, and immersion of bare-rooted seedlings for 4 weeks in water followed by transplanting to media mix in polybags. The study was arranged using a single factor a completely randomized block design, and quantitative data was analyzed using Microsoft Excel 2013 and the SAS. Stomatal density and photosynthetic rate were not significantly different between treatments. In contrast, the sugar content of immersed seedling without polybags for one month followed by transplanting to polybag had the best growth compared to those from other treatments, both at the nursery phase and post-transplanting phase. All planting methods resulted in good quality planting materials. At the nursery phase, sago seedlings immersed in water method grew the best; at the post-transplanting phases seedlings immersed bare-rooted for one month followed by transplanting to polybags grew better than those with other treatments

Keywords: photosynthesis, sago, seeding, starch, sucker, sugar

Introduction

Indonesia's population in 2020 will reach more than 270 million people (BPS, 2021). The increase in population in Indonesia demands a stable food security. Sago palm is a very rich staple food that is commonly found and consumed in the eastern parts of Indonesia. Mature sago palms are very productive and starch rich staple. Sago palm has the potential to provide substantially to food security in Indonesia.

Indonesia is a country with the largest sago area in the world, i.e. 5.5 million ha out of 6.5 million ha of sago in the world (Djoefrie et al., 2014). Sago are carbohydrate-producing tree with high productivity and can be used as food and non-food raw materials. According to Ayulia et al. (2021) sago 'Beremban' in Kepulauan Meranti Regency, Riau, can produce dry starch of 81.65-318.64 kg per tree, sago 'Sangka' 82.12-232.51 kg per tree, and sago 'Meranti' 105.01-174.49 kg per tree. Pratama et al. (2018) stated that sago plants in Mioko Village, Central Mimika District, Mimika Regency, Papua Province, had a potential to produce dry starch of 143.87-402.09 kg per ha, or productivity of 27 tons per ha per year.

Sago starch has various uses including basic foods, food industry raw materials, pharmaceutical industry, for production of ethanol and liquid sugar (Rahman et al., 2021). The development of sago needs to be followed by efforts to develop sago and forest management that fit naturally into sago plantations. The development requires good quality seedlings with continuous availability. Sago can be propagated vegetatively using sago saplings (sucker), and generatively using seeds. Prior to planting, sago saplings can be immersed directly in a flowing pond (raft system) or planted in polybags as sago adapt well to water-saturated environment. Papilaya (2009) reported that sago saplings can be soaked directly in water or small rivers or flowing ponds prior to planting

to the field, whereas according to Bintoro et al. (2010) sago seedlings can be kept on rafts in the canals for up to three months prior to planting in the field. Sago seedlings kept on rafts result in a high seedling survival of 80% (Jong, 2007).

Plant growth is controlled by many physiological processes, one of which is the photosynthesis. The photosynthesis rate is a key that has a significant role in the sustainable growth and development of sago plants during the nursery phase and the post-transplant phase. The energy obtained from photosynthesis is essential for plant growth, and energy availability significantly affects plant's survival.

A study on the photosynthesis rate, the intercellular concentration of CO_2 , stomatal conductance, transpiration, sugar and starch content of sago leaves was conducted using several planting methods of sago saplings, and the best method to obtain the highest survival after being transplanting in the field was determined.

Materials and Methods

The nursery and the post-transplant study was conducted from January 2020 to February 2021 at the Cikabayan Experimental field of IPB University, Darmaga, Bogor. The leaf identification study was conducted at the Microtechnical Laboratory and Testing Laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. Several methods of sago saplings planting were studied:

- 1) Sago saplings were immersed in water bare-rooted on a raft in a tarpaulin pond for three months (P1).
- 2) Sago saplings were planted in media mix consisting

of manure and husk charcoal in polybags, then immersed in water for three months (P2).

- 3) Sago saplings were planted in media mix consisting of manure and husk charcoal in polybags, without water immersion (P3).
- 4) Sago saplings were immersed bare-rooted in a water pool for one month, then planted in media mix consisting of manure and husk charcoal in polybags, followed by soaking in the water pool for two months (P4).

In all water immersion treatment, water level is kept 10-cm from the base of the seedlings.

Forty-eight sago saplings that have been sowed for three months were grown in the nursery, and 20 were transferred to the field. Observations and measurements were made on three plants for each treatment, both in the nursery phase and the post-transplant phase. The samples for measurement was sago saplings that have fully expanded leaves, with leaf age of 37-45 days after the leaves emerged (Figure 1 and 2), according to the method and criteria by Yamamoto et al. (2006). Measurements were conducted on the photosynthesis rate, intercellular concentration of CO_2 , stomatal conductance, transpiration, sugar and starch content in leaves from the plants in the nursery phase and the post-transplant phase.

Data Collection

Measurements of the photosynthesis rate, the intercellular concentration of CO_2 , stomatal conductance, transpiration, sugar and starch content were carried out on new leaves that have fully opened perfectly. Measurements were conducted in July, September, and November (Figure 3). Stomatal density per mm^{-2} was measured using Pangaribuan et al. (2000) method where lower surface of the leaf

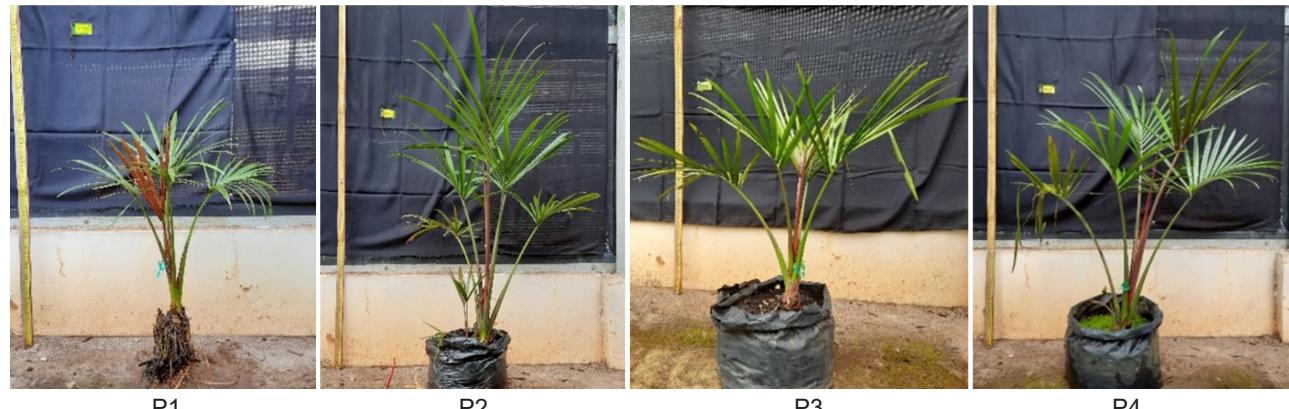


Figure 1. Sago saplings at the nursery phase. P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

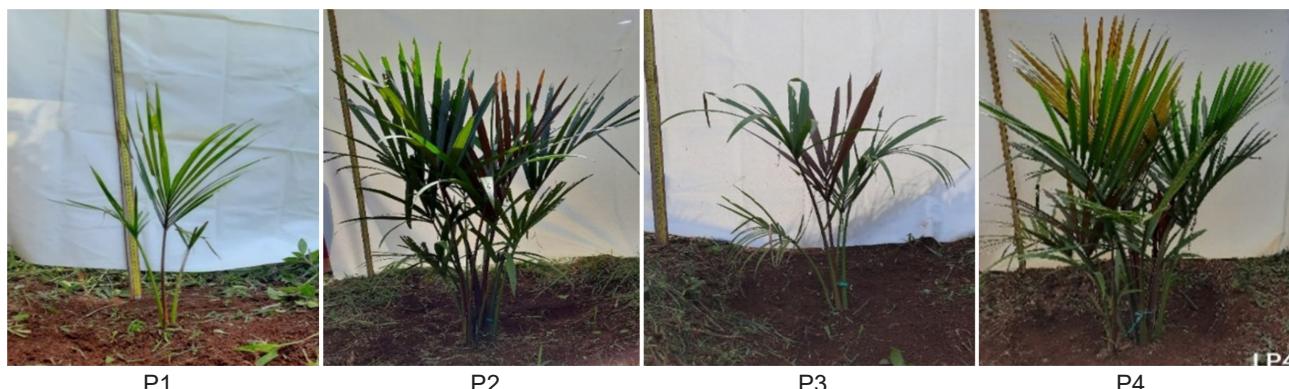


Figure 2. Sago saplings after transplanting to the field. P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

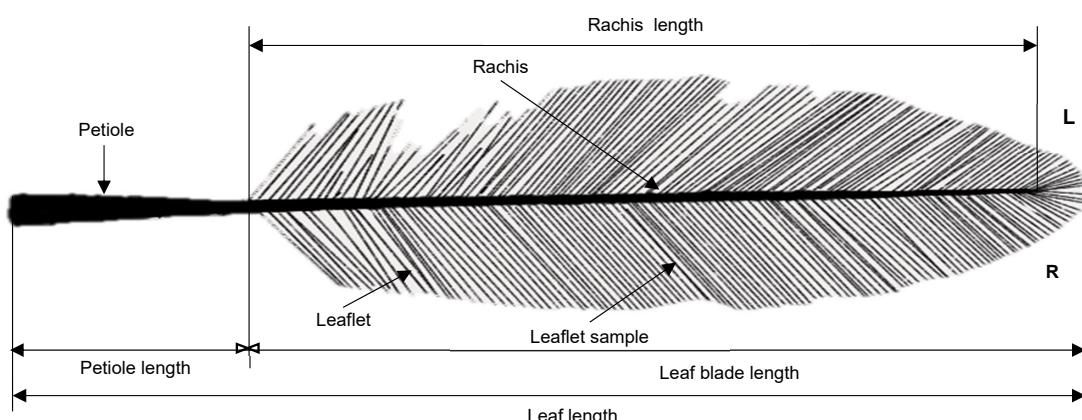


Figure 3. Illustration of a sample of sago leaves that has fully opened (Nakamura et al., 2015)

samples was smeared with clear nail polish (cellulose acetate), clear plaster is prepared and cut according to the size of the object glass of 8×1.2 cm, then samples were observed under a microscope with a magnification of 40x. Rate of photosynthesis ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), the intercellular concentration of CO_2 ($\mu\text{mol} \cdot \text{mol}^{-1}$), stomatal conductance ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), and transpiration ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) were measured using a portable LI-COR 6400 XT. Measurements were carried out at 0900-1000 hours according to Azhar et al. (2019) method; the optimum photosynthetic rate of sago plants at 700-800 μmol of light radiation is from 0900 hours and decreased after 12.00. Leaf sugar and starch content was measured using the AOAC method (1970) and (1971), respectively.

Data Analysis

The study was designed using a single factor in a completely randomized block design with four treatments (described above). Each treatments were repeated three times to obtain 12 experimental units for each seedling phase and the phase after transplanting to the field. Data analysis used Analysis of Variance (ANOVA); further tests were carried out

using the t-test with a level of 5%. Data analysis was carried out using Microsoft Excel 2013 and SAS application version 9.0.

Results and Discussion

Stomatal Density

The stomata density significantly affects the absorption and exchange of carbon dioxide (CO_2) rate for photosynthesis (Haworth et al., 2011). The part of the leaf observed was on the lower surface because stomata are generally located on the lower surface of the leaves (Figure 4). According to Suwarto et al. (2014) stomata on the lower surface of the leaves are about twice the number of stomata found on the upper surface of the leaves.

The analysis showed that the methods of sago seedling preparation did not affect the stomatal density, both at the seedling phase and the phase after transplanting to the field. The density of stomata at the nursery phase was $155.33-160.43 \text{ mm}^{-2}$, while

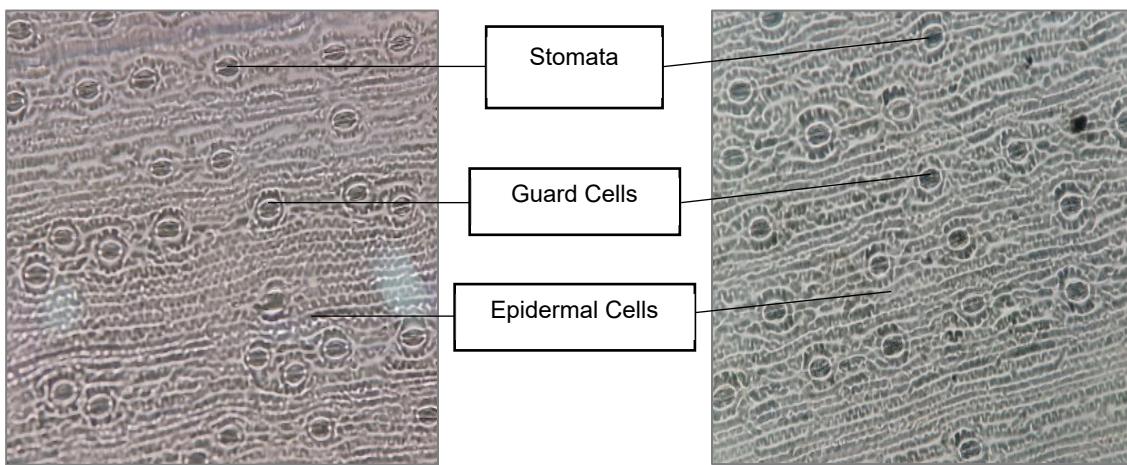


Figure 4. Stomata on the abaxial sago leaves (40x magnification)

in the post-transplant phase was 141.72-168.37 mm⁻² (Figure 5).

The sago leaf stomatal density was relatively low, likely because of the young age of the saplings (less than 12-month-old). Omori et al. (2000) reported that the stomatal density on the abaxial surface can increase from the age of 1 to 3 years, i.e. 400 to 900 mm⁻², then gradually increasing to around 1,000 mm⁻² in 5-year-old saplings or at the rod formation phase. Similarly, Ahyuni (2014) reported the stomatal density of the 2-year-old seedlings of 50.88-101.76 mm⁻² on the upper surface (adaxial) 251.40-369.20 mm⁻² on the lower surface (abaxial). The stomatal density in sago leaves increases with the age, for example, Dewi et al. (2016) reported stomatal density of sago saplings in the lower epidermis of 354.13-588.53 mm⁻². Ehara et al. (2008) reported that the stomatal aperture might be small and lead to a decrease in the photosynthetic rates. The stomatal opening has important roles in maintaining the plant water status, e.g. by restricting the water loss in

sago palm during water or salinity stress. According to Amarillis et al. (2011) the stomatal density varies greatly with sago accessions, and this variation can affect the photosynthesis capability. Although CO₂ assimilation rate and net photosynthesis are reduced due to stomatal closure, a low transpiration rate and inhibition of water loss from leaves can be a useful trade-off in exchange for growth, and affect seedling survival after transplanting (Pirasteh-Anosheh et al., 2016). Since stomatal closure has negative effects on CO₂ uptake and photosynthesis, and uptake of water and nutrient, it is important to close the stomata only when the benefit of water retention outweighs the negative effects.

Photosynthesis Rate

The photosynthesis of the sago saplings between treatments did not differ significantly, both at the nursery and the post-transplant phases. The photosynthesis rate of the plants at the post-transplant phase was higher than those during

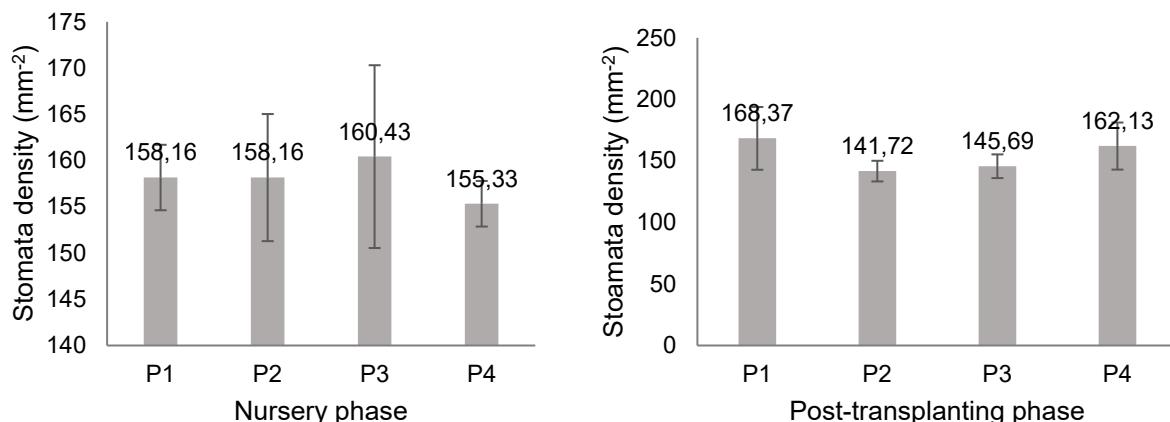


Figure 5. Stomatal density in sago at the nursery phase and the post-transplant phase. (Mean \pm SE, n = 3).
 P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

the nursery phase. The photosynthesis rate at the nursery phase was 20.39-22.96 $\mu\text{mol.CO}_2\text{m}^{-2}\text{s}^{-1}$, stomatal conductance was 0.17-0.20 $\text{mol.m}^{-2}\text{s}^{-1}$, the intercellular concentration of CO_2 was 159.95-212.92 $\text{mol.m}^{-2}\text{s}^{-1}$ and the transpiration rate was 5.20-6.30 $\text{mmol.m}^{-2}\text{s}^{-1}$ (Figure 6). At the post-transplant phase the photosynthesis rate was 20.94-24.25 $\mu\text{mol.CO}_2\text{m}^{-2}\text{s}^{-1}$, the stomatal conductance was 0.19-0.24 $\text{mol.m}^{-2}\text{s}^{-1}$, the intercellular concentration of CO_2 was 177.23-193.90 $\text{mol.m}^{-2}\text{s}^{-1}$, and the transpiration rate was 5.50-6.96 $\text{mmol.m}^{-2}\text{s}^{-1}$ (Figure 7). In Azhar et al. (2019) study, the photosynthetic rate of sago seedlings was in the range of 9.00-10.00 $\text{mol.CO}_2\text{m}^{-2}\text{s}^{-1}$, while in Flach (1983) study it was 8.00-12.00 $\text{mg.CO}_2\text{dm}^{-2}\text{h}^{-1}$ in young plants, and 16.00-18.00 $\text{mg.CO}_2\text{dm}^{-2}\text{h}^{-1}$ in older plants. After the sago forms stems, the rate of photosynthesis became higher, i.e. is 25-27 $\text{mg.CO}_2\text{dm}^{-2}\text{h}^{-1}$ (Miyazaki et al., 2007).

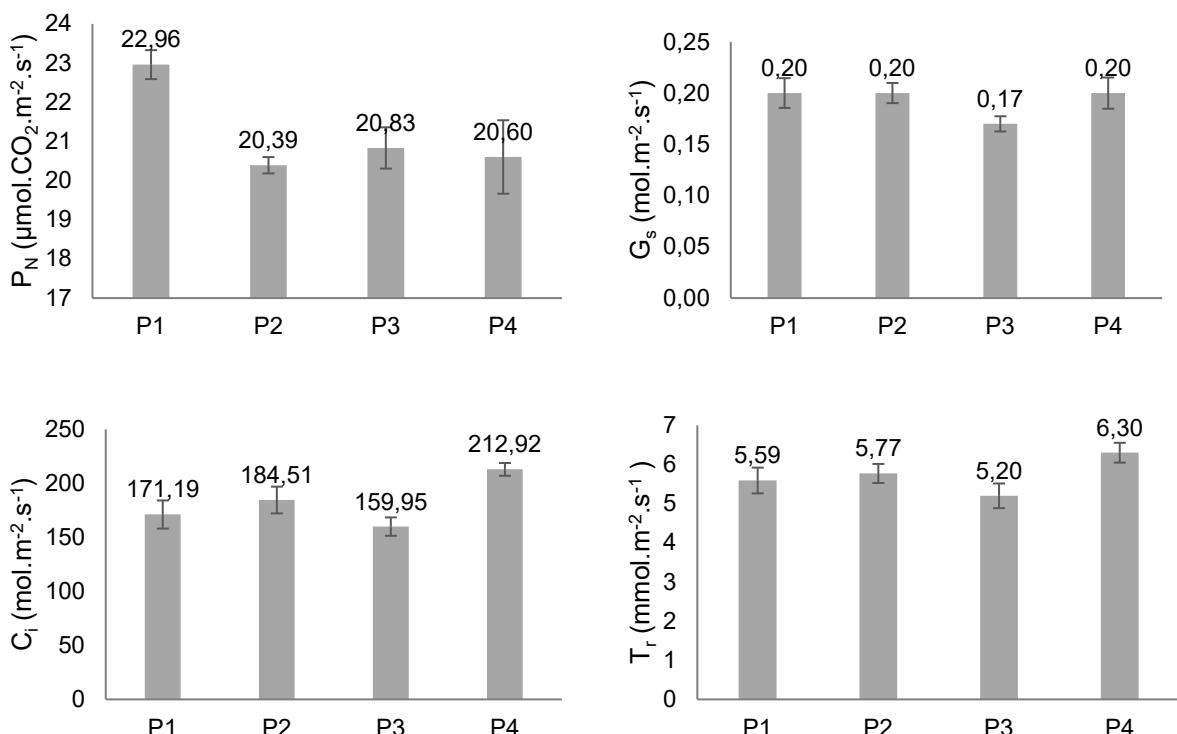


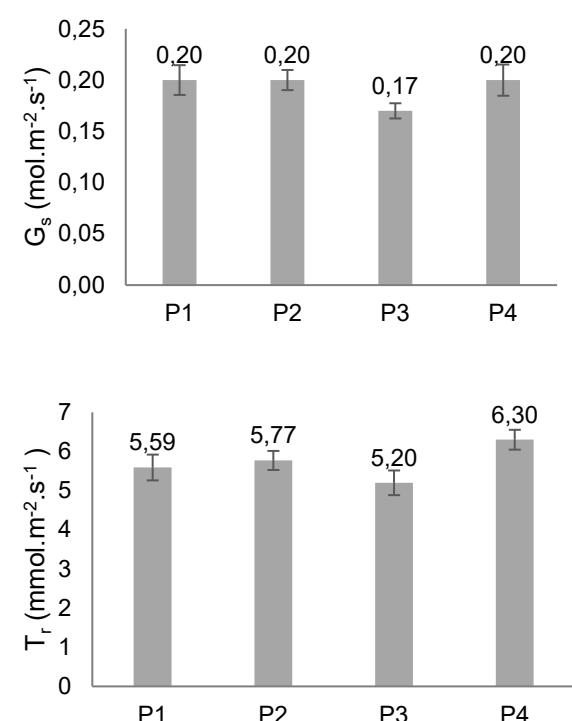
Figure 6. Photosynthetic rates (P_n), stomatal conductance (g_s), the intercellular CO_2 concentration (C_i) and transpiration rate (T_r) in sago palms at the the nursery phase. (Mean \pm SE, $n = 3$). P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

The intercellular concentration of CO_2 and transpiration did not differ significantly in each treatment, both in the nursery and the post-transplant phases. During the nursery phase, the stomatal density was not significantly different between treatments, so the transpiration rates were also not significantly different. The photosynthesis and intercellular concentration of CO_2 between treatments was also not significant. At

the post-transplant phase, the difference in stomatal density did not affect the transpiration rate and the absorption of CO_2 and O_2 , then the photosynthesis and intercellular concentration of CO_2 rates were similar between treatments.

The photosynthesis rate was strongly influenced by the size and density of stomata. The width and length of stomata opened were closely related to the absorption rate of CO_2 from the air as the raw material for photosynthesis. The larger the size of the stomata opening will be followed by the absorption rate of CO_2 from the more substantial air so that the concentration of the intercellular CO_2 leaves became higher, and increases the photosynthesis rate (Medlyn et al., 2013; Singh et al., 2013).

The agroclimatic conditions of the sago nursery and



after transplanting into the field during the study was based on the BMKG Dramaga data in 2020 and can be seen in Table 1. The average temperature and humidity of the study sites ranged from 25.66-26.90°C and 77.48-89.21%, respectively, the rainfall was 89.10-705.30 mm, and the average duration of irradiation was 2.32-7.44 hours per day. Djoefrie et al. (2014) stated that sago palms grow well under

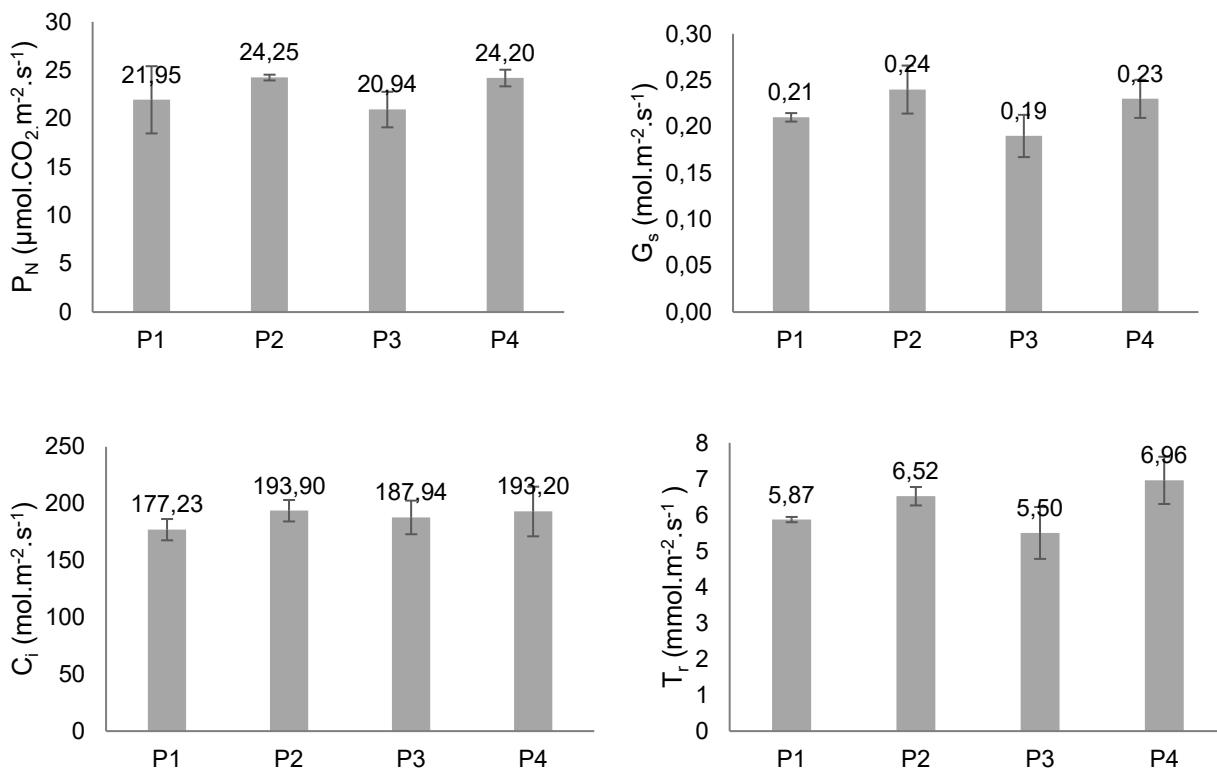


Figure 7. Photosynthetic rate (P_N), stomatal conductance (g_s), the intercellular CO_2 concentration (C_i) and transpiration rate (T_r) in sago palm plants in the the post-transplant phase. (Mean \pm SE, $n = 3$). P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

rainfall of 2,000-4,000 mm per year, irradiation duration of 5-6 hours per day, air humidity of 80-90%, and daily temperature of 25-29°C. Similarly, Okazaki et al. (2015) reported an optimal rainfall for sago palm of 2,000 mm per year. Azhar et al. (2018) conducted a study on the photosynthetic response of sago seedlings to air temperature, and 29-33°C is the optimum range for photosynthesis, and temperature of 25-29°C reduces the rate of gas exchange (net photosynthesis, stomata conductance, the intercellular CO_2 concentration, and transpiration rate), thus inhibiting the photosynthesis process.

During the nursery phase, the sugar content of the leaves was 6.75-7.77%, while the starch content was 0.83-1.28%. The sugar content during the post-transplant phase was 8.46-10.76%, while the starch content 0.88-1.18% (Table 2). Ahyuni (2014) reported the sugar content in sago leaf of 21.12-26.14% with the starch content of 2.99-5.80%. Another study by Takemori et al. (2013) reported the total sugar content in sago leaves of 10-45% of the total sugar in one sago tree, while the starch content was 1-5%. The sugar and leaf starch content in this study were lower than those reported by Ahyuni (2014) and Takemori et al. (2013), likely because of the young age of the seedlings (less than 12-month-old).

The sugar analysis were different between the nursery and the post-transplant phase; sugar content increased from the nursery phase (younger seedlings) to the post-transplant phase (older seedlings) (Table 2).

Sago seedlings that were water-immersed with or without medium in polybags, and immersed bare rooted for one month then transplanted to media in polybags can conduct photosynthesis optimally, likely because of good water availability. In contrast, seedlings without water immersion only receive water from its regular watering. After transplanting to the field, the seedlings bare-rooted treated with water immersion for one month then transferred to media mix in polybag had the highest starch value.

Table 1. Climatic data on the research location in 2020.

| Month | Temperature (°C) | Humidity (%) | Rainfall (mm) | Long exposure (hours/day) |
|-----------|------------------|--------------|---------------|---------------------------|
| January | 25.99 | 88.93 | 399.80 | 3.40 |
| February | 25.66 | 89.21 | 525.40 | 2.60 |
| March | 26.24 | 86.26 | 705.30 | 4.84 |
| April | 26.68 | 85.00 | 478.50 | 5.94 |
| May | 26.90 | 84.19 | 350.50 | 5.49 |
| June | 26.73 | 82.43 | 246.20 | 6.82 |
| July | 26.15 | 81.19 | 186.30 | 6.47 |
| August | 26.47 | 77.48 | 89.10 | 7.44 |
| September | 26.87 | 78.33 | 178.30 | 6.80 |
| October | 26.30 | 83.96 | 583.70 | 5.29 |
| November | 26.50 | 83.20 | 189.50 | 5.17 |
| December | 25.86 | 84.23 | 149.70 | 2.32 |

Source: Meteorology, Climatology and Geophysics Agency (BMKG 2020)

Table 2. Sugar and starch content of the sago leaves at the nursery and the post-transplant phases

| | Sugar (%) | Starch (%) |
|----------------------------------|-----------|------------|
| Nursery Phase | | |
| P1 | 7.35b | 1.21a |
| P2 | 6.75c | 1.28a |
| P3 | 6.84c | 0.83c |
| P4 | 7.77a | 1.05b |
| F-test | ** | ** |
| The post-transplant phase | | |
| P1 | 10.23a | 0.91b |
| P2 | 9.61ab | 0.88b |
| P3 | 8.46b | 0.99b |
| P4 | 10.76a | 1.18a |
| F-test | * | * |

Note: The values followed by different letters in the same column showed real differences according to Duncan Multiple Range Test at $\alpha = 5\%$; * and ** (significant). P1: bare-rooted water immersion; P2: water immersion with media mix in polybags; P3: transplanted to media mix in polybags without immersion; P4: bare-rooted water immersion for 1 month followed by transplanting to media mix in polybags.

Conclusion

The stomatal density and the photosynthesis rate of the sago seedlings were similar between different methods of planting. The sugar content of the seedlings immersed bare-rooted for one month followed by transplanting to polybags had the best growth compared to those from the other treatments, both at the nursery phase and the post-transplant phase. All methods of seedling preparation resulted in seedlings with similar quality. Sago seedlings at the nursery phase tended to grow better when immersed in water, but after transplanting to the field

the seedlings immersed bare-rooted for one month then transplanted to media mix in polybags had better growth than those from other treatments.

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