Does sago palm have a high $\delta^{13}C$ value?

Masanori Okazaki, Koyo Yonebayashi, Naoya Katsumi, Fumi Kawashima, and Tomoe Nishi

Ishikawa Prefectural University 1-308, Suematsu, Nonoichi, Ishikawa 921-8836 Japan

Abstract: Sago palm (Metroxylon rumphii MARTIUS) has been regarded as a typical C3 plant because of a low apparent photosynthetic rate, low light saturation, high CO$_2$ compensation point, and warm optimum temperature. However, there has been no report on the carbon stable isotope ratio ($\delta^{13}C$) of sago palm (Metroxylon sagu Rottb.). In the present study, sago palm leaflets in different growth stages and different leaf positions and sago starch samples were collected in Sungai Talau, Sarawak of Malaysia, Kendari, Sulawesi of Indonesia, and Pangasugan and Hilsig, Leyte of the Philippines, and their $\delta^{13}C$ values were determined. The $\delta^{13}C$ of sago samples ranged from -27.6 to -28.8‰, with a mean value of -28.4‰, indicating that sago palm is a C3 plant by means of $\delta^{13}C$.

Key words: C3 plant, C4 plant, $\delta^{13}C$, sago palm

1. INTRODUCTION

Isotopic discrimination of $^{12}C$ and $^{13}C$ occurs in the photosynthetic process (Hoefs, 2004). The $\delta^{13}C$ is calculated according to the following formula:

$$\delta^{13}C = \frac{\left(\frac{^{13}C}{^{12}C}_{\text{Sample}} - \frac{^{13}C}{^{12}C}_{\text{Standard}}\right) \times 1000}{\frac{^{13}C}{^{12}C}_{\text{Standard}}} \text{ (‰).}$$

The step model of carbon discrimination in photosynthesis consists of (1) the difference between the binary diffusivity of $^{13}$CO$_2$ in plant cells and that of $^{12}$CO$_2$ in air and (2) the difference between the kinetic constants for the reaction of $^{13}$CO$_2$ and $^{14}$CO$_2$ with ribulose bisphosphate carboxylase-oxygenase (Rubisco). It gives the $\delta^{13}C$ of -17 to -40‰ (O’Leary, 1981) and -9 to -35‰ for about 1,000 samples of plant tissue (O’Leary, 1988). Sakai and Matsuhisa (1996) introduced -23 to -33‰ for C3 plants using Rubisco (Ribulose-2-phosphate carboxylase/oxygenase) carboxylation (the Culvin-Benson metabolic pathway) during photosynthesis, -9 to -16‰ for C4 plants using Hatch-Slack metabolism (phosphoenolpyruvic acid carboxylase), and -12 to -34‰ for Crassulacean acid metabolism (CAM) plants. The mean $\delta^{13}C$ for terrestrial C3 plants is -27‰, while the value for terrestrial C4 plants, which became abundant in grasslands within the last 7 to 8 million years (Kohn, 2010), is -12‰.

The $\delta^{13}C$ value has been used, e.g., as an index of water and salt stress of plants (Guy et al., 1980; Bai et al., 2008). As for water stress, Kohn (2010) reviewed a broad compilation of carbon isotope compositions in all C3 plants and concluded a monotonic increase in the $\delta^{13}C$ with decreasing mean annual precipitation, as the $\delta^{13}C$ is positively related to water availability, leading to a proportionately lower $\delta^{13}C$ (Simpkins et al., 2000). Christensen et al. (2011) reported that the winter wheat grain $\delta^{13}C$ significantly increased from -28 to -22‰ with increasing with water balance (the sum of precipitation minus potential evapotranspiration from May to July). Meanwhile, Guy et al. (1980) reported that the $\delta^{13}C$ of halophytes Salicornia europaea and Puccinellia nuttalliana was from -29 to -23.5‰ and -27 to -24‰, respectively, with decreasing salt concentrations. Mangrove species living further inland were enriched in $^{13}C$ (from -25.9 to -29.1‰) relative to those living near the shoreline, as shown by Muzuka and Shunula (2006). Kao et al. (2001) reported the effect of NaCl and nitrogen availability on the growth and photosynthesis of seedlings of a mangrove species (Kandelia candel (L.) Druce).
The $\delta^{13}C$ values also vary among tissue types of plants, but there were no consistent patterns in variation. Ellison et al. (1996) reported no significant differences in $\delta^{13}C$ between leaves, branches, and twigs of *Rhizophora mangle*, while the $\delta^{13}C$ values of cable roots and small rootlets were significantly higher than those of leaves. Lee et al. (2001), however, reported that the twig and bark tissues of *Kandelia candel* and *Aegiceras corniculatum* were slightly depleted in $\delta^{13}C$ (by <2‰) relative to the leaf tissues.

Sago palm (*Metroxylon sagu* Rottb.) accumulates a huge amount (about 120 kg per plant) of starch in its trunk. Uchida et al. (1990) concluded that sago palm has a Calvin-Benson cycle and is classified as a C3 plant based on its low light saturation, high carbon dioxide compensation point, and warm optimum temperature for attaining maximum photosynthetic ability.

However, there has been no report on the $\delta^{13}C$ of sago palm, although Kuramoto and Minagawa (2001) reported the $\delta^{13}C$ of nipa (*Nypa fruiticans*) to be -26.7‰ in tropical forests of Thailand.

The objective of this study is to elucidate the $\delta^{13}C$ of sago palm as a C3 plant. The sago palm leaflet and starch samples were taken from Talau (Malaysia), Kendari (Indonesia), and Pangasagan and Hilsig (Philippines) to evaluate the $\delta^{13}C$ values affected by the slightly different water regimes of soil and autotrophic and heterotrophic plant organs.

### 2. MATERIALS AND METHODS

#### 2.1 Site description

The study site in Malaysia was at the Sungai Talau Peat Research Station (Ministry of Agriculture, Sarawak), which was developed for sago plantation experiments on shallow and deep peats. The mean annual rainfall and the mean annual temperature at Sibu, which is 60 km from the Sungai Talau Peat Research Station, are 3,194 mm and 26.3 °C, respectively (Yamaguchi et al., 1997). A detailed site description was recorded in Yamaguchi et al. (1998).

The soil profile at the Sungai Talau Peat Research Station is as follows: 0–24 cm; 10 R 2/2 (dark reddish brown), wet, humic, von Post grade 3, many medium roots, many charcoals, Electrical conductivity (EC) 0.425 mS cm$^{-1}$ (8/1000 of EC for mean sea water), clear boundary to, 24–65 cm; 10 R 3/2 (dark reddish brown), wet, fibric, von Post grade 1, many large roots and stems, irregular graduate boundary to, 65–cm; 10 R 3/3 (dark reddish brown), wet, fibric, von Post grade 1, abundant large stems and roots, classified as a Histosol (TUAT, 1998). The low salt concentration and wet water regime of this soil may not influence carbon assimilation by C3 plants (Kao et al., 2001; Kohn, 2010; Christensen et al., 2011).

The sampling site in Indonesia was located in Tobimeita in the alluvial plain of the Wanggu River, Kendari, Sulawesi (Okazaki, 1998). Inceptisols and Entisols were distributed in the area. The soil profile is as follows: 0– 7 cm; 10 YR 5/5 (yellowish brown), moist, coarse sand, structureless, few medium and fine weed roots, pH 7.8, EC 0.30 mS cm$^{-1}$ (6/1000 of EC for mean sea water), clear smooth boundary to, 7–35 cm; 5Y 5/2 (grayish olive), moist, coarse sand, structureless, few organic debris with original shape and decomposed shape, pH 6.5, EC 0.32 mS cm$^{-1}$, clear smooth boundary to, 35 – 70 cm; 2.5 YR (brownish black) for matrix and 2.5 Y 2/1 (black) for spots, wet, silty clay, structureless, common medium and fine sago roots, very sticky, plastic, pH 6.8, EC 0.32 mS cm$^{-1}$, gradual wavy boundary to, 70– cm; 2.5 YR 5/2 (dark grayish yellow), wet, silty clay, structureless, sticky, pH 6.7, EC 0.35 mS cm$^{-1}$, classified as an Inceptisol. This soil profile shows a drier moisture condition compared to the Talau soil profile.

The study site in the Philippines was located in Pangasagan and Hilsig, Leyte (Okazaki et al., 2005). The mean annual precipitation was 2,951 mm from 1995 to 2004. The mean maximum temperature from 1995 to 2004 was 33.6 °C, and the mean minimum temperature from 1995 to 2004 was 22.4 °C (Kimura and Okazaki, 2006). The soil distributed in this area
was Eutropept (BSWM, 1993; Baynes et al., 2007). The soil profile at Pangasugan is as follows: 0–20 cm; 10 YR 4/3 (dull yellowish brown), moist, silt loam, kaolin, pH 6.4, EC 0.342 mS cm⁻¹ (6/1000 of EC for mean sea water), gradual wavy boundary to, 20–31 cm; 10 YR 5/3 (dull yellowish brown), moist, silty clay loam, slightly sticky, gradual boundary to, 31–71 cm; 10 YR 5/3 (dull yellowish brown), moist, silt loam, slightly sticky (Lina et al., 2008).

The soil profile at Hilsig, 31 km southeast of Pangasugan, which has no climatic data, is described as follows: 0 – 24 cm; 7.5 YR4/3 (brown), moist, sandy loam, slightly hard, pH 5.9, EC 0.295 mS cm⁻¹ (5/1000 EC for mean sea water), gradual wavy boundary to, 24 – 46 cm; 7.5 YR 5/3 (dull brown), moist, sandy loam, slightly hard, gradual boundary to, 46–78 cm; 10 YR 5/3 (dull yellowish brown), moist, sandy loam, slightly hard. This soil was the intermediate moisture condition among four.

2.2 Sago leaflet and starch samples

Sago leaflet and starch samples were taken from the selected four experimental fields in 1994–2010. The leaflet samples of a sago palm more than 10 years after transplanting were collected in August 1994 in the Talau field and in August 1995 in the Kendari field. The leaflet samples of a sago palm 4 years after transplanting were collected in 2009 in the Pangasugan field. The longest leaflet of the middle leaf (the fourth leaf from the top) was collected for stable isotope analysis with three replications.

Mature sago palms (more than 10 years after transplanting) were cut down to take sago starch (a heterotrophic organ) to compare to the autotrophic organ (leaflet) in 2009 in the Hilsig field. Sago starch samples were taken from the 10 logs of trunks using a wet extraction method (Yamamoto, 1998). Starch samples were washed with distilled water three times and dried in a ventilated oven-drying apparatus at 70 °C for 48 hours. After drying, the starch samples were powdered using a mill (Retsch MM 301) and stored in the dark at 10 °C.

2.3 δ¹³C determination

About 0.5~ 0.7 mg of the samples weighed in a small tin cup was used for determination of the δ¹³C. Analysis of the δ¹³C was performed using a CN analyzer (Euro EA 3028-HT Elemental Analyzer, EuroVector, Milan, Italy) connected to an isotopic ratio mass spectrometer (IsoPrime IRMS, GV Instruments, Cheadle, UK). USGS 40 (δ¹³C: -26.2 ‰ (U.S. Geological Survey, Reston, VA, USA)) was used as a secondary standard sample. The analytical condition was as follows: combustion column temperature, 1030 °C; reduction column temperature, 670 °C; GC oven temperature, 115 °C; He gas flow, 110 mL min⁻¹.

3. RESULTS AND DISCUSSION

3.1 δ¹³C of sago leaflet

The δ¹³C of sago leaflet samples taken from Talau (n = 9) and Kendari (n = 6) in August 1994 and 1995 ranged from -29.19 ‰ (minimum value) to -26.25 ‰ (maximum value) (Fig. 1), with a mean value of -27.88 ‰, standard deviation of 0.94 ‰, and variant coefficient of 3.36%. Sago leaflet samples (n = 12) taken from Pangasugan throughout 2009 showed -27.47 ‰ of the mean δ¹³C value. The δ¹³C value of sago leaflets from three sites was -28.4 ‰. These values were comparable to other C3 plants (Kohn,
increasing MAP (Table 1). Wooller et al. (2003) exhibited that the δ\textsubscript{13}C values of *Laguncularia racemosa* (white mangrove) from Florida (mean: -26.4 ‰) were slightly higher than those from Belize (mean: -27.4 ‰) due to higher salinity. The results, however, showed no distinctive trend between δ\textsubscript{13}C values and EC among the four study sites (Table 1).

In Fig. 1, *Miscanthus sinensis* (n=5), one of the C4 plants taken from Kawatabi of Miyagi (n=2), Nodayama of Ishikawa (n=2), and Hakusan City of Ishikawa (n=1) indicated a higher mean δ\textsubscript{13}C value (-12.4 ‰) (Katsumi, 2011), which corresponded to the 2010; Katsumi, 2011) and indicated that sago palm is certainly a C3 plant.

The δ\textsubscript{13}C values of C3 plants change due to C discrimination depending on the moisture conditions, which are affected by mean annual precipitation (MAP). According to Diefendorf et al. (2010), the δ\textsubscript{13}C values of C3 plants increased with decreasing MAP because the global carbon dioxide uptake and fixation (\(\Delta_{\text{leaf}}\)) values positively correlated to the MAP (p < 0.0001, R\(^2\) = 0.55).

However, the δ\textsubscript{13}C values of sago leaf samples did not show a tendency to be enriched in δ\textsubscript{13}C with increasing MAP (Table 1). Wooller et al. (2003) exhibited that the δ\textsubscript{13}C values of *Laguncularia racemosa* (white mangrove) from Florida (mean: -26.4 ‰) were slightly higher than those from Belize (mean: -27.4 ‰) due to higher salinity. The results, however, showed no distinctive trend between δ\textsubscript{13}C values and EC among the four study sites (Table 1).

In Fig. 1, *Miscanthus sinensis* (n=5), one of the C4 plants taken from Kawatabi of Miyagi (n=2), Nodayama of Ishikawa (n=2), and Hakusan City of Ishikawa (n=1) indicated a higher mean δ\textsubscript{13}C value (-12.4 ‰) (Katsumi, 2011), which corresponded to the

<table>
<thead>
<tr>
<th>Site of origin</th>
<th>Site description</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Mean monthly temperature °C</th>
<th>Precipitation mm yr(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaysia Talau</td>
<td>Tropical, Af Lowland Haplofibriz</td>
<td>2°46'N</td>
<td>112°5'E</td>
<td>Max: 37 Min: 20</td>
<td>3662</td>
</tr>
<tr>
<td>Indonesia Kendari</td>
<td>Tropical, Af Lowland Haplaquent</td>
<td>3°43'S</td>
<td>122°36'E</td>
<td>Max: 30.5 Min: 24.1</td>
<td>2403</td>
</tr>
<tr>
<td>Philippines Pangasugan</td>
<td>Tropical, Am Lowland Haplaquent</td>
<td>10°45'N</td>
<td>124°47'E</td>
<td>Max: 33.6 Min: 22.4</td>
<td>2996</td>
</tr>
<tr>
<td>Philippines Hilsig</td>
<td>Tropical, Am Hilly land Dystrudept</td>
<td>10°33'N</td>
<td>124°89'E</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>
Contrasting δ¹³C values arise in different biosynthetic pathways and physiological pools (Gleixner et al., 1998; Dungait et al., 2011). To compare the δ¹³C value of leaflet (autotrophic organ) samples, starch samples from trunks (heterotrophic organ) were analyzed. Hilsig’s sago palm starch gave -25.75‰ of the mean δ¹³C value (n=10), which was similar to the δ¹³C starch samples from the buli palm (Corypha elata Roxb.), -25.64‰ (Fig. 2) (Kawashima, unpublished data). Although the δ¹³C of sago starch was slightly higher than that of the sago leaflet samples (autotrophic organ; Fig. 1), there was no significant difference between them (Figs. 1 and 2).

CONCLUSION
Sago palm is a C3 plant, as determined from the result of its carbon discrimination in leaflet samples: -27.88‰ for Talau and Kendari and -27.47‰ for Pangasugan.

REFERENCES


Does sago palm have a high $\delta^{13}$C value?

CO$_2$ evolved from incubated maize residues and maize-derived sheep faces. Soil Biology & Biochemistry 36: 99-105.


Okazaki, M. 2009 Function and utilization of sago starch as a pharmaceutical excipient, pp. 96, Tokyo University of Agriculture and Technology.


Okazaki, M., K. Toyota and D. S. Kimura 2005 Sago Project in Leyte, pp. 96, Tokyo University of Agriculture and Technology.


