CONTRASTING SUGARS IN COCONUT AND OIL PALM: CAN CARBOHYDRATE PATTERNS BE USED AS CHEMOTAXONOMIC MARKERS?

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ABSTRACT

The coconut and oil palm are members of the Arecaceae. Despite a recent revised classification of this family, uncertainties still remain. We evaluated the taxonomic potential of the carbohydrate reserves by comparing these two palm species, belonging to different subtribes within the same Cocoseae tribe. We showed that both palms share features with all palm taxa but differ by others. We showed that the coconut and oil palm exhibit common but also distinct characteristics leading to different carbohydrate patterns as potential markers. Indeed, both palms, like all palms, store their reserves mainly in the stem but, in contrast to numerous palms, do not use starch as major reserve pool. Both palms share the use of soluble sugars as reserves but differ in the nature of the dominant sugar (sucrose or glucose), the amount of starch and in the total amount and distribution of the reserve pool. Each palm, thus, exhibits a particular carbohydrate profile, based on similarities and differences, reflecting their relationships within the family and within the Cocoseae tribe such as their distinct biological features. These profiles might be an aid for taxonomic and evolutionary studies of Cocoseae palms, combined with anatomical, morphological and DNA markers.

Keywords: *Cocos nucifera, Elaeis guineensis*, taxonomy, palms, Cocoseae, non-structural carbohydrates

INTRODUCTION

Coconut and oil palms are perennial arborescent monocotyledonous species, typical of the tropics. Both play a vital role at many different economic levels. The coconut (*Cocos nucifera* L.) is often referred to as the "tree of life" because of its multitude of subsistence and commercial uses. The African oil palm (*Elaeis guineensis* Jacq.) is the most important oil-producing plant, also used locally as a source of wine, thatch and building materials.

Both palms belong to the Arecaceae, a complex family of nearly 185 genera and 2500 species (Dransfield *et al.,* 2008; Baker *et al.*, 2011). More precisely, they belong to the largest sub-family, the Arecoideae. Initially, Uhl and Dransfield (1987) resolved six tribes within this subfamily, with the coconut and oil palm included in the Cocoeae tribe. However, Dransfield *et al.* (2008), in the new edition of the family monograph *Genera Palmarum,* present a revised classification. Fourteen tribes are now described within the Arecoideae; the Cocoseae being one of them.

Palm classification mainly relies on morphological and anatomical features. Members of the Cocoseae, such as the coconut and oil palm, are characterised, overall, by the presence of an endocarp with three, or more, clearly defined pores. Recently, the classification of the Cocoseae tribe has been modified. Indeed, Dransfield *et al.* (2008) resolved the Cocoseae into three subtribes instead of the five subtribes previously described (Uhl and Dransfield, 1987). In this new classification, Dransfield *et al.* (2008) have broadened the delimitation of the subtribe Attaleinae to include all members of the tribe Cocoseae except the spiny cocosoids (Bactridinae subtribe) and *Elaeis* and *Barcella* (Elaeidinae subtribe).

Cocos is one of the eleven genera of the Attaleinae while *Elaeis* is one of the two genera of the Elaeidinae.

However, uncertainties still remain in terms of taxonomy and evolutionary relationships within this family and even within the Cocoseae tribe, despite the contribution of several DNA-based studies (Asmussen *et al.,* 2006; Dransfield *et al.,* 2008; Horn *et al.,* 2009; Meerow *et al*., 2009; Baker *et al.,* 2009, 2011; Noblick *et al.,* 2013). For example, the exact position of *Cocos* within the Attaleinae and the Cocoseae sister relationships are not fully resolved despite several studies, including one based on a combination of all molecular markers commonly used in palm systematics plus morphological data (Hahn, 2002; Gunn, 2004; Baker *et al.*, 2009, 2011). Though, Meerow *et al.* (2009) and Noblick *et al.* (2013) described sister relationships between *Cocos* and *Syagrus*. In addition, the biogeographic origin of the coconut and its wild relatives are still matter of debates (Gunn, 2004; Meerow *et al.*, 2009). Similarly, regarding *Elaeis*, most of the studies resolved the subtribe Elaeidinae as sister to the Bactridinae subtribe (Asmussen *et al.*, 2006; Baker *et al.*, 2009; Eiserhardt *et al.*, 2011); but for Gunn (2004) *Barcella* appears basal in the Cocoeae tribe rather than closely related to *Elaeis*.

Emerging data from the few surveys of palm chemistry suggest that chemical data may be useful for refining the palm taxonomy and phylogenetic (Dransfield *et al.*, 2008). For example, the few studies addressing the flavonoids and phenolic acids in palms showed their potential as chemotaxonomic markers and provided some insight into relationships among palm taxa (Harborne and Wiliams, 1994; Chakraborty *et al*., 2006). Instead, the anthocyanins have limited value for palm chemotaxonomic study while the potential of the alkaloids remains to be assessed

(Harborne and Williams, 1994; Dransfield *et al.*, 2008). Regarding the carbohydrates, starch was retained by Van Die (1974) to classify the Arecaceae in three groups: (i) species that do not store starch in the trunk (e.g. *Cocos nucifera*); (ii) species which store starch in small quantities (e.g., *Arenga* spp.), and (iii) species which store large quantities of starch (e.g., *Corypha* and *Metroxylon*). Besides, Simas *et al.* (2004, 2006) suggested that the polysaccharides from palm gum exudates might have some potential.

However, the potential of these chemotaxonomic markers, to our knowledge, has not been further assessed in palms. By contrast, carbohydrates have been exploited in angiosperm groups as chemotaxonomic and evolutionary markers. For example, differences in cell wall carbohydrate composition and structure were used as biomarkers for chemotaxonomic studies in several groups of plants (Kim *et al.*, 2004; Hoffman *et al.*, 2005). In Leguminosae, the level of galactomannans and the mannose:galactose ratio in seeds reflect the systematic but also the pattern of evolution (Hegnauer and Gpayer-Barkmeijer, 1993; Buckeridge *et al.*, 2000).

Therefore, our objective was to evaluate the taxonomic potential of the carbohydrates by comparing two important palm species, the coconut (Attaleinae) and the oil palm (Elaeidinae), belonging to different subtribes within the same Cocoseae tribe. The carbohydrate reserves stored in each palm were thus compared for their chemical nature, amount and distribution within the plant vegetative organs. We expected to determine a carbohydrate pattern for each palm species based on similarities and differences, reflecting their relationships within the palm family and within the Cocoseae tribe such as their distinct biological features.

MATERIALS AND METHODS

Experimental sites and plant materials

For the coconut, the experiment was conducted on the coconut plantation of the Vanuatu Agricultural Research and Training Centre (VARTC). The coconut cultivar studied was a hybrid between the Vanuatu Red Dwarf and the improved Vanuatu Tall. Coconut plants were 19 years old at the onset of the study. For the oil palm, the experiment was conducted at the Smart Research Institute (SMARTRI, Smart Tbk.) located in the Kandista estate (Riau Province, central Sumatra, Indonesia). The oil palm cultivar studied was a tenera hybrid (called 64, a *Deli* × *Avros* hybrid*)* and the plants were nine years old. Both experiments were previously described (Mialet-Serra *et al.,* 2008; Legros *et al.,* 2009a, 2009b).

For both palm species, twelve non-neighbouring plants (i.e. 12 replicates) were randomly selected from a large population of plants with a known individual production history, and felled.

Sampling procedure for sugar analyses

Stem, leaf, persistent leaf base (only for oil palm) and root samples were taken on each plant, in the morning, as described by Mialet-Serra *et al.* (2008) and Legros *et al.* (2009c).

Briefly, on the stem, one or two radial core samples were taken in the meristem, at the top (sub-apical area), at mid-height, at the base (200 mm from the ground) and in the stump, using a Pressler drill (6.6 mm diameter × 300 mm length). Each core was divided into four subsamples (‘bark’, outside, inside and ‘core’) in the stump, at the base and at mid-height, or three subsamples (‘bark’, outside and ‘core’) at the

top. From these subsamples, cubes (0.5–1g fresh biomass) were cut, representing the three or four radial zones that have a decreasing density of woody, vascular strands from the periphery (i.e. 'bark') to the centre (i.e. 'core').

Leaves were collected on one of the five spirals for the coconut and one on the eight spirals for the oil palm, corresponding, for the coconut, to rank 4, 9, 14, 19, 24 and (if present) 29 and for the oil palm, to rank 9, 17, 25, 33 and (if present) 41 (leaf rank 1 being the youngest fully-expanded leaf out of about 30 or 40 leaves present on the plant for coconut and oil palms, respectively). Subsamples of 20–30 g (fresh biomass) were taken from the base, middle portion and end of the petiole, from the middle and end portions of the rachis, and from leaflets at the base, middle portion and end of the rachis.

Persistent leaf bases were collected at the base, at mid-height and at the top of oil palm stems. Subsamples of 20-30 g (fresh biomass) were taken.

On the root system, large roots (R1 type, 10 mm < diameter < 15 mm), medium size roots (R2 type, 1 mm < diameter < 9 mm) and fine roots (R3 type, diameter ≤ 1 mm) were distinguished. Samples were taken from the white portion in the course of differentiation located close to the meristem (zone 1), and the mature, differentiated zone that includes a suberified rhizodermis and a lignified endodermis (zone 2) for both, R1 and R2 root types.

All samples were stored at -20°C until freeze-drying using a Cryodos -50°C lyophilizer (Telstar, Spain). Samples were then kept in vacuum-sealed bags at -20°C until grounding.

Sugar analyses

Freeze-dried samples were ground with liquid nitrogen to 100 µm particle size using a knife grinder (Thomas Scientific, United States) for large samples or a ball grinder (Mixer Mill MM 200, Retsch, Germany) for small samples. Sugars were extracted from 20 mg samples with 1 ml 80% ethanol for 30 min at 80 °C, and then centrifuged at 9000 g for 15 min. This procedure was repeated once with 80% ethanol and once with 50% ethanol and the supernatants were pooled.

Soluble sugars (glucose, fructose and sucrose) were contained in the supernatant and starch in the sediment. The supernatant was filtered in the presence of polyvinyl polypyrrolidone and activated charcoal to eliminate pigments and polyphenols. After evaporation of ethanol with Speedvac (RC 1022 and RCT 90, Jouan SA, Saint Herbain, France), soluble sugars separately were quantified by high performance ionic chromatography (HPIC, standard Dionex) with pulsed amperometric detection (HPAE-PAD). Starch was solubilised with 0.02 N soda at 90 °C for 2 h and then hydrolysed with α-amyloglucosidase at pH 4.2 for 1.5 h. Glucose was quantified as described by Boehringer (1984) with hexokinase and glucose-6-phosphate dehydrogenase, followed by spectrophotometry of NADPH at 340 nm (spectrophotometer UV/VIS V-530, Jasco Corporation, Tokyo, Japan).

Soluble sugars and starch together are referred to non-structural carbohydrates (NSC). The results were expressed either as organ concentrations (mg g^{-1} dry matter) or as the total contents for each organ (g) on a single-plant basis.

Standing dry biomass measurements

Stem standing biomass was calculated from stem dimensions (thickness and height) and the specific density (dry mass:wet volume ratio) at different locations on the stem: in the stump, at the base, at mid-height, at the top and in the meristem. Standing biomass of the petioles, rachis, leaflets and persistent leaf bases (for oil palm) was estimated by weighing the entire organs after drying at 104 °C and then multiplying by the total number of such organs present on the plant.

Root sampling was performed for one-twelfth of the theoretical, hexagonal soil surface available to a plant, measuring 5.20 m^2 for coconut and 5.83 m^2 for oil palm. Roots were collected from a 1-m deep hole for coconut (Navarro *et al.*, 2008) and a 0.8-m deep hole for oil palm (Nodichao, 2008), sieved, sorted by type, dried at 104 °C and weighed. The biomass was extrapolated to the full hexagon associated with the plant. The results were then multiplied by 1.1 to take deep roots into account, based on previous observations on the same plots for both species.

RESULTS

Sugar concentrations in the vegetative organs

In the stem

For the coconut, the mean NSC concentrations in the stem gradually increased from 89.4 mg g^{-1} (in the stump) to 365 mg g^{-1} (in the meristem) on a dry biomass basis (*Figure 1*). The mean sucrose concentrations followed the same increasing pattern from the bottom to the top of the stem. Sucrose was the major sugar as it accounted for 77% or more of the NSC in all parts of the stem. Starch was practically absent in the lower stem parts, then slightly increased up to 12% of

NSC in the apical meristem. The monosaccharide (glucose + fructose) concentration never exceeded 9% of NSC.

For the oil palm, the mean NSC concentrations in the stem ranged from 164.5 mg g^{-1} (in the stump) to 390.1 mg g^{-1} (in the meristem) on a dry biomass basis (*Figure 1*). Compared to the coconut, the NSC concentration at each height of the oil palm stem was higher, except in the meristem. Glucose, with a minimum of 64% of the NSC concentration, was the dominant sugar up to the mid-height of the stem. At the top of the stem, starch became the major NSC compound, representing 55% of NSC, followed by glucose (24% of NSC) and sucrose (18% of NSC). Along the stem, fructose was only present in low proportion. In the meristem, the NSC distribution was more similar to the data observed in the coconut stem with 67% of sucrose, 29% of starch and 2% of glucose.

In the radial zones of the coconut stem, the mean NSC concentrations gradually increased from the periphery ('bark') to the centre ('core') whether at mid-height or at the top of the stem (*Figure 2*). Sucrose represented more than 60% of the NSC in each radial zone. Only low concentrations of starch and glucose were detected, reaching 12% and 7%, respectively, in the stem core. In the radial zones of the oil palm stem, the mean NSC concentrations revealed no particular pattern, although a slight increase was measured from the periphery to the centre at the top of the stem (*Figure 2*). Differences were observed for the NSC components. At mid-height of the stem, glucose was the dominant component, gradually increasing from 44% of NSC in the outside zone to 80% in the stem centre. Starch was mainly detected in the external part at the mid-height of the stem where it represented 31% of the NSC. The mean sucrose concentration slightly decreased from the outer

(19% of NSC) to the inner parts (12% of NSC). At the top of the stem, starch became the dominant compound, followed by glucose and sucrose. Starch represented 60% of NSC in the 'bark' and 'outside' zones. The mean concentration of the soluble sugars (glucose + fructose + sucrose) increased from the outer (40% of NSC in the 'bark') to the inner (63% of NSC in the 'core') parts. In the 'core' zone, glucose (38% of NSC) and starch (37% of NSC) were equally represented.

In the leaves

In the coconut leaves, we observed that sucrose was always the dominant NSC compound. Figure 3 shows the pattern of sucrose distribution with a high concentration in the petioles (74%) and rachis (68%) and a moderate one in leaflets (49%). The monosaccharides were equally represented in the petioles and rachis (glucose: 13%; fructose: 12%) and in the leaflets (glucose: 24%; fructose: 22%). In the oil palm leaves, glucose was the major NSC component in the petioles (77%) and rachis (74%) while we detected lower concentrations in leaflets (40%) (*Figure 3*). In oil palm, the fructose concentration was similar to the glucose concentration in the leaflets but in the petiole and rachis, the fructose concentration was limited to 9%. Low sucrose concentrations were detected in the petiole (14%) and rachis (15%). For both species, we measured very low concentrations of starch. The NSC concentrations in the petiole and rachis of the coconut leaves were nearly half the NSC concentrations in the petiole and rachis of the oil palm leaves.

In the roots

For both species, the mean NSC concentration showed a strong gradient from large (R1) to fine (R3) roots, the latter having very low concentrations (*Figure 4*).

Soluble sugars were dominant and starch was found only in traces. For both species, differences were observed between topological zones. Zone 1 (near the meristem) showed higher NSC concentrations than zone 2. Monosaccharides dominated in zone 1. In zone 2, sucrose dominated in coconut and monosaccharides dominated in oil palm. Overall, the mean sucrose concentrations were respectively higher in each root types and zones of the coconut compared to the oil palm.

In the persistent leaf bases (for oil palm)

The dominant NSC compound in the persistent leaf bases was, by far, the glucose (83% of NSC), followed by sucrose (9%), as in the stem (*Figure 5*). The mean concentrations of soluble sugars gradually decreased from the bottom to the top of the stem. We only detected starch in the persistent leaf bases located at the top of the stem.

Therefore, we observed that the soluble sugars, instead of starch, showed the highest concentrations in tissues of both palm species, mainly sucrose for the coconut and glucose for the oil palm. The glucose/fructose concentration ratio varied from 0.9 to 1.7 in all coconut organs. Instead, this ratio reached 6.9 to 14.4 in all major oil palm organs except in roots and leaflets.

Standing dry biomass

The mean total plant dry biomass, including roots, was 331 kg for the coconut and 680 kg for the oil palm (*Table 1*). For both species, the stem was the most imposing compartment; it accounted for 45% of the total dry matter for the coconut and 52% for the oil palm. The leaf compartment contributed for 29% in the coconut and

38% in the oil palm, adding the persistent leaf bases along the stem. The root system contributed for 26% in the coconut and only 10% in the oil palm.

Sugar content in the plant

The NSC pool accumulated in the vegetative organs of the coconut totalled 32 kg plant^{-1} and about 141 kg plant^{-1} for the oil palm (*Table 2*). These pools represented 10% of the total dry vegetative standing biomass of the coconut and 21% of the oil palm biomass. For both species, the carbohydrate reserves were predominantly in the stem, with nearly 18 kg plant^{-1} for the coconut stem and 99 kg plant^{-1} for the oil palm stem. Instead, the root compartment stored 5.6 kg plant^{-1} of NSC for the coconut and 3 kg plant^{-1} for oil palm.

Sucrose was the main reserve compound in the coconut, representing nearly 80% of the NSC in the entire plant. In the oil palm, glucose was the main reserve compound with nearly 51% of NSC. In the coconut, glucose represented only 8% of NSC, located mainly in the leaves. In contrast in oil palm, sucrose constituted 20% of the NSC and was present in all vegetative compartments. Therefore, sucrose was not a minor compound in the oil palm while glucose was a minor compound in the coconut. For both species, starch was mainly located in the top of the stem. For the coconut, the starch pool represented 6% of the NSC while for the oil palm, it corresponded to 23% of the NSC. Fructose was present in similar proportion in both palm species, representing 6% of the NSC.

DISCUSSION

In this study, we show that the coconut and the oil palm have in common the storage in their vegetative organs of soluble sugars, instead of starch, as the main carbohydrate reserves. For the coconut, indeed, the soluble sugars represent more than 90% of the non structural carbohydrates (NSC) in the stem, leaves, and roots. For the oil palm, the same proportion of soluble sugars is in leaves and roots while in the stem, they account for 70% of NSC. Both palms, thus, store starch as a minor component, mainly at the stem apex as a transitory reserve. In addition, we show that the coconut and oil palm differ in the nature of the dominant sugar, sucrose versus glucose, respectively, and in the amount and distribution of NSC within the plant. Hence, both palm share features with all palm taxa but differ by others. In addition, these two palms exhibit common but also distinct characteristics leading to different carbohydrate patterns as potential chemotaxonomic markers.

For both palms, like for all palms, allocation of the NSC to the stem is a logical pattern of reserve distribution. Palms are, indeed, distinctive in that they can make long lived trees entirely by primary developmental processes (Tomlinson, 2006). The growing stem only results from continually active shoot apical meristem. It lacks a peripheral vascular cambium and, thus, capability for secondary growth. Palms, therefore, retain the longest-lived differentiated cells in their stems (Tomlinson, 2006; Dransfield *et al.*, 2008). For both palms, the high NSC concentration through the stem indicates a storage function. Indeed, nearly 55% of the NSC reserves for coconut and 70% for oil palm are in the stem. This function of storage is probably a permanent one, considering the perennial character of this vegetative compartment. For both palms, the highest NSC concentration is at the

top and in the core of the stem. Concentration of the carbohydrates at the top of the stem correlates with palm biology to sustain growth and development of palm leaves, inflorescences and fruits.

For both palms, the carbohydrate reserves are mainly stored as soluble sugars while the small amounts of starch at the top of the stem serve as a transient pool. Soluble sugars are advantageous because they are readily available to supply sink demands. Thus, storage of soluble sugars seems well adapted to the biology of these palms, i.e. large quantities of water in the stem, meristem continuously active, many sinks in simultaneous and extended development with high energy needs, absence of physiological dormancy (Tomlinson, 2006; Adam, *et al.* 2007). Our findings agree with previous studies of the coconut (Mialet-Serra *et al.*, 2005; Ranasinghe and Silva, 2007) and oil palm (Henson *et al.*, 1999; Legros *et al.*, 2006; Yamada *et al.*, 2010).

However, this feature is not shared by the palm family (Arecaceae) since several palms are known to store starch. For example, McPherson and Williams (1998) reported high levels of starch in the stem of three species from the Coryphoideae subfamily, the cabbage palm (*Sabal palmetto*), the date palm (*Phoenix dactylifera*) and the saw-palmetto (*Serenoa repens*). Besides, Van Die (1974) recognized two groups of palms with significant starch content in the trunk. The first group included species forming lateral inflorescences few years before their end of life and which store starch in small quantities (60 to 100 kg) (e.g., *Arenga* spp.). The second group included species flowering only once and which store up to several hundred of kilograms of starch (e.g., *Corypha* and *Metroxylon* (i.e. sago palm).

Van Die (1974) identified a third group with species that do not store starch in the trunk (e.g. *Cocos nucifera*).

For both palms, starch is, thus, a minor NSC component but its amount varies between these two palms. For coconut, our results agree with its position in the group of palms not storing starch. In contrast, for the oil palm, which stores 30 kg of starch per stem, the criteria of Van Die are not fulfilled. Indeed, in contrast to the palms in the first group of Van Die, the mature oil palm forms inflorescences continuously (Adam *et al.,* 2007; Dransfield *et al.,* 2008). Therefore, both the starch content and flowering characteristics of oil palm suggest that oil palm might represent an intermediate type (a fourth possible type of Arecaceae) in this classification.

Sucrose is, by far, the dominant sugar in coconut while glucose is the major sugar in oil palm. Indeed, we showed that in coconut, sucrose represents 80% of the NSC in the whole plant; it is the major sugar at all heights of the stem, in the leaves and in the roots. In contrast, in oil palm, glucose represents 51% of NSC in the whole plant; it is the dominant sugar in the stem, up to the mid-height, in the leave petioles and rachis and in the leaf bases. Yet, the oil palm also contains sucrose, up to 20% of NSC in the whole plant (except in leaflets), but also starch, in the stem apical zones, and fructose, in the leaflets and roots. For both species, high monosaccharide concentrations are found near the root apexes. In this site with high metabolic activity, these monosaccharides might result from an invertase activity. Here, these sugars might be substrates for local use rather than storage sugars for the whole plant. By contrast, in oil palm, the high amount of glucose compared to fructose suggests that glucose results from starch hydrolysis but not

from sucrose hydrolysis. In previous oil palm studies, sucrose, followed by glucose, was identified as the main reserve compound in the stem (Mansor and Ahmad, 1990; Henson *et al.*, 1999). In contrast, Legros *et al.* (2006) reported glucose as the largest fraction of the NSC reserves in the oil palm and Lamade *et al.* (2009) showed the high concentration of glucose and fructose compared to sucrose in the oil palm leaves.

As a general rule, the carbohydrate reserves vary among plant species. In many tree species, the reserve functions are mainly covered by starch. However, sucrose can also be an important component and glucose, fructose, raffinose, stachyose or sugar alcohols, can occur as minor compounds (Hoch *et al.*, 2003; Wurth *et al.*, 2005; Regier *et al.*, 2010). Some trees store particular sugars like sorbitol in most temperate-zone fruit trees (apple, apricot, plum, peach) and mannitol in coffee and olive trees (Stoop *et al.*, 1996; Cheng *et al.*, 2005). Several large grass species (monocots) are known for storing sucrose or fructan in their stem, such as the sugar cane (Komor, 2000), sorghum (Kouressy *et al.*, 2007), wheat and barley (Scofield *et al.*, 2009). Also, the root of sugar beet (dicots) and the leaves of spinach are known to store sucrose (Goldschmidt and Huber, 1992; Getz, 2000). Instead, plants storing glucose are, to our knowledge, uncommon.

The composition, level and distribution of the plant carbohydrates might thus have a chemotaxonomic potential. From this point of view, in this study we show that the coconut and oil palm, like all palms, store the main fraction of their carbohydrate reserves in the stem but, in contrast to numerous palms, do not use starch as a major form of reserve pool. We also show that the coconut and oil palm share the use of soluble sugars as carbohydrate reserves but differ in the nature of

the dominant sugar, in the amount of starch and in the total amount and distribution of the reserve pool. Therefore, in the coconut and oil palm stems, the storage, respectively, of mostly sucrose or of a combination of mainly glucose followed by sucrose and low amount of starch, might represent a chemotaxonomic profile.

Both palms, thus, have in common the use of soluble sugars as carbohydrate reserves, which might illustrate their relationships as members of the Cocoseae tribe. There is little evidence that storage of soluble sugars is shared by other members of the Cocoseae tribe. However, studies have shown soluble sugars stored in the stem of palms, such as *Jubaea* (Gonzalez *et al.*, 2009) and *Syagrus* (Uzelac and Trigueiro, 1978), both members of the Attaleinae subtribe, and as the peach palm (*Bactris gasipaes* Kunth), from the Bactridinae subtribe (Clement *et al.*, 1993). Besides, the Cocosoide group is characterized as a "sweet group"; as a result, species like the coconut and the peach palm are used for the production of heart-of-palm (Clement *et al.*, 1993; Johnson, 2010). Whether the nature and distribution of the carbohydrate pool in palms from the Attaleinae subtribe are similar to the coconut profile, or from the Elaeidinae subtribe similar to the oil palm profile deserve further investigation.

The coconut and oil palm thus differ in their carbohydrate profile and in the total amount and distribution of the reserve pool. Indeed, the oil palm stem stores a high amount of NSC, representing almost 30% of the stem dry matter. In contrast, in the coconut the NSC represent only 12% of the stem dry matter. To compare, Wurth *et al.* (2005) analysed 17 tropical trees and showed that the NSC ranged from 2.3 to 20.4% of the stem dry matter. Similarly, Hoch *et al.* (2003) showed a 1.5-7% range of NSC in the stem dry matter of 10 temperate trees and Cunningham (1997)

showed an average of 6.9% (± 3.1%) of NSC in the stem dry matter of 60 understory palms. On a whole tree basis, oil palm exhibits a two times larger NSC concentration (nearly 20% of dry biomass) than coconut (nearly 10% of dry biomass). While the coconut allocated 17% of NSC to the roots, in oil palm all the NSC are distributed in the above-ground vegetative part, except 2% of NSC in the roots.

In coconut, Mialet-Serra *et al.* (2008) showed that the NSC storage is mainly driven by physiological demand and thus, NSC mobilization contributes moderately with a short-term compensatory function. In contrast, Legros *et al.* (2009c) showed in oil palm that, due to the absence of negative feedback on photosynthesis, storage of the NSC in the stem is the main buffer mechanism. Under favourable climatic conditions, the NSC pool may reflect an over-abundance of photoassimilates and might have a vital function in situations of stresses. We estimate that the NSC are sufficient, in the absence of fresh assimilates, to sustain full growth rate for nearly one or two months in the coconut and seven months in the oil palm (Mialet-Serra *et al.,* 2005; Legros *et al*., 2009c).

These differences between the coconut and oil palm probably reflect functional differences and might illustrate their assignment to different subtribes. Indeed, beside the anatomical differences at the basis of the two subtribes Attaleinae and Elaeidinae, the coconut and oil palm exhibit morphological and growth pattern differentiation. While the coconut continuously produces inflorescences with female and male flowers, the oil palm produces separate male and female inflorescences on the same palm in alternating cycles (Moore and Uhl, 1982; Adam *et al.*, 2005; Perera *et al.*, 2010). Recently, Horn *et al*. (2009) showed that the oil

palm leaf has a mechanically stronger lamina than in coconut and hypothesized that, by limiting the need for lignified tissues; this might reduce the cost in energy. Moreover, the coconut is a slow maturing palm, producing new leaf almost every month and needing five to seven years to fully develop (Perera *et al.*, 2010). Instead, oil palm development from seed germination needs nearly three years with leaf production almost every two weeks and it produces twice as much vegetative dry matter than coconut (Adam *et al.*, 2005; Mialet-Serra *et al.*, 2008; Legros *et al.*, 2009c). Therefore, the distinct carbohydrate profiles for the coconut and for the oil palm could be interpreted as additional evidence for different palm biology.

Carbohydrates have not been used, to our knowledge, for palm taxonomy or phylogeny. By contrast, beside anatomical and morphological studies, several DNA-based phylogenetic analyses contributed to resolve relationships within the palm family (Hahn, 2002; Gunn, 2004; Asmussen *et al.*, 2006; Meerow *et al.*, 2009; Baker *et al.*, 2009, 2011). Yet, several uncertainties still remain. Regarding the coconut, for example, its exact sister relationships, its origin and wild relatives are still matter of debates (Gunn, 2004; Meerow *et al.*, 2009). Genomic data may generate robust phylogenetic hypothesis, but functional biomarkers should also be valuable in palm chemotaxonomy.

There have been few chemical studies in palms. The most extensive investigations concerned phenolic compounds. For example, the wide range of variations in leaf flavonoid composition among palm taxa provided insight into relationships within the family (Williams *et al.*, 1983; Harborne and Williams, 1994; Dransfield *et al.*, 2008). Regarding the Cocoseae tribe, the flavonoid profiles were shown to differentiate this tribe from other tribes of the Arecoideae subfamily (Williams *et*

al., 1983; Dransfield *et al.*, 2008). Moreover, the specificity of certain components within this tribe showed the potential of flavonoids to refine the phylogeny of this tribe (Williams *et al.*, 1983; Dransfield *et al.*, 2008). Some phenolic acids were also characterized in several palm fruits, including coconut and oil palm, but a common pattern between all palms rather suggests a chemotaxonomic marker at the level of the family (Dey *et al.*, 2005; Chakraborty *et al.*, 2006; Neo *et al.*, 2010). But, the value of carbohydrates in the chemotaxonomy of palms has not been evaluated.

This study aimed, therefore, to compare the carbohydrate reserves between two important palm species, the coconut and the oil palm, belonging to different subtribes within the same tribe. Our findings show that each palm exhibits a particular carbohydrate profile. These profiles might, thus, represent chemotaxonomic markers for these palms.

However, since this study was limited to these two palms, the potential of these biomarkers in chemotaxonomy has to be confirmed. Therefore, we suggest exploring the carbohydrate patterns of other palm species from the Cocoseae tribe, focusing on closely related species, to determine the common and distinct characteristics with the coconut (Attaleinae) and oil palm (Elaeidinae). Another possible limitation might be that the carbohydrate patterns could be influenced by both sink demand and source supply, both being sensitive to environmental factors. Yet, studies showed that, in the stem, seasonal variations of the NSC pool were non-significant (Hoch *et al.*, 2003; Wurth *et al.*, 2005). For the coconut and oil palm, we found similar results (Mialet-Serra *et al.*, 2008; Legros *et al.*, 2009c).

The carbohydrate profiles might, thus, be an aid to the taxonomic and evolutionary studies of the Cocoseae palms, in combination with anatomical, morphological and

DNA markers. Applications could lead to improved taxonomic position of these palms and better understanding of their relationships and could also be of biogeographic interest. Besides, further characterization of the carbohydrate metabolism, including key enzymes and minor metabolites, will increase the potential for discrimination and the understanding of palm biological functions.

CONCLUSION

The coconut and oil palm exhibit common but also distinct characteristics leading to different carbohydrate patterns as potential markers. We show that the coconut and the oil palm have in common the storage in their vegetative organs of soluble sugars, as the main carbohydrate reserves. In addition, the coconut and oil palm thus differ in the nature of the dominant sugar, sucrose versus glucose, respectively. Both palms store starch as a minor component, mainly at the stem apex as a transitory reserve. In addition, the coconut and oil palm differ in the total amount and distribution of the reserve pool. On a whole tree basis, oil palm exhibits a two times larger NSC concentration (nearly 20% of dry biomass) than coconut (nearly 10% of dry biomass). While the coconut allocated 17% of NSC to the roots, in oil palm all the NSC are distributed in the above-ground vegetative part, except 2% of NSC in the roots.

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Figure Legends

Figure 1. Concentrations of carbohydrates in longitudinal compartments of the coconut and oil palm stems. Vertical bars indicate standard error of mean (s.e.m.) for 4 replications

Figure 2. Concentrations of carbohydrates in radial compartments of the coconut and oil palm stems at the top and at mid-height. Vertical bars indicate standard error of mean (s.e.m.) for 4 replications

Figure 3. Concentrations of carbohydrates in various compartments of the coconut and oil palm leaves. Vertical bars indicate standard error of mean (s.e.m.) for 4 replications

Figure 4. Concentrations of carbohydrates in various compartments of the coconut and oil palm roots. R1, R2 and R3 represent root diameter classes in decreasing order. Zone 1 is the sub-apical area and zone 2 the proximal area. Vertical bars indicate standard error of mean (s.e.m.) for 4 replications

Figure 5. Concentration of carbohydrates in persistent leaf bases along the stem of the oil palm. Vertical bars indicate standard error of mean (s.e.m.) for 4 replications