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PHOTOSYNTHETIC ASSIMILATION, CARBOHYDRATES IN VEGETATIVE ORGANS AND CARBON REMOVAL IN NUT-PRODUCING AND SAP-PRODUCING COCONUT PALMS

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ABSTRACT

The net assimilation rate, carbohydrate content in leaf and trunk tissues and quantum of carbon removed in sap-producing (SP) and nut-producing (NP) coconut palms were compared. The correlations between sap (toddy) yields of SP palms, and their leaf and trunk carbohydrate content; net assimilation rate; and the pre-tapping phase nut yields, were investigated as possible criteria for selecting coconut palms with potential for high toddy yields. Thirty-five-year-old coconut palms of the Tall variety (*Cocos nucifera* L., *var. typica*), at Bandirippuwa Estate, Lunuwila, Sri Lanka were used for the study. Total soluble sugar content (TSS) in leaf and trunk tissues was higher (62-73 mg g dw^{-1}) than their starch content (24-41 mg g dw^{-1}) in both SP and NP palms. In SP palms, TSS of leaf tissue was higher, and trunk tissue was lower, than in NP palms. The total carbohydrate (TC) content in the trunk was generally higher than in leaves of both SP and NP palms. In SP palms, the ratio of TSS : starch was higher in leaves, and lower in the trunk, than in NP palms. Net assimilation rates and carbon removal by the produce (nut or sap) was similar in NP and SP palms.

There was no significant correlation between sap yields and TSS and starch contents of leaf or trunk tissues of palms, before and during tapping; and the nut yields before; and the NAR during tapping. These parameters are therefore not of predictive value for selecting coconut palms for high sap yield.

Key words: *Cocos nucifera* L., coconut, inflorescence sap, toddy, soluble sugars, starch, physiology, assimilate storage

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an arborescent, monocotyledonous species with indeterminate growth, producing inflorescences continuously over several decades (Menon and Pandalai, 1958). The inflorescences develop into mature coconut fruits (nuts) after pollination. The unopened 'spadix' can

also be used to extract inflorescence sap (toddy). Coconut inflorescences are produced throughout the year, and with 12-14 emerging annually they are a substantial drain on plant assimilates. This continuous demand is met by the production of photo assimilates. Crop yields depend on the efficiency of dry matter production and its partitioning into economic produce; a phenomenon applicable to coconut as well (Jayasekara *et al*., 1996). Perennial plants accumulate non-structural carbohydrates in vegetative organs, during periods of excess production of photo assimilates, to be used when demand exceeds production (Dickson, 1991; Kozlowski, 1992). A study of this phenomenon could help to understand the physiological and biochemical processes involved in the production of coconut fruit (nuts) and inflorescene sap (toddy).

Toddy is obtained by artificially stimulating (tapping) the fully extended, unopened tender spadices (inflorescences), just before the spathes split and the spikelets emerge. Freshly collected toddy which contains 12-18% sugar, mainly as sucrose, is used as a beverage or converted to products such as alcoholic drinks (fermented toddy and arrack), sugar, treacle, jaggery and vinegar (Nathanael, 1966; Pethiyagoda, 1978). Toddy production brings much higher profits to growers than nut production (Ranasinghe *et al.,* 1999). Toddy yields can be increased by applying Ethrel to the axil of the inflorescence (Ranasinghe and Waidyanatha, 2003). Coconut varieties, seasons and management factors have a considerable influence on toddy yields (Nathanael, 1966; Ranasinghe, 1997). In addition, there is wide palm to palm variation within a variety, with yields ranging from 200 ml to 1500 ml toddy palm^{-1} day^{-1}. This variability may be attributed to genetic differences among the coconut palms. Currently, selection of coconut palms for toddy production is based only on qualitative criteria. Nathanael (1955) lists long internodes, uniform inflorescence production and relatively thin inflorescence sheaths as criteria used for selecting coconut palms for high sap yield. Toddy tappers in Sri Lanka traditionally use visual criteria such as high number of fronds, long and relatively thin inflorescence sheaths, which facilitates the bending of flower stalks without splitting, to select palms for 'sustainably high toddy yields'. However, these qualitative selection criteria do not give consistent results. Quantitative scientific information on criteria to select coconut palms with potential for high toddy yields has not been reported. The physiological and biochemical parameters of a palm measured during nut production preceding tapping, or during tapping itself, may well be correlated with its toddy yields. Therefore, the objectives of this study were *a)* to compare the net assimilation rate, removal of carbon in the produce, and carbohydrate content in the leaf and trunk tissues of nut-producing and toddy or sap-producing palms and *b)* to determine the

predictive value of correlations between toddy yield and leaf or trunk carbohydrates, and net assimilation rate of palms, before and during tapping, to select high toddy yielding palms.

MATERIALS AND METHODS

Selection of palms

Eighteen coconut palms of the Tall variety (*Cocos nucifera L.* var. typica), 35 years of age, were selected randomly from a large population at Bandirippuwa Estate, Lunuwila, situated in the north-west of Sri Lanka. The palms had been managed uniformly in accordance with agronomic and cultural practices recommended by Coconut Research Institute of Sri Lanka (Liyanage, 1999).

Application of treatments and data collection

The experiment was conducted over a period of two years. Two sets of treatments were applied, on nine palms each, as follows (Figure 1):

- Nut production for one year followed by sap production for one year (NP-I and SP).
- Nut production continuously for two years (NP-II)

In the first year, nut yields of all 18 selected palms (NP-I and NP-II) were recorded. Then, the nine NP-I palms were switched over to toddy or sap production (SP) and the balance nine palms continued on nut production (year-2 NP-II).

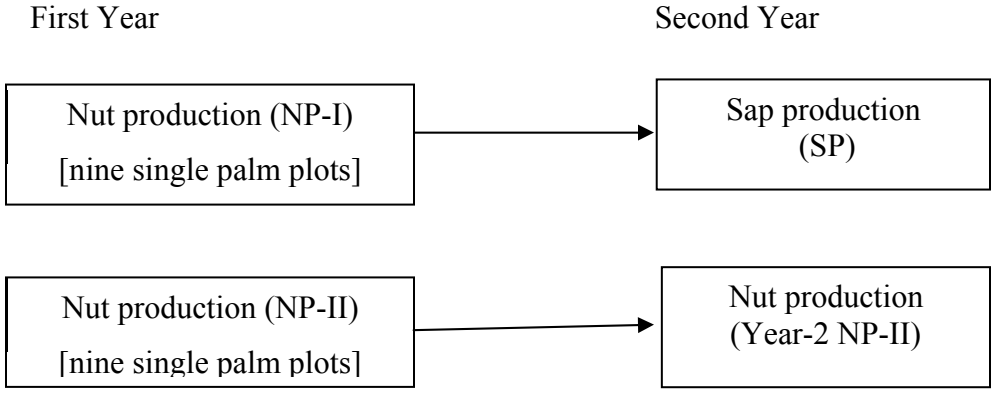

Fig. 1: Diagram showing the treatments applied in the first and second years

All parameters were measured and recorded separately for each palm. The nut yield of NP-I and NP-II palms was recorded at monthly intervals. SP palms were tapped according to the technique described by Ranasinghe and Waidyanatha (2003). A thin slice of the spadix was pared transversely, twice a day, morning (7.00–9.00 hrs) and evening (16.00–18.00 hrs). All the spadices were tapped, and the toddy yield was measured at the time of paring.

To compare net assimilation rate, carbon removed by the produce, and carbohydrate content of vegetative tissues, of sap-producing and nut-producing palms, data were collected from SP and Year-2 NP-II palms. To test the correlation of sap yields with net assimilation rate and carbohydrate content of vegetative tissues, before and during tapping, data were collected from NP-I and SP palms, respectively.

Determination of Net Assimilation Rate

The net assimilation rate (NAR) of leaves was measured using LI-6200 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA), from 9.00 to 12.00 hrs under a clear sky with full sun (PAR 1200-1400 μmol cm^{-2} s^{-1}). The ninth leaf (taking the youngest fully expanded leaf as one, and counting downwards) was used to measure NAR of NP-I and Year-2 NP-II palms, and the leaf subtending the youngest spadix in tapping (eighth or ninth leaf) for SP palms.

Determination of carbohydrate content

Starch and total soluble sugar (TSS) content in the trunk and leaves of NP-I and Year-2 NP-II palms and SP palms were measured at three monthly intervals. The ninth leaf of NP-I and Year-2 NP-II palms and the subtending leaf of the tapping spadix (eighth or ninth leaf) of SP palms were sampled for starch and sugar analysis. Two leaflets from the middle portion of the selected leaf were taken for analysis. Trunk tissues were collected from just beneath the leaf canopy where the highest trunk-carbohydrate content is found (Mialet-Serra *et al.*, 2005). Two core samples were drawn from opposite sides of the trunk, at a depth of 6.0 cm from the surface, using an electric drill with a 5 mm drill bit, causing minimum damage to the trunk. The leaf and trunk tissues were collected between 9.00 and 11.00 hrs, and immediately stored in ice to minimize formation of polyphenolic compounds. Later they were oven dried for 48 hours at 60^{o}C in a fan forced oven. The dried samples were finely powdered using a high speed micro mill

(Model: Retsch-MS, Germany). A sample of 0.5 g was placed in 10 ml of 80% ethanol (analytical reagent, BDH Laboratory Supplies, UK) for 15 min. in a water bath at 60^{o}C and transferred to a centrifuge tube with subsequent washing with 2 ml of 80% ethanol. The suspension was then centrifuged (Kubota – 5100 table top centrifuge, Kubota Corporation, 29-9, Tokyo, Japan) for 10 min. at 3500 rpm and the supernatant decanted. The residue was boiled again with another 5 ml of 80% ethanol for 10 min. and the supernatant was again collected after centrifuging for 10 min. at 3500 rpm. The two supernatants were combined and the residue was kept aside for analysis of starch. The consolidated supernatant was mixed with 5.0 ml of chloroform (Analytical reagent, BDH Laboratory Supplies, UK) and 10 ml of distilled water, shaken well and kept for 10 min. to separate the aqueous layer and the organic layer containing chlorophyll. The organic layer was discarded and the aqueous layer containing water soluble compounds in leaf and trunk samples was concentrated to a volume of 5.0 ml in a roto-evaporator (Rotavapor RE-111 with Buchi 461 water bath, Laboratoriums - Technik, Flawil, Switzerland) for sugar analysis. The total sugar content was determined by the Phenol Sulphuric method (Dubois *et al.,* 1956).

The residue was suspended in 10 ml of distilled water and boiled for 20 min. in a water bath and allowed to cool to room temperature. Two ml of 1% α-Amylase (1 g of α-Amylase from *Bacillus subtilis*, Fluka Chemical, Switzerland, dissolved in 100 ml of 0.2 M sodium acetate at pH 4.5) was added to the suspension and allowed to stand overnight at 42^{o}C in a water bath. The suspension was then centrifuged at 3500 rpm for 10 min. and the supernatant was analysed for total sugars (Dubois *et al*., 1956) to determine the starch content.

Determination of carbon removal by nuts and sap

Estimation of carbon removal by nuts of Year-2 NP-II palms was based on the carbon content of each fruit component: husk, shell, kernel and nut water (L L W Somasiri, personal communication), and the number of nuts produced by these palms. Sucrose being the predominant carbon source (12-18%) in the sap, the total carbon removed as sucrose was taken as the total carbon removed by the sap or toddy (Ranasinghe and Waidyanatha, 2003).

Statistical analysis of data

A completely randomized design was used for the experiment, with single tree plots. The data was analysed using the SAS statistical package, with

one-way ANOVA. Correlations between parameters were examined with Pearson correlation analysis.

RESULTS

Yield, net assimilation rate and removal of carbon by the produce of Year-2 NP-II and SP palms

The mean annual yield of Year-2 NP-II and SP palms were 67 nuts and 266 liters sap per palm, respectively. The NAR and the amounts of carbon removed by the produce (nuts and sap) were similar in nut–producing and sap-producing palms (Table 1).

Table 1: Net assimilation rate of nut-producing (Year-2 NP-II) and sap-producing (SP) coconut palms and amount of carbon removed in the nuts and sap (toddy). Number of palms per treatment = 9

Type of production	Net assimilation rate ($\mu mol\ m^{-2}\ s^{-1}$)	Carbon removed by the produce (Kg/palm/year)
Year-2 NP-II palms	9.76	20.50
SP palms	10.23	16.75
s.e.	0.81	1.13

Carbohydrate content in leaf and trunk tissues of Year-2 NP-II and SP palms

Total soluble sugar (TSS) content (62-73 mg gdw^{-1}) was considerably higher than the starch content (24-41 mg gdw^{-1}) in leaf and trunk tissues in both nut-producing (Year-2 NP-II) and sap-producing (SP) palms. TSS content of leaf tissues was significantly higher ($p \leq 0.001$) in SP palms than in Year-2 NP-II palms. However, TSS content of trunk tissues was significantly higher ($p \leq 0.05$) in Year-2 NP-II palms than in SP palms. Starch content in trunk tissues was significantly higher ($p \leq 0.001$) than in leaf tissues, of both Year-2 NP-II and SP palms. However, in SP palms, the leaf starch content was lower, and the trunk starch content higher, than in Year-2 NP-II palms, though not statistically significant (Table 2).

Table 2: Carbohydrate content (total soluble sugars and starch) in leaf and trunk tissues of nut-producing (Year-2 NP-II) and sap-producing (SP) coconut palms. Number of palms per treatment = 9

Type of production	Total soluble sugars (mg gdw^{-1})		Starch (mg gdw^{-1})	
	Leaf	Trunk	Leaf	Trunk
Year-2 NP-II palms	61.64	72.33	26.07	37.08
SP palms	72.54	62.11	23.53	41.42
s.e.	1.70	2.83	0.99	3.06

Total soluble sugar and starch are referred to collectively as total carbohydrates (TC). TC content in trunk tissues was generally higher than that of leaves in both nut-producing and sap-producing palms. In SP palms, total soluble sugar:starch ratio in leaf tissue was higher, and in trunk tissue lower, than in Year-2 NP-II palms (Table 3).

Table 3: Total carbohydrate content (TC) and ratio of total soluble sugar (TSS) to starch, in leaf and trunk tissues, of nut-producing (Year-2 NP-II) and sap-producing (SP) palms. Number of palms per treatment = 9

Type of production	TC (mg gdw^{-1})		TSS : starch	
	Leaf	Trunk	Leaf	Trunk
Year-2 NP-II palms	87.7	109.41	2.41	2.24
SP palms	96.07	103.53	3.12	1.64
s.e.	1.53	2.55	0.08	0.15

Testing potential selection criteria for high sap yielding coconut palms

(a) Correlation of sap yield of SP palms with physiological and biochemical parameters of these palms before they were tapped i.e. of NP-I palms.

Analyses were performed to test the correlations between sap yield of SP palms and the TSS and starch contents of leaf and trunk tissues and the nut yields of these palms before they were tapped, i.e. of the NP-I palms. Sap yield was positively correlated with the TSS content in leaf and trunk tissues; and negatively correlated with starch content in leaf and trunk tissues, and the nut yields. However, these correlations were not statistically significant (Table 4).

Table 4: Correlation coefficients for the association between sap yields of SP palms and total soluble sugars (TSS) and starch, in the leaf and trunk tissues, and the nut yields prior to tapping (i.e. of NP-I palms). No. of palms per treatment = 9

	TSS (mg gdw^{-1})		Starch (mg gdw^{-1})		Nut yield (No./palm/yr)
	Leaf	Trunk	Leaf	Trunk	
Sap yield (ml/palm/day^{1})	0.3499	0.0004	-0.0342	-0.0824	-0.2076
Significance	ns	ns	ns	ns	ns

ns: not significant

(b) Correlation of sap yield of SP palms with physiological and biochemical parameters of these palms during tapping

Analyses were performed to test the correlations between sap yield of SP palms and the TSS and starch contents in their leaf and trunk tissues, and the NAR of these palms during tapping. Sap yield was negatively correlated with TSS and starch content of leaf tissues and positively correlated with TSS and starch content of trunk tissues, and the NAR. However, all these correlations were not statistically significant (Table 5).

Table 5: Correlation coefficients for the association between sap yields of SP palms and TSS and starch in the leaf and trunk tissues, and net assimilation rate (NAR) of these palms during the tapping period. Number of palms per treatment = 9

	TSS (mg gdw^{-1})		Starch (mg gdw^{-1})		NAR (μmol m^{-2} s^{-1})
	Leaf	Trunk	Leaf	Trunk	
Sap yield (ml/palm/day)	-0.0354	0.3143	-0.2763	0.1183	0.1169
Significance	ns	ns	ns	ns	ns

ns: not significant

DISCUSSION

In the present study, the NAR, leaf and trunk carbohydrates, and removal of carbon in the produce of sap–producing (SP) and nut-producing (NP) palms were compared. TSS of leaf and trunk tissues was approximately twice their starch content in both NP and SP palms. The starch content of the trunk was always higher than that of leaves. Furthermore, in the SP palms, total carbohydrate (TC) content, TSS content, and sugar:starch ratio of leaves were higher, and starch content lower than in NP palms. However, this is reversed in trunk tissues, with TC content, TSS content and sugars:starch ratio being lower, and starch content being higher in SP palms. The rate of net assimilation, the main process of carbohydrate accumulation in plants, and the removal of carbon from the palm were similar in NP and SP palms. Therefore, it was evident that the pattern of assimilate partitioning, storage and utilization of food reserves in vegetative organs vary with the type of produce according to the fluctuating demands of active 'sinks'.

Sucrose, the transport form of carbohydrates in many plants, was found to be the dominant sugar in the vegetative parts of coconut (Mialet-Serra *et al*., 2005). Under favourable environmental conditions, 17 year-old Vanuatu Red Dwarf (VRD) x Vanuatu Tall (VT) hybrid palms, contained little starch but had large quantities of sucrose, mainly located in the trunk. In addition to sucrose, they found large glucose and fructose pools in the leaves near the apex of the trunk, and in the terminal portions of large roots. In oil palm too, a relatively high concentration of sugar was found in the sub apical region of the trunk (285 mg g^{-1}, Henson *et al*., 1999). As in the present study, starch content in the trunk was quite low in both oil palm (24 mg g^{-1}) and VRD x VT hybrid coconut (between 11-40 mg g^{-1}) (Henson *et al*., 1999; Mialet-Serra *et al*., 2005). In contrast to most higher plants (Glerum, 1980; Kozlowski, 1992), starch is not the major carbohydrate storage product in coconut. Coconut palms seem to accumulate sugars (sucrose) as the main reserve, with small quantities of starch, as a transient pool.

In comparing the two production systems, nuts and sap, a prominent feature was the higher level of soluble sugar in leaf tissues of sap-producing palms. The leaf sampled for sugar analysis was that subtending a spadix in tapping. It is likely that the subtending leaves, with 'tapping spadices' in their axils, were the main source of sugars in the exuded sap. The rise of sugar levels in the subtending leaf may be due to sugars imported from other leaves of the canopy, and the conversion of starch reserves of the subtending leaf into soluble sugars, as evidenced by the higher leaf sugar:starch ratio in SP palms compared to NP palms. Furthermore, the relatively lower sugar level of

trunk tissues in SP palms suggests less sugar is being transported from the canopy to the trunk, as food reserves in SP palms.

The source of the copious flow of sap that occurs in a 'tapped' tree is not clearly understood. The exudation of inflorescence sap in coconut, and probably some other palms too, may be regarded as the mobile aqueous phase of the sieve tube system of these trees, flowing to an artificial sink, the bleeding site. Pethiyagoda (1978) suggested that the large volume of exudates produced during tapping and the high sugar concentration of sap indicate that the material is drawn from stored resources and is in excess of currently synthesized sugars. In a coconut palm used for nut production, there are about 12-14 bunches of developing fruits at a time. Therefore, as the 'sink demand' is very high, low storage of starch in the trunk can be expected. In palms used for sap production, however, there are only one or two spadices exuding sap at a given time. Therefore, the conversion of some photo assimilates of canopy leaves (other than the subtending leaves) to starch, and storage in trunk tissues, is likely. This contention is supported by the relatively high trunk starch content recorded in SP palms than in NP palms (Tables 2 and 3). However, it is also important to note here that the rate of bleeding from a single inflorescence is several times higher than the rate of assimilation flow into a single bunch during fruit maturation (Van Die, 1974).

A positive correlation of coconut yield with rate of photosynthesis, number of leaves in the canopy and chlorophyll content has been observed in coconut (Chacko Mathew and Ramadasan, 1974; 1975; Shivashankar *et al*., 1982). However, correlations between inflorescence sap yield and such physiological factors, which could be used as selection criteria for potential high toddy yielders, have not been reported to date. In the present study, there was an attempt to correlate plausible biochemical and physiological factors with the yield of sap. It was revealed that there is no significant correlation between sap yield and the carbohydrate content in vegetative parts before and during tapping, rate of net assimilation during tapping, and the yield of coconut before tapping. Therefore, these parameters cannot be recommended as criteria for selecting coconut palms with high sap yield. Furthermore, this reconfirms the fact that the high nut yielding palms are not necessarily the high sap yielders as proposed by Pethiyagoda (1978).

CONCLUSION

Total soluble sugar content in the leaves and trunk of coconut palms is twice the magnitude of their starch content, in both nut-producing and sap-producing palms. In sap-producing palms, the content of total soluble sugar in leaves is higher, and in the trunk lower, than in nut-producing palms. Net assimilation rates and carbon removal by palm produce (nuts or sap) is similar in the two production systems. Thus carbohydrate reserves in leaf or trunk tissue, net assimilation rate or the nut yield of a palm, are not acceptable as selection criteria to screen coconut palms for high sap yield.

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