Identification of metabolites involved in leaf and bunch edification using $^{13}C/^{12}C$ on oil palm trees in North Sumatra

JESIUM 2012 Leipzig, September 2-7, 2012

Emmanuelle Lamade[1], Eko Setiyo[2], Rosario Lobato Rodrigues[3], Chantal Fresneau[4], Marlène Lamothe[5], Caroline Mauve[5], Diana Sketriene[4], Guillaume Tcherkez[5] and Jaleh Ghashghaie[4]

1. CIRAD-PERSYST, UPR34 Performance of Perennial Cropping Systems, Montpellier, France, corresponding author: emmanuelle.lamade@cirad.fr,
2. Indonesian Oil Palm Research Institute, IOPRI, 53, Jalan Katamaso, North Sumatra, Medan, Indonesia.
3. Embrapa Amazonia Ocidental Rodovia AM-010, km 29, Manaus, Brasil.
4. ESE, University of Paeis XI-Sud, CNRS-UMR 8079, Orsay Cedex F-91405, France.
5. Metabolism-Metabolom Plateform, IFR 87, IBP, University of Paris XI Sud, Orsay Cedex F-91405, France.

Introduction

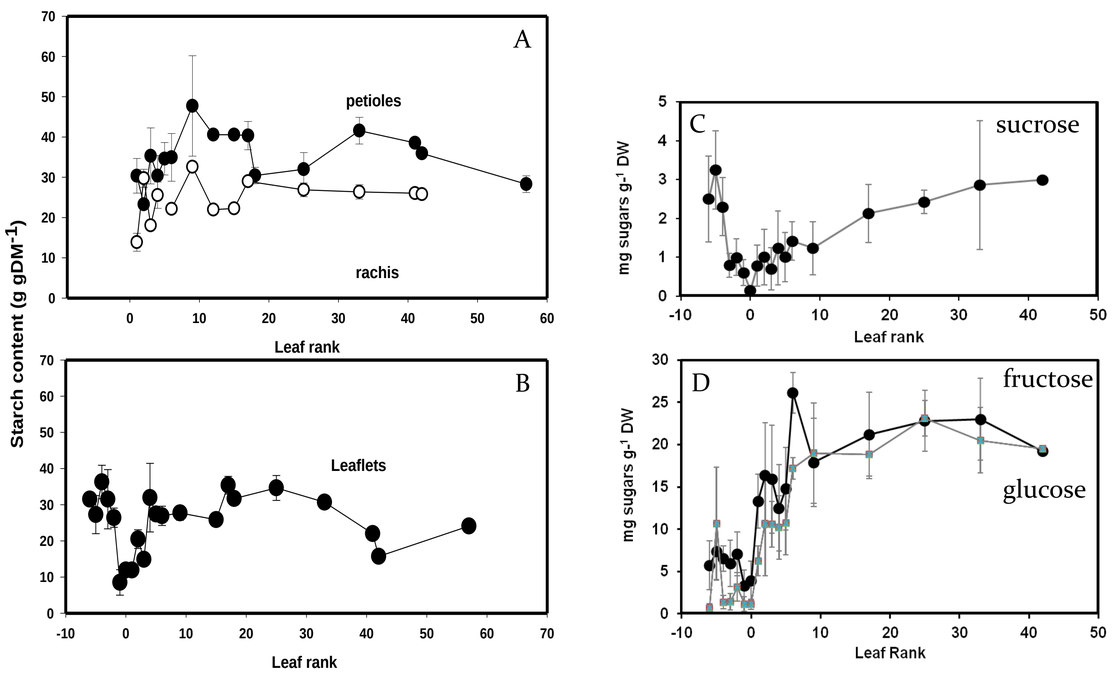
Oil palm (*Elaeis guineensis* Jacq.is one of the most productive perennial crops in the world with maximal yield at around 40 t ha-1 of fresh fruit bunches. To ensure such a high yield oil palm owns high photosynthetic rate (until 30 µmol m^{-2} s^{-1}) related to a continuous leaf emission all along the year, important standing biomass (50 t ha^{-1}) and high carbon allocation to the fruits. The undestanding of the main metabolic pathways involved in bunch elaboration is a very important goal in oil palm agronomical research. Meristem zone (palm heart) trunk and roots seem to be very important sinks for carbon reserves, which could be remobilized during fruit development and leaf emission. In that context, the use of the carbon isotope discrimination ($^{13}C/^{12}C$) could be a usefull tool for identifying the main metabolites responsible for vegetative and reproductive growth.

Materials and Method

Important samplings were done since 2003 to 2007 in Aek Pancur Research Station (3°30'N,98 °48'E, North Sumatra, Indonesia) on ten trees issued from crossing "*tenera x pisifera*", belonging to clonal material "MK60". Organic matter samplings were done on leaves (composed by petiole, rachis and leaflets) from rank -6 to rank 62 , on trunks (palm heart, terminal buds, meristem zone, up part, middle part and bottom part) , on roots (primary, secondary, tertiary+absorbant roots) and on bunch components (rachis, fruits and spikelets). Metabolic (soluble sugars and starch contents)as well as ^{13}C isotope analyses were performed at the Metabolism-Metabolom plateform in Orsay . Lipids quantification were done at ESE.

Results

The Leaves



From starch and sugar contents and the respective $\delta^{13}C$ values as well as $\delta^{13}C$ of bulk organic matter of all organs studied, some hypotheses concerning general pattern of carbon allocation at tree level have been merged : at the leaf level, from "rank -6" to "rank 1" (rank 1 : full expansion of the leaflets, passage from heterotrophy to autotrophy), clearly sucrose and starch (and their transitory phases) are mostly used for leaf growth between rank-1 and rank 0 (Fig. 1 B and D). At the same time $\delta^{13}C$ values of OM and starch are showed ^{13}C-depletion indicating a change in the carbon pool utilization. From leaf rank -6 to -1, the ^{13}C-depletion of OM is due to the rapid use of sucrose (Fig. 1C, which is known to have a ^{13}C-enrichment signature) and the starch (Fig.1. F). When the leaf becomes heterotrophic at rank 1, soluble sugars as glucose and fructose are mainly produced (Fig. 1.D) at the same time as starch (Fig. 1 B). Petiole compared to rachis could be one accumulated zone for the starch used during leaf edification (Fig. 1 A).

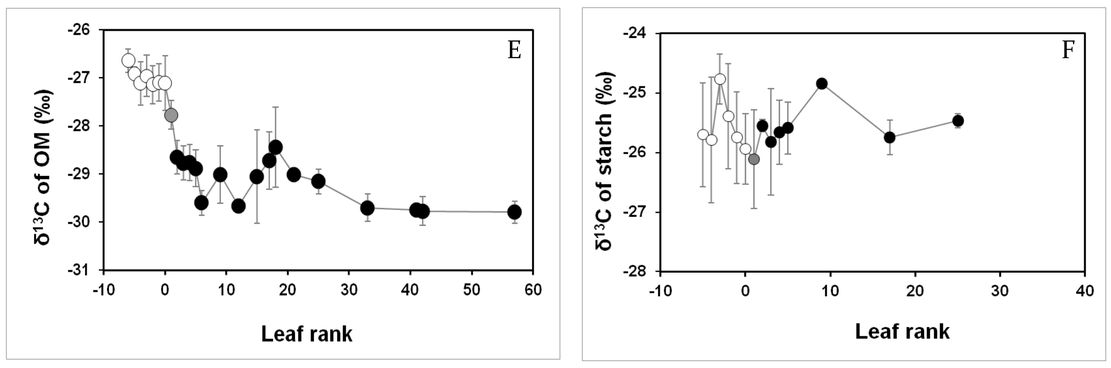


Fig. 1. A,B,C,D,E,F. A: Evolution of starch content in rachis and petioles from rank 1 to rank 57 (means and standard errors in bars). B: evolution of starch content in leaflets from rank -6 to rank 60 (means and standard errors in bar). C: sucrose content in leaflets from rank -6 to rank 42. D: glucose and sucrose content in leaflets. E: variation of $\delta^{13}C$ of OM in leaflets from rank -6 to rank 60 with heretrophic stage in white circle, autotrophic stage in black and in grey the passage between the two stages. E: variation of $\delta^{13}C$ of starch in leaflets from rank-6 to rank 27.

The Roots

Roots do not contain that much of starch (Fig. 3 A) and soluble sugars. $\delta^{13}C$ values of OM are specific to heterotrophic organs. A tendency is oberved for the type roots II which seems to be elaborated with more ^{13}C-enriched products. (Fig. 3 B)

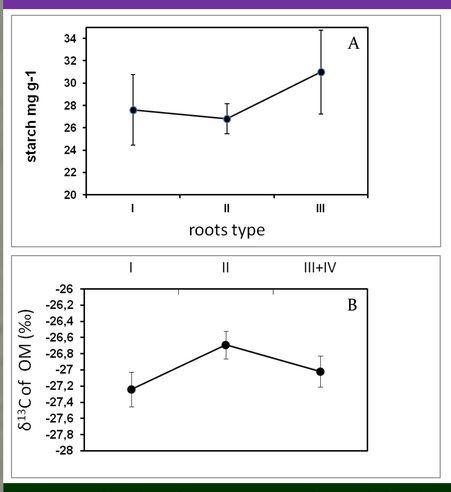
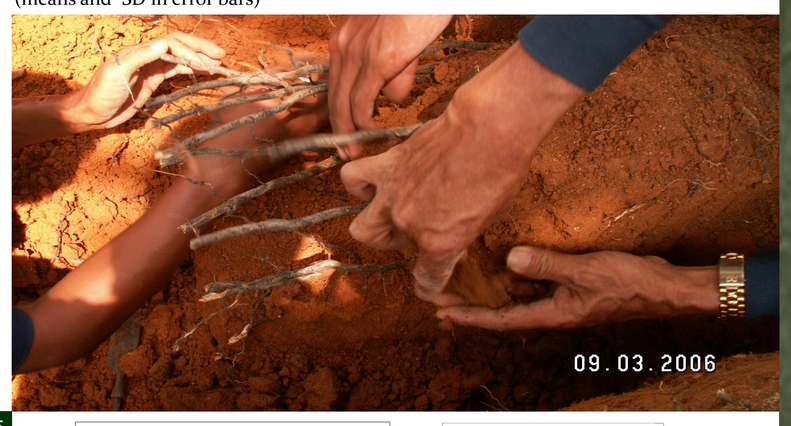
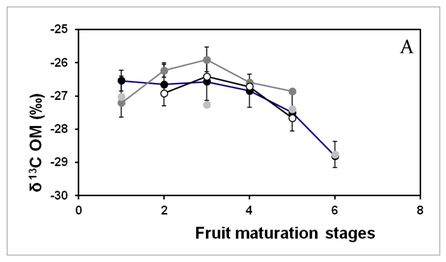


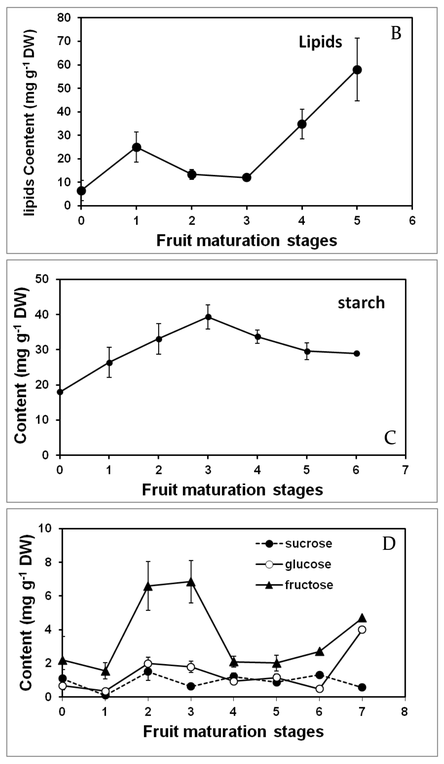
Fig. 3 A B. A: evolution of starch content in different roots types (I: primary, II: secondary, III: absorbant roots. B: variation of $\delta^{13}C$ values of OM in different roots types (means and SD in error bars)



The trunk: the C reserve pool used for leaf elaboration is located at the trunk heart (Meristem: mainly sucrose, Fig. 4 A). It could be filled directly by assimilats ($\delta^{13}C$ values of OM comparatively more ^{13}C-depleted). Then starch (important in the terminal bud, Fig. 4 C) is mobilized in the leaf development just before autotrophic transition (rank -6 to 0): its ^{13}C signature quite depleted indicating a photosynthetic source (Fig. 3 B). A second reserve pool is located at the top of the trunk fulled by starch presenting a ^{13}C signature relatively more enriched (Fig. 3 B). This second reserve could be responsible for the fruits filling.

The Fruits





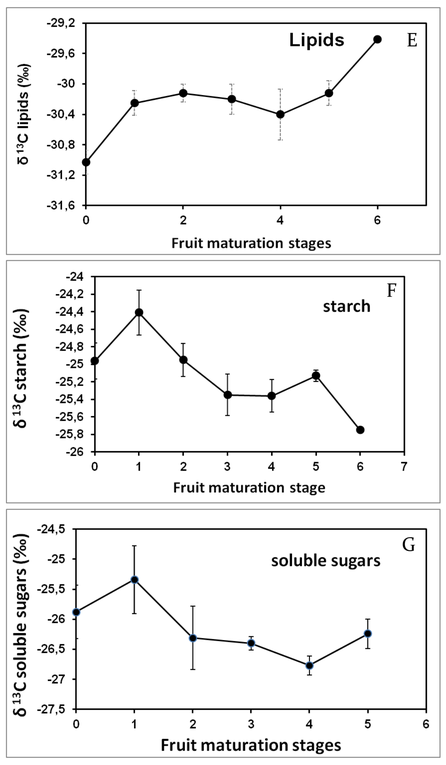
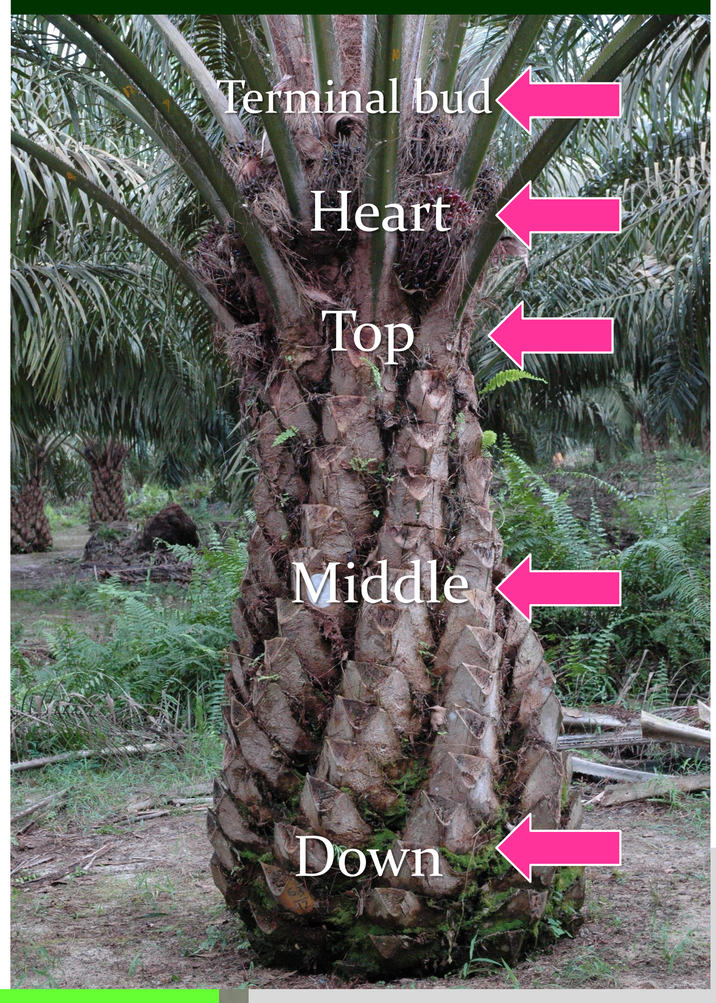
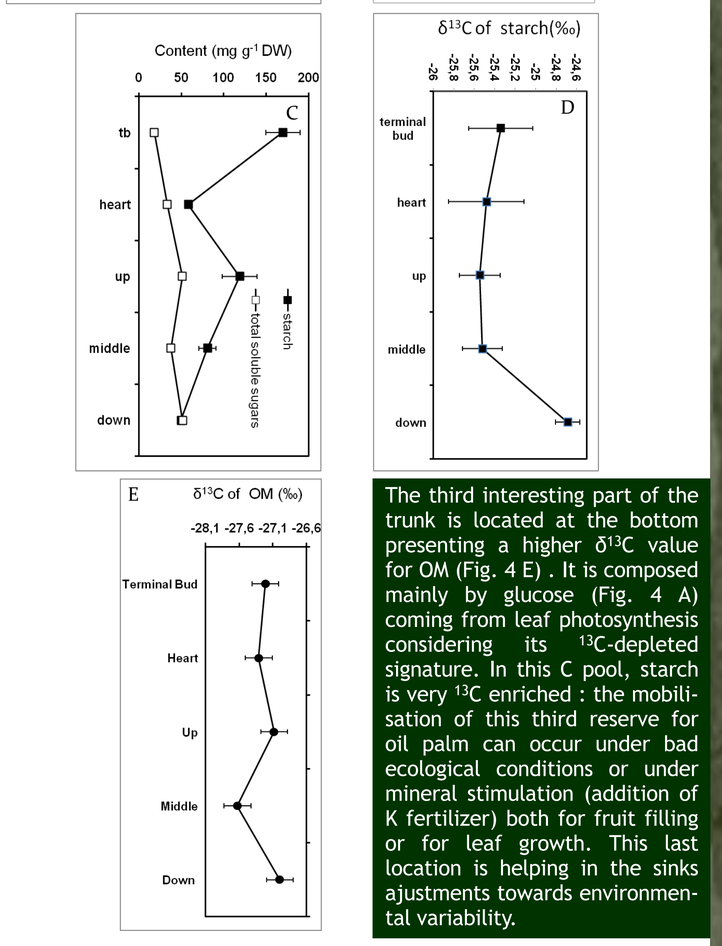


Fig. 2 A,B,C,D,E,F,G. A: evolution of $\delta^{13}C$ of OM for different bunches at 6 different developments stages; B: variations of lipids content during fruit develoment stages; C: variations of starch content during fruits development stages. D: variations of soluble sugars during fruit development stages. E: evolution of $\delta^{13}C$ of lipids during fruits development stages. F: evolution of $\delta^{13}C$ of starch during fruits development stages. G: evolution of $\delta^{13}C$ of soluble sugars during fruits development stages (all with means with SD on error bars).

Concerning fruits and bunches (Fig. 2.A,B,C,D,E,F,G) it is possible to see on graphes the transformation at stage 3 (3 months after pollination) of the soluble sugars (Fig.1 D) and starch (Fig. 1 C) in lipids (Fig. 1 B). This is the oleosynthesis process. $\delta^{13}C$ of OM is becoming more negative (Fig. 1. A) indicated ^{13}C-depletion during fruits maturation. This due to the important proportion of lipids which show (Fig. 1 E) ^{13}C-depleted signature at any development stage. The evolution of ^{13}C signature of starch from stage 1 to 6 shows ^{13}C depletion again during fruits maturation. This point ehanced what is observed on total OM. Apparently, starch is the main reserve sugar used for fruit filling. For the beginning of the fruit development at stage 1: this starch could come from the basal part of the trunk where similar starch $\delta^{13}C$ signature (-24.4 ‰) is found. Then during the last month of maturation, fruits are apparently filled with more ^{13}C-depleted sugars coming from new elaborated assimilat pool.

The Trunk





The third interesting part of the trunk is located at the bottom presenting a higher $\delta^{13}C$ value for OM (Fig. 4 E) . It is composed mainly by glucose (Fig. 4 A) coming from leaf photosynthesis considering its ^{13}C-depleted signature. In this C pool, starch is very ^{13}C enriched : the mobilisation of this third reserve for oil palm can occur under bad ecological conditions or under mineral stimulation (addition of K fertilizer) both for fruit filling or for leaf growth. This last location is helping in the sinks ajustments towards environmental variability.

Fig. 4. A,B,C,D,E. A: variations of soluble sugars along trunk height. B: **$\delta^{13}C$ values of soluble sugars along trunk height. C: evolution of starch and total soluble sugars along trunk height. D: $\delta^{13}C$ values of starch along trunk height. E: $\delta^{13}C$ values of OM along trunk height. All with means and SD with errors bars.**

Conclusion

$^{13}C/^{12}C$ is an interesting tracer for identifying oil palm C reserves pools and metabolites mobilized for leaf growth and bunches edifications. It is the first time that a complete scheme is proposed with at least 3 reserves pool all located in trunk. The first one (heart) is responsible for sustain the indefinite leaf emergence, the second (top of the trunk) for bunches development, the third (at the bottom of the trunk) for sink ajustment during last month of oleosynthesis and leaf edification under bad ecological conditions.