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Molecular networking on wild "Tali" (*Erythrophleum*, Fabaceae) from Central tropical Africa

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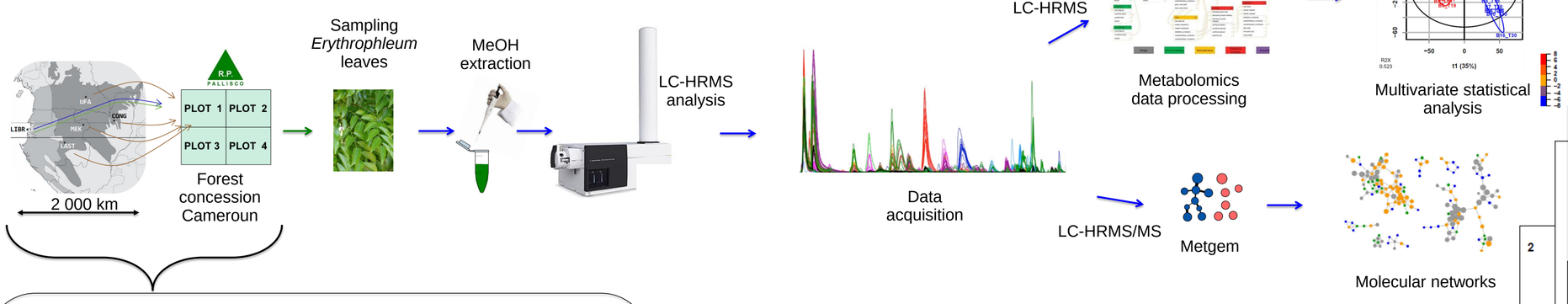
Plant secondary (or specialized) metabolites (SM) are essential for plants which rely on specific set of molecules to handle basic life functions like reproduction (e.g. pollinator attraction) or defense (e.g. herbivore deterrence). As metabolites are the result of a complex network of gene expression, protein expression and interactions and other regulatory processes, they are therefore closer to the phenotype than transcriptome or proteome. Untargeted metabolomics allows to investigate the metabolic phenotypes of plants including the diversity of SM. As SM are produced by specific biosynthetic pathways, most metabolites have characteristic structural features and often show a clear phylogenetic signature.

The tropical woody genus ***Erythrophleum*** (Fabaceae, Caesalpinioideae) contains original cassaine diterpenes, a subfamily of the cassane diterpenes restricted to various Fabaceae genera. The cassane skeleton is structurally characterized as a tricyclic diterpene with a substitution by ethyl group in C-13 position and a methyl group at C-14 position.

Cassaine diterpenes are known for their non-steroidal inhibition of Na^+/K^+-ATPase in a way similar to that of the digitalis glycosides. In addition to this cardiotonic effect, the traditional use of *Erythrophleum* is based on emetic, antimalarial, antibacterial and anti-inflammatory properties.

In this study, we used two closely related species of *Erythrophleum*, *E. suaveolens* and *E. ivorense*, to investigate the metabolic diversity of their cassaine diterpenes. After a metabolomic exploration by LC-HRMS and multi-block correlation of neutral genetic diversity with metabolome, we have developed a molecular networking dereplication pipeline based on MS/MS focusing on the cassane diterpenes family. For molecular network construction the user-friendly Metgem software has been used. Principal results of dereplication will be presented in the poster and interpretations of the metabolome will be proposed for the different genotypes.



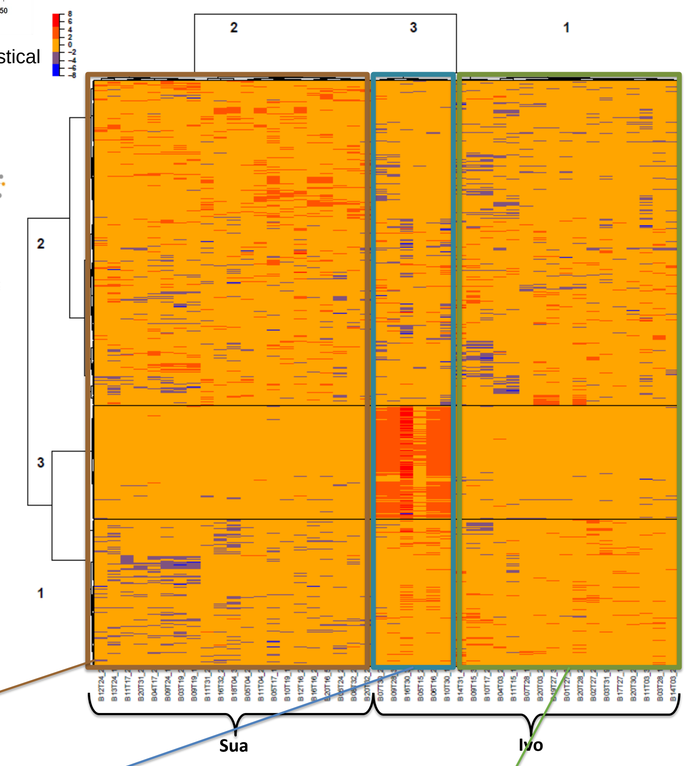
Species	Population/ Country (*)	Population abbrev.	GPS coordinates (long/lat)	Genetic groups (**)
E. ivorense	Libreville/C	LIBR	9.3727/0.6187	IvoS
E. suaveolens	Ouesso/RC	CONG	16.4892/1.2926	SuaN
	UFA30/C	UFA	13.9128/3.4287	SuaN
	Lastourville/G	LAST	12.952/-0.5345	SuaS
	Mekambo/G	MEK	13.9396/0.9618	SuaS

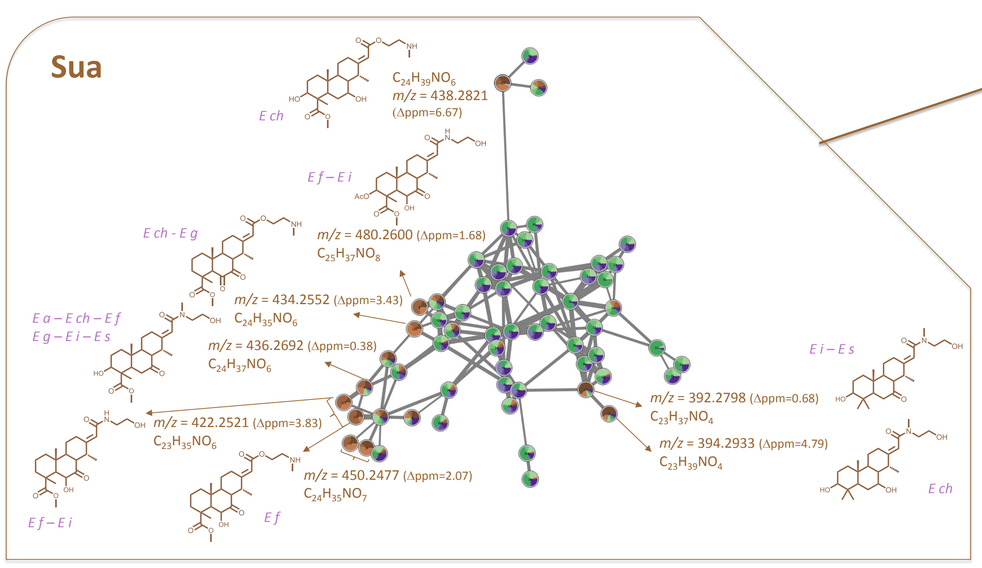
(*) C : Cameroon, RC : Republic of the Congo, G : Gabon
(**) Genetic groups refer to the assignment of the different populations to larger genetic groups based on microsatellite markers. *IvoS: ivorense South, SuaN: suaveolens central North, SuaS: suaveolens central South* based on Duminil et al (2013).

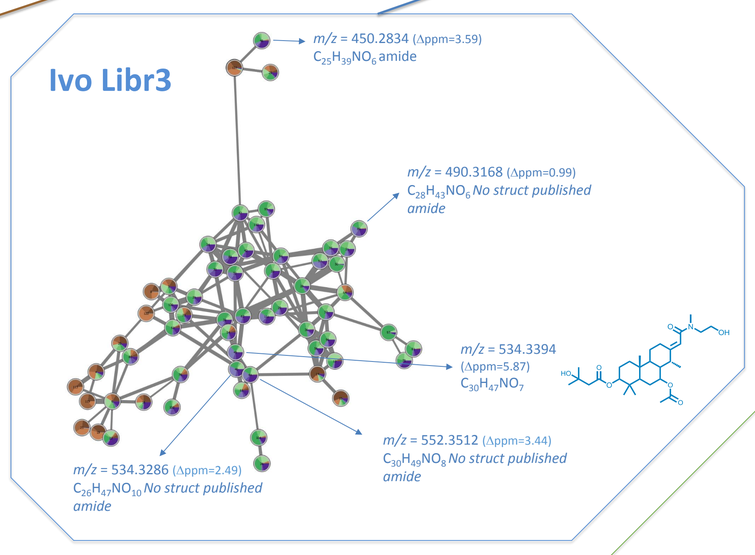
Material and methods

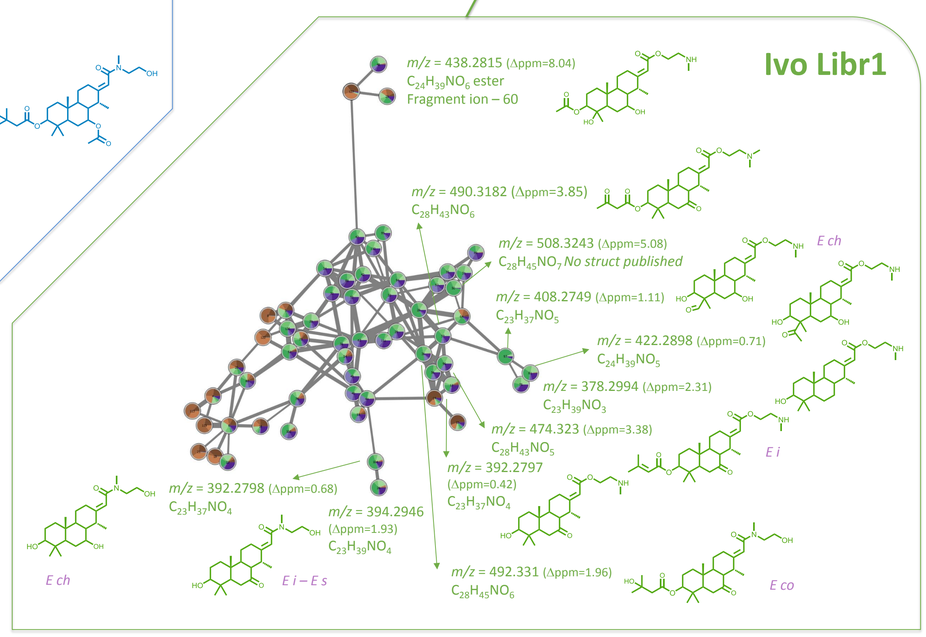
Metabolites were extracted in methanol using ultrasonication from grounded dried leaves. Reverse phase LC-ESI-HRMS and HRMS/MS were performed using a RRLC system coupled to an ESI-QTOF from Agilent Technologies. Solvent were (A) water and (B) acetonitrile, both acidified with 0.1% formic acid. MS ionisation mode was positive. MS/MS analyses were performed in a data dependent mode (Auto-MS/MS) with a fixed collision energy (25 V) after optimisation. The MS/MS data were used to build the molecular networks.

Heatmap visualization constructed based on the differential metabolites of importance. Heatmap represents unsupervised hierarchical clustering of samples (columns) and variables (rows).





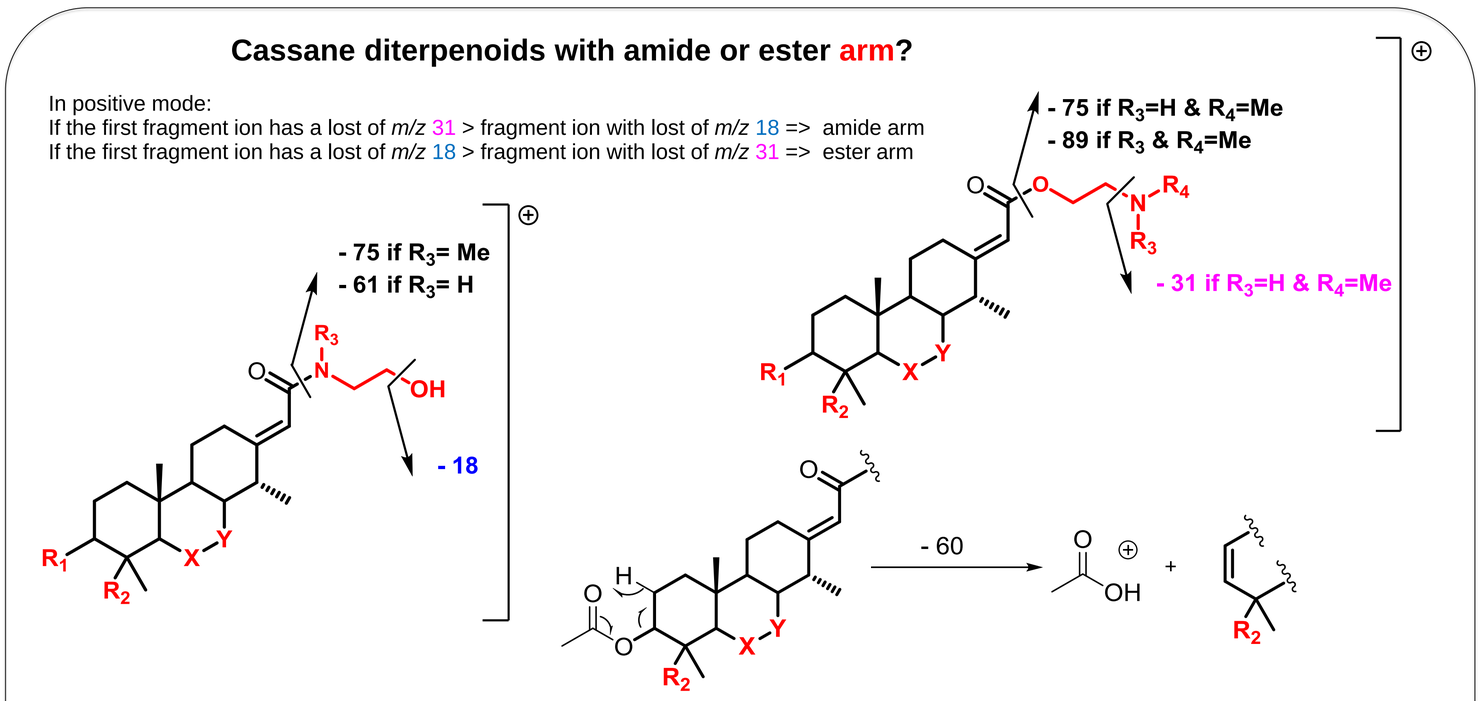




This structure has been described before in E a = E africanum – E ch = E cholostachys – E co = E couminga – E f = E fordii – E g = E guineense – E i = E ivorense – E s = E suaveolens

Cassane diterpenoids with amide or ester arm?

In positive mode:
If the first fragment ion has a lost of *m/z* 31 > fragment ion with lost of *m/z* 18 => amide arm
If the first fragment ion has a lost of *m/z* 18 > fragment ion with lost of *m/z* 31 => ester arm



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Conclusions

MS/MS conditions have been optimised for molecular networking interpretations.

Thanks to literature on *Erythrophleum* genus putative chemical structures have been proposed:
In *E suaveolens*, we identified 8 structures with a cassane diterpenoid skeleton are already known in other *Erythrophleum* species
In *E ivorense-Libr1, we proposed* 10 structures including 5 already described in literature
In *E ivorense-Libr3, no specific cassane diterpenoids have been described before. This group might be* ***undescribed localized cryptic*** *specie.*