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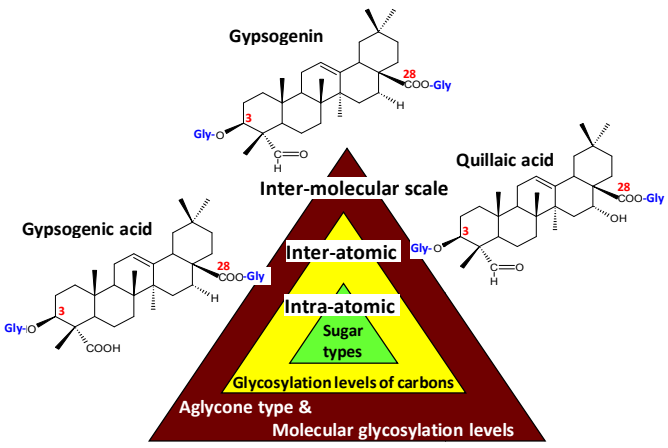
Multi-scale analysis of structural variability of *Caryophyllaceae* saponins by a simplex machine learning approach

Soumaya CHEIKH ALI (E-mail: cheikhalisoumaya@gmail.com) [a,d], Muhammad FARMAN (E-mail: farman@qau.edu.pk)[b], Asma HAMMAMI-SEMMAR (E-mail: asma.hamami@gmail.com)[c], Nabil SEMMAR (E-mail: nabilsemmar5@gmail.com)[d,*].

[a]*University of Carthage, Faculty of Sciences of Bizerte, Tunisia*
[b]*Quaid-i-Azam University, Department of Chemistry, Islamabad 45320, Pakistan*
[c]*University of Carthage, Institut National des Sciences Appliquées et Technologies, Tunis, Tunisia*
[d]*University of Tunis El Manar, Institut Pasteur de Tunis, Laboratory of BioInformatics, BioMathematics & BioStatistics, Tunisia*

Graphical Abstract



Abstract. A mass conservation law-based chemometric approach was developed to extract smoothed processes governing inter- and intra-molecular variability of structural diversity in metabolic pools. The approach consisted of a machine-learning method using simplex rule to calculate a complete set of smoothed barycentric molecules from iterated linear combinations between molecular classes (glycosylation classes). An application to four glycosylation levels (*GLs*) of Caryophyllaceae saponins highlighted aglycone-dependent variations of glycosylations, especially for gypsogenic acid (*GA*) which showed high 28-glucosylation levels. Quillaic acid (*QA*) and gypsogenin (*Gyp*) showed closer variation ranges of *GLs*, but differed by relationships between glycosylated carbons toward different sugars. Relative *GLs* of carbons C3 and C28 showed associative (positive), competitive (negative) or independent (unsensitive) trends conditioned by the aglycone type (*GA*, *Gyp*) and molecular (total) *GLs* (the four classes): 28-glucosylation and 28-xylosylation showed negative global trends in *Gyp* vs *GLs*-depending trends in *QA*. Also, relative levels of 3-galactosylation and 3-xylosylation varied by unsensitive ways in *Gyp* vs positive trends in *QA*. These preliminary

	results revealed higher metabolic tensions (competitions) between considered glycosylations in *Gyp* vs more associative processes in *QA*. In conclusion, glycosylations of *GA* and *QA* were relatively distant whereas *Gyp*(common precursor) occupied intermediate position.

Introduction

The Caryophyllaceae plant family was proved to be a wide source of saponins essentially based on three triterpenic skeleton (aglycones or sapogenins) including gypsogenin (*Gyp*), quillaic acid (*QA*) and gypsogenic acid (*GA*) [1].Apart from the sapogenin type, structural variability of Caryophyllaceae saponins showed multi-factorial and multi-scale aspects due to different glycosylation levels (*GLs*) and glycosylation types essentially occurring at the carbons C3 and C28.

By considering a wide dataset of 205 Caryophyllaceae saponins based on*Gyp*, *QA* and*GA*with different *GL*(2 to 9), a machine learning approach was applied to extract key information on inter- and intra-molecular regulatory processes governing the observed structural diversityin relation to aglycones (a), glycosylation levelsand types (b, c) and substitution carbons(d) [2]. *In silico* combinations between saponin structures belonging to different molecular classes (*GLs*) provided a complete set of simulated theoretical molecules from which significant trends within and between glycosylated carbons were revealed to govern structural variability at inter-molecular scale. This helped to better understand hierarchical and sequential glycosylation orders responsible for diversification of saponins in Caryophyllaceae.

Materials and Methods

Machine learning approach was applied to the three aglycones separately(*Gyp*, *QA*, *GA*). It consisted in combining structural variabilities of saponins belonging to *q*molecular classes (concerning one aglycone)representing*q*increasing glycosylation ranges:for *Gyp* and *QA*, saponins were stratified into *q*=4 classes of glycosylation levels (*GLs*)(*GLs*= 1, 2, 3, 4) representing saponins with 3-4, 5-6, 7 and 8-9 substituted sugars, respectively; for GA, *q*=3 classes were considered (*GLs* = 1, 2, 3) corresponding to saponins with 3, 4, 5 substituted sugars, respectively. Saponins of different *GL* classes wereinitially characterized by the relative *GLs* of different sugars substituted at different carbons (C3, C16, C23, C28).Combinations between the *q*molecular classes were applied using Scheffé's simplex matrix (*N* rows x *q* columns) which provides a complete set of *N* mixturesvarying gradually by different weights w_j(from 0/5 to 5/5) of the *q*mixed *GL*classes*j* (with Σw_j=1)[2].

In output of each combination, a barycentric molecular profile was calculated by averaging the relative levels of glycosylation (*G*) profiles of the *n* randomly sampled contributive saponins. The mixture design was iterated 30 times by bootstrap technique then the 30 resulting response matrices (containing *N*elementary barycentric*G*-profiles) were averaged leading to a final response matrix containing *N*smoothed barycentric *G*-profiles and representing a deep regulatory machinery of the whole studied structural system.

The smoothed response matrix was used for graphical analysis of regulatory trends between glycosylated carbons.For two given glycosylated carbons, different regulatory trends were highlighted by considering successions of weight ellipses associated to different *GL* classes [2].

Results and Discussion

Data smoothing by simplexmachine learning approach helped to highlight regulatory processes of glycosylation in Caryophyllaceae saponins at inter-molecular, inter-atomic and intra-atomic scales. Illustrations are provided by xylose (*Xyl*), glucose (*Glc*) and galactose (*Gal*) substituted at carbons C3 and/or C28.

Inter-molecular scale variations 1 (aglycone effect). The three simplex plots associated to the three aglycones-based saponins showed strong differentiation spaces of relationships between the same glycosylated carbons (**Figure 1a, b**). Illustrations are given for 28-*Xyl* vs 28-*Glc* (**Figure1**) and 3-*Xyl* vs 3-*Gal* (**Figure2**):

GA was markedly distant from *Gyp* and *QA* indicating some specific glycosylation orders (in *GA*). This could be linked to the occurrence of two carboxylic groups in *GA*(23- and 28-COOH) vs only one (28-COOH) in both *QA* and *Gyp*. Specific space of *GA* was characterized by strong relative 28-*Glc* levels (0.65-0.75) without competing (comparable) levels from other sugars in both C3 and C28 (**Figure1a**)[1, 3, 4]. *Gyp* occupied intermediate position between *GA* and *QA* whereas *QA* was the most distant from *GA* (**Figure1a, 2b**).Relative locations of different aglycones-based saponins in simulated graphicswere compatible with the metabolism: *Gyp* is metabolically precursor of both *QA* and *GA* (by 16- and 23-hydroxylation, respectively); this is compatible with intermediate position of *Gyp*between *QA* and *GA* (competing for *Gyp*) [3].

Although *Gyp* and *QA* plots showed spatial neighboring (compared to *GA*), they differed by dispersions, inclinations and internal organization of corresponding clouds of points: relationship between 3-*Xyl* and 3-*Gal* showed significantly higher dispersion in *QA* than in *Gyp* (**Figure1b**). This aspect indicated wider regulation range of 3-*Gal* in *QA* compared to *Gyp*[3]. However, for (28-*Xyl* vs 28-*Glc*), the two aglycones showed opposite relationship leading to inversely inclined clouds of points: (global positive trend in *QA* against negative trend in *Gyp*) (**Figure1a**).

Inter-molecular scale variation 2 (molecular glycosylation effect). For a same aglycone, global variation trends between substituted sugars significantly varied with molecular *GLs*:

In *Gyp*, 28-*Glc* showed a strong peak under low molecular *GL* (*GL*=1) followed by rapid decrease to minimum in higher *GLs* (*GL*=2, 3, 4) (**Figure 1b**). This highlightedstrong contribution of early 28-*Glc* in ramification process (monodesmosylation) of*Gyp* leading to preliminary structural diversification of saponins [3, 5, 6]. In *QA*, 28-*Glc* seemed to play intermediate modulatory role due to its low relative levels in *GLs* = 1 and 4 (**figure 1c**).

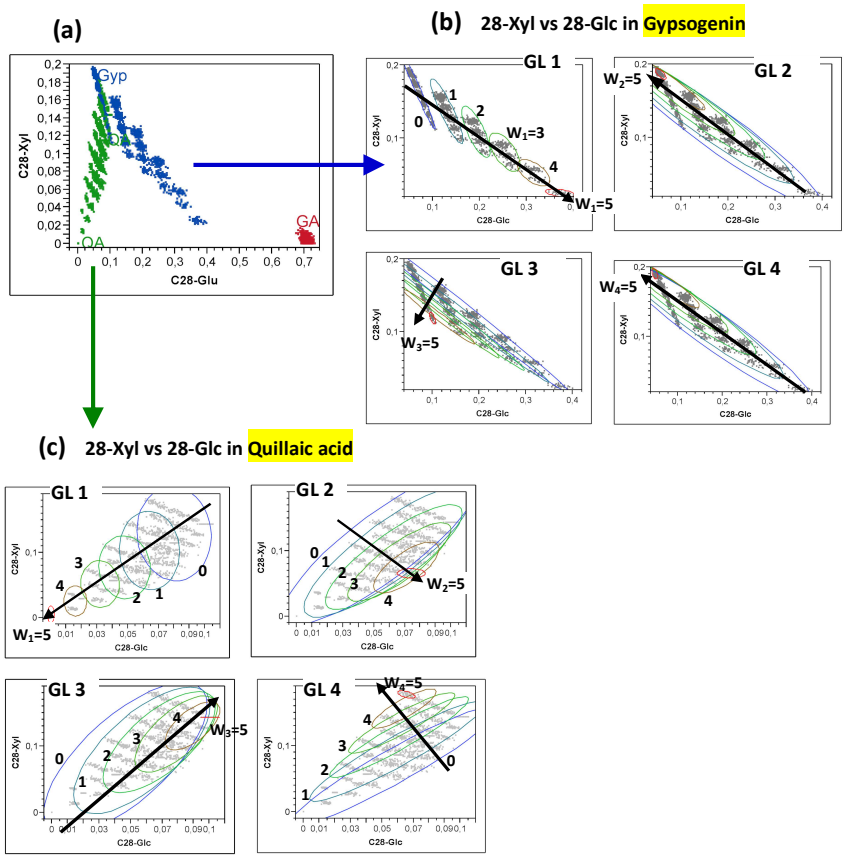


Figure 1. Multidirectional smoothed plots given by simplex machine learning for aglycone-dependent relationships between C28-xylosylation (28-*Xyl*) and C28-glucosylation (28-*Glc*). (**a**) Three plots corresponding to quillaic acid (*QA*), gypsogenin (*Gyp*) and gypsogenic acid (*GA*). (**b**, **c**) Four plots associated to four glycosylation levels (*GLs*) in *Gyp* and *QA*, respectively.

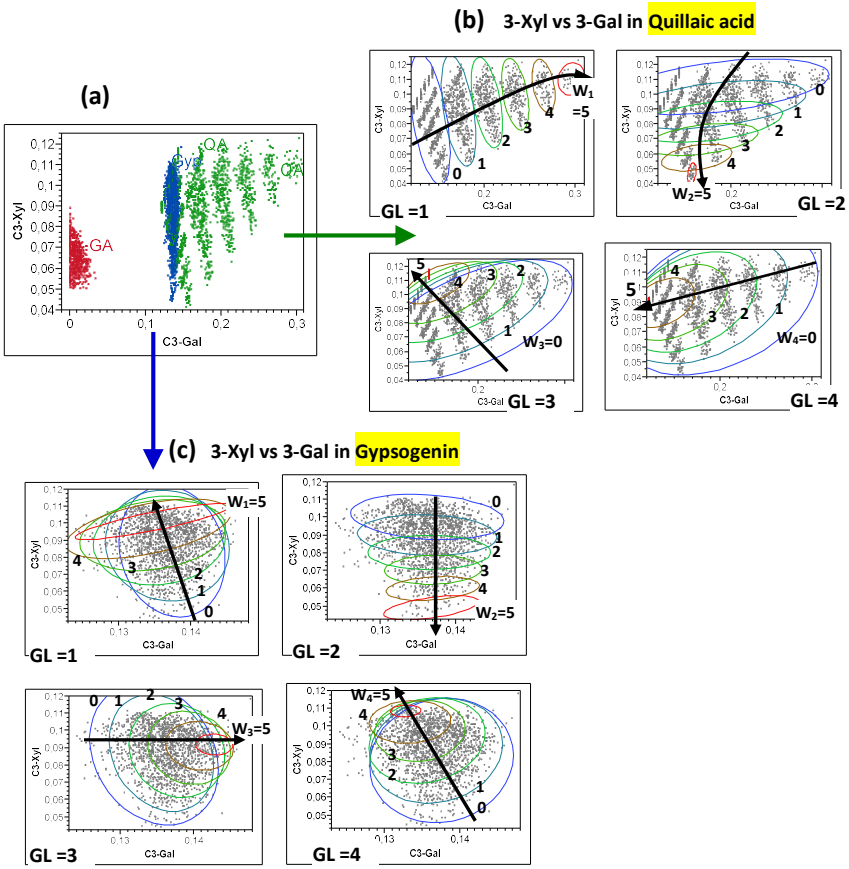


Figure 2. Multidirectional smoothed plots given by simplex machine learning for aglycone-dependent relationships between C3-xylosylation (3-*Xyl*) and C3-galoactosylation (28-*Gal*). (**a**) Three plots corresponding to quillaic acid (*QA*), gypsogenin (*Gyp*) and gypsogenic acid (*GA*). (**b**, **c**) Four plots associated to four glycosylation levels (*GLs*) in *QA* and *Gyp*, respectively.

However, in *Gyp*, relative levels of 28-*Xyl* showed alternated states with molecular *GLs*: global minimal regulations in the less glycosylated saponins (*GL*=1), followed by maximal global regulations in *GL*=2 and 4 via slight decrease in *GL*=3 (**Figure 1b**). This highlighted key role of 28-*Xyl* in

elongation process for the synthesis of *Gyp*-based saponins with high *GL*s. Also, in *QA*, 28-*Xyl* showed gradual (step-by-step) increases of relative levels with molecular *GLs*. This was indicative of key role of xylosylation in elongation process at C28 in *QA* (**Figure 1c**).

Inter-atomic scale variation. By considering xylosylation between C3 and C28 in *QA*, 3-xylosylation seemed to be initially favored while 28-*Xyl* was at its lowest levels (in *GL*=1) (**Figures 1c, 2b**). This could be indicative of key role of 3-xylosylation in molecular ramification of *QA* [3]. For higher *GL* (2 to 4), relative level of 28-*Xyl* showed continuous increase vs alternated variation for 3-*Xyl*. This highlighted increasing affinity of C28 vs alternated affinity of C3 for xylosylation with *GLs* in *QA*.

Intra-atomic scale variation. By considering the inclinations of weight ellipses of different *GLs*, further regulatory processes were highlighted between 28-*Xyl* and 28-*Glc* conditionally to the aglycone type (*Gyp* vs *QA*) (**Figures 1a, 2**):

In *Gyp*, weights' ellipses showed negative inclinations indicating systematic competitions between *Xyl* and *Glc* for substitution at C28 in all the *GLs* (**Fig. 1b**). Such a competition could suggest the implication of different glycosyl-transferases (*GT*) in the two glycosylations of C28 [3]. Hypothesis on specific *GT* in *Gyp* could be also locally indicated by not clearly inclined weight ellipses in the relationship 3-*Xyl* vs 3-*Gal*.

However, in *QA*, weights' ellipses in (28-*Xyl* vs 28-*Glc*) showed positive inclinations indicating some associative process between these two sugars for their 28-substitution despite some global negative trends (for *GLs*=2, 4). Such associative substitution process could occur under the effect of a same *GT* having a promiscuity character and leading to sequential glycosylations by different sugars [5, 7-10]. Also, weight ellipses in 3-*Xyl* vs 3-*Gal* showed some positive inclination (except in *GL*=1) leading to further indication about intra-atomic associations between considered glycosyls in favor of shared (promiscuity) *GT* hypothesis.

Conclusions

Simplex-based machine learning applied to structural variability of Caryophyllaceae saponins highlighted strong differentiation in metabolic glycosylation governed by the aglycone type, molecular *GL* and substituted carbon. *GA* was strongly characterized by high levels of 28-*Glc* followed by *Gyp* then *QA*. Effect of *GLs* was partially associated to key role of 28-*Xyl* in elongation in both *Gyp* and *QA*. At inter-atomic scale, preliminary 28-*Glc* and 3-*Xyl* seemed to be responsible for molecular ramification in *Gyp* and *QA*, respectively. At intra-atomic scale, 28-*Xyl* and 28-*Glc* showed competition in *Gyp* and association in *QA* that could be indicative of different and shared *GTs*, respectively. Finally, metabolic tensions for glycosylation seemed to decrease from *GA* to *QA* via *Gyp*.

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