Chemosystematics of the Rosiflorae

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(With 9 figures)

Abstract

The superorder Rosiflorae (sensu Dahlgren, 1980) belongs to the Angiospermae. It comprises twelve orders and thirty-eight families formed of species with varied habits widely distributed in temperate regions. The chemistry of Rosiflorae species is highly diversified; nevertheless it shows clearly phylogenetic affinity among the orders, except for Buxales. Flavonoids and triterpenoids are the real taxonomic markers for the superorder, due not only to the great number of occurrences, but also to the high structural diversity. On the other hand, the alkaloids are suitable as chemical markers only for the order Buxales. For orders and families of Rosiflorae, analysis of correlations among chemical parameters based on flavonoids and triterpenoids, with themselves and with the morphological and chemo-morphological parameters, showed evolutionary gradients among these taxa in which Trochodendrales occupy a primitive position while Saxifragales have the outpost. According to the types of flavonoids found in the superorder, there is clearly a higher incidence of flavonols than flavones, suggesting a primitive status of the Rosiflorae. Evolutionary advancement parameters relative to flavonoid hydroxyl protection show preferential protection mechanisms of glycosylation against methylation as well as a high percentage of free hydroxyl groups. The order Buxales has an isolated position in the superorder Rosiflorae with a high alkaloid production, which is quite exclusive to this taxon.

Keywords: chemosystematics, Rosiflorae, flavonoids and triterpenoids.

Quimiossistemática de Rosiflorae

Resumo

A superordem Rosiflorae (sensu Dahlgren, 1980), Angiospermae, é composta por doze ordens e trinta e oito famílias. Em geral são plantas de hábito variado e muito freqüentes em regiões temperadas. A química das espécies de Rosiflorae é muito diversificada, mas evidencia a proximidade filogenética entre as ordens, com exceção de Buxales. Os flavonóides e os triterpenóides mostram-se como verdadeiros marcadores quimiossistemáticos em nível de superordem, devido, não somente ao seu grande número de ocorrências, mas também pela sua elevada diversidade estrutural; contudo os alcalóides são os marcadores para Buxales. Avaliação das correlações dos parâmetros químicos entre si e com os parâmetros morfológico e químico-morfológico, para ordens e famílias de Rosiflorae, com base nos seus flavonóides e triterpenóides, evidenciaram uma grande proximidade filogenética entre esses táxons, além de mostrar gradientes evolutivos, em que Trochodendrales e Saxifragales são posicionadas como a ordem mais primitiva e a ordem mais evoluída, respectivamente. Com relação aos tipos flavonoídicos produzidos na superordem, verifica-se uma maior produção de flavonóis em relação às flavonas, o que acarreta uma baixa relação favona/flavonol, confirmando o posicionamento primitivo da superordem, indicado pelos índices morfológico e químico-morfológico. A proteção das hidroxilas flavonoídicas mostra uma nítida preferência na proteção por glicosilação e desproteção em detrimento à proteção por metilação. A ordem Buxales praticamente encontra-se isolada na superordem com grande produção de alcalóides muito característicos, sendo bastante diferenciada da produção alcaloídica relativamente pobre da superordem.

Palavras-chave: quimiossistemática, Rosiflorae, flavonóides e triterpenóides.

1. Introduction

Today there are many plant classification systems. The major botanical authorities often disagree with respect to the positioning of certain taxa (Goldberg, 1986). Such discrepancies frequently concern the position of subtribes in tribes, of families in orders, and of subclasses or orders in superorders. This situation is due to the utilization of different morphological markers, as well as chiefly to the absence of unique criteria for the taxonomic evaluation of the markers (Gottlieb et al., 1996).

Recent developments in plant chemosystematics, represented by the demonstration of basic principles (Gottlieb, 1982), confirmed the relevance of special metabolites in phylogenetic studies. Thus correlation among chemical characters and numerical parameters referring to oxidation level, skeletal specialization and protection of flavonoid hydroxyls with plant taxonomic positioning is a promising subject for study.

The superorder Rosiflorae (Dahlgren, 1980) belongs to the Angiospermae and comprises twelve orders (Fagales, Cunoniales, Saxifragales, Rosales, Juglandales, Myricales, Balanopales, Casuarinales, Trochodendrales, Hamamelidales, Gunnerales and Buxales) and thirty-eight families formed by species with varied habits and a wide distribution in temperate regions. Dahlgren, in his first system of classification for Angiospermae (Dahlgren, 1975), placed the families of Rosiflorae in three superorders: Hamamelidanae, Rosanae and Saxifraganae allied with other families and orders. In 1989, an important system of classification for the flowering plants appeared showing modifications in the neighbourhood in Rosanae orders former (Rosiflorae) (Dahlgren, 1989; 1995). In Cronquist's system of classification (Cronquist, 1981), Dahlgren's Rosiflorae is separated in two Subclasses: Hamamelidae (15 families in 7 orders) and Rosidae (16 families in 4 orders).

The positioning of the Rosiflorae (or Rosanae) families shows great controversy according to recent botanists (Goldberg, 1986). In order to analyse the validity of the positioning of the families and orders into superorders in Rosiflorae, a detailed study of micromolecular composition of the Rosiflorae families has been made.

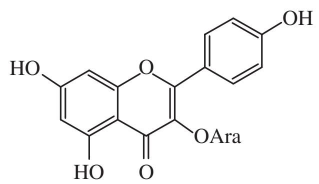
The present work attempts to look for the phylogenetic affinities in Rosiflorae. The flavonoids and triterpenoids were selected as chemosystematic markers for this purpose, due to their general occurrence and diversification in this superorder.

2. Material and Methods

Chemical data were collected from Chemical Abstracts and from specific literature surveys. Selected micromolecules were tabulated with the occurrence number (ON = number of compounds of a chemical class produced by a taxon) and correlated with respect to their evolutionary parameters determined according to chemosystematic methodology (Gottlieb, 1982). The occurrence numbers were used as a base for calculation of flavonoid chemical indexes (Figure 1 and 2). Evolutionary advancement parameters related to the orders were evaluated by the weighted average of their flavonoid chemical indexes (Barreiros, 1990). These parameters were further correlated to each other and to the chemo-morphological and morphological indexes: the herbaceousness index (HI) (Borin, 1993) and the Sporne index (SI) (Sporne, 1980). The systems of classification used for taxa of flowering plants circumscription were those of Dahlgren (1980) and of Brummitt (1992).

3. Results and Discussion

Among the botanists specialized in plant taxonomy, there are differences in opinion with respect to the systematic positioning of some families in Rosiflorae. However, for many families, total agreement is observed in their classification systems. The families Fagaceae, Betulaceae and Corylaceae are invariably positioned in the order Fagales. The families Casuarinaceae and



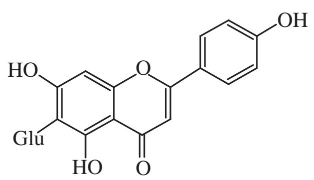
MI = 0/4 = 0

GI = 1/4 = 0.25

MGI = 1/4 = 0.25

$$EA_M \text{ or } EA_G \text{ or } EA_{MG} = \frac{\sum \text{MI or GI or MGI of all flavonoids in the taxon}}{\text{Number of occurrence of flavonoids in the taxon}}$$

Figure 1. Methylation index (MI), glycosylation index (GI) and methylation/glycosylation index (MGI) of juglanine and the corresponding evolutionary advancement parameter (EAM, EAG and EAMG) of flavonoids in the taxon.



Aditional substituents * = 1

Loss substituents = 0

ATI = 1

*C-glucosylation in C-6

$$EA_{AT} = \frac{\sum \text{ATI of all flavonoids in the taxon}}{\text{Number of occurrence of flavonoids in the taxon}}$$

Figure 2. A-ring transformation index (ATI) of isovitexin and the corresponding evolutionary advancement parameter (EA_{AT}) of flavonoids in the taxon.

Rosaceae are classified, respectively, in the orders Casuarinales and Rosales. Neuradaceae for modern botanists is positioned in Rosales, however Emberger (1960), Hutchinson (1973), Benson (1979) and Thorne (1983), consider *Neurada* a genus of Rosaceae. The same happens with all genera of Chrysobalanaceae, which Hutchinson (1973) maintains included in Rosaceae (Goldberg, 1986).

By contrast, most of the Rosiflorae families exhibit different positionings outside the superorder. Melchior (1964), Benson (1979), Cronquist (1981) and Rouleau (1981) classify Buxaceae in Euphorbiales; Takhtajan (1983) in Hamamelidales, suborder Buxineae; Thorne (1983) in Pittosporales, suborder Buxineae; Emberger (1960) in Terembinthales; Melchior (1964) in Celastrales, suborder Buxineae; Dahlgren (1980) and Young (1981) in Buxales. Grubbiaceae is another example of uncertain positioning. According to Emberger (1960), Melchior (1964), Hutchinson (1973) and Benson (1979), it is classified in Santalales; while for Stebbins (1974), Cronquist (1981), Rouleau (1981) and Takhtajan (1983) it is in Ericales, whilst for Young (1981) and Thorne (1983) it is in Pittosporales, but for Dahlgren (1980) it is in Cunoniales (Goldberg, 1986).

3.1. Chemical profile of Rosiflorae

In Rosiflorae families, approximately 60% of the families have undergone some chemical study. The main special metabolites of these plants are biosynthesized through the shikimic acid pathway but very representative terpenoids are also produced (Castilho, 1997).

The special metabolism of Rosiflorae to date is characterized by: flavonoids, condensed and hydrolysable tannins, cinnamic and benzoic acid derivatives, alkaloids, mono and triterpenes, carotenes and steroids, among others (Figure 3).

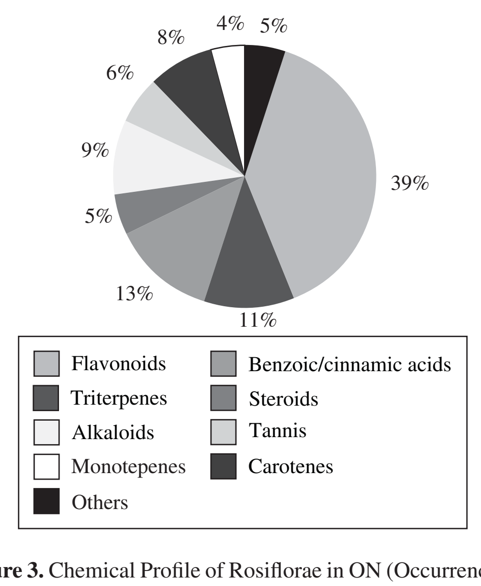


Figure 3. Chemical Profile of Rosiflorae in ON (Occurrence Number).

Monoterpenes have been isolated from eight families of Rosiflorae. In Rosaceae they are the largest representatives, belonging mainly to canfeno, pineno and cimeno types. These metabolites appear mainly in Bruniaceae, Hamamelidaceae, Saxifragaceae, Fagaceae, Myricaceae, Betulaceae and Juglandaceae.

Iridoids are very rare in Rosiflorae: Buxaceae having only one representative, buxeletin; Daphniphyllaceae, with three representatives, such as: asperuloside, daphylloside and geniposidic acid; and Hamamelidaceae with two representatives from the genus *Liquidambar*, asperuloside and monotropein.

The sesquiterpenes of Rosiflorae are sparsely distributed in five families and they are represented mainly by caryophyllene, humulene, eudesmene, cadinene and germacrene.

In Rosiflorae the presence of diterpenes is rare, registered only in three families, Crassulaceae with one labdane representative, Rosaceae with representatives of the kaurene and abietane types and Chrysobalanaceae with representatives of the kaurene types.

Triterpenes are found in almost all studied Rosiflorae families, with a large occurrence and great structural diversity in Rosaceae and Betulaceae.

The Rosiflorae flavonoids are represented mainly by flavonols, which constitute more than 50% of all isolated flavonoids from this taxon.

Tannins are commonly found in Rosiflorae, having predominant distribution of polygalloyl-ellagoyl glucose in all families. Condensed tannins are recorded for the superorder occurring mainly in Rosaceae, Myricaceae, Saxifragaceae and Crassulaceae. Another group of substances usually found in the Rosiflorae families, mainly in Rosaceae, are aromatic acids, such as: cinnamic, caffeic, ferulic, vanillic, sinapic and benzoic acids (Geissman and Crout, 1969).

Steroid chemistry is simple involving fifteen families. The more common structural types are: colestane, stigmastane and ergostane, apart from the Crassulaceae family which produces highly oxydised bufadienolides.

Ten Rosiflorae families produce alkaloids. They belong to the types: spermidine/guanidine/piperidine/pyrrolizidine/indolic/Daphyniphyllum/triterpene and diterpene alkaloids. Attention should be given to the presence of a pyrrolizidine alkaloid in Casuarinaceae of the same type as produced by *Crotalaria*, Leguminosae (Castilho et al., 1999).

Other more restricted chemical classes of compounds produced by some Rosiflorae families are: diarylheptanoids in Myricaceae; phloroglucinol derivatives (common in ferns) in Rosaceae (Murakami and Tanaka, 1988); lignans are restricted to three families: Casuarinaceae, Gunneraceae and Rosaceae; coumarins to Rosaceae, Crassulaceae, Buxaceae and Saxifragaceae; hydroquinone glycosides as arbutin in Fagaceae, Juglandaceae, Casuarinaceae, Buxaceae, Saxifragaceae and Rosaceae; anthraquinones as emodin and chrysophanol in Saxifragaceae and Rosaceae.

Analysis of the chemical profile for Rosiflorae orders (Table 1), excluding Buxales, shows a good homogeneous production of secondary metabolites. On the basis of these data, flavonoids and triterpenoids were shown to be real taxonomic markers for the taxon, due not only to the large number of occurrences, but also to their high structural diversity. On the other hand, alkaloids may be suitable as chemical markers only for the order Buxales (Castilho, 1997).

3.2. Flavonoid evolution in Rosiflorae

Flavonoids constitute a special category of metabolite derived by a mixed (acetate-mevalonate/shikimate) biosynthetic pathway (Torssell, 1983). The potentiality of these compounds as systematic markers is due not only to their ubiquity in all Metaphyta but also to their great structural diversity (Barreiros, 1990).

Flavonoid chemistry is quite diversified in Rosiflorae and it reveals some evolutionary polarizations of great chemosystematic value. Rosaceae is the family that presents the largest flavonoid occurrence number (ON) in the superorder, followed by Saxifragaceae and Crassulaceae. The other families are not so expressive, presenting ON less then 100 (Figure 4).

Among twenty-three Rosiflore families chemically studied, twenty were shown to have produced flavonoids, nineteen of them were shown to have a preponderance of flavonols and in only nine families flavone production has been recorded. The flavonoid profile for Rosiflorae shows flavonols (54%), anthocyanidins (20%), flavones (8%), flavanones (7%), dihydroflavonols (4%), flavan-3-ols (4%) and others (3%) including chalcones, dihydrochalcones, isoflavonoids, biflavonoids and flavans (Figure 5). The occurrence of flavonols generally prevails in the most primitive plants (Harborne and Turner, 1984; Gottlieb and et al., 1996). This happens for the superorder Rosiflorae, suggesting a low "evolutionary status" for the taxon. This proposal can be strengthened by analysis of the flavone/flavonol ratio (Figure 6), which is very low for most of the studied families, and also for the whole superorder (Soares and Kaplan, 2001). Analysis of the hydroxyl protection mechanism for the Rosiflorae flavonoids states that 69% of hydroxyls are protected. Among them 49% are protected by glycosylation only, 11% by methylation only and 9% receive double protection (methylation and glycosylation) (Figure 7). Once again, this high percentage of protection by glycosylation (49%), together with the percentage of unprotected glycosyls (31%), indicates a more primitive character for the superorder.

In the order Trochodendrales, the family Eupteleaceae produces flavonols and dihydroflavonols with free and protected hydroxyl groups in close percentages; Cercidiphyllaceae exhibit only dihydroflavonols with unprotected hydroxyl groups, but that order does not show transformation of the A-ring flavonoids (Table 2).

In Hamamelidales, the family Hamamelidaceae biosynthesises anthocyanidins, flavonols, dihydroflavonols and flavan-3-ols all have a high proportion of unprotected hydroxyls and when they are protected, the mechanism is through glycosylation. Platanaceae only produce flavonol glycosides.

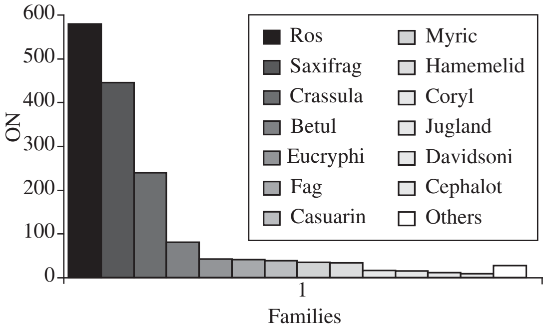


Figure 4. Ocurrence number (ON) of flavonoids in families (aceae) of Rosiflorae.

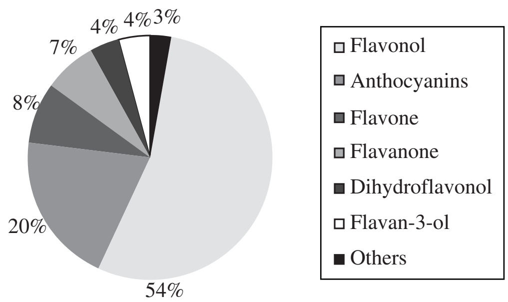


Figure 5. Types of flavonoids in Rosiflorae (ON).

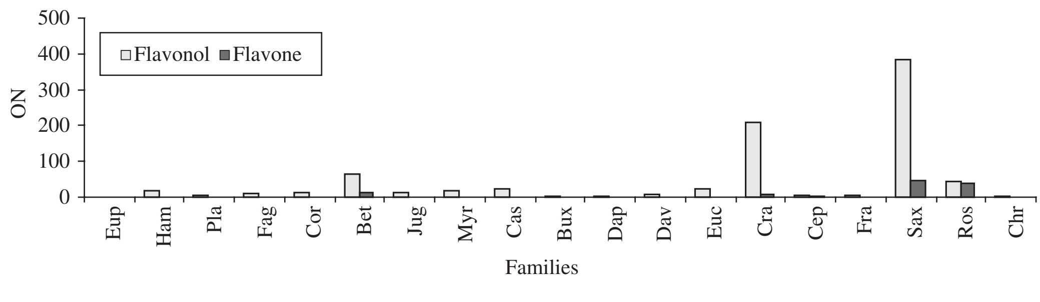


Figure 6. Relationship Flavonol/Flavone for the families (aceae) of Rosiflorae.

Table 1. Chemical Profile (ON) of families (aceae) of superorder Rosiflorae.

Chemical Class/ Families	MON	IRI	SES	DIT	TRI	CAR	STE	ALK	TAN	BCD	FLA	COU	HYD	ANT	LIG
Trochodendr	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Euptele	-	-	-	-	24	-	2	-	-	-	2	-	-	-	-
Cercidiphyll	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-
Hamamelid	14	2	3	-	6	3	6	-	22	-	34	-	-	-	-
Platan	-	-	-	-	8	-	2	-	-	-	7	-	-	-	-
Fag	11	-	-	-	218	19	30	4	70	25	41	-	-	-	-
Coryl	-	-	-	-	1	12	1	2	5	-	16	-	-	-	-
Bet	2	-	7		44	38	3	17	8	5	81	-	-	-	-
Jugland	3	-	-	-	1	1	2	-	9	9	15	-	26	-	-
Myric	11	-	9	-	22	1	-	1	10	-	35	-	-	-	-
Casuarin	-	-	1	-	8	-	7	1	12	-	49	-	1	-	-
Bux	-	1	-	-	17	-	3	242	-	-	-	3	1	-	-
Daphniphyll	-	3	-	-	-	-	1	49	-	-	2	-	-	-	-
Davdisoni	-	-	-	-	3	-	-	-	7	-	11	-	-	-	-
Eucryphi	-	-	-	-	-	-	-	-	-	-	42	-	-	-	-
Bruni	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Crassul	-	-	-	1	15	1	80	59	8	64	240	13	-	-	-
Cephalot	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-
Franco	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-
Saxifrag	14		-	-	11	-	3	-	-	34	445	23	11	2	-
Gunner	-	-	-	-	24	-	8	-	-	-	-	-	-	-	1
Ros	75	-	7	4	61	301	52	29	86	486	579	29	1	5	4
Chrysobalan	-	-	-	-	2	-	3	-	1	-	3	-	-	-	-

MON = Monoterpenes, IRI = Iridoids, SES = sesquiterpenes, DIT = Diterpenes, TRI = Triterpenes, CAR = Carotenoids, STE = Steroids, ALK = Alkaloids, TAN = Tannins, BCD = Benzoic and Cinnamic acids derivatives, FLA = Flavonoids, COU = Coumarins, HYD = Hydroquinone, ANT = anthraquinones and LIG = Lignins.

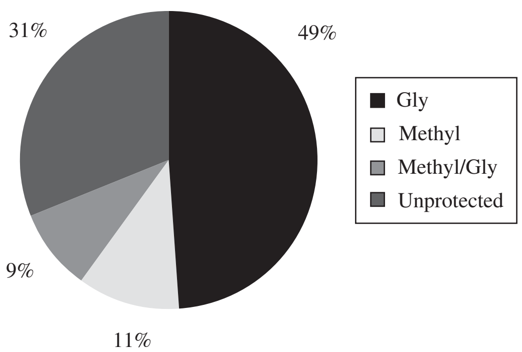


Figure 7. Protection of hydroxyls of Rosiflorae flavonoids. Gly = Glycosylation, Methyl = Methylation, Methyl/Gly = Methylation/glycosilation (double protection).

Fagaceae is one family of Fagales with a high diversity of flavonoid structural types with low hydroxyl protection and when this happens it is by glycosylation. On the other hand, in Betulaceae, 84% of flavonoid hydroxyls are protected by methylation and/or glycosylation. Corylaceae follows the same pattern, but in this case the protection is only made by glycosylation.

Saxifragales is the order that presents the largest index of A-ring transformation. The flavonoids found in Saxifragaceae have their hydroxyl groups mainly protected by methylation, glycosylation, or for double protection (glycosylation and methylation). In this family, the methylation protection mechanism is preferentially used in the genus *Chrysosplenium*, justifying the highest evolutionary advancement methylation index (EAM) indicating its advanced positioning. The flavonoids of Crassulaceae have the largest A-ring transformation in the order, but its hydroxyl groups are protected mainly by glycosylation.

In Superorder Rosiflorae, Rosaceae stands out for being the family that shows the largest flavonoid number of occurrence and produces the greatest variety of structural types. These flavonoids have their hydroxyl groups protected by glycosylation (53%) and 34% being free, proving the low “evolutionary status” of the family.

Table 2. Sporne index (SI) and herbaceousness index (HI) and flavonoid chemical index (EA_M, EA_G, EA_{MG}, EA_U e E_{AT}) for Rosiflorae taxa.

Orders/Families	HI	SI	EA_M	EA_G	EA_{MG}	EA_U	EA_{AT}
TROCHODENDRALES							
Trochodendraceae	1,0	33	-	-	-	-	-
Tetracentraceae	1,0	33	-	-	-	-	-
Eupteleaceae	25,0	35	0,00	0,10	0,10	0,90	0,00
Cercidiphyllaceae	1,0	26	0,00	0,00	0,00	1,00	0,00
HAMAMELIDALES							
Hamamelidaceae	25,0	37	0,02	0,13	0,15	0,85	0,04
Platanaceae	1,0	41	0,00	0,23	0,23	0,77	0,00
Myrothamnaceae	50,0	33	-	-	-	-	-
Geissolomataceae	50,0	33	-	-	-	-	-
FAGALES							
Fagaceae	25,0	43	0,01	0,11	0,19	0,80	0,18
Corylaceae	25,0	41	0,02	0,19	0,21	0,79	0,00
Betulaceae	25,0	38	0,30	0,04	0,34	0,65	0,07
BALANOPALES							
Balanopaceae	1,0	38	-	-	-	-	-
JUGLANDALES							
Rhoipteliaceae	1,0	40	-	-	-	-	-
Juglandaceae	12,5	50	0,03	0,18	0,20	0,80	0,00
MYRICALES							
Myricaceae	25,0	50	0,08	0,09	0,20	0,80	0,32
CASUARINALES							
Casuarinaceae	25,0	50	0,06	0,09	0,15	0,85	0,17
BUXALES							
Buxaceae	75,0	36	0,75	0,08	0,08	0,17	1,00
Daphniphyllaceae	25,0	38	0	0,10	0,10	0,90	0,00
CUNONIALES							
Cunoniaceae	25,0	40	-	-	-	-	-
Baueraceae	50,0	42	-	-	-	-	-
Ribesiaceae	50,0	42	-	-	-	-	-
Brunelliaceae	1,0	43	-	-	-	-	-
Davidsoniaceae	1,0	37	0,00	0,14	0,14	0,86	0,00
Eucryphiaceae	25,0	35	0,11	0,16	0,25	0,74	0,00
Bruniaceae	37,5	54	-	-	-	-	-
Grubbiaceae	50,0	42	-	-	-	-	-
SAXIFRAGALES							
Crassulaceae	75,0	55	0,07	0,21	0,30	0,70	0,48
Cephalotaceae	100,0	60	0,00	0,13	0,13	0,86	0,00
Iteaceae	25,0	54	-	-	-	-	-
Francoaceae	100,0	54	0.07	0,18	0,25	0,75	0,00
Saxifragaceae	75,0	48	0,23	0,15	0,36	0,60	0,35
Vahliaceae	100,0	54	-	-	-	-	-
Greviaceae	25,0	54	-	-	-	-	-
GUNNERALES							
Gunneraceae	100,0	54	-	-	-	-	-
ROSALES							
Crossosomataceae	50,0	43	-	-	-	0,67	-
Rosaceae	50,0	43	0,14	0,11	0,28	-	0,18
Neuradaceae	100,0	60	-	-	-	0,93	-
Chrysobalanaceae	25,0	48	0,00	0,06	0,07	-	0,00

Correlation of the flavonoid chemosystematic parameters with the herbaceousness index (HI) and Sporne index (SI), allows visualization of valuable evolutionary tendencies inside the superorder. A positive correlation is observed when the herbaceousness index (HI) and Sporne index (SI) are compared, with the chemosystematic evolutionary advancement parameter of the flavonoid hydroxyl protection by glycosylation (EAG) for the of Rosiflorae families (Figure 8a,b). These Figures demonstrate that taxa of the Rosiflorae preferentially use the glycosylation mechanism to protect their flavonoid hydroxyls. The families of Saxifragales are a closely re-

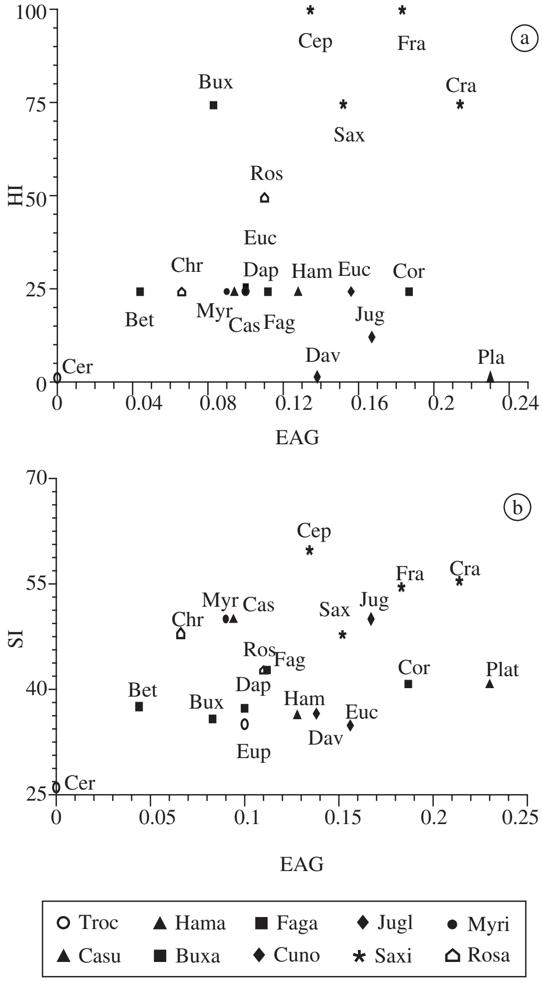


Figure 8. Relationship between herbaceousness index (HI) and sporne index (SI) and the protection of the flavonoids hydroxyls for glycosylation (EAG) for the families of Rosiflorae. a) herbaceousness index (HI) and the protection of the flavonoids hydroxyls for glycosylation (EAG), b) sporne index (SI) and the protection of the flavonoids hydroxyls for glycosylation (EAG). Tro =Trochodendrales; Hama = Hamamelidales; Faga = Fagales; Jugl = Juglandales; Myri = Myricales; Casu = Casuarinales; Buxa = Buxales; Cuno = Cunoniales; Saxi = Saxifragales; Rosa = Rosales

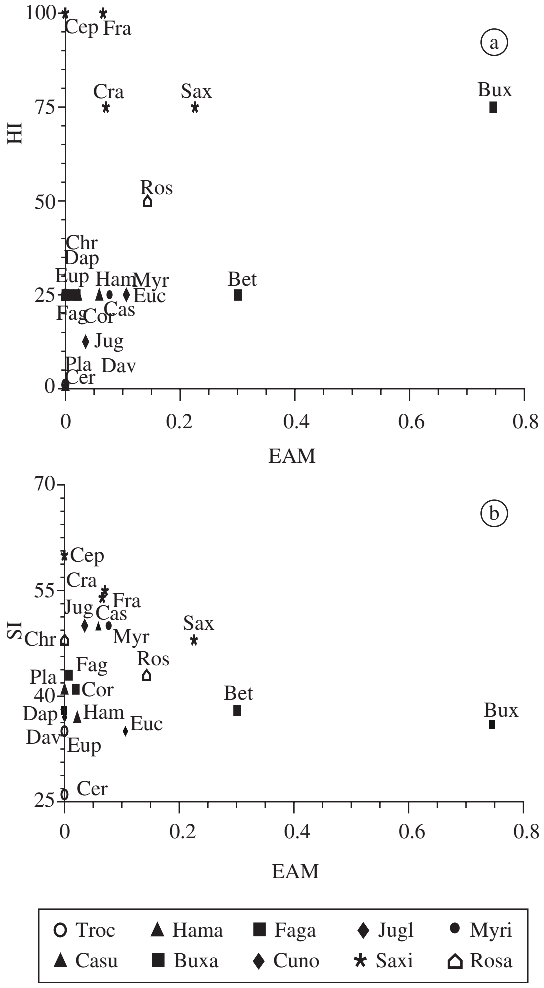


Figure 9. Relationship between herbaceousness index (HI) and sporne index (SI) and the protection of the flavonoids hydroxyls for methylation (EAM) for the families of Rosiflorae. a) herbaceousness index (HI) and the protection of the flavonoids hydroxyls for methylation (EAM), b) sporne index (SI) and the protection of the flavonoids hydroxyls for glycosylation (EAM). Tro = Trochodendrales; Hama = Hamamelidales; Faga = Fagales; Jugl = Juglandales; Myri = Myricales; Casu = Casuarinales; Buxa = Buxales; Cuno = Cunoniales; Saxi = Saxifragales; Rosa = Rosales.

lated and more developed group, while the families of Trochodendrales were shown to be less developed. The families of the other orders have an intermediary positioned.

The systematic indexes: herbaceousness index (HI) and the Sporne index (SI) were compared with the flavonoid protection parameter. When evolutionary advancement parameters for methylation index (EAM) are compared, it is observed that Buxaceae presents a prominent position, showing that the protection of its flavonoid hydroxyls is mainly by means of methylation. This fact separates Buxaceae from Daphniphyllaceae. Evidence of Betulaceae is also shown by being separated from Fagaceae and Corylaceae. The positioning of Saxifragales should be noted in that its families form a closed group, positioned at a higher evolutionary level. The other families are grouped together in a small homogeneous range, demonstrating that the Rosiflorae flavonoid hydroxyl groups in general are not protected by methylation (Figures 9a,b).

Correlations of the herbaceousness index (HI) and the Sporne index (SI) with the total protection evolutionary advancement parameters (EATP), revealed the prominence of the family Buxaceae relative to the other families of the superorder. The families of Saxifragales, were still shown to be closely related and positioned in the highest advancement level.

4. Conclusion

The results of chemosystematics analysis: the ubiquitous production of flavonols in detriment of other flavonoid type metabolites resulted in a very low flavone/flavonol ratio; the high degree of unprotected flavonoid hydroxyls (31%) and preferential hydroxyl protection by glycosylation mechanism (49%) in contrast to methylation (11%) and double protection (9%) mechanism; besides the low level of A-ring transformation, confirm the primitive positioning of the superorder Rosiflorae according to the botanists.

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