

## Distribution of Leaf Flavonoid Aglycones and Glucuronides in the Genus *Phaseolus* and Related Genera

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### Abstract

Ninety-six species and hybrids of the genus *Phaseolus* and related genera, *Dysolobium*, *Macroptilium*, *Strophostyles*, and *Vigna* are surveyed for their leaf flavonoid (flavone/flavonol) aglycone and glucuronide distribution patterns. The aim is to determine whether the distribution patterns of certain flavonoid aglycones and/or glucuronides are restricted in each genus and hence, could be used to delineate the genus. The results show that flavonol aglycones (kaempferol and quercetin) are more frequent in the entire group than the flavones (luteolin and apigenin). Kaempferol is more prevalent than quercetin in the genus *Macroptilium*, while *Strophostyles* accumulates mainly quercetin. There is generally no restricted pattern for a particular type of flavonoid aglycone in *Phaseolus* and *Vigna* genera. Results also show the presence of flavonoid glucuronides in six out of 18 species of *Phaseolus* surveyed, but are absent in all the species of *Vigna*, *Macroptilium*, and *Strophostyles*. The situation in *Dysolobium* is inconclusive. A flavonoid band moved toward the anode during electrophoresis (a positive test for the presence of glucuronide) but glucuronic acid was not detected during subsequent analysis. In the genus *Phaseolus*, all the species and cultivars that accumulate flavones such as *P. coccineus*, *P. ricciardianus* and *P. parviflorus* accumulate flavone glucuronides, while *P. vulgaris*, *P. tuberosus* and *P. zebra* accumulate flavonol glucuronides.

**Keywords:** *Phaseolus*, flavonoid, quercetin, kaempferol, luteolin, apigenin, glucuronide, flavone, flavonol

### 1. Introduction

Species of the plant genus *Phaseolus* and its relatives are generally known as “beans”. They form important staple food crops in many countries especially in some tropical areas where protein rich beans could be substituted for meat proteins. Few species are cultivated. Two of the most important ones are *P. vulgaris*, which thrives in the temperate regions and *P. lunatus*, which prefers the tropical regions. The majority of the species is wild and is distributed in all the climatic regions. The potentials of these wild species are not yet fully realized.

The precise definition of the genus has long been unclear, mainly because it is difficult to distinguish it clearly from *Vigna* (Evans, 1980; Onyilagha, 1993; Onyilagha & Shahidul, 2009). This confusion may have started early in Linnaean taxonomy. Linnaeus included eleven species in his genus, namely, *Phaseolus vulgaris*, *P. coccineus*, *P. lunatus*, *P. inamoenus*, *P. farinosus*, *P. vexillaatus*, *P. helvulus*, *P. alatus*, *P. caracalla*, *P. radiatus* and *P. max* (Linnaeus, 1753).

De Candolle divides the genus *Phaseolus* into two sections, *Euphaseolus* and *Strophostyles* with five subgroupings in the first and two in the second. These are however heterogeneous and do not provide a satisfactory basis for classification (Candolle, 1825). Bentham gives a synopsis of the genus. He describes 85 species distributed in seven sections: *Drepanospron*, *Euphaseolus*, *Leptospron*, *Strophostyles*, *Lasiospron*, *Microcochle* and *Macroptilium* (Bentham, 1837). In his later work, he dropped *Lasiospron* and *Microcochle* and added *Dysolobium*. Since that time, *Dysolobium* and *Macroptilium* are recognized as distinct genera by Prain and Urban respectively.

In his review, Piper recognized eight sections: *Sigmoidotropis* Piper, *Ceratotropis* Piper, *Lasiospron*, *Macroptilium*, *Microcochle*, *Cochlianthus* (Trew) Piper, *Leptospron* and *Euphaseolus* (Piper, 1926). In another revision of the genus, Verdcourt recognized *Macroptilium* as a genus after Urban and the treatment of *Sigmoidotropis*, *Ceratropis*, *Lasiospron* and *Cochlianthus* as sub-divisions of *Vigna* (Verdcourt, 1970). *Phaseolus* was therefore limited to only 50 species. To date, this treatment serves a practically useful classification

suitable for most plant breeders, but there are still some American lowland types which are *Vigna*-like and some African species which have *Phaseolus* type characteristics (Marechal, Mascherpa, & Stainier, 1978). The latest revision of Marechal et al. recognized three sections, which include a large *Euphaseolus* and smaller *Alepidocalyx* and *Minkelersia* (Marechal, Mascherpa, & Stainier, 1978). They maintained earlier elevations of *Macroptilium*, *Dysolobium* and *Strophostyles* to distinct generic levels. It is important to note, however, that the above revisions of the genus were based largely on morphology and related characters. Phytochemical data were absent or rare.

Some accounts have appeared in literature where biochemical data have been employed to help resolve taxonomic problems in the Leguminosae. For example, seed extracts of *Lathyrus* yielded amino acid patterns which reflect species differences (Bell, 1962). Lackey presented a classification of the tribe Phaseoleae based on canavanine amino acid distribution in seeds of species (Lackey, 1977). Ingham studied the systematic significance of phytoalexin compounds in the Phaseoleae (Ingham, 1990). Webb and Harborne studied the sectional classification of the genus *Vicia* based on leaf flavonoid aglycones patterns (Webb & Harborne, 1991). Seneviratne and Harborne gave an account of the taxonomic usefulness of constitutive flavonoids and induced isoflavonoids in the genus *Vigna* (Seneviratne & Harborne, 1992). Other more recent applications of biochemistry in taxonomic studies include Williams et al. (1995), Onyilagha and Shahidul (2009), Onyilagha et al. (2003, 2009), etc.

The actual function(s) of glucuronides in plants is yet to be elucidated and there is doubt as to whether it is a true sugar. However, we speculate that it may be one of the defense molecules, which may protect the plant species against herbivores. Our application of the presence and/or absence of flavonoid glucuronides in a taxonomic study is novel and it is hoped that it will help to delineate species or generic boundaries in the legumes.

The aim of this study is to bring to life the taxonomic problem of delineating *Phaseolus* from other relatives by presenting the distribution pattern of leaf flavonoid aglycones and glucuronides in the entire group, and this new information may provide useful taxonomic data during any future revision of the genus *Phaseolus* or the entire group.

## 2. Materials and Methods

Most of the seed samples were obtained from the National Botanic Gardens, Belgium, the United States Department of Agriculture (Plant Introductions unit), and CIAT. In the text/Table, all samples from Belgium have the letters 'N.I.' prefixed to their accession numbers; those from U.S. have the letters 'P.I.'; while those from CIAT are prefixed with the letter 'G'.

### 2.1 Planting of Seeds

The seeds were initially scarified and soaked in deionized water overnight (Onyilagha, 1993). The scarification allowed easy water penetration into the cotyledons thereby helping to break dormancy. Soaked seeds were transferred into petri dishes containing a small quantity of wet vermiculite. They were covered and placed in dark isothermal conditions of 25 °C for up to three days. The seeds or seedlings were transferred into six inch pots containing high nutrient soil and the pots placed in the glasshouse. Watering was normal and was done every two days. Nutrients (N.P.K., ratio 1:1:1) were fed from 2-3 weeks after germination. A one week feeding interval was maintained throughout the growing period (Onyilagha, 1993). Healthy leaves were harvested from mature plants. Samples were completely air dried, and stored in the laboratory. This served as the source of materials for biochemical analyses.

### 2.2 Biochemical Extractions and Analyses

Biochemical compounds were studied using standard procedures (Holt et al., 2010; Onyilagha et al., 2012). Dried leaf samples were boiled in 70% ethanol for about 10 minutes and left to stand overnight for completed extraction. The extract was filtered and evaporated to dryness using a rotary evaporator. Purification of glycosides was done by 1-D paper chromatography (3 MM paper) in at least three solvents: Butanol-Acetic acid-Water (BAW 4:1:5, top layer), 15% Acetic acid and then Water. In some cases it was necessary to further purify the glycosides in Chloroform-Acetic acid- water (CAW 1:1).

Ultraviolet spectral measurements of methanolic glycosides (neutral) were obtained using the HPLC. The glycosides were then hydrolyzed with 2 M HCL in a boiling water bath for about 30 minutes. The sample was allowed to cool. Aglycones were extracted in ethyl acetate, dried in fume cupboard, and chromatographed on cellulose thin layers (100 mm) against authentic markers in 50% Acetic acid, BAW, and Forestal solvents (Onyilagha et al., 2003).

Paper electrophoresis was carried out to determine the presence or absence of glucuronides in all the pure samples. A small amount of pure glycoside was applied as separate spots on a 3 MM paper (11" × 8"). Glucuronide is not present in rutin and therefore it was used as a negative marker plus a positive marker such as quercetin or

kaempferol 3-glucuronide or apigenin or luteolin 7-glucuronide. Electrophoresis was carried out for 2.5 hours at 400 V in acetate buffer pH 4.4. Dried electrophoretic papers were examined under UV/NH<sub>3</sub> for any migration toward the anode (+). Rutin does not migrate due to absence of glucuronide.

### 3. Results

Results of this survey are presented in Table 1. Apart from *P. coccineus*, *P. parviflorus*, and *P. ricciardianus*, which accumulate flavonoids based on flavone (apigenin and/or luteolin), all other species of *Phaseolus* surveyed so far accumulate flavonoids based on flavonols (quercetin and/or kaempferol) (Figure 1). In the genus *Vigna*, only the species *V. praecox* has so far been found to accumulate flavones. Hence, the results tend to suggest that the entire group is dominated by flavonols. Myricetin flavonoid was found absent in all the genera surveyed. There is no consistent agreement in flavonoid aglycone distribution in *Phaseolus*, even among the cultivated species such as *P. coccineus*, *P. vulgaris*, *P. acutifolius*, *P. lunatus* and *P. polystachyus* (Table 1). The situation is different in *Vigna* where there is apparent dominance of kaempferol aglycone in most of the cultivated species (*V. angularis*, *V. trilobata*, *V. aconitifolia*, *V. mungo*, *V. radiata*, *V. subterranea* and *V. unguiculata*).

This survey reveals that glucuronides are not universally distributed in plants. Its absence is scored when glucuronic acid cannot be detected on paper chromatograms after sugar analyses and also when there is no movement of spots to the anode during electrophoresis. We present the presence/absence of flavonoid glucuronide in Table 1. Similar to flavonoid aglycones, there is no consistency in accumulation of glucuronides in *Phaseolus* genus. It is present in 6 out of 18 species of *Phaseolus*, but absent in all surveyed species of *Vigna*, *Macroptilium*, and *Strophostyles*. The situation in *Dysolobium* is confusing. There was obvious movement of one of the flavonoid bands toward the anode during electrophoresis but glucuronic acid was not detected on the paper chromatograms after sugar analysis.

Table 1. Distribution of leaf flavonoid aglycones and glucuronides in *Phaseolus* and related genera

Species	Q3'Me	Q	K	L	A	Glucuronides
<i>Phaseolus coccineus</i> L.						
Ssp. Polyanthus:						
G35089	-	-	-	+	+	+
G35877	-	-	-	+	+	+
Ssp. Coccineus:						
Var. coccineus N.I. 0132	-	-	-	+	+	+
Var. silvester N.I. 0726	-	-	-	+	+	+
<i>P. ricciardianus</i> Tenore						
Gradina 901	-	-	-	+	+	+
<i>P. parviflorus</i> G. Freytag						
N.I. 812	-	-	-	+	+	+
<i>P. vulgaris</i> L.						
Ssp. Aborigineus						
G19892	-	+	+	-	-	+
Var. vulgaris:						
Giessen 220	-	+	+	-	-	NT
N.I. 0613 (weedy type)	-	+	+	-	-	NT
N.I. 0928 (wild type)	-	+	+	-	-	NT
<i>P. tuberosus</i> Eaton & Wright						
Kn 850114	-	+	+	-	-	+
<i>P. zebra</i> Savi						
Dijon 1306	-	+	+	-	-	+

<i>P. acutifolius</i> A. Gray						
Var. acutifolius N.I. 0576	-	+	+	-	-	-
Var. latifolius G 40066A	-	+	+	-	-	-
<i>P. lunatus</i> L.						
N.I. 823	-	+	+	-	-	-
Dijon 1303	-	+	+	-	-	-
<i>P. limensis</i> Macfadyen	-	+	+	-	-	-
<i>P. leptostachyus</i> Benth.						
N.I. 1037	-	+	+	-	-	-
<i>P. polystachyus</i> (L). Britt.						
P.I. 264607	-	?	+	-	-	-
P.I. 264608	-	+	+	-	-	-
<i>P. microcarpus</i> Mart.						
N.I. 708	-	-	+	-	-	-
<i>P. filiformis</i> Benth.						
P.I. 535300	-	+	+	-	-	-
N.I. 0587	-	+	+	-	-	NT
F 1147	-	+	+	-	-	NT
<i>P. oligospermus</i> Piper						
N.I. 1116	-	+	+	-	-	-
<i>P. xanthotrichus</i> Piper						
N.I. 1239	-	+	+	-	-	-
<i>P. micranthus</i> Hooker & Arn.						
N.I. 1107	-	+	+	-	-	-
<i>P. angustissimus</i> A. Gray						
N.I. 0788	-	+	+	-	-	-
<i>P. bolivianus</i>						
Kew 10968	-	+	+	-	-	NT
<i>P. polymorphus</i> S. Wats.						
Kew, Parry & Palmer	-	+	+	-	-	NT
<i>P. wrightii</i> A. Gray						
Kew 1347	-	+	+	-	-	NT
<i>P. anisotrichus</i> Schlecht						
Kew 13163	-	+	+	-	-	NT
<i>Strophostyles helvula</i> (L)						
Elliott	?	+	?	-	-	-
P.I. 215295						
<i>S. leiosperma</i> (Torrey & A. Gray) Piper	+	+	-	-	-	-
P.I. 215298						
<i>S. umbellata</i> (Willd.) Britt.						
N.I. 0317	+	+	?	-	-	-
<i>Dysolobium apiodes</i>						

(Gagnepain) Marechal	-	-	-	-	+	?
CIAT 4596						
<i>Macroptilium atropurpureum</i>						
(DC.)Urb.	-	-	+	-	-	NT
N.I. 101	-	-	+	-	-	-
N.I. 0328	-	-	+	-	-	NT
Q 883						
<i>M. bracteatum</i> (Nees & Mart.)						
Marechal & Baudet	-	+	+	-	-	-
N.I. 0327	-	+	+	-	-	NT
Kew 23251						
<i>M. lathyroides</i> L. Urban						
N.I. 0384	-	-	+	-	-	NT
Ksukuba 587	-	-	+	-	-	-
<i>M. fraternum</i> (Piper) Lackey						
N.I. 1307	-	-	+	-	-	-
<i>M. erythroloma</i> (Benth.)						
Urban	+	+	+	-	-	-
N.I. 698						
<i>M. gibbosifolium</i> (Ort.)						
Delgado	-	+	?	-	-	-
P.I. 322595	-	+	?	-	-	NT
N.I. 697						
<i>M. gracile</i> (Poepp.) Urban						
Kew 793	-	?	+	-	-	NT
<i>M. Supinum</i>						
N.I. 872	-	?	+	-	-	-
<i>Vigna luteola</i> (Jacq.) Bentham						
P.I. 196813	-	-	+	-	-	-
<i>V. Caracalla</i> L. Verdc.						
P.I. 146800	-	+	+	-	-	-
<i>V. glabrascens</i> Marechal						
P.I. 207655	-	+	-	-	-	-
<i>V. longifolia</i> (Benth.) Verdc.						
Kew-Irwin	-	+	+	-	-	NT
<i>V. parkeri</i> Baker						
N.I. 1315	-	?	+	-	-	-
Archbold 1456	-	+	+	-	-	NT
<i>V. marina</i> (Burman) Merrill						
Q 821	-	+	+	-	-	NT
<i>V. reticulata</i> Hooker						
Flock 77A	-	+	-	-	-	NT
<i>V. frutescens</i> A. Richard						

Kew 846	-	+	+	-	-	NT
<i>V. Oblongifolia</i> A. Richard						
NI 124	-	+	-	-	-	-
NI 389	?	-	?	-	-	-
<i>V. praecox</i> Verdc.						
Kew 19065	-	-	-	+	+	NT
<i>V. juncea</i> Milne-Redhead						
Kew 1947	-	+	+	-	-	NT
<i>V. triphylla</i> (R. Wilczk)						NT
Verdc.	-	?	+	-	-	NT
Kew 2817	-	+	+	-	-	
N.I. 100						
<i>V. catjang</i> Walp						
Dijon 1359	-	+	-	-	-	NT
<i>V. angularis</i> (Willd.) Ohwi & Ohashi						
AA1	-	-	+	-	-	-
5125	-	-	+	-	-	-
5152	-	-	+	-	-	-
Ahevi 936						
<i>V. trilobata</i> L. Verdc.						
N.I. 0251	-	+	+	-	-	-
N.I. 0258	-	+	+	-	-	-
<i>V. aconitifolia</i> (Jacq.) Marechal						
MoA1	-	-	+	-	-	-
502	-	-	+	-	-	-
512	-	-	+	-	-	-
PG-I						
<i>V. mungo</i> L. Hepper						
BA1	-	-	+	-	-	-
TVm1	-	-	+	-	-	-
T-9	-	-	+	-	-	-
3118	-	-	+	-	-	-
<i>V. subterranea</i> L. Verdc.						
Roger 3	-	-	+	-	-	-
Ankpa-Nigeria	-	-	+	-	-	-
Tali-Ghana	-	-	+	-	-	-
<i>Vigna umbellata</i> (Thunb.) Ohwi & Ohasi						
436	-	-	+	-	-	-
4022	-	-	+	-	-	-
4042	-	-	+	-	-	-

TVum1						
<i>V. unguiculata</i> L. Verdc.						
Ssp. Unguiculata:						
CA I	-	-	+	-	-	-
362	-	-	+	-	-	-
Ssp. Cylindrical:						
360	-	-	+	-	-	-
Hawarimae	-	-	+	-	-	-
Ssp. Sesquipedalis:						
361	-	-	+	-	-	-
Bushitawo	-	-	+	-	-	-
Ssp. Sinensis:						
P.I. 29354	-	+	?	-	-	-

Key to Table 1: Q3'Me = quercetin 4' methyl ether; Q = quercetin; K = kaempferol (M, Q, K = flavonols); L = luteolin; A = apigenin (L, A = flavones); NT = not tested; (+) = present; (-) = absent; (?) = undetermined.

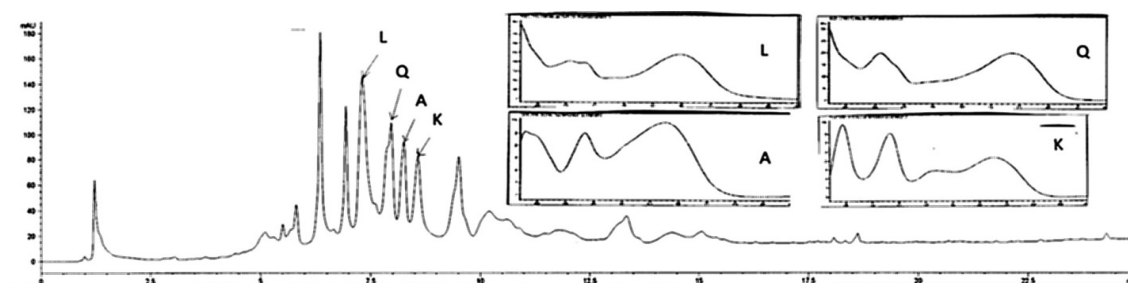


Figure 1. Chromatogram showing luteolin (L), quercetin (Q), apigenin (A), and kaempferol (K) as well as corresponding uv-spectrum

#### 4. Discussion

This study appears to support earlier separations of the genera *Macroptilium* and *Strophostyles* from *Phaseolus*. While there is biochemical relationship between the three genera on account of flavonoid accumulation, however, flavonol aglycones are restricted in *Macroptilium* and *Strophostyles* genera, a characteristic which could be used in defining both genera. Furthermore, kaempferol appears to dominate in the genus *Macroptilium* while *Strophostyles* seems to accumulate more of quercetin aglycones. Hydrolysis was not achieved in *Dysolobium* because of the presence of flavonoid C-glycosides (Williams, 1995). Distribution of flavonoid aglycones agrees in the existence of a complex relationship between *Phaseolus* and *Vigna* (Marechal, Mascherpa, & Stainier, 1981). There is apparently no defined pattern of flavonoid distribution in the two genera, an observation which is consistent with Williams et al. (1995). Most of their species contain flavonol (quercetin and kaempferol) aglycones while few others contain either of the two aglycones. In the genus *Phaseolus*, it is interesting to note that all the species and cultivars that accumulate flavone (apigenin and/or luteolin) glucuronides, while *P. vulgaris*, *P. tuberosus*, and *P. zebra* accumulate flavonol glucuronides.

We observe that although leaf flavonoid aglycone and glucuronide distribution are not adequate enough to resolve the complex relationship between *Phaseolus* and *Vigna*, however, their distribution patterns in *Vigna*, *Strophostyles*, and *Macroptilium* suggest that they may be useful in the characterization and/or revision of the genera. Further investigations are necessary in the presence/absence of glucuronides in *Dysolobium*. We surveyed only one species (*D. apiodes*), but the true situation will be known when many more species are surveyed.

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