INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
08–140/LCS/2009/11–3–231–234
http://www.fspublishers.org

Full Length Article

Flavonoids and other Polyphenols of the Cultivated Species of the Genus *Phaseolus*

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ABSTRACT

Phaseolus, a member of the legume subtribe, Phaseolinae, is an economically important genus comprising of several food crop species such as *Phaseolus vulgaris* (the red kidney bean), *P. lunatus* (butter bean) and *P. coccineus* (runner bean). Flavonoid and proanthocyanidin polyphenols play significant role in human health as antioxidants. To complement earlier studies on constitutive flavonoids in the leaves, we decided to investigate different flavonoid structures present in the stems of five cultivated species, *P. vulgaris, P. coccineus, P. lunatus, P. polyanthus* and *P. zebra*. Since the seed is the major edible part, and the leaves are used as vegetables in many countries, this study also investigated the presence and/or absence of proanthocyanidins in the seeds and leaves. Results indicated that proanthocyanidins are absent in the leaves of all the species, but present in their seeds. Flavonoids in the stems generally agreed with leaf flavonoids and placed the species into two groups, the flavone group (*P. coccineus* & *P. polyanthus*) and the flavonol group (*P. vulgaris, P. lunatues* & *P. zebra*). The biochemical data were used to assess the relationships between the five cultivated species. This biochemical information would be useful in plant selection and also in future breeding programs.

Key Words: *Phaseolus* spp.; Flavonoid; Kaempferol; Quercetin; Apigenin; Luteolin; Proanthocyanidin

INTRODUCTION

The plant genus *Phaseolus* is a member of the legume tribe Phaseoleae, subtribe Phaseolinae. The genus is made up of 50 species (Marechal *et al.,* 1978), a few of which are cultivated, while majority are wild. Seeds from the cultivated species are consumed worldwide and constitute a vital source of protein and fiber for humans. Some of the cultivated species are often confused with those belonging to *Vigna*, another important genus of the subtribe and the seeds from both genera are together referred to as "beans". In an attempt to discriminate between cultivars of the two genera, beans within *Phaseolus* are often referred to as "dry beans" and are distinguished by their shape, color and growth characteristics. They include the common bean or red kidney bean for *P. vulgaris*, butter bean or lima bean for *P. lunatus*, runner bean for *P. coccineus* and tepary bean for *P. acutifolius*.

Although several domesticated species of *Phaseolus* exist, however, *Phaseolus vulgaris* remains the most cultivated and most eaten of all (Phaseomics, 2004). Over 12 million tons of dry beans are produced annually worldwide, with a total production value of $5717 US million; and of this production, about 81% occurs in tropical countries. Brazil is the most important country for production and consumption of beans in the world (Janssen *et al.*, 1992), followed by Mexico and the East Africa region. Beans are naturally low in fat and are a significant source of both soluble and insoluble fibers, protein, essential vitamins and minerals and phytochemicals (Geil & Anderson, 1994). In terms of nutritional quality, the common bean, *P. vulgaris* may be regarded as "perfect food" on account of its high protein content (16 g/cup/serving), fiber (8 g/cup), complex carbohydrates (40 g/cup) and other dietary necessities (Anonymous, 2008). It is estimated that a single serving (1 cup) of beans provides at least half the US Department of Agriculture's recommended daily allowance of folic acid, 25 to 30% of iron and meets 25% of the daily requirement of magnesium and copper as well as 15% of the potassium and zinc (Anonymous, 2008).

In the United States, high quality beans are produced mostly in the states of Michigan and North Dakota. In the southern states, *P. vulgaris* is regarded as an "alternative crop" and most commonly produced by small-scale (limited-resource) farmers who cannot compete with the large-scale farmers on major crops such as cotton, soybean, rice and corn.

Several findings on the biochemicals of the bean have been reported. The work of Williams *et al.* (1995) focused on the flavonoid profiles in leaves of 17 *Phaseolus* species (cultivated & wild) and in flowers of nine species. Some

To cite this paper: Onyilagha, J.C. and S. Islam, 2009. Flavonoids and other polyphenols of the cultivated species of the genus *Phaseolus. Int. J. Agric. Biol.*, 11: 231–234

recent biochemical studies center on the most economically important species, *P. vulgaris*, including profiles of seed coat anthocyanin glycosides (Choung *et al.,* 2003) and polyphenols (Espinosa-Alonso *et al.,* 2006). Efforts have not yet been made to study the phytochemicals of other cultivated species for useful biochemicals, which could be exploited for human benefit.

Since the flavonoid profiles in leaves and flowers of some wild and cultivated *Phaseolus* species have been studied (Williams *et al.*, 1995), the aims of this study are to provide additional information on the flavonoid profiles in the stems of five cultivated species and also the proanthocyanidin profile in seeds and leaves of the five species. This information would be useful in understanding of the chemistry and nutritional benefits of the cultivated species, as well as in the analysis of relationships among these species.

MATERIALS AND METHODS

Seeds of five cultivated *Phaseolus* species, including *P. vulgaris*, *P. lunatus*, *P. polyanthus*, *P. coccineus* and *P. zebra* were obtained from the International Center for Tropical Agriculture (CIAT), Colombia, the National Botanic Gardens, Belgium and the Botanic Gardens, France. The seeds were scarified and planted in 6 inch pots filled with high nutrient soil and placed in isothermal conditions of 25^{o}C for three days (Onyilagha, 1993). The pots were then transferred to the greenhouse maintained at optimal temperature and humidity, 16 h light and 8 h darkness. Healthy leaves and mature seed-pods were harvested for analysis.

Flavonoids in the stems of the five species were extracted and identified using standard methods (Harborne & Harborne, 1998; Onyilagha *et al.,* 2003). About 50 g dry stems of each of the five species were extracted with 70% ethanol in a boiling water bath for 10 min. The extract was allowed to stand overnight for complete extraction and then evaporated to dryness using a rotary evaporator. The residue was redissolved in 25 mL 70% ethanol and chromatographed on cellulose thin layer and developed in Butanol-Acetic acid-Water (BAW, 4-1-5, top layer). Flavonoids were identified as dark bands under the UV light, turning to yellow when fumed with ammonia. The flavonoids were eluted and further purified in 15% acetic acid in water and in 100% water. Flavonoid glycosides were identified using information obtained from their Rf values, UV-visible absorption spectra (Beckman DU640) and their specific behaviors with five shift reagents (2 M NaOH, 5% aluminum chloride in ethanol, aluminum chloride-HCl, hydrated sodium acetate salt & powdered boric acid).

The presence of proanthocyanidin (prodelphinidin & procyanidin) in leaves and seeds was assayed using standard methods (Harborne, 1984; Onyilagha, 1993; Harborne & Harborne, 1998). Specifically, 5 g of macerated dry leaves and ground seeds were covered in approximately 20 mL of 2 M HCl in a glass tube, followed by heating in a boiling water-bath for 10 min. Red, orange, magenta, mauve or purple coloration indicated the presence of proanthocyanidins (Harborne, 1984; Harborne & Harborne, 1998). The tube was allowed to cool and interfering flavonoids were removed with equal volume of ethyl acetate. The tube was reheated (5 min) to remove remaining traces of ethyl acetate. On cooling, proanthocyanidins were extracted from aqueous solution in isoamyl alcohol, evaporated to dryness under the fume hood and chromatographed on cellulose thin layer plates (Sigma-Aldrich, 100µm thick on polyester support). TLC plates were developed in BAW, 50% Acetic acid in water, and FORESTAL reagent (water: acetic acid: conc. HCl in ratio of 10: 30: 3 v/v, respectively) (Harborne, 1984; Onyilagha, 1993; Harborne & Harborne, 1998). Presence of prodelphinidin and procyanidin was confirmed by comparing the color and Rf values of the samples with standard markers (Onyilagha, 1993).

RESULTS

The flavonoids in stems of the five cultivated *Phaseolus* species are shown in Table I along with those found in leaves as detailed in earlier study (Williams *et al.*, 1995). The species accumulate diverse flavonoid structures in their stems; while *P. vulgaris, P. lunatus* and *P. zebra* accumulate flavonols, *P. coccineus* and *P. polyanthus* accumulate flavones. In the three species that accumulate flavonols, kaempferol and quercetin glycosides appear equally represented. The two flavone species, *P. polyanthus* and *P. coccineus* accumulate glucuronic acid at C_7 position. Both species accumulate Lu-7- *O*- glucuronide in their stems; however, Ap-7- *O*- glucuronide seems to be absent or of undetectable concentration in the stems of *P. coccineus*. Apigenin-and luteolin-7- *O*-glucuronides are present in the leaves of the two species. *P. lunatus* is apparently the only cultivated species here examined that does not accumulate glucuroninc acid.

Proanthocyanidins are absent in the leaves, but present in the seeds of all the five species (Table II). Although *P. polyanthus* and *P. coccineus* both accumulate flavones, however, they differ in their abilities to accumulate proanthocyanidins. *P. polyanthus* accumulates low (+) concentrations of prodelphinidin and procyanidin, while *P. coccineus* accumulates low concentration of procyanidin only. The other three species which accumulate flavonol glycosides appear to accumulate higher concentrations of proanthocyanidins. Cultivars of *P. vulgaris* accumulate varying amounts, ranging from low (+), through medium (++) to high (+++) concentrations (Table II).

DISCUSSION

Results from this study show that most of the five cultivated *Phaseolus* species accumulate similar flavonoids

Table I. Flavonoids in stems (from this study) and leaves (Williams et al., 1995) of cultivated species of *Phaseolus*

Species	Flavonoids in stems	Flavonoids in leaves
P. vulgaris (Burk.) Baudet		
G. 19892	Robinin	Qu-3- *O* -glucuronide
	Km-3- *O* -triglycoside	Km-3- *O* -glucuronide
	Qu-3- *O* -glucuronide	Km-3- *O* -rutinoside
Burkat 220	Km-3-*O*-glucuronide	Qu-3- *O* -glucuronide
	Km-3- *O* -triglycoside	Qu-3- *O* -rutinoside
	Qu-3- *O* -glucuronide	Km-3- *O* -glucuronide
	Qu-3- *O* -rutinoside	Km-3- *O* -rutinoside
P. lunatus L.		
NI 823	Robinin	Qu-3- *O* -digalactoside
	Qu-3- *O* -glycoside	Km-3- *O* -robinobioside
Dijon 1303	Robinin	Km-3- *O* -robinobioside
	Qu-3- *O* -glycoside	
P. polyanthus (L.) Greenman		
G. 35089	Ap-7- *O* -glucuronide	Ap-7- *O* -glucuronide
	Lu-7- *O* -glucuronide	Lu-7- *O* -glucuronide
P. coccineus L.		
NI 0132	Lu-7- *O* -glucuronide	Lu-7- *O* -glucuronide
		Ap-7- *O* -glucuronide
P. zebra Savi		
Dijon 1306	Robinin	Qu-3- *O* -glucuronide
	Qu-3- *O* -glucuronide	Km-3- *O* -glucuronide

*Km = kaempferol; Qu = quercetin; Ap = apigenin; Lu = luteolin

Table II. Distribution of proanthocyanidin in seeds and leaves of cultivated *Phaseolus* species

Species (accession #)	*Proanthocyanidin		
	Delphinidin (seed)	Cyanidin (seed)	Leaf
P. vulgaris (Burk.) Baudet			
G. 19892	++	+	-
Burkat 220	+++	++	-
NI 0613	-	+	-
NI 0928	++	+	-
P. lunatus L.			
NI 823	-	++	-
Dijon 1303	-	+	-
P. polyanthus (L.) Greenman			
G. 35089	+	+	-
G. 35877	+	+	-
P. coccineus L.			
NI 0132	-	+	-
NI 0726	-	+	-
P. zebra Savi			
Dijon 1306	++	++	-

*high concentration = +++; medium concentration = ++; low concentration = +; absent = –

in their leaves and stems. The flavonoid survey has distinguished between two groups of cultivated species, those containing flavones (lacking a C_3 hydroxy group) and those containing flavonols (possessing a C_3 hydroxy group). Within the flavonol group, *P. lunatus* appears distinct from others due to the absence of glucuronic acid and its ability to accumulate different flavonoid glycosides in the stems and leaves. Robinin and Qu-3- *O*- glycoside accumulate in the stems, while Km-3- *O*- robinobioside and Qu-3- *O*- digalactoside accumulate in the leaves (Williams *et al.*, 1995). Accumulation of robinin is an important characteristic of the flavonol group, although this compound is lacking or accumulates in undetectable low concentration in Burkat 220, one of the two cultivars of *P. vulgaris*.

The flavonoid survey lends support to the close genetic relationship between *P. vulgaris* and *P. zebra* on one hand and between *P. coccineus* and *P. polyanthus* on the other. Indeed, Marechal *et al.* (1978) treated *P. polyanthus* as a subspecies of *P. coccineus* based on morphological characteristics and *P. zebra* is often considered a synonym for *P. vulgaris* (Anonymous, 2004). The flavonoids in leaves and stems of *P. vulgaris* and *P. zebra* agree with these classifications. Similarly, the accumulation of apigenin and luteolin glucuronides in the leaves and stems of *P. coccineus* and *P. polyanthus* agree with the treatment of *P. polyanthus* as a subspecies of *P. coccineus* (Marechal *et al*., 1978).

None of the cultivated *Phaseolus* species surveyed in this report accumulate proanthocyanidins in their leaves (Table II). This uniformity could be an important taxonomic marker, although other species need to be surveyed. Seed proanthocyanidin could provide some useful information on the relationship of the cultivated *Phaseolus* species. Whereas the relationship between *P. zebra* and *P. vulgaris* is evident on account of their flavonoid and seed proanthocyanidin accumulations, the same cannot be said of *P. coccineus* and *P. polyanthus*, as the latter (*P. polyanthus*) accumulates both prodelphinidin and procyanidin, but the former (*P. coccineus*) accumulates only procyanidin. However, this dissimilarity may not be enough strong evidence to warrant disagreement with the treatment of Marechal *et al*. (1978).

CONCLUSION

Cultivated *Phaseolus* species are rich in flavonoids. The species accumulate similar flavonoids in their stems and leaves. Two groups of cultivated species (the flavone group & the flavonol group) can be distinguished on account of their flavonoid structures. Apart from *P. lunatus*, all other species studied accumulate glucuronic acid. Since the species are important sources of high quality nutrients, the effect of flavonoid glucuronide in human diet is worth investigating.

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(Received 08 May 2008; Accepted 29 December 2008)