SEED GALACTOMANNAN IN THE CLASSIFICATION AND EVOLUTION OF THE LEGUMINOSAE

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Abstract—The galactomannans from seeds of 28 Brazilian legume species were extracted and analysed, and these data were added to those of 23 others previously presented. Among the species of the subfamily Caesalpinioideae studied, 84.6% contain galactomannan in their seeds, whereas this polysaccharide was found in 52 and 46.6% of the Mimosoideae and Faboideae, respectively. Higher galactomannan yields were found in seeds of Caesalpinioideae than in those of Mimosoideae and Faboideae. Furthermore, the galactomannans of Caesalpinioideae presented higher mannose:galactose ratios. This pattern did not change when further data available for 71 species were added. Considering the evolutionary pattern accepted for the Leguminosae in which Mimosoideae and Faboideae are derived from Caesalpinioideae, the data obtained suggest that in the course of evolution there was a decrease in galactomannan content, together with an increase in the proportion of galactose present. These features of galactomannan are also reproduced both at subfamilial and tribal levels. The yield and composition of seed galactomannans from tropical legume species are discussed in terms of the possibility that this multifunctional polysaccharide could be an important factor for the adaptation (through different strategies) of these species to the tropical humid, semi-arid and arid climates.

INTRODUCTION

Galactomannans are polysaccharides which occur in the endosperm cell walls mainly of leguminous seeds [1–3]. They are formed by a linear chain of β-1,4-D-mannopyranosides branched with single units of D-galactopyranosides through α-1,6 linkages [2]. It has been experimentally demonstrated that galactomannan initially takes up relatively large amounts (*ca* 60%) of the total water imbibed by the whole seed [4]. After germination, galactomannan is completely catabolized and its breakdown products are transferred to the embryo where they supply carbon and energy to the growing plantlet [5, 6]. This polysaccharide is therefore considered a 'multipurpose macromolecule' [4].

In the family Leguminosae, species from the subfamilies Caesalpinioideae and Mimosoideae evolved towards adaptation to tropical environments, whereas those from Faboideae mainly to the temperate and semi-arid climates [7]. The taxonomic potential of the presence of galactomannans in legume endosperms was firstly stressed by Hegnauer in 1957 [8]. Several authors have suggested that two features of galactomannan, the yield and mannose:galactose ratio, could be used as markers in the taxonomy of Leguminosae [1, 2, 9, 10]. In 1971, Bailey [9] proposed that high galactomannan contents and high mannose:galactose ratios are characteristics of the Caesalpinioideae, whereas low contents and low mannose:galactose ratios are features of the Faboideae subfamily. Therefore, it can be seen that the galactomannan distribution throughout Leguminosae might reflect the systematics, as well as the pattern of evolution of the family.

In a review, Dea and Morrison [2] presented the contents and mannose:galactose ratios of 71 species belonging mainly to the Caesalpinioideae and Faboideae subfamilies. According to the authors, only two species of Mimosoideae had been studied. Farooqi and Kapoor [11] investigated the seeds of 14 Mimosoideae, and only four contained 15–30% of endosperm yielding 10–20% in galactomannans. Due to the low number of species of Mimosoideae for which yield and composition of the galactomannans have been obtained, it has not been possible to analyse the distribution and composition of galactomannans from the evolutionary viewpoint in the Leguminosae family as a whole.

Buckeridge and Dietrich [3] analysed seeds of 23 species of Leguminosae including Mimosoideae. Although the possibility of using galactomannan as a taxonomic marker was pointed out by these authors, the number of species (mainly from Mimosoideae) was still too small to have an accurate view of the distribution of galactomannan throughout the Leguminosae.

The present work describes a distribution analysis of the yield and composition of galactomannans from 28 further Brazilian leguminous species, together with data

for those 23 already studied by Buckeridge and Dietrich [3], and the 71 reviewed by Dea and Morrison [2], in order to ascertain the possibility of using galactomannans as chemotaxonomic markers for the Leguminosae.

RESULTS

Table 1 shows the yield and mannose:galactose ratios of seed galactomannans of 28 tropical legume species. The data from Table 1 were added to those presented by Buckeridge and Dietrich [3], and analysed through diagrams of occurrence and frequency distribution of the yield and mannose:galactose ratios (not shown). It was calculated that among the Brazilian species studied 84.6% of the species belonging to Caesalpinioideae contain galactomannan in their seeds against 52% of Mimosoideae and 46.6% of Faboideae. Literature data could not be used to increase the sample, since apart from the data by Buckeridge and Dietrich [3], the species which do not

Table 1. Occurrence, yield and mannose:galactose ratio of galactomannans from selected Brazilian legume seeds

Subfamily species	Yield %	Ratio man:gal
Caesalpinioideae		
Cassia grandis	37.5	1.7
Senna reticulata	17.2	1.4
Mimosoideae		
Acacia farnesiana	*	—
Anadenanthera colubrina	*	—
Anadenanthera falcata	*	—
Calliandra selloi	*	—
Calliandra bracteosa	*	—
Enterolobium sp.	*	—
Inga marginata	*	—
Inga uruguensis	*	—
Leucaena sp.	14.9	1.3
Leucaena pulverulenta	15.4	1.2
Mimosa sp.	26.4	0.9
Mimosa platyphylla	18.5	0.9
Mimosa scabrella	27.1	1.0
Pithecellobium incurialli	*	—
Pithecellobium langsdorfii	*	—
Piptadenia gonoacantha	*	—
Prosopis juliflora	27.0	1.2
Faboideae		
Aeschynomene paniculata	*	—
Bowdichia virgilioides	8.4	0.9
Centrolobium robustum	*	—
Crotalaria micans	16.6	2.1
Crotalaria juncea	25.6	2.5
Indigofera suffruticosa	18.2	1.1
Machaerium sp.	*	—
Mucuna sp.	*	—
Sophora tomentosa	*	—

*Species that seem not to contain galactomannan as a reserve polysaccharide in the endosperm. In these cases either no mannose and galactose were detected, or their proportions were very low in relation to other monosaccharides.

present galactomannan in their seeds have hardly been mentioned.

Analysing the distribution of galactomannan yield among Brazilian species from the three subfamilies (i.e. the data shown in this work plus data published by Buckeridge and Dietrich [3]), it was observed that species with higher yields occur amongst Caesalpinioideae (mode 25.1–30%), the lowest yields amongst Faboideae (mode 15.1–20%), whereas the Mimosoideae subfamily presents a bimodal frequency distribution of yields (modes 15.1–20% and 25.1–30%). The distribution of frequencies of yields remains almost unchanged with the addition of data from the review by Dea and Morrison [2] mentioned above (Fig. 1A–C).

A relationship was also observed between the taxonomic position (subfamily) of the species and the mannose:galactose ratios of their galactomannans. It was found that the galactomannan occurring in Caesalpinioideae (mode in 3.01–3.5) has less galactose in the molecules than those from Mimosoideae (mode in 1.01–1.5) and Faboideae (0.5 to 2.5). When the literature data, i.e. 67 species whose mannose:galactose ratios were presented by Dea and Morrison [2] were added, the mannose:galactose ratio distributions (Fig. 1D–F) showed a clear cut mode for Faboideae (1.01–1.50, Fig. 1F), the others remaining practically unchanged.

DISCUSSION

Considering these data, which represent the majority of the leguminous species already studied for galactomannan content and composition, it is possible to demonstrate that there is a correlation between the taxonomic position (at the subfamily level) and the galactomannan yields and mannose:galactose ratios among the Leguminosae.

These data strongly confirm the suggestion made by several authors [1, 2, 8–10] that galactomannans are relevant for the taxonomy of Leguminosae. These data particularly extend the proposition of Bailey [9] that there is a tendency for a decrease both in galactomannan yields and mannose:galactose ratios from Caesalpinioideae to Faboideae.

Considering the evolutionary pattern accepted for the Leguminosae in which Mimosoideae and Faboideae are derived from Caesalpinioideae, with the Faboideae being the more advanced subfamily [7], these data suggest that in the course of evolution there was a decrease in galactomannan content together with an increase in the proportion of galactose present.

The subfamily Caesalpinioideae, which presented the highest yields and mannose:galactose ratios in relation to the other two subfamilies, occurs mainly in tropical areas: South America, tropical Africa and Southern Asia. This subfamily is thought to have originated in warm and moist environments [7].

From the evolutionary viewpoint, the same pattern observed at subfamily level was also found at genus level. In Mimosoideae, which showed intermediate values of
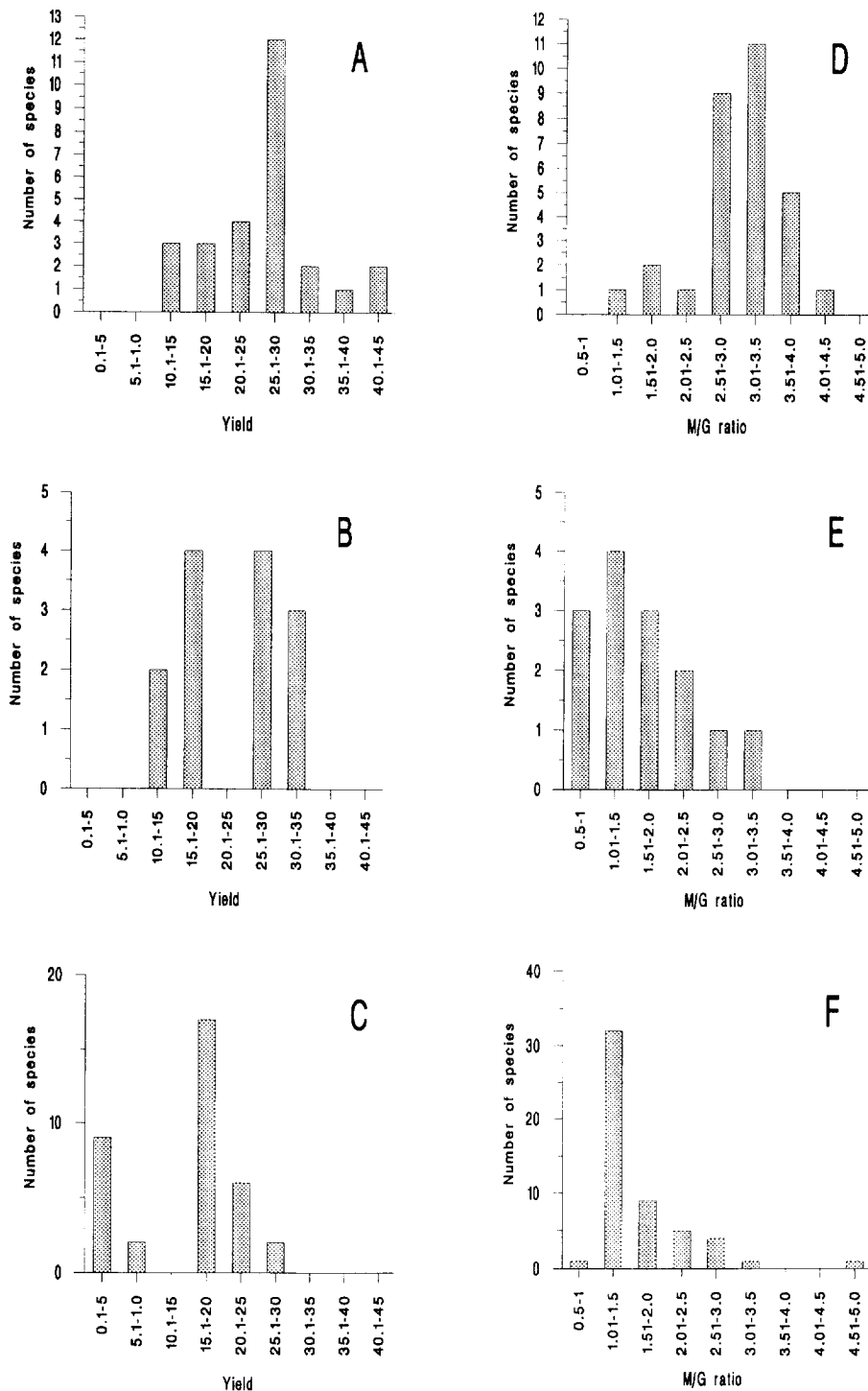
Fig. 1. Frequency distribution of galactomannan yields (percentage of the dry weight of the seed) (A–C) and mannose: galactose ratios (D–F) in species of the three subfamilies of the Leguminosae. Numbers given are a compilation of data from this work, Dea and Morrison [2], and Buckeridge and Dietrich [3]; A and D: Caesalpinioideae; B and E: Mimosoideae; C and F: Faboideae.

yields and low mannose:galactose ratios, three tribes (Mimosae, Acaciae and Ingae) comprise *ca* 1/3 of the species of all genera in the subfamily [7]. Galactomannan was detected only in genera of the tribe Mimosae (*Leu-*

caena and *Mimosa*) which is supposed to be the most primitive. It is noteworthy that Farooqi and Kapoor [11] studied the distribution of galactomannans in 14 species of Mimosoideae and found the same tendency. Thus, the

proposition stated above that the galactomannan content decreased during the course of evolution might also be valid below the subfamily level.

Faboideae is thought to be relatively ubiquitous in comparison to the two other subfamilies. According to Polhill et al. [7] the members of this subfamily are widely distributed from rain-forests to the edges of dry and cold deserts. This might explain the greater variability in galactomannan yield observed for the Brazilian species of Faboideae (Fig. 2C).

As suggested by Reid [10], the presence of galactomannan as a major component in the endosperm of leguminous seeds could represent an adaptation to the arid and/or semi-arid environment. The presence of galactomannan would enable the seed to imbibe more water, hence protecting the embryo against possible dry periods. Furthermore, a galactomannan molecule containing more galactose branches is more soluble in water compared to less branched molecules [2]. This feature could allow the seed to imbibe water quickly, which would be important in a soil with low water availability during the dry season. Preliminary results from our laboratory (not shown) suggest that seeds containing high galactomannan yield and low galactose branching degree are able to imbibe more water at the end of the first phase of imbibition than the others, and that the rate of imbibition seems to be inversely correlated to the mannose:galactose ratios.

It should be considered that not only the function as imbibing substance might have been important for the adaptation of galactomannan-containing legume seeds to new environments, but also its nutritional function, i.e. as a reserve. In fact, seeds with higher galactomannan yield provide more energy for the growing plantlet. Reid and Bewley [4] showed that the conversion of galactomannan in the endosperm has an essentially quantitative effect on the initial growth of the plantlet so that the final lengths reached by the radicles and hypocotyls which did not have access to galactomannan hydrolysis products during germination were, respectively, 30 and 35% shorter than those from the intact seeds. This could favour the establishment of those plantlets in a shadowy microenvironment like the tropical forest grounds. As has been pointed out by Stebbins [12], the establishment of the seedling may have been very important for the adaptation to new environments since this step is one of the weakest links in the chain of colonization of new territory by plant species.

EXPERIMENTAL

The seeds used in this work were from the following sources and species: (i) seeds collected in the campus of the University of Campinas, São Paulo, Brazil. Caesalpinioideae: *Cassia grandis* L.f., *Senna reticulata* (Willd.) Irwing & Barneby. (ii) Seeds collected from plants cultivated or naturally occurring in the forest of the Instituto de Botânica, São Paulo, Brazil. Mimosoideae: *Acacia farnesiana* (L.) Willd., *Anadenanthera colubrina* (Vell.) Brenan, *Calliandra bracteosa, Calliandra selloi* (Spreng.) Macbr., *Inga uruguensis* Hook & Arn., *Mimosa platy-*

phylla Benth., *Mimosa scabrella* Benth., *Pithecellobium incurialli* (Vell.) Benth., *Pithecellobium langsdorfii* Benth., *Piptadenia gonoacantha* (Mart.) Macbr. Faboideae: *Aeschynomene paniculata* Willd. ex Vog., *Crotalaria micans* Link (= *Crotalaria anagyroides* Kunth = *Crotalaria brachystachya* Benth.), *Machaerium* sp., *Mucuna* sp., *Sophora tomentosa* L. (iii) Seeds collected by the authors from plants naturally occurring in the savanna at Campininha Farm, Mogi-Guaçú, São Paulo, Brazil. Faboideae: *Anadenanthera falcata* (Benth.) Speg., *Bowdichia virgilioides* Kunth, *Centrolobium robustum* Mart. (iv) Seeds obtained from the Empresa Brasileira de Agropecuária (EMBRAPA). Mimosoideae: *Leucaena* sp., *Leucaena pulverulenta* (Schlecht.) Benth.; Faboideae: *Crotalaria juncea* L. (seeds kindly provided by Dr Avilio A. Franco). (v) Seeds collected from several other places: Mimosoideae: *Mimosa* sp. (Serra do Cipó, Minas Gerais, Brazil), *Prosopis juliflora* (SW.) DC. (bought at the local market), *Enterolobium* sp. (Goiás, Brazil), *Inga marginata* Willd. (unknown provenance); Faboideae: *Indigofera suffruticosa* Mil (Cardoso Island, São Paulo, Brazil).

Galactomannan extraction and analysis. Whole seeds were ground to a powder by milling and dried at 80° to constant wt. The dry powder was extracted with benzene–EtOH, 2:1 at 60° for 8 hr, dried at 80° and weighed.

The defatted powder was extracted with hot H_2O (80°) for 8 hr, filtrated through cheese cloth and centrifuged (10 000 g, 30 min, 5°). The H_2O extract was pptd with 3 vols of EtOH and left overnight at 5°, collected by centrifugation, dried (80°) and weighed. This was considered as the crude polysaccharide extract (adapted from ref. [13]). The polysaccharide yields were always expressed as a percentage of the dry wt of the seed.

Ten milligrams of the crude polysaccharide were hydrolysed with 3 N trifluoroacetic acid for 3 hr at 100°. The optimal conditions for hydrolysis were previously established for *Senna cernua* (= *Cassia sulcata*) and *Stryphnodendron adstringens* galactomannans [3]. The polysaccharide composition was determined by descending PC and by GC according to the procedures described by Buckeridge and Dietrich [3]. When using values of yield and mannose:galactose ratios of galactomannans together with those already reported by Dea and Morrison [2], or in cases in which 2 or more values were presented by those authors for a single species, an average was calcd and used as 1 species for the construction of the diagrams.

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