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Cover: *Greslania* circinata: part of leafy branches, showing leaf blades without midribs, Mont *Dzumac,* SD1490 (Photograph by W. Baker). See the first article of this issue: *"Greslania circinata* and *Greslania rivularis* (Poaceae-Bambusoideae) from New Caledonia" by *Soejatmi Dransfield.*

Greslania circinata and *Greslania rivularis* (Poaceae-Bambusoideae) from New Caledonia

Soejatmi Dransfield

The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK

The bamboo genus *Greslania* Balansa is endemic to New Caledonia, and comprises three or four species. Two of them, *G. circinata* Balansa and *G. rivularis* Balansa, are discussed.

The genus *Greslania* was described by Balansa in 1872 with three species: *G. montana*, *G. circinata*, and *G. rivularis* all from New Caledonia. They are found only in the southern part of the island where the soil contains heavy metals, such as iron. Balansa stated that the genus was related to *Nastus* Juss. in having one-flowered spikelet and a pedicel-like rudimentary second floret (or 'rachilla extension', see Holttum 1958), but differed from the latter in having a caryopsis with a relatively thick pericarp and free seed (attached only along the hilum). Pilger (1906) described another species, *G. multiflora* (conspecific with *G. circinata*), and in 1945 Pilger mentioned the presence of sheaths (bracts) and prophylls in the inflorescence of *Greslania*.

In 1966 McClure noted the unusual structure of the inflorescences in *Greslania* and *Glaziophyton* (a genus from South America), when he described bamboo inflorescences and proposed two types of inflorescences in bamboos, i.e. iterauctant and semelauctant for indeterminate and determinate inflorescences respectively. In indeterminate or iterauctant inflorescence the basic unit is a pseudospikelet, which comprises a prophyll at the base, one or more bracts subtending a bud (or another pseudospikelet), and a proper spikelet. In this form the spikelet of different phases (from buds to mature spikelets) are produced almost indefinitely. In a determinate or semelauctant inflorescence the basic unit is a spikelet; prophylls and subtending bracts are absent. In this form, the spikelets are mature almost simultaneously. *Greslania* and *Glaziophyton* posses determinate inflorescences but the branches bear well-developed prophylls and are subtended by well-developed bracts; a dormant prophyllate bud occasionally is found in the inflorescence, especially in *Greslania*. *Greslania* appears in fact to resemble *Hickelia*

A. Camus, a genus of four species found in Madagascar and Tanzania, in having bracteate, paniculate, determinate inflorescence with one-flowered spikelets and a rachilla extension in the spikelet (see Dransfield 1994). An investigation on the phylogenetic relationship between *Greslania* and *Hickelia*, and other related genera is being carried out. In this paper the morphological structures of the plants and the inflorescences of *G. rivularis* and *G. circinata* are described and discussed. This paper is not intended to be a revision of the genus, but aims to provide an extended descriptions of two of its species.

Greslania circinata and *G. rivularis* are small clumping bamboos and possess pachymorph or determinate rhizomes with necks that are moderately short. The inflorescences are determinate, with the presence of subtending bracts/sheaths and prophylls. The spikelet consists of two glumes (usually), one fertile floret and a rachilla extension bearing a rudimentary or vestigial floret. *Greslania circinata* is characterized by the unbranched culms, the absence of midribs in the leaf blades, curled branches in the inflorescence and slender spikelets. On the other hand, the culm in *G. rivularis* has a single branch at each node, the leaf blade has a prominent midrib and the spikelet is broader than that of *G. circinata*.

Greslania circinata Balansa, in Bulletin de Société Botanique de France 19: 320 (1873); Type: New Caledonia, Mont Humboltd, *Balansa* 3580 (P holotype).

Sympodial bamboo 0.70 – 1 (–3) m tall, forming dense clumps. Culm unbranched, but the two lowermost nodes each bearing an undeveloped branch bud (buds triangular), hollow, with relatively thin walls, glabrous and smooth, 4-8 mm diam., internodes about 7 cm long. Culm sheath and blade indistinguishable

from the proper leaf blades and sheaths. Leaf blades rigid (stiff), leathery, 7-29 × 1.5-8.5 cm, tapering to long stiff and sharp tips, base cordate, glabrous, bluish green when fresh, petiole not prominent, midrid not visible or not present; sheaths smooth, glabrous; ligule very short, entire; auricles not present. Flowering branches terminating leafy culms, inflorescence about 50 cm long, paniculate, highly branched, branches of higher order about 4 cm long, slender, curled, axis glabrous, lower nodes of the main axis bearing complete leaf blades (consisting of sheaths and blades proper) subtending spikelet-bearing lateral branches, the nodes distal to these nodes each bearing an undeveloped lateral branch bud, buds ovate-lanceolate, upper nodes of the main axis and of higher order branches having subtending bracts, with much reduced blades, bracts glabrous, ciliate along the margins near the apex, prophylls split. Spikelets slender, 6-8 mm long, glumes 2, rachilla extension 5 mm long, slender, vestigial floret very short; lower glume 3 mm long, 1 mm wide, acuminate, glabrous, ciliolate along the margins near the apex, 7-nerved; upper glume 5 mm long, 1.5 mm wide, acuminate, glabrous, ciliolate along the margins near the apex, 7-nerved; lemma 6 mm long, 1.5 mm wide, glabrous, acuminate, 5-nerved; palea 6 mm long, glabrous, 4-nerved (2 at each side of the sulcus), sulcus shallow, glabrous; lodicules 3, membranaceous, about 1.5 mm long, ciliolate along the apex (often joined to form a tube); stamens with free filaments, anthers with blunt or lobed apex; ovary cylindrical, glabrous, style very short, with 3 stigmas. Caryopsis cylindrical (probably not fertilized), about 4.5 mm long, pericarp thin, easily removed from the seed; seed flat. (Figs. 1-2 and the cover).

Distribution. Endemic to southern part of New Caledonia.

Specimens examined. New Caledonia. Mt Dzumac, alt. 1250 m, fl., 29 Sept. 2000, *Dransfield et al. SD1490* (K, P); l.c., *Le Rat 2639* (K, P); Montagne des Sources, alt 900 m, fl., 24 July 1982, *McPherson 4743* (MO, K, P);

Habitat. Mountain forest in marquis.

I have not seen the holotype of *G. circinata*, but I believe *G. multiflora* Pilger could be conspecific with this species. Demoly (2001) regards *G. multiflora* as a synonym of *G. circinata*. Nevertherless in this paper I do not include plants assigned as *G. multiflora* in the discussion.

The population of this bamboo on Mt. Dzumac seemed just to have finished flowering in September 2000, because there were many (if not all) dead clumps, with culms bearing dead inflorescences. The plant is thus monocarpic. However, some non-flowering clumps, small seedlings, and clumps with fresh inflorescences were found, so it seems there are different states of age in this population.

This extraordinary bamboo has no branches in the culms, and each culm bears more than 24 leaf blades. The lower blades fall off during the growing season, and the culm bears about 10 to 33 leaf blades, in the same way the leafy branches of other woody bamboos drop the lowermost blades.

Undeveloped branch buds can be found at the lower nodes of the culm. It is interesting to note that this sort of bud is also found at nodes below the nodes bearing flowering branches of a flowering culm. The shape of these branch buds varies from triangular to lanceolate (from the lower nodes upwards).

The flowering branches of the specimens deposited at K (see above) have spikelets with no stamens. In these spikelets the lodicules are often joined forming a tube. During my field trip in 2000 we collected flowering branches from various clumps. Most of these flowering branches bear spikelets, like those in the specimens at Kew, containing caryopses but no stamens. The pericarp of the caryopses is not attached to the seed, and the seed is unusually flat. It is not certain whether this seed is viable; fresh caryopsis were not collected, so at present it is not possible to show its viability.

One flowering branch with larger spikelets in the inflorescence was found and collected. Each spikelet possesses a fertile floret consisting of three lodicules, six stamens and an ovary. Caryopses are not found, and have probably not developed yet in this inflorescence.

Greslania rivularis Balansa, in Bulletin de Société Botanique de France 19: 320 (1873); Type: New Caledonia, Revière du Pamboui, *Balansa 1742* (K iso, P holotype).

Sympodial bamboo about 3 m tall, forming loose and open or dense clumps, with few to

many culms in a clump. Culms about 1 cm diam., internodes 10-20 cm long, smooth, terminating in a leafy branch. Branch bud one at each node, triangular, developing into one lateral branch, branched again, producing leafy branches, intravaginal, bearing 10 to 26 blades. Leaf blades on non-flowering branches relatively thick and rigid, with prominent midribs, 13-20 cm long, 2-3 cm wide (leaf blades on flowering branches much smaller, 2-3.5 cm long, 5-12 mm wide, less rigid), base rounded, tapering to long pointed tips, yellowish green or light green, glabrous, smooth, petiole 2-5 mm long; sheaths smooth, glabrous, covered with white wax when young; ligule very short, entire; auricles not present, curly bristles present, up to 7 mm long, easily shed. Flowering branches terminating leafy branches, inflorescence paniculate, very much branched, each branch subtended by a sheath/bract with a modified blade and with the presence of a prophyll, prophylls split or not split, sheaths glabrous, margins hairy towards the apex, many nerves, blades broadly ovate to triangular or vestigial on upper (uppermost) sheaths, base cordate to rounded, glabrous, midrib not prominent, axis slender, usually glabrous. Spikelets 6 mm long, containing a fertile floret, but the lowermost ones of the lateral branches often lacking either stamens or ovary in the floret; rachilla extension slender, 4 mm long, glabrous, except ciliolate near the apex, vestigial floret very short; glumes usually 2, lower 2.5 mm long, about 2 mm wide, membrano-chartaceous, obtuse, glabrous, 9-nerved; upper glume 5 mm long, 3.5 mm wide, chartaceous, margins membrano- chartaceous, truncate, glabrous, 11-nerved; lemma 6 mm long, 3 mm wide, chartaceous, margins membrano-chartaceous, acuminate, glabrous on the back, pubescent inside near the apex, 9-nerved; palea 5.5 mm long, 2 mm wide, glabrous, sulcus not very deep, glabrous, 6-nerved (3 at each side of the sulcus); lodicules 3, membranaceous or hyaline, glabrous, entire; stamens 6, filaments free, anthers with blunt apex; ovary glabrous, style short, stigmas 3, plumose. Caryopsis ovate, about 4 mm long, pericarp relatively thick but porous, seed flatened (probably abortive or not fertilized). (Figs. 3-4)

Distribution. Endemic to southern part of New Caledonia.

Specimens examined. New Caledonia. Yaté, fl., 2 Sept. 1963, *Blanchon 377* (K); route de Yaté, alt. 200 m, fl., 31 July 1966, *McKee 15413* (K) & sterile, 29 Jan. 1970, *McKee 21492* (K); Nouméa-Yaté road, alt. 225 m, 24 July 1980, *McPherson 2804* (MO, K, P); north of Tontouta, alt. 100 m, 14 Jan. 1945, *Virot 1472* (K, P); Revière Bleue Reserve, alt. 150 m, 24 July 1980, *McPherson 2888* (MO, K, P) & alt. 160 m, 8 Jan. 1984, *McPherson 6252* (MO, K, P); l.c., sterile, 10 Nov. 1978, *Moore 10470* (BH, K); l.c., alt. 200 m, sterile, 22 Febr. 1951, *Guillaumin et al. 10848* (K, P); between Rivière Bleue and Yaté, alt. 350m, fl., 30 Sept. 2000, *Dransfield et al. 1491* (K, P); Riv. Blanche, alt. 300 m, sterile, 14 Aug. 1951, *Baumann-Bodenheim et al. 15174* (K, P); near the Riv. la Couleé, about 17 km east of Nouméa, alt. 10 m, fl., 29 Nov. 1963, *Green 1711B* (K, P); Vallée du Pins, fl., 30 March 1951, *Guillaumin et al. 11727* (K, P).

Habitat. Marshy places or river banks in marquis on serpentine soil.

Greslania rivularis is found mainly at low altitude to about 300 m above sea level, growing especially near water/rivers/streams or in marshy places. It can be found growing abundantly locally, such as along the nature trails in the Rivière Bleue Reserve and along the road to Yaté, in open or shaded parts.

In September 2000 in the Rivière Bleue Reserve various states of plant were observed, from seedlings to dying clumps. (Note: collecting is not allowed in the Reserve, so there is no voucher specimen to give a precise record of this situation). Clumps, in which all culms bear inflorescences, seem to die after flowering. It would be very interesting to know if a clump with both flowering culms and sterile culms also dies after flowering. I believe that the rhizomes will continue producing new culms bearing inflorescences, until the rhizomes are exhausted and die. The duration of flowering is not known.

Clumps with several culms bearing small leaf blades can also be found in the Reserve. One can tell that a culm bearing only small leaf blades will become a flowering culm, because this culm will eventually produce flowering branches only.
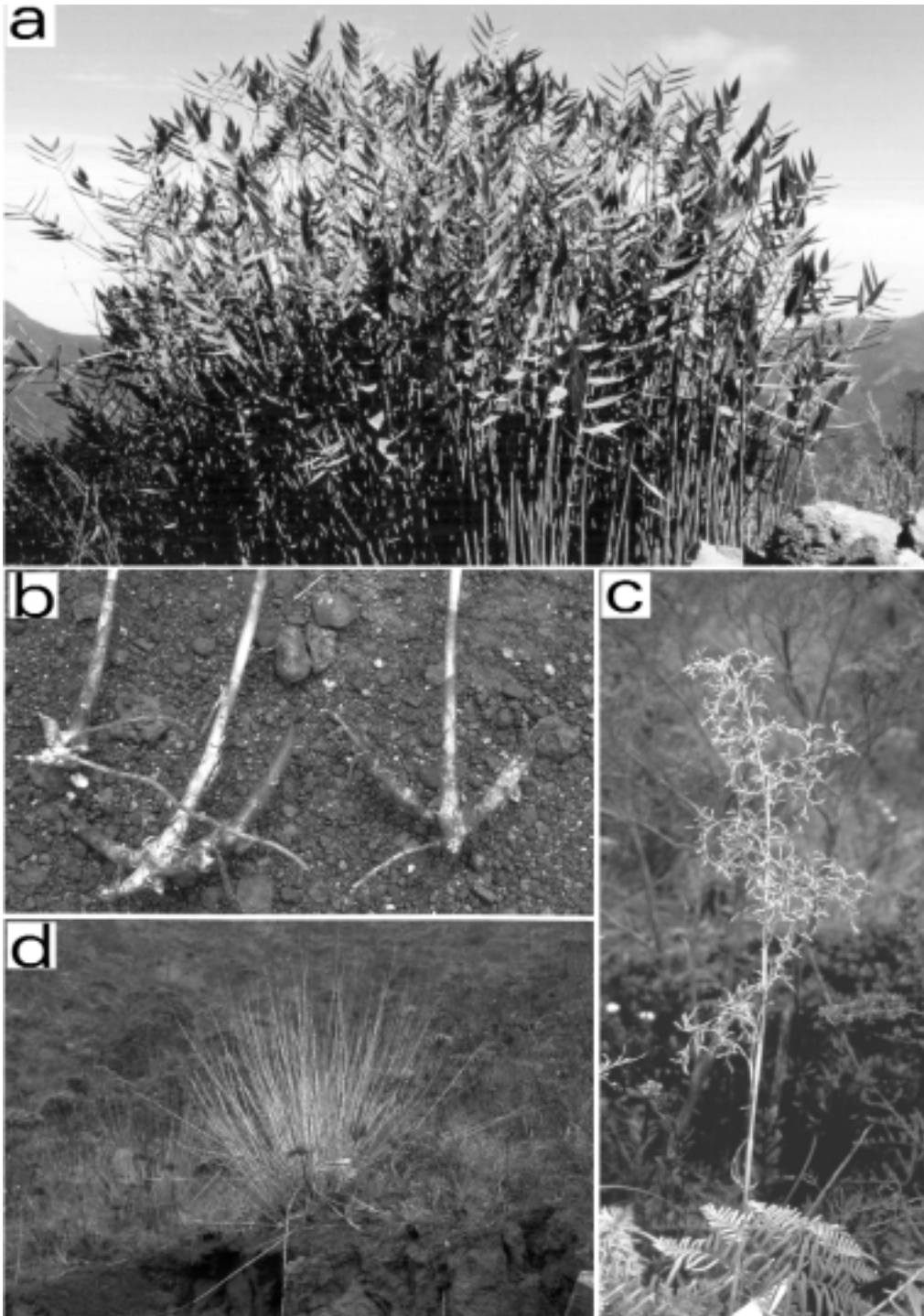
Figure 1. *Greslania circinata:* a. culms (in a clump) without branches, Mont Dzumac (Photograph by J.-C. Pintaud); b. part of the rhizomes, Mont Dzumac, SD1490 (Photograph by J. Dransfield); c. part of flowering branch/culm, Mont Dzumac, SD1490 (Photograph by J. Dransfield); d. dead clumps after flowering, Mont Dzumac (Photograph by J. Dransfield).
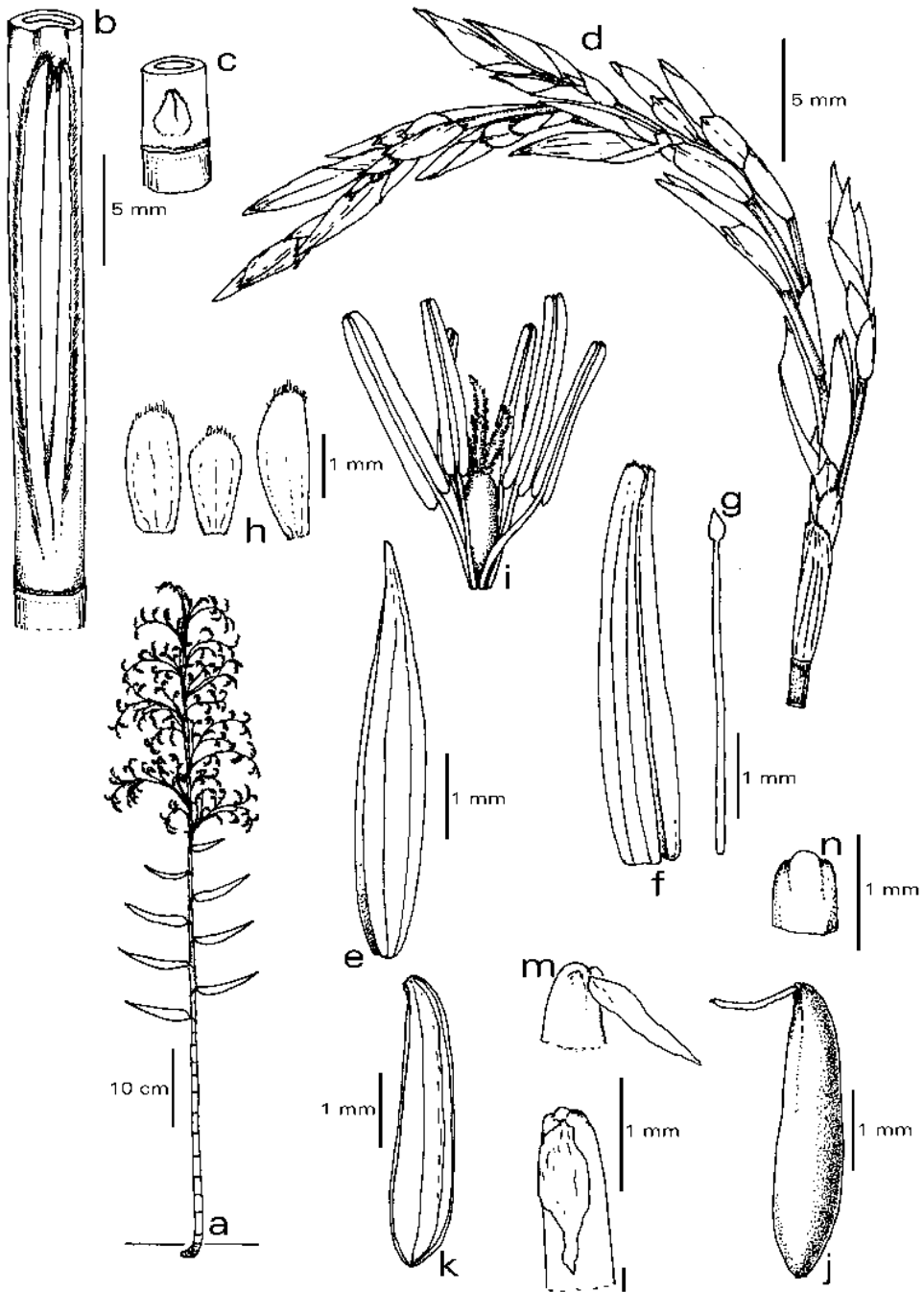
Figure 2. *Greslania circinata* line drawing: a. habit, showing inflorescence terminating a culm; b-c undeveloped branch buds, found below the inflorescence; d uppermost part of a lateral floret; h lodicules; i ovary and stamens; j caryopsis; k section of caryopsis showing thin pericarp and flat-tened seed; l-m caryopsis apex showing the remnant of a stigma; n back side of caryopsis apex. All from *Dransfield SD1490*, drawn by the author.
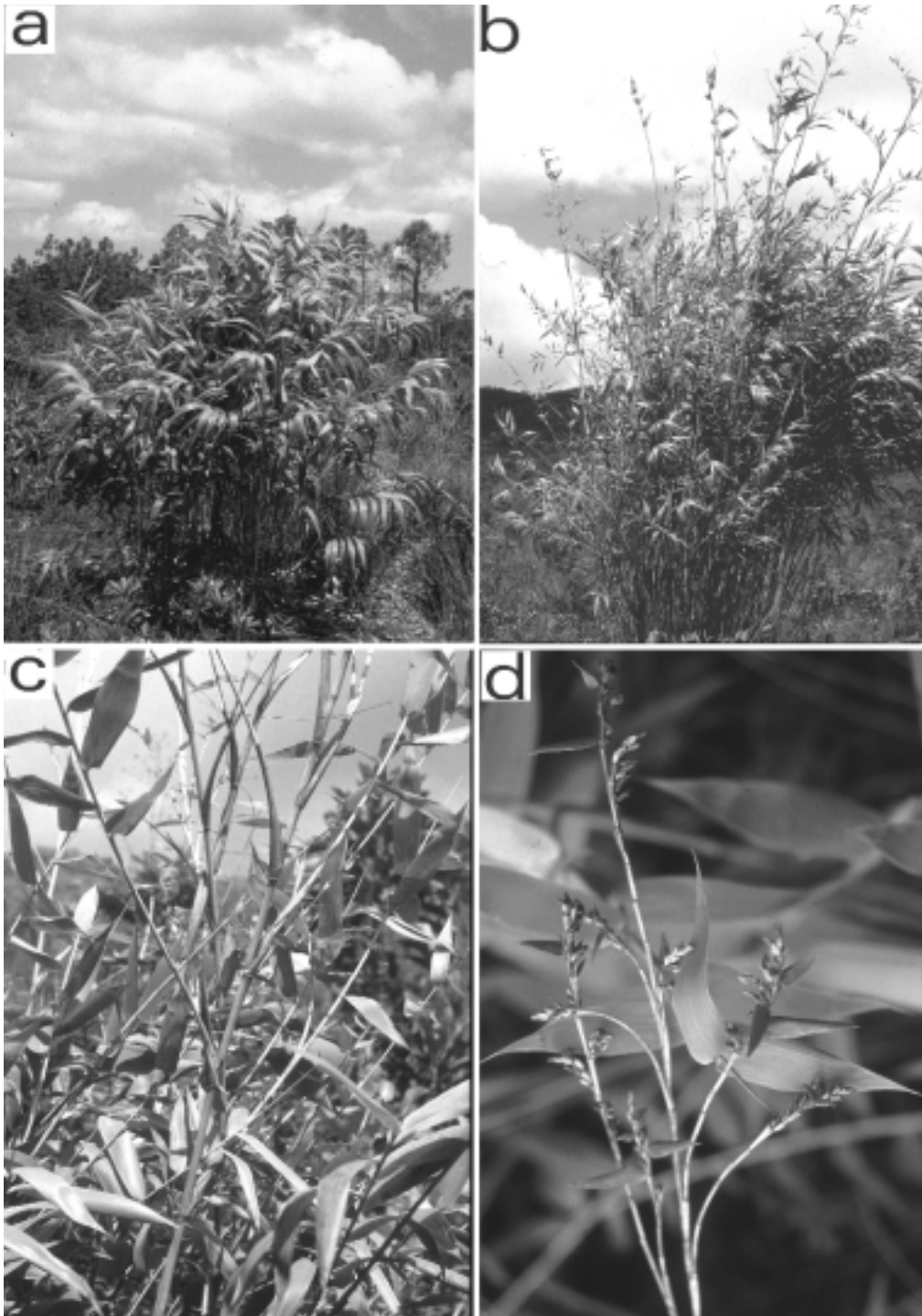
Figure 3. *Greslania rivularis:* a. sterile/non-flowering clump (Phtograph by S. Dransfield); b. Clump with several culms bearing young inflorescences (Photograph by S. Dransfield); c. branches, Yaté, *SD1491* (Photograph by S. Dransfield); d. Part of flowering branch, Yaté (Photograph by J. Dransfield)
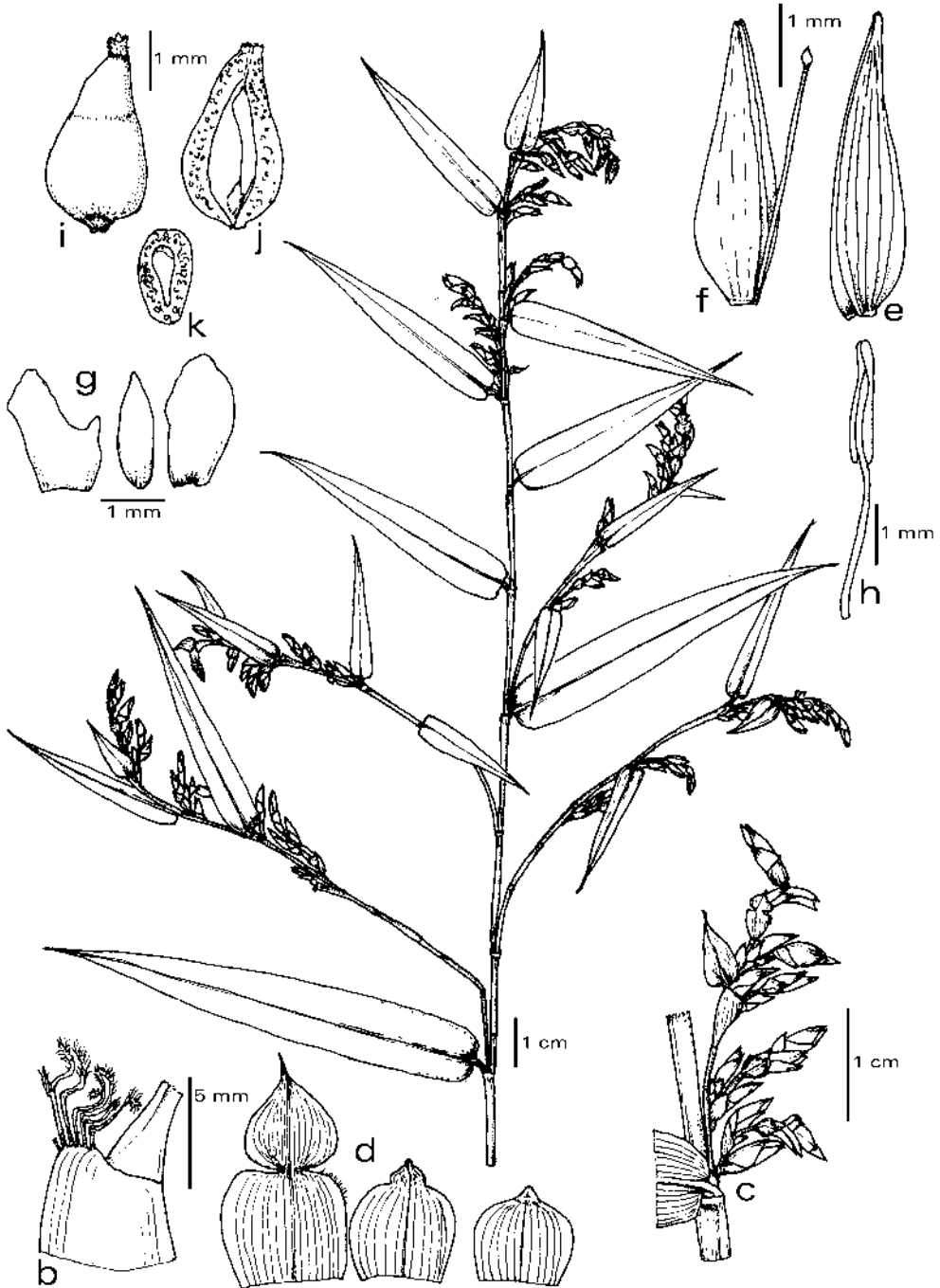
Figure 4. *Greslania rivularis* line drawing: a. uppermost part of the inflorescence; b. fimbrae/bristles on sheath of leaf-blade, showing the simplest type; c. part of the inflorescence; d. various types of sheaths or subtending bracts in the inflorescence; e. lemma; f. palea with a rachilla extension bearing a rudimentary floret; g. lodicules; h. stamen; i. caryopsis; j-k section of caryopsis showing porous pericarp and flattened seed. Drawings a and c from *McPherson 2888,* b and d-l from *Dransfield SD1491.* All drawn by the author.

There are usually two glumes, but occasionally the lower one is missing and is replaced by a sheath-like structure bearing a small modified blade (Fig.). The spikelets of the material deposited at Kew are all at nearly the same age and have almost mature caryopses. I tried very hard to collect material with spikelets early their in development but with very little success.

The caryopsis has a very interesting structure. The pericarp is thick, but porous, and is easily removed from the seed, as described by Balansa in the generic descriptions (1872). As in *G. circinata*, the seed is unsually flat, and there is a large empty cavity between the pericarp and the seed. The anatomical preparation of the caryopsis has not been made yet, so it is not possible to explain whether the seed has an embryo (or whether the ovary has or has not been fertilized).

The above descriptions of two species include only a few characters to add to the previous ones on the genus (cf. Balansa 1873); there are more features/characters that are still to be examined and described. In the case of flowering habit in these two species, for example, the information is not complete. It is not easy to decide which type of flowering habit occurs in *Greslania*. It could be sporadic, with the death of clumps after flowering (monocarpic) in a population. I suggest *G. rivularis* has a sporadic flowering habit, followed by the death of the clumps after flowering. Likewise, in Mont Dzumac the majority of the clumps of *G. circinata* died after flowering, and the whole hill is almost covered by these dead clumps giving the impression that this bamboo has a gregarious flowering habit. However, in this population, young clumps, seedlings and clumps with young inflorescences can also be found. It is possible that the dead clumps are of the same age, and they flowered at the same time, then died. I believe that *G. circinata* also has sporadic flowering habit.

This information was a result of two day's observations and is not complete. Therefore it would be very interesting to observe and investigate this situation again critically and continuously. The bamboos are found growing in protected areas, on soil which is not suitable for growing/building anything, so they will not disappear. Further study could provide more information on the flowering behaviour/habit of the woody bamboos.

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Genetic diversity and population structure of *Pseudosasa japonica* (Bambusaceae) in Korea

Hong Wook Huh[2] and Man Kyu Huh[1]

[2]Department of Biology Education Pusan National University, Pusan, 609-735,
The Republic of Korea
[1]Department of Molecular Biology, Dong-Eui
University, Pusan, 614-714, Korea
The corresponding author: Man Kyu Huh

Enzyme electrophoresis was used to estimate genetic diversity and population genetic structure of *Pseudosasa japonica* (Bambusaceae) in Korea. Twelve of the 28 loci (71.4%) showed detectable polymorphism. Genetic diversity (0.204) was higher than average values for species with similar life history traits. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a substantial deficit of heterozygotes relative to Hardy-Weinberg expectations. This deficit is expected when inbreeding is due to vegetative spread. The average GST for polymorphic loci was 0.152, indicating that most (84.8%) of the genetic diversity occurred within populations. The indirect estimate of gene flow based on mean GST was moderate (*Nm* = 1.39). Given limited gene flow, reduced populations are expected to diverge genetically due to drift and the random loss of alleles due to sporadic cutting.

During the past 20 years, enzyme electrophoresis has been used to describe the population genetic structure of over 700 plant taxa (Hamrick and Godt 1989). This information has contributed greatly to an understanding of the evolutionary history of individual species and related groups of species (Haufler 1987), and has provided insights into the relationships between allozyme diversity and life-history traits (Loveless and Hamrick 1984). Despite the importance of knowledge on genetic variation for providing information for conservation purposes, detailed studies of genetic variation are not available for most native taxa in China and Korea, particularly woody plants (Huh and Huh 2000). In addition, almost no information is available from flora-rich countries in Africa and in China (Bennett and Leich 1995).

While a great deal of information on allozyme variation of magnoliopsida (dicots) has been accumulated over the last 2 or 3 decades, information for liliopsida (monocots) is rudimentary and, almost no information is available from the Asia. Increased efforts to broaden our understanding of allozyme variation in different plant species from these regions is needed. One important group of liliopsida are the bamboos

and they have been extensively used for thousands of years. *Pseudosasa japonica var. koraiences* (Siebold & Zuccarini) Makino is distributed in eastern Asia, primarily Japan and the southern part of the Korean peninsula where it forms a northern boundary line in Korea. This boundary has moved northward over the past 30 years (Fig. 1) and one aim of this study is to investigate the differences between populations in the newly colonized zone (between A&B) and those from the old range (below A).

The species has been an abundant plant over its range in Korea, but reduction of populations is serious. Many manufacturers substitute bamboo for plastic or iron goods. Fields of bamboo in Korea are turned over to agricultural land to raise products for industry because Korea is a developing country. All of this leads to isolated, smaller populations. Populations that are reproductively isolated may gradually lose effective population sizes to be viable. The rapid loss of new plants results in the permanent loss of gene pools with the potential for species conservation.

P. japonica can reproduce extensively by rhizomes and potentially by sexually produced
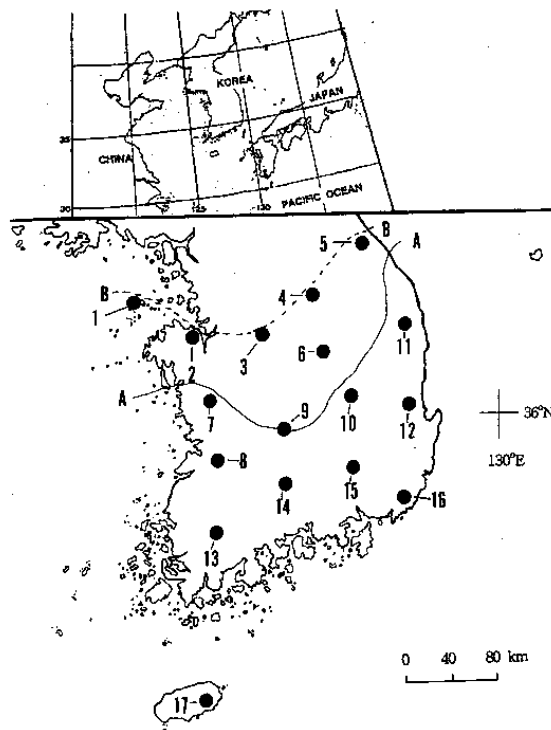
Figure 1. Collection localities for populations of *Pseudosasa japonica* as source for isozyme analysis. A – A: The North boundary line of bamboo-growth in Korea (1970s). B – B: The North boundary line of bamboo-growth in Korea (2000s).

seeds although flowering may not happen for many years. The purposes of this paper are; 1) to estimate how much total genetic diversity is maintained in the species and 2) to describe how genetic variation is distributed within and among populations.

MATERIALS AND METHODS

Sampling procedure and enzyme electrophoresis

Pseudosasa japonica var. koraiences was collected from seventeen natural populations in Korea during 1997 to 1998 (Fig. 1). Twenty to 35 sexual mature individuals were collected from each population and one leaf per each plant was used in this study.

Electrophoresis was performed using 10% starch gel. Buffer systems and enzyme staining procedures from Soltis et al. (1983) were used to assay thirteen enzyme systems; acid phosphatase (ACP), fluorescent esterase (FE), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malic enzyme (ME), malate dehydrogenase (MDH), menadione reductase (MNR), peroxidase (PER), 6-phosphoglugonate dehydrogenase (PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and superoxide dehydrogenase (SOD) (Table 1). For enzymes which resolved more than one zone of activity, the most anodal isozyme is arbitrarily designated 1, with the others sequentially assigned higher numbers. Likewise, alleles were designated sequentially with the most anodally migrating allozyme designated 'a' and progressively slower forms 'b', 'c', and so on. The *P. japonica* isozymes expressed phenotypes that were consistent in subunits structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel (1989).

Data analysis

Four standard genetic parameters were estimated using LYNSPROG developed by M.D. Loveless and A. Schnabel and POPGENE (Yeh et al. 1999). The percent of polymorphic loci (*P*), mean number of alleles per locus (*A*), effective number of alleles per locus (*A*$_E$), and gene diversity (*He*) were calculated according to Hartl and Clark (1989). A_E is calculated as the reciprocal of the sum of squares of allele frequencies. Subscripts refer to species (s) or population (p) level parameters. Observed heterozygotes (*Ho*) were compared to Hardy-Weinberg expected value using Wright's fixation

Table 1. Gel and electrode buffer systems that gave optimal activity and resolution in *Pseudosasa japonica*

Enzyme	Number	Symbol	Locus	Buffer System[a]
Acid phosphatase	E.C. 3.1.3.2	ACP	2	9
Fluorescent esterase	E.C. 3.1.1.2	FE	2	3
Glutamate oxaloacetate transaminase	E.C 2.6.1.1	GOT	2	7
Isocitrate dehydrogenase	E.C. 1.1.1.42	IDH	2	3
Leucine aminopeptidase	E.C. 3.4.11.1	LAP	1	9
Malic enzyme	E.C. 1.1.1.40	ME	1	11
Malate dehydrogenase	E.C. 1.1.1.37	MDH	4	3
Menadione reductase	E.C. 1.6.99.-	MNR	2	7
Peroxidase	E.C. 1.1.1.7	PER	4	3
6-phosphoglugonate dehydrogenase	E.C. 1.1.1.43	PGD	2	3
Phosphoglucomutase	E.C. 2.7.5.1	PGM	2	3
Shikimate dehydrogenase	E.C. 1.1.1.25	SKD	2	11
Superoxide dehydrogenase	E.C. 1.15.1.1	SOD	1	3

[a]According to Soltis et al. (1983).

index (*F*) or inbreeding coefficients (Wright 1922). These indices were tested for deviation from zero by c^2-statistics (Li and Horvitz 1953).

Nei's gene diversity formulas (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations (Nei 1977). In addition, c^2-statistics were used to detect significant differences in allele frequencies among populations for each locus (Workman and Niswander 1970). Nei's genetic identity (I) was calculated for each pairwise combination of populations (Nei 1973). PC-SAS program (SAS Institute Inc. 1989) was used to conduct a cluster analysis on genetic identities via the unweighted pairwise groups method using arithmetic average (UPGMA).

The genetic structure within and among populations was also evaluated using Wright's (1965) F-statistics F_{IT}, F_{IS}, and F_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire samples and within populations, respectively. The F_{ST} coefficient estimates relative population differentiation. Deviations of F_{IT} and F_{IS} from zero were tested using c^2-statistics (Li and Horvitz 1953). Two indirect estimates of gene flow were calculated. One estimate of Nm (the number of migrants per generation) was based on G_{ST} (Wright, 1951) and the other was based on the average frequency of "rare" alleles found in only one population (Slatkin 1985; Barton and Slatkin 1986). The absolute population differentiation (*D*m) was calculated using the formulae: $Dm = sD_{ST} / (s-1)$ where s is the number of subpopulation in the analysis and D_{ST} is the genetic diversity among populations (Nei 1973).

RESULTS

Genetic diversity

Twenty-eight loci encoding 13 enzyme systems were screened. Twenty of them (71.4%) showed detectable polymorphism in at least two populations. The remaining eight loci (*Per-4,*

Mdh-3, Mdh-4, Pgd-2, Pgm-2, Acp-2, Skd-2, and *Idh-1*) were monomorphic in all populations. An average of 52.4% polymorphism was found within populations, with individual population values ranging from 42.9% to 57.1% (Table 2). The average number of alleles per locus (A) was 1.81 across populations, ranging from 1.64 for the population with the lowest number of alleles to 1.93 for the population with the highest number of alleles. The effective number of alleles per locus, or the number of alleles needed within a locus to maintain the current level of heterozygosity was similar at the species and the population level (Aes = 1.37; Aep = 1.28). The mean genetic diversity within population was 0.167. The population 17 had the highest expected diversity (0.208), while population 1 had the lowest (0.128). Overall, mean observed heterozygosity at the population

levels different to the expected value (Hop = 0.099; Hep = 0.167).

Although there were not any significant differences between two groups for the P, A, A_P, and H_{OP}, the genetic diversity (mean Hep = 0.148) of populations north of the 1970's boundary was significantly lower than the pre-existing southern populations (mean Hep = 0.178) (Table 4). The effective number of alleles showed the same trend: mean A_E = 1.30 for old (southern) populations and A_E = 1.24 for new (northern) populations.

Genetic structure

In general, genotype frequencies do not conform to Hardy-Weinberg expectations. Chi-square tests indicated significant deviations from Hardy-Weinberg. As expected from the chi-square tests, F_{IS}, a measure of the deviation

Table 2. Percentage of polymorphic loci (P), mean number of alleles per locus (A) and polymorphic locus (A_P), effective number of alleles per locus (A_E), observed heterozygosity (Hop), Hardy-Weinberg expected heterozygosity or genetic diversity (Hep) for seventeen populations of *P. japonica*

Pop[a]	N[b]	*P*	*A*	*A_P*	*A_E*	*Hop (SD)*	*Hep (SD)*
1	35	42.86	1.64	2.50	1.20	0.072 (0.009)	0.128 (0.036)
2	35	53.57	1.79	2.47	1.21	0.072 (0.009)	0.139 (0.039)
3	38	53.57	1.79	2.47	1.22	0.100 (0.010)	0.147 (0.037)
4	36	53.57	1.86	2.60	1.30	0.100 (0.010)	0.152 (0.043)
5	35	50.00	1.82	2.64	1.26	0.134 (0.011)	0.154 (0.041)
6	39	57.14	1.93	2.63	1.25	0.085 (0.009)	0.166 (0.036)
7	35	57.14	1.79	2.38	1.29	0.085 (0.009)	0.168 (0.040)
8	35	57.14	1.89	2.56	1.28	0.108 (0.010)	0.174 (0.038)
9	35	51.85	1.89	2.71	1.32	0.110 (0.010)	0.181 (0.044)
10	35	53.57	1.86	2.60	1.29	0.100 (0.010)	0.177 (0.041)
11	36	46.43	1.71	2.54	1.25	0.102 (0.010)	0.153 (0.039)
12	37	50.00	1.82	2.64	1.29	0.088 (0.009)	0.166 (0.040)
13	35	53.57	1.75	2.40	1.30	0.115 (0.010)	0.187 (0.038)
14	37	53.57	1.75	2.40	1.29	0.102 (0.010)	0.180 (0.039)
15	37	50.00	1.89	2.79	1.31	0.097 (0.009)	0.176 (0.041)
16	37	57.14	1.86	2.50	1.32	0.105 (0.010)	0.188 (0.042)
17	39	50.00	1.75	2.50	1.38	0.100 (0.010)	0.208 (0.043)
Mean		52.42	1.81	2.55	1.28	0.099 (0.002)	0.167 (0.010)

[a]Numerical codes as in Figure 1.

[b]Number of individuals in the sample.

from random mating within 17 populations, was 0.457, and ranged from -0.755 for *Sod* to 0.829 for *Idh-1* (Table 3). The observed high, significant, and positive F_{IS} value (0.457) indicates that there was a significant deficit of heterozygotes in the populations.

At the level of the sample as a whole, however, Wright's *F*; coefficients showed that significant deficiencies of heterozygosites exist at all polymorphic loci. Analysis of fixation indices, calculated for all polymorphic loci in each population, showed a substantial deficiency of heterozygotes relative to Hardy-Weinberg expectations. For example, 84.5% of fixation indices were positive (207/244), and 162 of them departed significant from zero (p < 0.05). Thirty-seven of these indices were negative and 18 of them departed significant from zero, indicating as excess of heterozygotes at those loci and in these populations. On a per locus basis, the proportion of total genetic variation due

to differences among populations (G_{ST}) ranged from 0.001 for *Sod* to 0.823 for *Per-3* with a mean of 0.152, indicating that about 15% of the total allozyme variation was among populations (Table 3). Thus, the majority of genetic variance (85%) resided within populations. The values of genetic distance (D) were below 0.1 in most populations. The estimated of gene flow based on G_{ST} was moderate (Nm = 1.39). Genetic identity values among pairs populations range from 0.880 to 0.998. The similarity among *P. japonica* populations can be seen in the UPGMA dendrogram, where total populations cluster at a below genetic distance 0.050. The UPGMA dendrogram provided a few insights into the genetic structuring of populations (Fig. 2). In addition, the correlation between genetic distance and geographic distance was low (r = 0.35, p < 0.05), and indicated that about 88% of the variation in genetic distance was caused by unknown factors other than distance.

Table 3. Total genetic diversity (H_T), genetic diversity within population (H_S), genetic diversity among population (D_{ST}), absolute population differentiation (Dm), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}) of *P. japonica*

Locus H_T		H_S	D_{ST}	Dm	F_{IS}	F_{IT}	G_{ST}
Fe-1	0.491	0.459	0.032	0.034	0.780	0.794	0.065***
Fe-2	0.495	0.441	0.054	0.057	0.667	0.704	0.109***
Fe-3	0.316	0.262	0.054	0.057	0.588	0.658	0.170***
Per-1	0.323	0.286	0.039	0.039	0.490	0.549	0.114***
Per-2	0.082	0.076	0.007	0.007	0.424	0.471	0.081***
Per-3	0.097	0.017	0.080	0.085	0.698	0.946	0.823***
Got-1	0.281	0.243	0.038	0.041	0.637	0.686	0.136***
Got-2	0.012	0.010	0.002	0.002	0.134	0.281	0.170***
Pgd-1	0.438	0.158	0.280	0.297	0.734	0.904	0.639***
Idh-1	0.258	0.251	0.007	0.008	0.828	0.833	0.027***
Mdh-1	0.061	0.056	0.005	0.005	0.356	0.406	0.077***
Mdh-2	0.253	0.242	0.011	0.012	0.295	0.326	0.044***
Acp-1	0.651	0.527	0.125	0.132	0.796	0.835	0.191***
Skh-1	0.024	0.018	0.006	0.006	0.613	0.711	0.253***
Mnr-1	0.536	0.528	0.008	0.008	0.124	0.137	0.014ns
Mnr-2	0.155	0.148	0.007	0.008	0.359	0.390	0.048***
Pgm-1	0.103	0.100	0.003	0.003	0.136	0.159	0.026***
Lap	0.488	0.470	0.018	0.019	0.494	0.512	0.037**
Me	0.137	0.135	0.002	0.002	0.745	0.749	0.013ns
Sod	0.500	0.499	0.001	0.001	-0.755	-0.753	0.001ns
Mean	0.285	0.246	0.039	0.041	0.457	0.515	0.152***

ns: Not significant; ** = P < 0.01; *** = P < 0.001

Table 4. Comparison of genetic variability between new (northern) populations
and old (southern) populations in Korea.

Group	P	A	A_P	A_E	Hop	Hep
New pop.	51.79	1.81	2.55	1.24	0.094	0.148
Old pop.	52.76	1.81	2.54	1.30	0.101	0.178
F-test	0.229ns	0.058ns	0.005ns	12.671**	0.874ns	18.858**

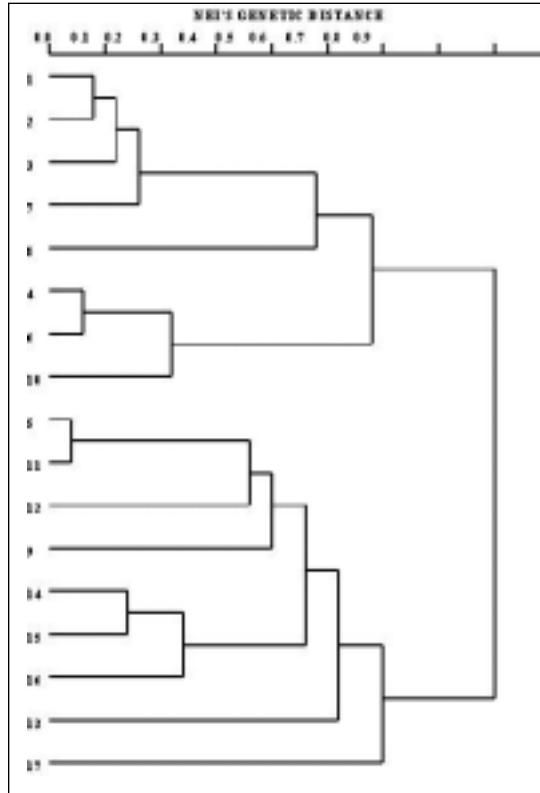ns: Not significant at the 0.05% level. **: Significant at the 0.01% level.

Figure 2. UPGMA dendrogram showing the phylogenic relationships among the seventeen populations of *P. japonica,* based on data of genetic distance obtained by starch gel electrophoresis.

DISCUSSION

Pseudosasa japonica maintains relatively high levels of allozyme variation and percentage of polymorphic loci compared to the average plant species of restricted distribution and those with both sexual and asexual reproduction but it is similar to many species with widespread distributions based on values given by Hamrick and Godt (1989). These comparisons suggest that genetic diversity levels of *P. japonic*a are as high as its associates with similar life history traits. Ellstrand and Roose (1987), in a review of studies of population genetic structure of primarily obligate clonal plant species, concluded that clonal plant species tend to have intermediate levels of genetic diversity which is consistent with our own results.

Populations with parameters like those in Korea are theorized to be less variable for several reasons, including small population sizes, reduced gene flow, and historical factors (Nei et al. 1975; Cwynar and MacDonald 1987; Schnabel and Hamrick 1990). Although

levels of allozyme diversity within populations of many species are not correlated with geographical boundaries (Levin 1977; Yeh and O'Malley 1980; Wendel and Parks 1985), significantly reduced variability in marginal populations has been observed (de Arroyo 1975; Rick et al. 1977). The northernmost populations of P. japonica in Korea are relatively small and indeed maintain less genetic variation than the other populations, probably due to the founder effect. Although there were not any significant difference between latitudes for the A_P, A_E, and H_{OP}, the three statistics (A, P, and Hep) were negatively correlated with the increase of latitude at which the populations were collected (Table 4). The expansion of north boundary line in Korea may be explained by global warming but this needs to be tested by future work.

The relatively high level of genetic variation found in *P. japonica* is consistent with several aspects of its biology. First, geographic range has been shown to be strongly associated with the level of variation maintained within populations and at the species level (Hamrick and Godt 1989; Hamrick et al. 1992). Widely distributed plant species tend to maintain more variation than more narrowly distributed species. Although *P. japonica* in Korea is distributed patchily, the species has a wide geographic range. Second, long-lived perennial species, like *P. japonica*, generally maintain relatively higher levels of variation than annuals. Observation of *P. japonica* has revealed that some clones had possibly been harvested for at least several hundred years. As populations of *P. japonica* become older, opportunities for the accumulation of mutations should be high (Ledig 1986). This is insufficient to explain the high genetic diversity in P. japonica. At least, we think that evidence of outcrossing is necessary. Because outcrossing species generally maintain relatively higher genetic diversity than selfing species or obligately asexual species (Godt and Hamrick 1991).

Bamboo reproduce asexually rhizomes. When bamboo does flower, which may not happen for many years, all numbers of the clone flower at the same time and then die. *P. japonica* has rarely flowered, probably only once or twice in a century. The rhizomes spread about 2 m a year and produce offspring that are genetically identical to each other and to the maternal plant. Therefore, vegetative reproduction and spread can also affect the genetic structure of populations (Murawski and Hamrick 1990). If a small amount of gene flow and/or mutation adds new genotypes to a population from time to time, variation may be maintained. Furthermore, *P. japonica* in Korea can be characterized as weedy, at least in recent times. Thus, if clonalization occurs by multiple genotypes, the ephemeral nature of populations may preclude significant loss of genetic variation while those populations are extant (Ellstrand and Roose 1987). Species with independent ramets could spread the risk of mortality among ramets, thus reducing the probability of genet death and preserving genetic diversity. Hartnett and Bazzaz (1985) have also argued that physiological independence among ramets may maintain genetic diversity by buffering clones against localized, patch specific selection forces. Nei et al. (1975) have also shown that the reduction in average heterozygosity per locus depends not only on the size of the population bottleneck, but also on the subsequent rate of population growth. If population growth is rapid, reduction in average heterozygosity is small, even given a small number of founders. This may be the situation with the populations in Korea. *Pseudosasa* is being used and heavily displaced but it also grows and spreads rapidly so diversity should decline slowly. Eventually decline may happen though because regardless of growth rate, populations undergoing bottlenecks tend to lose low frequency alleles, reducing polymorphism and the number of alleles per polymorphic locus (Godt and Hamrick 1991).

The only other issue left to be explained is our high F_{IS}. F_{IS} is a direct parameter of inbreeding, Wright's fixation indices (84.5% positive) as well as the observed high, significant, and positive F_{IS} value (0.457) indicate that homozygotes were significantly in excess. Because *P. japonica* is predominantly an asexual species, it is expected that high apparent inbreeding (FIS = 0.457), is actually due to vegetative mainly reproduction mainly.

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Characterization of the anatomy of *Guadua angustifolia* (Poaceae: Bambusoideae) culms

Ximena Londoño[1], Gloria C. Camayo[2], Néstor M. Riaño[2], and Yamel López[3]

[1]Sociedad Colombiana del Bambú, P O Box 661, Armenia, Colombia
[2]Centro Nacional de Investigaciones de Café – Cenicafé, Manizales, Caldas, Colombia
[3]Universidad Nacional de Colombia, Palmira, Colombia

ABSTRACT

Anatomical characterization of *Guadua angustifolia* (Poaceae: Bambusoideae) culms was carried out using material collected at four different localities in the Colombian Coffee zone. Anatomical features such as size, form and distribution of the vascular bundles, diameter and percentage of metaxylem, and percentage of fibers along the width and length of the culm's wall, were analyzed. The features evaluated showed variation in relation to the thickness of the culm's wall, with a higher concentration and smaller size of the vascular bundles towards the periphery. There is no relationship between anatomical features and the culm's age. We also observed the number of vascular bundles per unit of area (cross section) at different heights of the culm. Fiber percentage of wall area increases from the base to top of the culm. The culm of *Guadua angustifolia* is formed by 40% fiber, 51% parenchyma and 9% vascular tissue on average.

Bamboos (Bambusoideae: Poaceae) differ from the other grasses in leaf anatomyin the following ways: non radiated mesophyll with fusoid and arm cells; vascular bundles usually found in groups of than one, and over the mid nerve; and silica cells are vertically oriented (Soderstrom and Ellis, 1987). Studies such as those of Soderstrom and Ellis (1987) and Ding and Zhao (1994) show that there is a significant correlation between the anatomy of the bamboo leaves and the different taxa. Soderstrom and Ellis (1987), for example, grouped the woody bamboo of the world into nine sub-tribes based on anatomical characters of the leaves.

Research conducted by Grosser and Liese (1971) on 53 species of Asian bamboos from 14 genera showed that the anatomy of bamboo culms shows differences in the structure of the vascular bundles between genera and between species and thus has considerable value for taxonomy. Vascular bundles in bamboo culms are characterized by their shape, size and grouping in most of the species studied, thus supplying the basis for an anatomical classification system that includes four categories (Grosser (1971),

Grosser and Liese (1971, 1973), Grosser and Zamuco (1973) and Liese (1998). From Liese (1998). We know that the physical properties of bamboo culms are determined by their anatomy so these characteristics of the culm will ultimately reflects its usability. For example, mechanical properties of the culm, important in building materials, are determined by its specific gravity, which depends mainly on the density and diameter of the fibers and the thickness of the fiber cell walls (Liese, 1998). The length of the fibers is also an important feature for paper industry (Latif and Liese, 2001).

In Colombia, *Guadua angustifolia* is a sustainable natural resource with a high growth rate (11 to 21 cm per day). Several native and rural communities satisfy basic necessities with this material. It is an ideal construction material with a high percentage of fibers, a specific gravity of 0.5 to 0.6 and excellent structural properties such as a high resistance-to-weight-ratio, a high capacity to absorb energy and excellent flexability. Thus, Guadua houses are very resistant to failure in earthquakes, but

the anatomic characteristics of this American bamboo have not been studied in detail. This study broadens the basic knowledge about *G. angustifolia* culm anatomy, and correlates it to age and length (basal, middle and apical segments) and help to establish quantitative parameters for the delimitation of different culm growth stages for harvesting and use.

MATERIALS AND METHODS

Culm samples were collected at four sites in Colombia: Hacienda Nápoles (Montenegro, Quindío), Center for the study of Bamboo-Guadua (Córdoba, Quindío), Botanical garden of the Universidad Tecnológica de Pereira (Risaralda) and Centro Nacional de Investigaciones de Café-Cenicafé (Chinchiná, Caldas).

Samples were taken at breast height (1.5 m), from culms at ages of: 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 72-84, 96-108 and 120 months. All observations were done at middle of internode (Tables 2 & 4).

An additional single culm (48 months old) was selected and divided into three segments: basal (from internodes 0 to 16), middle (from internodes from 16 to 40), and apical (from internodes 40 to 72) (Table 4 & 5; Figs. 9-12). The samples were taken at middle of the internode and processed.

Samples of culm tissue (1 cm x 1 cm) were taken at different ages, at the middle of internode, and fixed in FAA (4% formaldehyde, 5% acetic acid, and 50% alcohol) for 24 h at 4°C then embedded in paraffin and softened with ethanol, glycerol (1:1) for three weeks at 60°C, and dehydrated in 50%, 70% and 96% ethanol for 1 h each.

Cross sections (10-30 μm) were made with a rotary microtome. They were stained with safranin and fast green, mounted in Canada Balsam, and observed with a light microscope (Zeiss-Axiophoto) (100X, 200X and 400X).

Vascular bundle dimensions (radial and tangential), diameter of the metaxylem, percentage of parenchyma, fiber and conducting tissue, were measured in cross sections of the culms using a magnification of 10X. Four fields were observed in each section to measure the thickness of the different zones in the culm's wall (periphery, transition, middle and inner) and the vascular bundle size, shape and concentration was also determined for each zone. The number of vascular bundles was counted for each field and then was calculated for an area of 1 cm^2.

Cross sections at middle of internode were made every 8 internodes along the culm, starting from internode 4. The size, shape and concentration of the vascular bundles were measured for each zone (periphery, transition, middle and inner). Because of the wide variation existing in the periphery and transition zones, 100 vascular bundles were also measured to calculate their average size.

RESULTS AND DISCUSSION

The anatomy of the culm is mainly composed of collateral vascular bundles embedded in parenchyma tissue. The size, shape, numbers and concentration of vascular bundles varies from the periphery towards the inner section of the culm, and from the base of the culm towards its apex. Close to the periphery, the vascular bundles are small, numerous and concentrated, while in the middle section of the culm they are larger and more widely spaced. In all bamboos, the size of the vascular bundles decreases noticeably from the base towards the apex with a corresponding increase in their density (Grosser and Liese, 1971).

As in other bamboos, the culm tissue of *G. angustifolia* is made up of: cortex (epidermis, hypodermis & cortical parenchyma), parenchyma cells, fiber, and vascular bundles, which in turn are made up of sclerenchyma cells, vessels (metaxylem, phloem and protoxylem) and sieve tubes with companion cells (Fig. 1). According to Liese (1998) the composition of the culm is (on average) 52% parenchyma tissue, 40% fiber and 8% conducting cells. These values vary among species. In *G. angustifolia,* the composition is 51% parenchyma tissue, 40% fiber and 9% conducting cells. (Table 1). Compared to other tropical and subtropical bamboo, *G. angustifolia* exhibits a typical percentage of fibers.

Bamboo Science and Culture

Table 1. Percentage of different tissues in the culm of *Guadua angustifolia* at 48 months old.

Tissue	Segment						Total (****)
	Basal	*	Middle	**	Apical	***	
Parenchyma	63	8	64	27	36	16	51
Fibers	29	4	26	11	56	25	40
Conductive	8	1	10	4	8	4	9

**** Conversion factors: *basal=0.1276; **middle 0.4186; ***apical 0.4537. (see Liese 1998 for comparison)

Cortex

The epidermis of *G. angustifolia* is made up of long cells that intermingle with short cells and stomata (Fig. 2a). The short cells, of cork and silica, are grouped in pairs between the long cells. The stomata, with their guard cells, have an ovoid shape and horizontally are longer than they are wide (Fig. 2b). The high concentration of silica cells in the epidermis of *G. angustifolia*'s culm contributes to the hardness of its wood (Fig 2c).

The hypodermis of *G. angustifolia* consists of 2-3 layers of thick sclerenchymatous cells. The cortical parenchyma is homogenous and formed of 8-10 layers of thin and thick-walled cells. The size of these cells increases from the periphery to the inside (Fig 2a).

In young culms, the external portion of the epidermis cells is covered by a cuticle of cellulose and pectin and unicellular hairs (Liese, 1998). In *G. angustifolia*, however, there is no evidence of the wax layer common in other bamboos such as *Phyllostachys heterocycla var. pubescens* Mazel ex H. de Lehaei.

Parenchyma

A longitudinal section of *G. angustifolia* shows long and short parenchyma cells (Fig. 3a). In cross section one can see that the parenchyma tissue is formed by both long cells and short cells that become lignified after the early stages of the shoot's growth. The long cells usually have thicker walls, while the short cells are small and cubic in shape. They are characterized by denser cytoplasm and thin walls because they do not lignify with age like the long cells. Simple pits are located in the longitudinal walls connecting the parenchyma cells amongst themselves. The function of these two types of parenchyma cells remains unknown (Grosser & Liese, 1971).

Vascular Bundles

A vascular bundle of *G. angustifolia* is formed by two large metaxylem vessels, one or two protoxylem elements, the phloem, the sclerenchyma sheath and fibers (Fig. 4).

Two large vessels separated by parenchyma tissue, with an intercellular space between them, form the metaxylem. Lignified parenchyma cells surround the vessels of the metaxylem, however, the parenchyma in the surrounding intercellular space always remains unlignified. The parenchyma cells that surround the vascular bundle are usually smaller than those of the general parenchymatous tissue and have a higher number of pits in their walls (Grosser and Liese 1971, Liese 1998). The diameter of metaxylem vessels in *G. angustifolia* varies from 0.02 to 0.22 mm with great variation along culm wall thickness. The periphery vessels of the metaxylem are smaller and increase their size in the middle and inner sections, a constant behavior during the culm's maturation process. We also observed longitudinal variation within internodes – the vessels are larger in the middle segment of the culm than in the basal and apical segments. The size and area of the metaxylem vessels are critical to the conductivity of water in bamboo and important for the preservation of fresh culms when using the Boucherie preservation method (Liese, 1998),

Phloem is formed by large thin-walled sieve tubes that are not lignified and always connected to companion cells (Grosser and Liese 1971). Sieve pores aligned with small callose platelets connect the sieve tubes. The companion cells are characterized by dense cytoplasm and a large nucleus (Liese, 1998). In *G. angustifolia*, the phloem is formed by 14 to 25 sieve tubes (Fig. 4). According to Liese and Weiner (1996), a bamboo culm might have a

lifespan of up to 15 years. In *G. angustifolia* the estimated lifespan of a culm is 10 years (Castaño 1985, Londoño 1992). The function of the conducting tissues (xylem and phloem) is continuous through the culm's lifespan, and no new conducting tissue is formed in contrast to that in the hardwoods and soft-woods of the dicotyledons. During ageing, these conducting vessels can become partially obstructed by gums (slime and tylosoids) limiting conductivity and inducing the death of the culm (Liese, 1998).
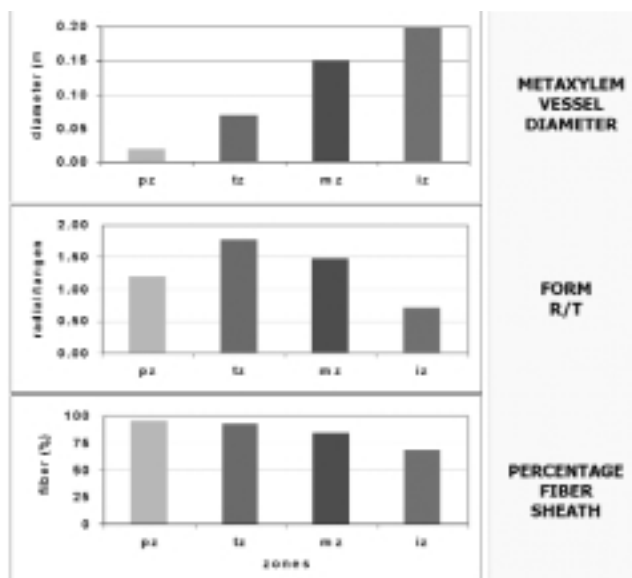

Figure 5. Anatomical characterization of the vascular bundles for the different zones in the culm wall of *Guadua angustifolia:* periphery zone (pz), transition zone (tz), middle zone (cz) and internal zone (iz).

Fibers

The fibers are characterized by their slender form, long and tapered at both ends and are sometimes forked. Their length influences the strength of the culm and its pulping properties (Liese, 1998). Fiber sclerenchyma sheaths surround the vascular bundles of the conducting tissue (phloem and xylem). They differ in size, shape and location depending on the species and the position within the culm. Considering the shape variation of the vascular bundles in 52 species of bamboo belonging to 14 genera, Grosser (1971) and Grosser and Liese (1971, 1973) established four basic types of vascular bundles. These have been used in later research for the anatomical characterization of the bamboo culm. Sekar and Balasubramanian (1994) added a subtype IIa to Liese and Grosser's vascular bundle typology (1971). *G. angustifolia* exhibits vascular bundle types II and IIa depending on their location through the culm wall. A central vascular bundle is surrounded by four schlerenchyma sheaths, two located at each side of the metaxylem vessels, another one around the protoxylem (intracellular space) and a final one around the phloem. The sclerenchyma sheaths of the phloem are more prominent in the middle and inner zone bundles, which are the ones used for anatomical classification. In the vascular bundles of the periphery and transition zones, the sclerenchyma sheath is more prominent towards the protoxylem (Fig. 4). One to two layers of thick walled parenchyma were also observed surrounding the metaxylem.

The relative area of fiber sheaths in a vascular bundle varies depending on the position through the culm's wall (Latif and Liese, 2001). In *G. angustifolia*, the fiber percentage fluctuates between 64.8% and 97.2% per vascular bundle, with a larger percentage in the vascular bundles of the peripheral and transition zones (89.9%-97.2%), than in the vascular bundles of the middle and inner zones (64.8%-87.2%). The lowest fiber percentage (64.8%) was recorded in the inner zone (Fig.5, Table 2). The fiber sheaths of the protoxylem, metaxylem and phloem of the middle and inner section of the culm wall of *G. angustifolia* do not touch each other, while in the periphery and transition zones they amalgamate between themselves, enclosing the conduction tissue (Fig. 4). No significant differences between fiber percentage and ageing where observed, therefore this parameter cannot be used to quantify the age of the culm (Table 2).
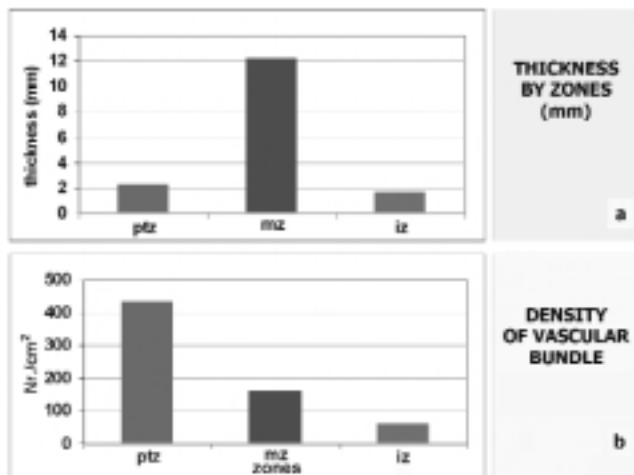
Figure 7. Thickness and density of vascular bundles across the culm wall zones in *Guadua angustifolia.*

Distribution of the vascular bundles through the culm wall.

In bamboo, the size, shape, amount and concentration of vascular bundles changes continuously along the wall of the culm. Four zones have been determined: periphery, transition, middle and inner zones (Grosser, 1971; Grosser and Liese, 1971, 1973). In *G. angustifolia*, the length of **periphery zone** ranges from 0.65 to 0.77 mm (Table 3) and is formed by vascular bundles adjacent to the cortex. These bundles are circular, small and numerous, having a radius/tangent ratio of 1.15 to 1.6, with scarce conduction tissue and few parenchyma cells between them. They are located in a chain, with a tangential orientation. The smallest are the metaxylem vessels, with a diameter of 0.02 mm (4.5 % of the thickness of the culm's wall) (Fig. 6). The length of the **transition zone** ranges from 1.2 to 2.55 mm (Table 3). It is formed by vascular bundles where the sclerenchyma sheaths amalgamate with the fiber bundles. These bundles are ovoid in shape, with a radial/tangential ratio from 1.5 to 2.7, and a metaxylem diameter of 0.07 mm. The vascular bundles of this zone are not typical (10.7% of the thickness of the culm's wall) and should not be used for the characterization of the species. The **middle zone** is the largest of the four zones. It has a length of 4.9-16.34 mm (Table 3) and round-shaped vascular bundles with a radial/tangential

ratio of 0.98 to 1.73, and a metaxylem diameter of 0.15 mm. The vascular bundles of this zone are the most diagnostic for anatomical characterizations of bamboo since they reach the optimum state of differentiation and size (73.9% of the thickness of the culm's wall). Length of the inner zone ranges from 1.3 to 2.0 mm (Table 3). The vascular bundles in this zone usually lack orientation and have an ovoid to circular shape with a radial/tangential ratio below 1 (0.62-0.94), and a metaxylem diameter of 0.20 mm (10.8% of the thickness of the culm's wall) (Figures 6 and 7).

According to Grosser and Liese (1971), the size of the inner zones differs with the species and with the position on the culm, and in some cases is not evident. They also point out that the middle zone is the one where most of the bamboo exhibits the largest vascular bundles. However, in *G. angustifolia* the largest appear in the inner zone. The length of the different zones varies with the species, the diameter and the thickness of the culm's wall. Generally, the external third of the culm's wall is formed by the periphery and by the transition zone (Grosser and Liese, 1971). In *G. angustifolia*, these two zones only represent 15% of the wall's thickness, which has a significant influence on the ultimate composition of the culm. The concentration of vascular bundles varies depending on the zone. In *G. angustifolia* the periphery and transition zone have the largest amount of vascular bundles per unit of area (346-530 vb cm-2) followed by the middle one (81-194 vb cm-2) and inner zones (52-96 vb cm-2) (Fig.7; Table 2).

Vascular Bundles according to age

Earlier stages of the culm show certain external characteristics useful as indicators to prevent premature exploitation. Some of these characteristics are the presence of cauline leaves over the culm, bud breaks of the branches, the branching patterns, the number

of leaf scars, and the color change of the culm which ranges from an intense green to a yellowish-gray (Figure 8) (Banik, 1993).

In G.angustifolia, the age of the culm is established using subjective parameters such as the culm's color and the concentration of lichens and fungi over the internode surface. However, no quantitative parameters have been established to identify the different growth stages of a culm for adequate harvesting purposes.

The structural changes during the maturation phases and the years that follow relate to the thickening of the fiber walls and of the walls of the parenchyma cells. This thickening of the walls occurs because of the deposition of additional lamellar material and not because of the thickening of the existing cell walls (Liese, 1998). Also, the presence of the tyloses and slime deposits in vessels and sieve tubes increases with the age of the culm. During the first months of growth, only minor anatomical changes can be observed as a result of the maturation of meristematic tissue within the internodes and along the length of the culm (Liese and Weiner, 1996).

According to Liese and Weiner (1996), the chemical and structural patterns in culms are the most significant when correlated with age. The percentage of holocellulose and *a*-cellulose tends to decrease in bamboos older than one year, while the lignin contents remains stable or increases slightly. It has been determined that lignification occurs in the early developmental stages and no increases are observed with age. Studies conducted by Chen et al, (1987) indicate that the ash composition changes significantly between culms of one (1) and seven (7) years old, observing a decrease in the copper, zinc, phosphorous, iron and potassium contents and an increase in calcium, magnesium and manganese.
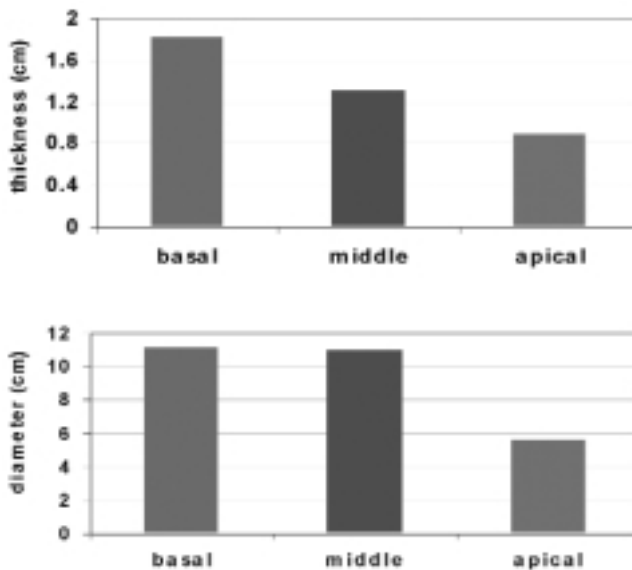

Figure 10. Wall tickness and diameter along the culm of *Guadua angustifolia.*

Age is also related to the storage and mobilization of carbohydrates. In young culms no starch is stored since it is used readily in the metabolic processes. However, in old culms starch grains located in the parenchyma tissue cells can be observed (Liese and Weiner, 1996).

The ageing of a bamboo culm influences certain properties but not the vascular bundles per unit of area, neither the diameter of the metaxylem, percentage of fiber and size of the vascular bundle. Tables 2 and 3 indicate that *Guadua angustifolia* has stable anatomical characteristics at the vascular bundle level without influence by ageing. Latif and Liese (2001), also point out that in bamboo in general the vascular bundles differ very little in size with age, but vary significantly with the location and the height of the culm.

Anatomical characteristics along the culm

Bamboos are fast growing monocotyledons. *Guadua angustifolia* in particular reaches its definite height six to seven months after the shoot emerges, growing up to 21 cm/day in the Coffee zone of Colombia. Culms reach maturity in four to six years (Castaño 1985, Londoño 1992). Because of the tapered morphology of the culm, the diameter and thickness of the
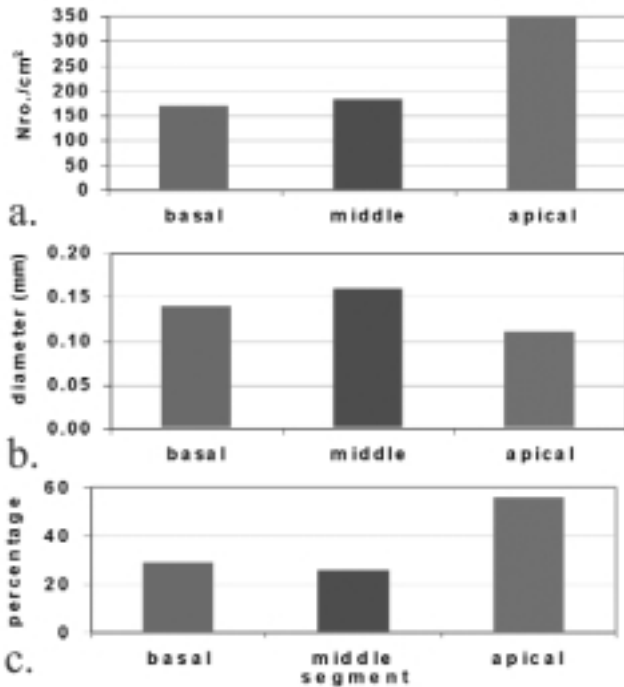
Figure 12. Anatomical characteristics of the vascular bundle along the culm of *Guadua angustifolia;* a. density, b. vessel diameter, c. % fiber.

than in the tangential diameter. Because of this, the shape of the vascular bundle in a cross section tends to be rounder towards the apical segment (radio/tangent 0.92), while in the basal segment (radius/tangent 1.04) it tends to be more elongated in the radial direction. The bigger the vascular bundle, the lower the number of vascular bundles per unit of area.

The number and density of the vascular bundles are characteristic for each species (Grosser and Liese, 1971). In *G. angustifolia*, the number of vascular bundles per unit of area increases towards the apical segment (350 vb cm^{-2}) and decrease towards the middle segments (184 vb cm^{-2}). This is a consequence of the reduction in the thickness of the culm's wall towards the apical segment. This reduction is directly related to the reduction of tissue in the middle zone. The periphery and transition zones are constant along the culm, while the inner zone does not show a significant variation (Table 5; fig. 12).

We have observed that the diameter of the metaxylem of the vascular bundles in *G. angustifolia* decreases slightly towards the apex, and is

related to the reduction in size of the vascular bundles along the culm. The percentage of fiber is higher in the apical segment (58%) followed by the basal segment (29%) and by the middle segment (26%) (Fig. 12; Tables 1 and 5). Also, the vascular bundles type II and IIa are constant along the culm. According to Grosser and Liese (1971), species of the genus *Bambusa, Dendrocalamus* and *Gigantochloa* show combinations of type III and type IV in the basal segment. However, no combination takes place in *G. angustifolia*.

From this study, we may conclude about *G. angustifolia* that: a) the anatomy of the culm can be used successfully for its identification; b) the shape, size and type of vascular bundle varies along the width and the length of the culm; c) it has stable anatomical characteristics that are not influenced by age factors; d) the composition of tissue in the culm is 51% parenchyma, 40% fiber, and 9% conductive tissue; e) in the epidermis there is a large concentration of silica cells that contribute to the hardness of its wood; f) the vascular bundles are classified as types II and IIa, being constant along the length and width of the culm; g) there is a direct correlation between the number of vascular bundles and the percentage of fiber; h) the relative percentage of fiber area is larger in the vascular bundles of the peripheral and transition zones than in the middle and inner zones; and i) the relative percentage of fiber area is higher in the apical segment (56%) than in the basal (29%) and middle (26%) segments of the culm. Results obtained from this work may be used to establish the mechanical strength characteristics of the guadua wood necessary to use for several purposes (furniture, agglomerates, floors, building, food, etc.)
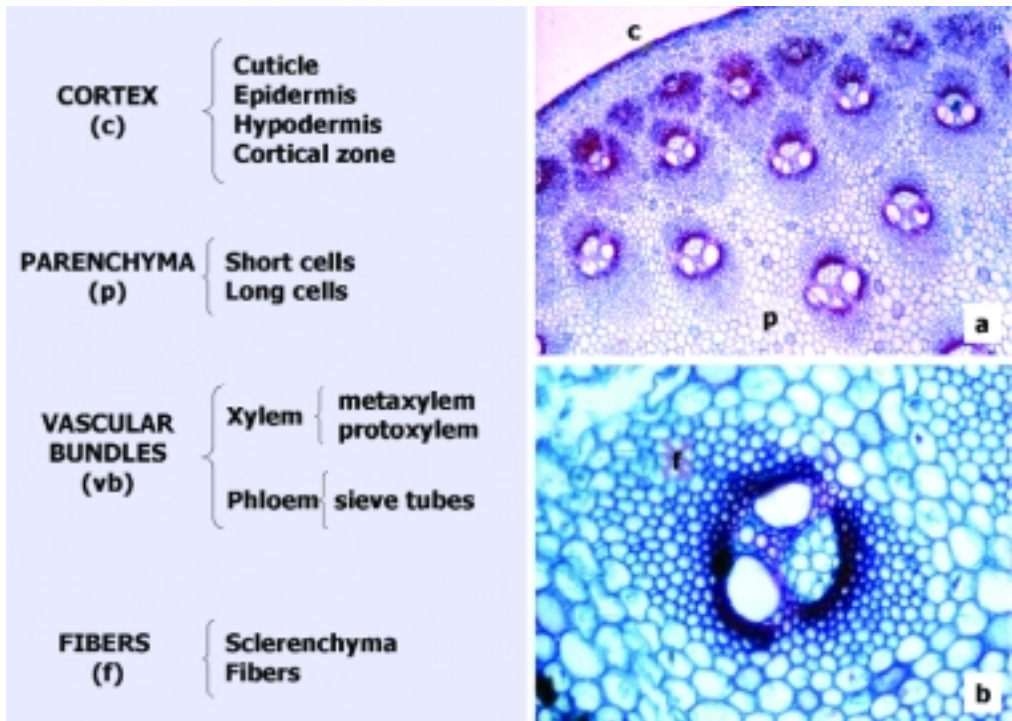
Figure 1. Anatomical structure of *Guadua angustifolia* culm: a. transversal section; and b . detail of vascular bundle.
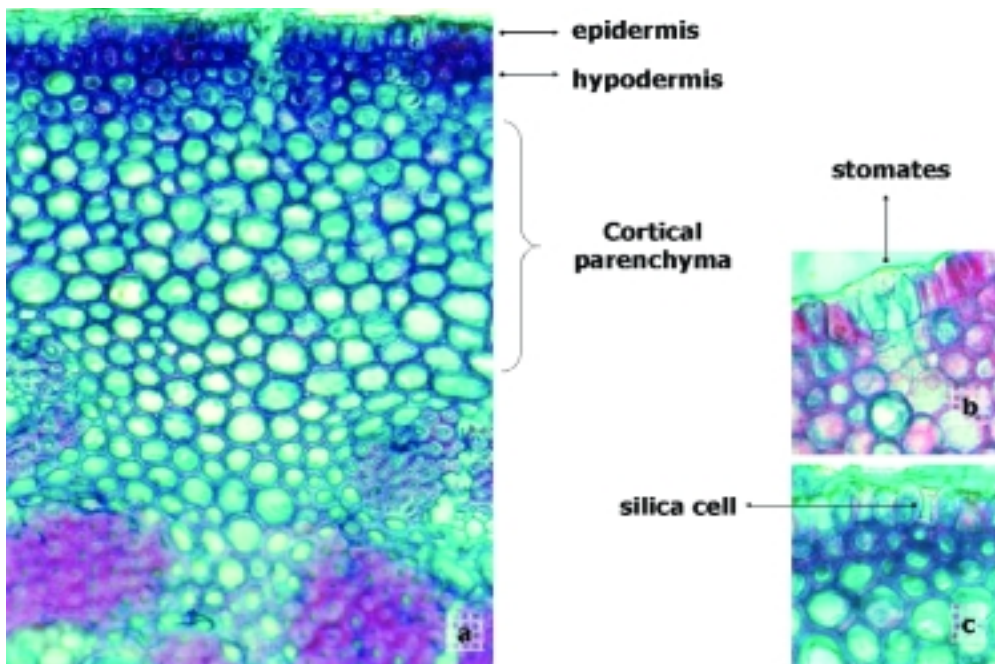

Figure 2. Cross section of *Guadua angustifolia* culm: a. cortex; b. detail of stomata; c. detail of silica cell (100X).
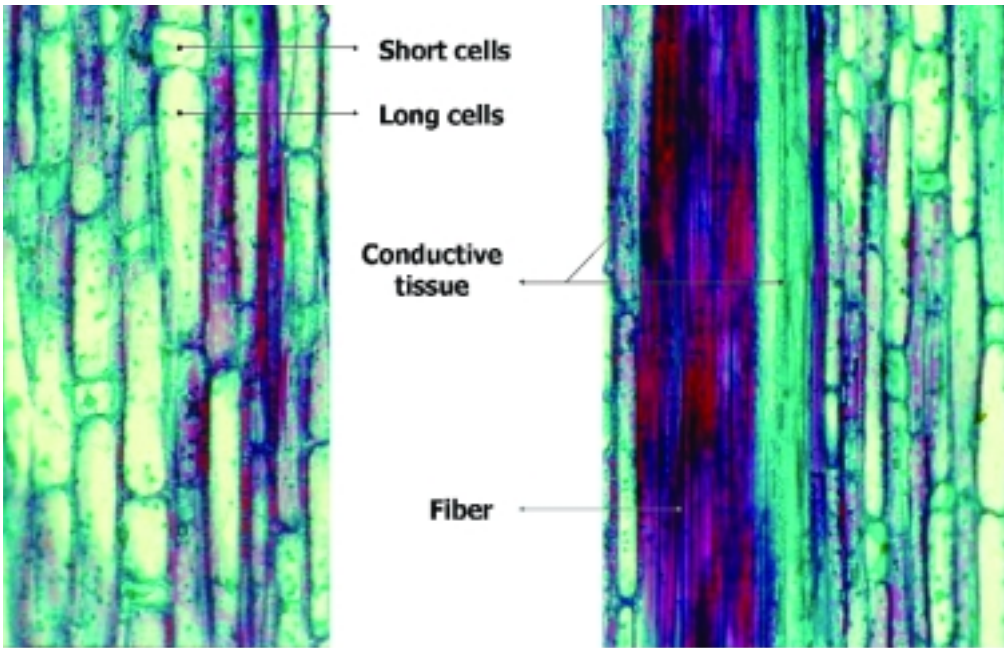
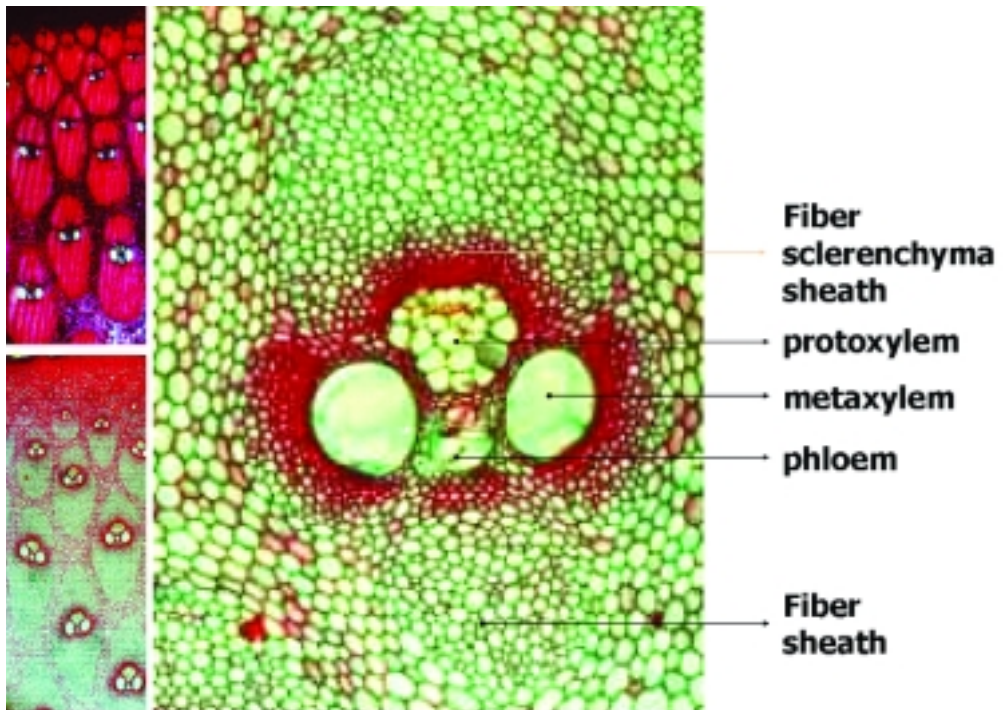Figure 3. Longitudinal section of *Guadua angustifolia* culm.

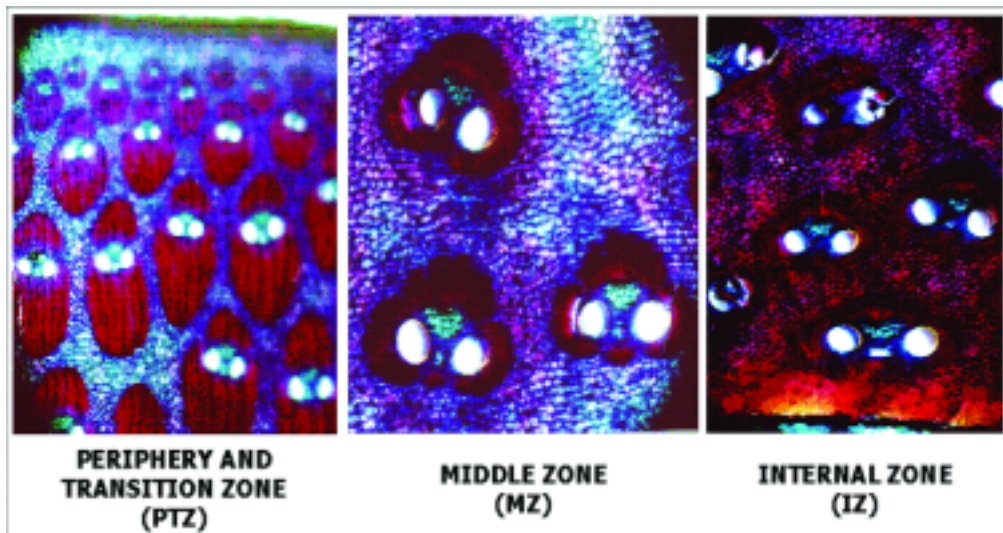Figure 4. Vascular bundle detail of *Guadua angustifolia.*

Figure 6. Composition of tissue in the diferent zones through a cross section of *Guadua angustifolia* culm: periphery and transition zone (ptz); middle zone (mz); internal zone (iz).

Figure 8. External characteristics of *Guadua angustifolia* culms according to age.

wall decrease from the base to the apex, while the internode length increases (Ueda 1960; McClure 1966, Hsiung et al 1980 and Latif and Liese 1998). The average height of a culm in the sites selected for this study was 23.65 m. Diameters and wall thickness were respectively basal segment (11.5 cm, 1.82 cm), middle segment (11.05 cm, 1.32 cm) and apical segment (5.84 cm, 0.89 cm) (Table 4; figures 9 and 10). In *G. angustifolia*, the internodes have an average length of 20 to 35 cm. The longest length between internodes was registered in the middle segment (35 cm) and the shortest in the basal segment (20 cm) (Table 4). The average diameter of the *G. angustifolia* culms studied ranged
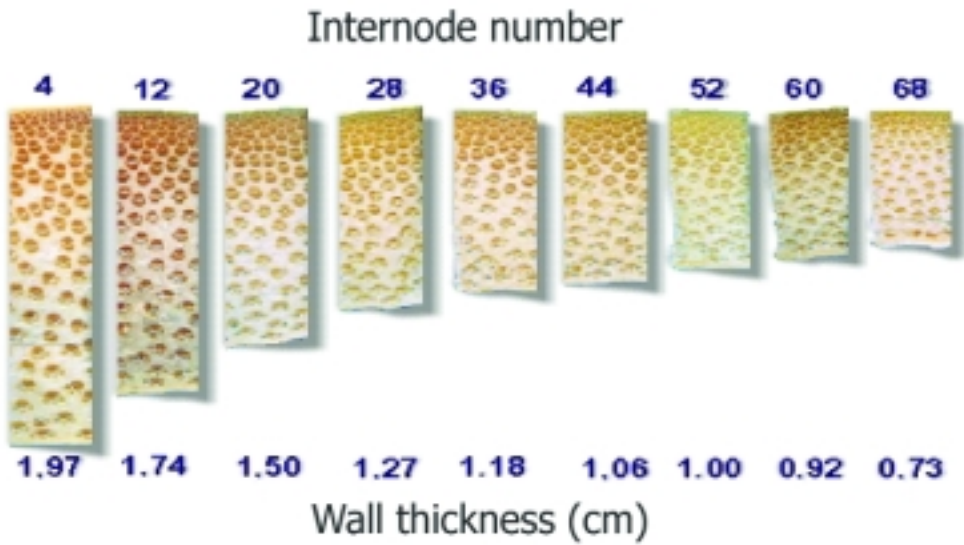
Figure 9. Culm wall thickness variation across 48 months (68 internodes) from a single culm.

from 5-11 cm. The largest diameter was measured in the basal segment (11 cm) and the smallest in the apical segment (5 cm) (Table 4 and Figure 10).

In *G. angustifolia*, the wall thickness of the culm decreases faster in the basal segment, which may explain why anatomical changes between one internode and the next are more pronounced in this segment than in the middle or apical segments (Fig. 9).

To make a representative characterization of the species, we must describe the tissue pattern in several internodes at different positions of the culm (Fig. 9). The height of the culm and the ratio of the decrease in the wall's thickness will ultimately determine the number of internodes that must be studied, and from this study we conclude that the analysis must be made every 8 internodes in a *G. angustifolia* culm.

In G.angustifolia, the size and shape of the vascular bundle differs from the base to the apex,

showing a mean surface of 0.19 mm^2 in the basal segment, 0.17 mm^2 in the middle segment and 0.11 mm^2 in the apical segment. The radial diameter of the vascular bundle decreases along the culm, and a proportionally larger decrease is observed in the radial diameter
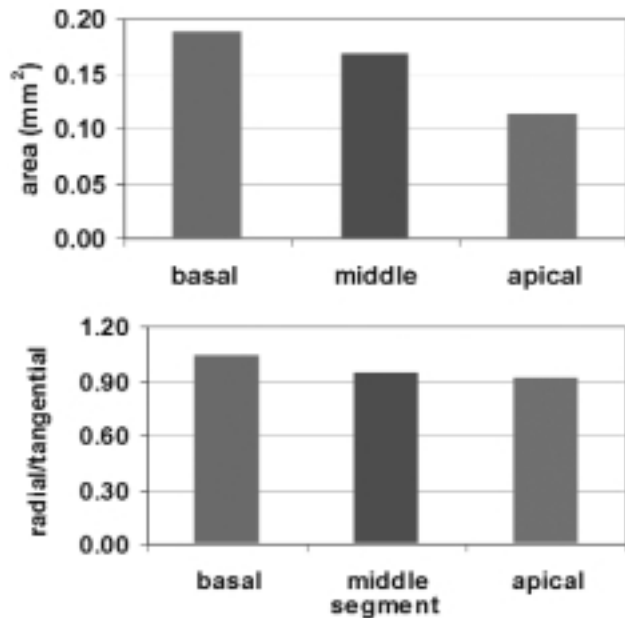

Figure 11. Size and form of the vascular bundles along *Guadua angustifolia* culm.

Table 2. Anatomical characteristics of the vascular bundles according to age and wall thickness zones.

Age (months)	Periphery Zone				Transition Zone				Middle Zone				Internal Zone		
	DVB (Nº/cm²)	R/T	Diam.mx (mm)	Fiber (%)	DVB (Nº/cm²)	R/T	Diam.mx (mm)	Fiber (%)	DVB (Nº/cm²)	R/T	Diam.mx (mm)	Fiber (%)	R/T	Diam.mx (mm)	Fiber (%)
6	476.0	1.57	0.02	95.47	81.6	1.93	0.06	92.58	53.9	1.49	0.11	85.09	0.67	0.20	64.81
12	346.0	1.23	0.03	96.71	142.8	2.05	0.05	90.15	58.8	1.63	0.16	84.09	0.67	0.22	66.18
18	367.2	1.15	0.02	95.99	136.0	2.67	0.10	90.29	52.3	1.61	0.17	82.30	0.79	0.20	67.03
24	387.6	1.20	0.02	97.20	132.6	1.77	0.07	95.71	58.8	1.70	0.15	86.55	0.93	0.21	86.67
30	408.0	1.33	0.02	97.00	183.6	1.56	0.07	96.51	65.4	1.73	0.13	89.23	0.82	0.20	69.41
42	408.0	1.28	0.02	96.50	163.2	1.55	0.10	94.65	56.6	1.54	0.16	84.67	0.87	0.22	66.97
48	448.8	1.43	0.02	94.53	183.6	1.70	0.09	89.98	65.4	0.98	0.15	66.28	0.64	0.21	66.89
54	489.6	1.35	0.02	94.14	193.8	1.71	0.09	92.33	71.9	1.16	0.16	78.60	0.62	0.21	67.33
60	530.4	1.30	0.02	94.10	176.8	1.74	0.03	93.39	65.4	1.50	0.15	87.26	0.65	0.20	64.93
84	489.6	1.18	0.02	95.46	163.2	1.68	0.08	92.90	65.4	1.41	0.16	85.77	0.94	0.19	68.52
108	448.8	1.28	0.02	95.26	163.2	1.52	0.07	92.55	69.7	1.51	0.16	86.91	0.77	0.20	66.92
120	448.8	1.25	0.02	95.16	190.4	1.66	0.06	91.14	95.8	1.41	0.12	85.09	0.75	0.20	68.75
AVG	435.2	1.20	0.02	95.63	159.5	1.77	0.07	92.94	64.0	1.48	0.15	83.96	0.70	0.20	68.70

DVB = Density of vascular bundles R/T = Radial/Tangential diam. mx = diameter of metaxylem vessel

Table 3. Internode wall thickness variation with age of the culm.

Age (months)	Total) Tissue (mm)	pz (mm)	pz (%)	tz (mm)	tz (%)	mz (mm)	mz (%)	iz (mm)	iz (%)
6	17.33	0.65	3.72	1.40	8.05	13.99	80.21	1.30	7.47
12	19.47	0.67	3.42	1.30	6.67	16.13	82.74	1.37	7.01
18	18.20	0.73	4.03	1.23	6.78	14.27	78.39	1.97	10.81
24	17.00	0.72	4.24	1.63	9.61	12.78	75.18	1.87	10.98
30	16.00	0.70	4.38	1.45	9.06	12.30	76.88	1.55	9.69
36	18.80	0.72	3.83	1.35	7.18	14.93	79.41	1.80	9.57
42	20.00	0.69	3.47	1.53	7.67	16.34	81.70	1.43	7.17
48	16.00	0.70	4.38	1.40	8.75	12.50	78.13	1.40	8.75
54	13.00	0.67	5.13	1.57	12.05	9.00	69.23	1.77	13.59
60	12.00	0.72	6.00	1.53	12.78	8.11	67.61	1.63	13.61
72	19.00	0.70	3.68	1.50	7.89	14.80	77.89	2.00	10.53
108	14.00	0.77	5.50	2.55	18.21	8.93	63.79	1.75	12.50
120	10.00	0.70	7.00	2.45	24.50	4.95	49.50	1.90	19.00
AVG	16.22	0.70	4.52	1.61	10.71	12.23	73.90	1.67	10.82

Table 4. Variation in the internode wall thickness, diameter, and internode length along a 48 month old culm.

culm segment	thickness zone (mm) ptz	thickness zone (mm) mz	thickness zone (mm) iz	Diameter (cm)	Internode length (cm)	wall thickness (cm)
basal	2.2	14.5	2.0	11.15	20.25	1.82
middle	2.0	8.9	2.3	11.05	34.83	1.32
apical	1.6	4.3	2.1	5.84	33.38	0.89

pz = periphery zone tz = transition zone mz = middle zone iz = internal zone

Table 5. Anatomical characteristics of the vascular bundle along the culm and through the wall thickness.

Vascular bundle	Segment Basal zone Avg.	ptz	mz	iz	Middle zone Avg.	ptz	mz	iz	Apical zone Avg.	ptz	mz	iz
DVB (N^0/cm^2)	169	377	68	61	184	392	82	77	350	612	224	215
R/T	1.05	1.41	0.91	0.82	0.95	1.40	0.92	0.52	0.91	1.33	0.85	0.56
Diam. mx (mm)	0.14	0.03	0.19	0.22	0.16	0.03	0.20	0.26	0.11	0.03	0.13	0.16
fb(%)	29	64.51	23.10	22.70	26	59.38	19.34	19.68	56	63.36	71.71	21.90

pz = periphery zone tz = transition zone mz = middle zone iz = internal zone
DVB = Density of vascular bundles R/T = Radial/Tangential diam. mx = diameter of metaxylem fb= fiber

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AFLP analysis of *Guadua angustifolia* (Poaceae: Bambusoideae) in Colombia with emphasis on the Coffee Region

Marta Leonor Marulanda[1], Pilar Márquez[1] and Ximena Londoño[2]

[1]Plant Biotechnology Lab., Universidad Tecnológica de Pereira, P.O. Box 097, Pereira, Colombia

[2]Sociedad Colombiana de Bambú, P.O. Box 661, Armenia, Quindio, Colombia

This study used the molecular markers known as Amplified Fragment Length Polymorphisms (AFLP) to establish the genetic relationship between accessions and biotypes of *Guadua angustifolia*, and compared them to other *Guadua* species existing in Colombia. Three primer combinations were selected because of the amplification efficiency and the number of polymorphisms. Fifty five individuals from the germplasm bank at the Botanical Garden Juan Maria Céspedes of Tulúa, Valle del Cauca, were studied. One hundred sixty two bands were found, which represent 70% of the polymorphisms. Two groups of individuals, G1 and G3, were found with the conglomerate analysis based on the estimates of genetic distance by molecular polymorphism. These correspond to two backgrounds of G. angustifolia genetic diversity, and a third group, G2, that includes *Guadua amplexifolia*, *Guadua.uncinata* and *Guadua sp*. Three accessions of *Guadua amplexifolia*, and the species *G. macrospiculata* and *G. superba,* show the largest genetic distances and were not grouped in the analysis. A clear genetic differentiation was seen between the different species of the *Guadua* genus. High diversity was also found within the accessions of the species *G. amplexifolia* in contrast to the low diversity observed between the accessions of *G. angustifolia*, even though this was the species that had greater representation in the study.

RESUMEN

En el presente trabajo se utilizaron los marcadores moleculares conocidos como longitud de fragmentos polimórficos amplificados (AFLP), para determinar las relaciones genéticas existentes entre accesiones y biotipos de *Guadua angustifolia* y compararlas con otras especies del genero *Guadua* presentes en Colombia. Se seleccionaron tres combinaciones de cebadores por la eficiencia de la amplificación y el número de polimorfismos. Se estudiaron 55 individuos del banco de germoplasma del Jardín Botánico Juan María Céspedes en Tuluá, Valle del Cauca. Se obtuvieron 162 bandas que representan el 70% del polimorfismo. Para el análisis de los resultados se utilizo el método de agrupamiento UPGMA mediante el paquete estadístico Ntsys – pc versión 2021 y un análisis de componentes principales. Con el análisis de conglomerado, basado en los estimados de distancia genética por polimorfismo molecular, se obtuvieron dos grupos de individuos, G1 & G3, que corresponden a dos fondos de diversidad de *G. angustifolia*, y un tercer grupo, el Grupo G2, donde se agrupan *Guadua amplexifolia*, *Guadua.uncinata* y *Guadua sp*. Tres accesiones de *Guadua amplexifolia* y las especies *G. macrospiculata* y *G. superba* presentaron mayores distancias genéticas y no se agruparon en el análisis. Se observo una clara diferenciación entre las diferentes especies del género *Guadua*, y se encontró además, una alta diversidad dentro de las accesiones de la especie *G. amplexifolia*, contrastante con una diversidad baja entre accesiones y biotipos de la especie *G. angustifolia*, de mayor representación en este estudio.

Woody bamboos belong to the tribe Bambuseae of the family Poaceae and the genus *Guadua*, American woody bamboo, belongs to the subtribe Guaduinae, which also includes *Apoclada, Eremocaulon, Olmeca* and *Otatea* (Judziewicz et al., 1999). *Guadua* stands out from other genera because it includes the largest bamboos in the Americas, and those that exhibit the greatest social, economic and cultural significance. It includes approximately 30 species distributed from Mexico to Argentina. It prefers humid habitats and lowlands, and is distributed from sea level to 1800 m (Londoño 1990).

Guadua angustifolia Kunth is perhaps the species in the genus with the highest economic potential. It grows wild in Colombia, Ecuador, and Venezuela and has been introduced to other countries in Central and South America, Europe and Asia (Londoño 2000) as well as to the Southern USA. To date, two varieties have been acknowledged: *G. angustifolia* var. *bicolor* Londoño and *G. angustifolia* var. *nigra* Londoño. According to Londoño (1989) and Londoño (Clark (1998), the *guadua* known as "cebolla", "macana", "cotuda" or "castilla" are biotypes or forms that react to specific climatic and soil conditions. However, Giraldo & Sabogal (1999), and Gómez et al. (2001) describe these four biotypes of *G. angustifolia*, characterized by morphological characteristics such as the size and thickness of the culm and the size of the cauline leaves. Other criteria such as the use given to it by farmers and vernacular terminology, were also considered.

In Colombia this species is widespread in the central-western region of the country known as the Coffee Region (main coffee growing area) where it covers an area of approximately 20,000 has. During recent years, the "*guadua*les" (area with *guadua*) have been affected by the indiscriminate felling and destruction of woodlands (Judziewicz *et al.* 1999). This could have a direct impact on the loss of genetic resources that remain unknown, and which could be of great significance in programs to manage and improve species with commercial value such as *G. angustifolia*.

However, the information required to develop and promote complementary preservation strategies does not exist (Stapleton & Ramanatha 1995). An important factor for the conservation of the genetic diversity in natural populations of any bamboo species is the understanding of its genetic structure, which becomes a basic tool when doing strategic planning for its preservation and sustainable use (Biswas 1994).

In this study, we aim to aid in the preservation of *Guadua angustifolia*, the most economically important bamboo in America, and to add depth to our knowledge of its genetic variation.

The only germoplasm bank of *Guadua* that exists in America, and which contributes to ensuring the genetic diversity of these bamboos, was established in 1987 in Mate*guadua*, Tulúa, Valle del Cauca, Colombia at the Juan María Céspedes Botanical Garden of the Scientific Research Institute of Valle del Cauca– INCIVA (Londoño 1991; Judziewicz et al. 1999). This bank has a collection of 45 accessions of *Guadua angustifolia* from 16 Departamentos (provinces) of Colombia. It also includes other species from the genus such as *Guadua amplexifolia* Presl, *Guadua macrospiculata* Londoño & Clark, *Guadua paniculata* Munro, *Guadua superba* Huber, *Guadua* weberbaueri Pilger and *Guadua uncinata* Londoño & Clark (Londoño & Clark 2002)

The use of molecular techniques for the study of the genetic diversity in bamboo has been relatively restricted although some has been done (For example see: Friar & Kochert 1991 & 1994, Gielis *et al.* 1995, Clark *et al.* 1995, Heng *et al.* 1996, Watanabe *et al.* 1994, Kobayashi, 1997, Kelchner & Clark 1997, Guala *et al.* 2000, & Suyama *et al.*, 2000). Loh *et al.* (2000) used AFLPs to analyze the relationships between four genera (*Bambusa, Dendrocalamus, Gigantochloa* and *Thyrsostachys*) using eight enzyme-primer combinations were used to generate AFLPs For 15 species.

Table 1. List of samples with their respective origin, collection number and herbaria

Code	Collection	Herbaria	Species	Origin
1	XL 076	TULV, US	*G. angustifolia*	Sasaima (Cundinamarca)
2	XL 109	TULV, US	*G. uncinata*	Morelia (Caqueta)
3	XL 144	TULV, US	*Guadua* sp$_1$	Florencia (Caqueta)
4	XL 206	TULV	*G. angustifolia*	Pto. Caicedo (Putumayo)
5	XL 214	TULV, US	*Guadua* sp1	Mocoa (Putumayo)
6	XL 233	TULV	*G. angustifolia*	Ricaute (Nariño)
7	XL 235	TULV	*G. angustifolia*	Ricaute (Nariño)
8	XL281	TULV	*G. aff. angustifolia*	Acacias (Meta)
9	XL 289	TULV	*G. angustifolia*	San Juan de Arama (Meta)
10	XL 291	TULV, US	*Guadua* sp$_2$	Río Guejar (Meta)
11	XL 303	TULV	*G. angustifolia*	Cumaral (Meta)
12	XL 343	TULV, US	*G. angustifolia*	Puente Nacional (Santander)
13	XL 344	TULV, US	*G. angustifolia*	Curiti (Santander)
14	XL 345	TULV, US	*G. angustifolia*	Bucarica (Santander)
15	XL 366	TULV, US	*G. aff. angustifolia*	Encizo (Santander)
16	XL 375	TULV, US	*G. angustifolia*	Mercaderes (Cauca)
17	XL 392	TULV	*G. angustifolia*	Neira (Caldas)
18	XL 397	TULV	*G. angustifolia*	Rio Armas (Caldas)
19	XL 399	TULV, US	*G. angustifolia*	Abejorral (Antioquia)
20	XL 413	TULV	*G. amplexifolia*	Cáseres (Antioquia)
21	XL 416	TULV	*G. angustifolia*	Sabaneta (Antioquia)
22	XL 418	TULV	*G. angustifolia*	Valparaiso (Antioquia)
23	XL 542	TULV, US	*G.* superba	Leticia (Amazonas)
24	JA 1003	TULV	*G. amplexifolia*	Monteria (Córdoba)
25	JA 1006	TULV	*G. amplexifolia*	Costa Atlántica
26	JA 1012	TULV	*G. angustifolia*	Venecia (Antioquia)
27	JA 1013	TULV	*Guadua* sp$_3$	Bolombolo (Antioquia)
28	JA 1016	TULV	*G. angustifolia*	Cañas gordas (Antioquia)
29	JA 1019	TULV	*G. angustifolia*	Mutata (Antioquia)
30	JA 1026	TULV	*Guadua* sp$_4$	Aipe (Huila)
31	JA 1027	TULV	*G. angustifolia*	San Juan de Rioseco (Antioquia)
32	JA 1028	TULV	*G. angustifolia*	*Guadua*s (Cundinamarca)
33	JA 1029	TULV	*G. angustifolia*	Rio Negro (Antioquia)
34	JA 1031	TULV	*G. angustifolia*	Florencia (Caldas)
35	sn	-	*G. angustifolia*	Ingenio Providencia (Valle)
36	sn	-	"macana"	El Eden, Quebradanegra (Quindio)
37	JA 1010	TULV	*G. aff. amplexifolia*	Turbana (Bolivar)
38	sn	-	*G. angustifolia var. bicolor*	Comfamiliar, Cerritos (Risaralda)
39	sn	-	"castilla"	Maracaibo, Caicedonia (Valle)
40	sn	-	"cebolla"	Santa Lucia, La Tebaida (Quindio)
41	sn	-	"cebolla"	La Hungria, Cerritos (Risaralda)
42	sn	-	"cebolla"	La Hungría, Cerritos (Risaralda)

XL= X. Londoño; JA = J. Adarve; sn = without number
TULV = Tuluá Valle; US = United States National Herbarium

Table 1. List of samples with their respective origin, collection number and herbaria *(Cont'd)*

Code	Collection	Herbaria	Species	Origin
43	sn	-	"cotuda"	Santa Lucia, La Tebaida (Quindio)
44	sn	-	"criolla"	Napoles, Montenegro (Quindio)
45	sn	-	"grandicaula"	Santa Lucia, La Tebaida (Quindio)
46	XL 536	TULV, US	*G. macrospiculata*	La Esmeralda, Montenegro (Quindio)
47	sn	-	"macana"	Napoles, Montenegro (Quindio)
48	sn	-	"macana"	La Hungria, Cerritos (Risaralda)
49	sn	-	"macana"	Club Campestre, Cerritos (Risaralda)
50	sn	-	"macana"	Club Campestre, Cerritos (Risaralda)
51	sn	-	*G. angustifolia var nigra*	Napoles, Montenegro (Quindio)
52	sn	-	*G. angustifolia var nigra*	La Sonora, Combia (Risaralda)
53	sn	-	"pepina"	La Gaucha, Morelia (Risaralda)
54	XL 887	TULV	*G. angustifolia*	San Calixto (Norte de Santander)
55	sn_2	-	*G. angustifolia*	La Esmeralda, Montenegro (Quindio)

XL= X. Londoño; JA = J. Adarve; sn = without number
TULV = Tuluá Valle; US = United States National Herbarium

In this paper, a group of new AFLP markers for the genus were made available and these were used to: a) analyze the genetic distance between, *G. angustifolia* var. *angustifolia* and the local varieties and biotypes, b) analyze the genetic distance between species in the genus, and c) establish the variability of *G. angustifolia* in the coffee region.

MATERIALS AND METHODS

Standardization of the AFLP technique for *Guadua*

The following extraction protocols were tested: Dellaporta et al. (1983), Gilbertson et al. (1991), and Hoisington (1992). The amount and purity of the DNA was measured using spectrophotometry, and its integrity was determined using electrophoresis on an 0.8% agar gel. A total of 55 samples were collected from the Germoplasm bank and from several farms of the Coffee Region (Table 1). Samples were collected in duplicate and all of them had the same treatment.

AFLP Analysis

The methodology described by Vos *et al.* (1995) was used, where the genomic DNA was digested with two restriction enzymes, one having a rare (bizarre) cut (EcoRI) and another one having a frequent cut (Msel). The digested fragment lengths were coupled with specific double strand adapters, then selective amplifications were made with specific primers of one (+1) and three (+3) selective bases. Three combinations of primers were analyzed. The amplified fragments were separated with electrophoresis on poliacrylamide gels and the bands were viewed with silver nitrate. The gels were read visually and the polymorphic bands were included in the statistical analysis. Ten combinations of primers were tested (Table 2) with the objective of selecting the ones exhibiting the best amplification, high polymorphism and good reading resolution of the bands.

Statistics

Only the polymorphic bands were used for the statistical analysis once their independence had been shown by means of the simple correlation coefficient of Pearson (P<0.05). They were evaluated in a binary form (0 = absence, 1 = presence). Genetic similarity (Gsij) between each pair of genotypes (Dice 1945) was calculated with the formula of and Nei & Li (1979) using the original data: $GS_{1j} = 2a/(2a+b+c)$ where: a = presence of the band in the I and j genotypes, b = presence of the band in genotype I, absence

Table 2. Primer combinations tested to evaluate the AFLP technique and selected combinations
for the *Guadua* characterization.

Combination	Selective Nucleotides	Total Bands	*Polimorphic* Bands	Level of polymorphism
1	AAG/CAC	–	–	High
2	AAC/CAA	–	–	High
3	AGC/CTG	–	–	High
4	ACT/CTC	–	–	High
5	ACA/CAG	–	–	High
6	ACC/CAT	–	–	High
7	AGC/CTA	–	–	High
8	AGG/CTT	–	–	Medium
9	AAC/AAC	–	–	High
10	ACA/CAA	–	–	Medium
*11	AAG/CAC	88	71	–
*12	AAC/CAA	75	48	–
*13	AGC/CTG	68	43	

(*) = Selected primer combinatios used for statistical data analysis.

in j, and c = absence of the band in genotype I, and presence in j.

The genotypes were grouped based on the dissimilarity values (1-Gsij) between every clone pair with the SAHN algorithm (Sneath & Sokal 1973). Grouping was by Unweighted Pair-Group Method with Arithmetic average (UPGMA) over 1000 bootstrap replicates using the NTSys-pc statistics package version 2.02I (1998). A Principal Components Analysis (PCA) and discriminate factorial analysis (using STATIFC version 4.0, 1988) was also done based on the paired distance matrix in the following groups: *Guadua angustifolia*, *G. amplexifolia*, *G. macrospiculata*, G. superba, *G. amplexifolia*, *G. uncinata* and *Guadua spp.*

RESULTS AND DISCUSSION

Standardization of the AFLP technique for *Guadua*

Hoinsigton's (1992) protocol provided the best results (high quality and DNA concentration) with developing leaves. The AFLP System I kit from Gibco BRL was used to standardize the AFLP technique. In total 10 primer combinations were tested (Table 2). Three primer combinations were selected due to the efficiency of the amplification of their sequences and the polymorphism level they exhibited. They were: E-AAG x M-CAC, E-AAC x M-CAA y E-AGC

x M-CTG. A total of 162 bands were found (Table 3) representing 70% of the polymorphism. Figure 1 shows an example of the band pattern found with the three combinations used in this study: 1) AAG/AC, 2) AGC/CTG, and 3) AAC/CAA. M: molecular weight marker (10bp).

A UPGMA tree (fig. 2) was generated and showed a pattern congruent with the PCA Analysis. If the truncating level mentioned (0.75) is considered, it is possible to differentiate six non-grouped accessions that show the greatest distance in relation to the groups mentioned: *Guadua superba* (XL542), *G. macrospiculata* (XL 536), *G. amplexifolia* (JA1003 & 1006), *G.* aff. *amplexifolia* (JA1010), and *G.* aff. *angustifolia* (XL366). The remaining accessions form the following groups:

- Group 1: *Guadua angustifolia* (XL76, JA1028 & JA1029).
- Group 2: *Guadua uncinata* (XL109), *Guadua* sp1 (XL144 & XL214) and *G. amplexifolia* (XL413).
- Group 3: *G. angustifolia* remaining in addition to the varieties *bicolor* and *nigra*, the biotypes "castilla", "cotuda", "criolla", "grandicaula", "macana", "pepina", and *Guadua sp.*

Figure 3 shows the relative distribution of the individuals studied in the axes 1 and 2. The first two principal components account for 75% of the total molecular variability, making the distribution very reliable.
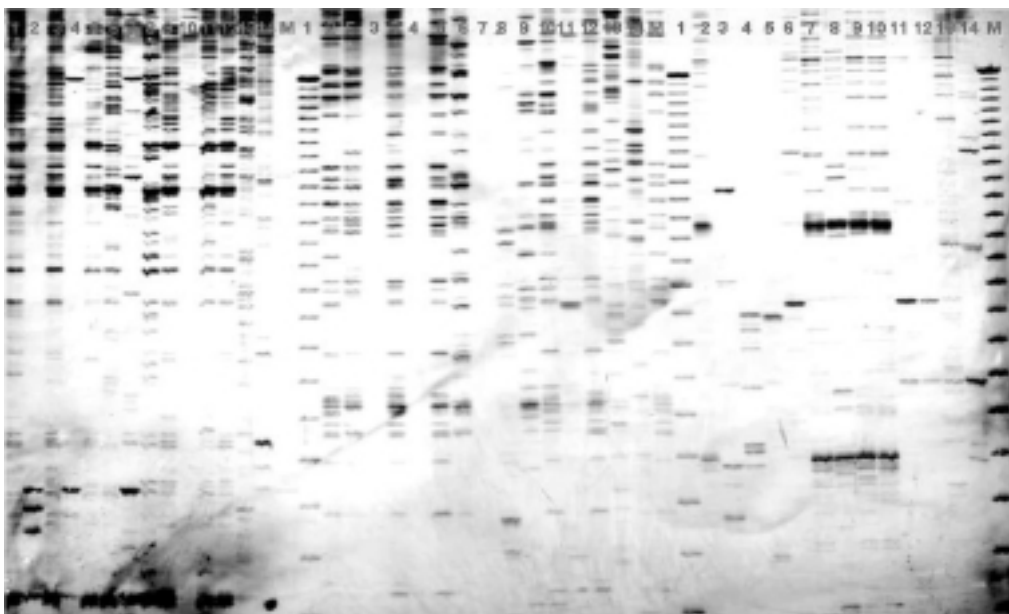
Figure 1. An example of the banding pattern found with the three combinations used in this study: 1) AAG/AC, 2) AGC/CTG, and 3) AAC/CAA. M: molecular weight marker (10bp).
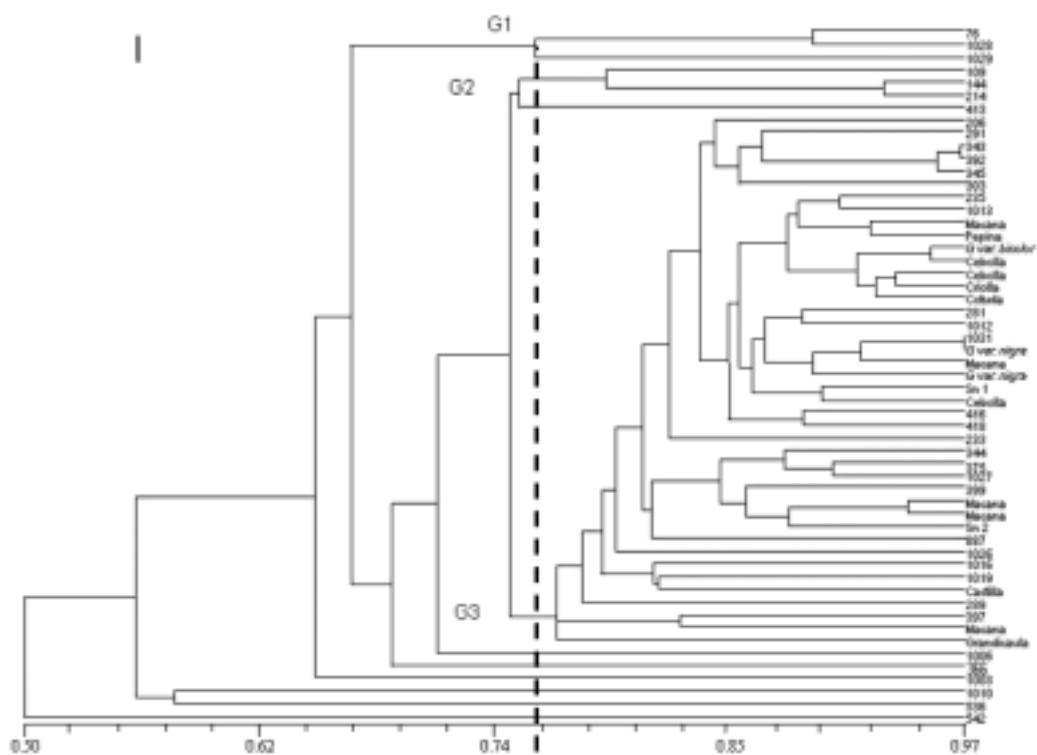

Figure 2. UPGMA dendrogram with 75% threshold (Dice Similarity Index) indicated.

Table 3. Estimates of average genetic distances of *Guadua angustifolia* and the species studied

	G1	G2	G3	G. angustifolia	G. amplexifolia (JA 1006)	G. aff. amplexifolia (JA 1003)	G. amplexifolia (JA 1010)	G. amplexifolia	G. macrospiculata (XL 536)	G. superba (XL 542)
G1	0.20									
G2	0.34	0.31								
G3	0.33	0.25	0.19							
G. angustifolia				0.209						
G. amplexifolia (JA1006)	0.44	0.32	0.29		–					
G. amplexifolia (JA1003)	0.47	0.33	0.35		0.33	–				
G. aff. amplexifolia (JA1010)	0.49	0.39	0.46		0.42	0.37	–			
G. amplexifolia				0.314				0.33		
G. macrospiculata (XL536)	0.55	0.40	0.42	0.429	0.43	0.42	0.42	0.42	–	
G. superba (XL542)	0.53	0.49	0.48	0.488	0.57	0.57	0.55	0.54	0.56	–
G. uncinata (XL109)				0.305				0.328	0.365	0.45
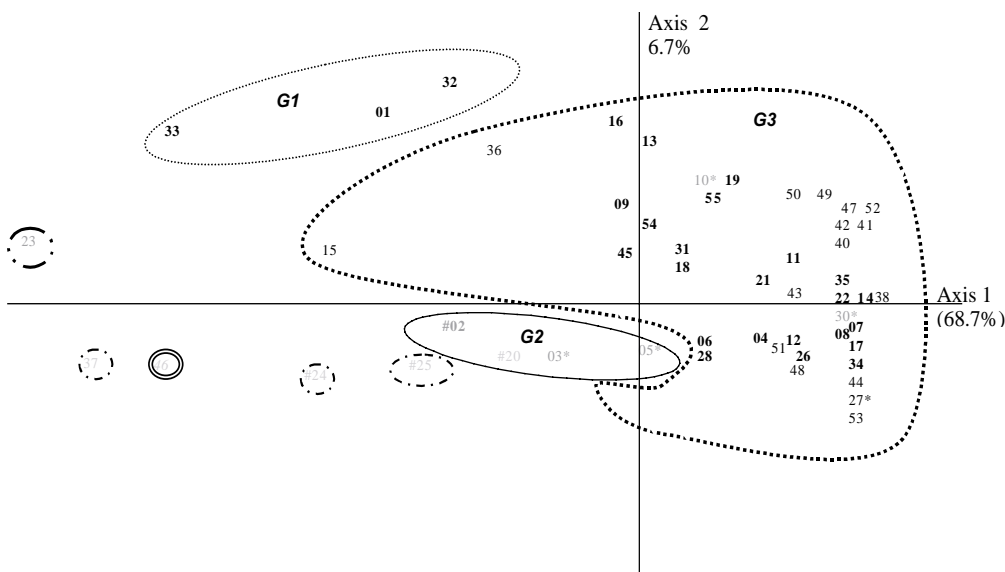
Species and Biotypes of *Guadua* spp.	Accession Numbers
– *Guadua angustifolia,* Germplasm bank	**01,04,06,07,08,09,11,12,13,14,15,16,17,18, 19,21,22,26,28,31,32,33,34,35,45,54,55**
– *G. angustifolia* - Biotypes	36,38,40,41,42,43,44,48,47,49,50,51,52,53
– *G. amplexifolia*	#02,#20,#24,#25
– *G. macrospiculata*	46
– *G. uncinata*	37
– *G. superba*	23
– *Guadua* sp	03*, 05*, 10*, 27*, 30*

The only difference between the groups that resulted from the analyses is due to the composition of groups 1 and 2 in the UPGMA. The PCA in the axes (1 & 2) only differentiates the JA1029 accession (33) from the remaining individuals in the *G. angustifolia* species studied. It also provides a site for the accession XL144 (3) and XL214 (5) of *Guadua* sp_1 closer to group 3 of *G. angustifolia* than to the remaining members of group 2 from the UPGMA.

The estimate of the average genetic distance within and between the groups considered, and also between species, is shown in Table 3. The greatest genetic distances were found between *G. superba* and *G. amplexifolia* and between *G. superba* and *G. macrospiculata. Guadua superba* showed the greatest average distance within the group (0.57) (Table 3). These estimates must be reviewed in the future increasing the number of accessions in these species. Notwith-

standing the higher number of individuals in group G3, this group showed the least average genetic distance within the group (0.19) caused by the high similarity of its members (Table 3). The AFLP polymorphism revealed shows:

1. A marked genetic diversity between *G. angustifolia* and the species *G. macrospiculata* and *G. superba*.
2. The presence of at least two backgrounds of genetic diversity within *G. angustifolia* (groups G1 y G3).
3. High diversity within the 5 accessions studied of *G. amplexifolia*.
4. Genetic similarity of the G3 group of accessions of *Guadua angustifolia* with the accessions of *Guadua sp*. This makes future confirmation advisable.
5. Greater genetic similarity between *Guadua amplexifolia* vs. *G. uncinata* and between *Guadua amplexifolia* vs. *Guadua* sp_1.

Establishing AFLP bands associated to groups of interest

The discriminate factorial analysis found a 100% good classification, indicating complete correspondence between the AFLP molecular diversity revealed by the three primer combinations and the previous taxonomic identification of the material.

The results allow us to expect the presence of band patterns associated with the 5 species represented and with the *Guadua* spp. group. Even though the members of the *Guadua* spp. group are distributed between the groups of molecular diversity 2 (*G. amplexifolia*) and 3 (*G. angustifolia*) according to the genetic distances, the group keeps its own molecular characteristics, either of a qualitative type expressed by the presence of its own bands, or of a quantitative type expressed by bands common to the groups mentioned, but having different frequencies.

The simple factorial analysis made to select the AFLPs as descriptors found an absence of single bands (those present in one accession) and of bands associated with the all the representatives of each of the five species, or with the three groups of molecular diversity (G1-G3) mentioned. This shows that the diversity groups based on the molecular distances have common bands present in frequencies that differ between each other. The differentiation of these groups resides in the quantitative and qualitative differences in the frequencies of the AFLP variants.

The results allow us to differentiate and identify the accessions of the same species, considering a set of AFLPs. Also, although the propagation of this species seems to be mainly asexual, an explanation for the molecular diversity found could be the existence of sexual reproduction periods that introduce variability to the genetic background of the species.

CONCLUSIONS

1. According to the molecular analysis done using three (3) combinations of primers in AFLP markers, the genetic diversity of the *Guadua angustifolia* species in the coffee region of Colombia is not significant. It records an average genetic distance of 0.209. It was not possible to make a molecular characterization of the biotypes using these three combinations.

2. For the species *Guadua angustifolia* two genetic diversity backgrounds, Group 1 (G1) and Group 3 (G3), were identified.

3. The genetic distance found within Groups G1 and G3 was 0.195. This value represents the lowest genetic distance in this study, indicating that there is high similarity among its members.

4. The G2 group includes species different from *G. angustifolia* such as: *G. uncinata* and *Guadua* sp$_1$. This group is genetically closer to Group 3 than to Group 1.

5. The AFLP markers revealed high molecular diversity between the species studied in the *Guadua* genus and within the accessions of the species *G. amplexifolia* in contrast to a relatively lower diversity observed within the accessions of the species *G. angustifolia* that had a higher representation in this study.

6. The species *G. amplexifolia, G. macrospiculata, G. superba,* and *G. uncinata* form separate groups and clearly show a wide genetic distance in relation to *G. angustifolia,* exhibiting values that go from 0.29 to 0.48. *Guadua superba* records the greatest genetic distance (0,488) vs. *G. angustifolia,* followed by *G. macrospiculata* (0.429), *G. amplexifolia* (0.314) and *G. uncinata* (0.305).

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Plant growth and biomass distribution on *Guadua angustifolia* Kunth in relation to ageing in the Valle del Cauca – Colombia

N.M.Riaño[1], X. Londoño[2], Y. López[3] and J.H. Gómez[1]

[1]Centro Nacional de Investigaciones de Café – Cenicafé, Manizales, Caldas, Colombia
[2]Sociedad Colombiana del Bambú, P O Box 661, Armenia, Colombia
[3]Universidad Nacional de Colombia, Palmira, Colombia

Samples from complete *Guadua angustifolia* clumps, with different ages, on the southeastern flank of the Cauca River valley (Colombia) were chosen for this study. Estimates of the accumulated biomass (>50 metric tons CO_2 ha^{-1} during 6 years) and its redistribution into different organs were obtained. The aerial portions (culm, branches, foliage leaves, and caulinar leaves) accumulate 80.1% of the total biomass and CO_2 fixation, and the rhizome 19.9% of these. Mathematical functions that describe its growth as a function of chronological time on number of organs, fresh and dry weight, and leaf area measurements were developed.

The atmospheric concentration of carbon dioxide has increased during the last five decades, mainly as a consequence of the combustion of fossil organic matter (coal, oil) and non-fossil matter (forests) resulting from antropogenic activity (IPCC 1996). According to Goodess *et al* (1992), the CO_2 rate increases 1.8 μmol (CO_2) mol^{-1}(air) year-1, equivalent to 0.5% year-1. Estimates indicate that in 100 years if the increasing rate is maintained, the environmental CO_2 will reach values of 650 -700 μmol (CO_2) mol^{-1}(air). This could cause an increase in the average global temperature of 1.5 C to 4.5 C (Saralabai *et al*. 1997; IPCC 1996).

Renewable biomass cultivated as a carbon sump can be managed in short rotation shifts, using it for the production of long-term consumption goods and for construction. Because of its low contents of sulfuric pollutants, plant biomass might be converted into energy, heat and liquid or gas fuels without great hazards to environment (GIECC 2000a).

The Clean Development Mechanisms (CDM) is the only instrument that links developed countries to the reduction of emissions, and which could lead those countries to invest resources by sowing plantations with high potential to fix atmospheric carbon dioxide.

G. angustifolia is one of the tropical species that have been identified as having great potential to fix atmospheric carbon dioxide. It is one of the 3 largest bamboo species and one of the most important in the world. Because of its physical/mechanical properties it may used to manufacture long lived products such as houses, furniture, handcrafts, agglomerates, veneers, floors, etc. (Londoño 1998a). *Guadua angustifolia* can increase its height up to 21 cm day- and emerges from the ground with a constant diameter up to 22 cm. Culms reach their final height in the first 6 months of growth, and come to maturity when they are 4 to 5 years old. Optimum growth is reached between 500 and 1500 m of altitude, with a rainfall of 1200-2500 mm per year-, temperatures between 18°C and 24°C, and 80-90% relative humidity. It adapts well to extreme rainfall conditions of Colombian tropical rain forests (more than 10000 mm per year-) but not under very dry conditions (<800 mm per year) (Londoño 1998b). The ideal composition of culms in a guadua plantation has been estimated to be 10% new shoots, 30% young culms, 60% mature and over mature culms and no dry ones, with a density between 3000 to 8000 culms per ha. The diameter diminishes as culm density increases (Londoño 1998b; CVC 2000).

In Colombia, *G. angustifolia* covers an approximate area of 51500 ha, 46261 ha of which are wild and 5260 ha are cultivated. The Valle del Cauca Department, has the largest

Table 1. Location and planting year of the sampling plots.

Plot	Municipality	Township	Sub-basin river	Altitude m.a.s.l	Planting Year	Semester
Katanovo	El Cerrito	San Antonio	Nima-Amaime	967	1995	B
B. Municipal	Palmira	Área Urbana	Nima-Amaime	1107	1996	A
Tarjo 2	El Cerrito	San Antonio	Nima-Amaime	964	1997	A
Canta Claro	Pradera	La Buitrera	Bolo-Fraile-Desb.	1189	1997	B
Las Vegas	Palmira	Guanabanal	Nima-Amaime	-	1998	B
Bella Vista	El Cerrito	El Paraíso	Nima-Amaime	1389	1999	A
El Palomar	Pradera	El Recreo	Bolo-Fraile-Desb.	1232	1999	B
La Playa	Florida	El Tamboral	Bolo-Fraile-Desb.	1415	2000	A
Normandía	Palmira	Aguaclara	Bolo-Fraile-Desb.	-	2000	B

area of wild guadua plantations (7960 ha), and has contributes the largest reforested area of this species (1830 ha) (Londoño 1998a; CVC 2000).

Biomass contribution of *G. angustifolia* to the soil biomass is around 10 tons per ha per year, and dry matter accumulation reaches 76.6 tons pe ha including culms, branches, foliage leaves and cauline leaves (De Wilde 1993). Most of the studies on nutrient extraction and composition of culms have been focused on recycling of nutrients and fertilization (Shanmughavel & Francis 1996; 1997; De Wilde 1993). No reports on growth analysis (accumulation and distribution of dry matter) or CO_2 fixation for photosynthetically active area were found in the literature.

The objective of this research is to study basic aspects of the biomass accumulation of *G. angustifolia* in order to establish the potential of this species as atmospheric carbon dioxide fixer, following classical growth analysis.

MATERIALS AND METHODS

Location and planting year of the sampling sites

Samples were taken from the southeastern flank of the Cauca River valley. The municipality, township, sub-basin river, altitude and establishing year from the 9 reforested sites of *G. angustifolia* are shown in Table 1. The measure was done during June and August of 2001, corresponding with a dry season in this zone.

During the 3 months period of sampling, 5 clumps of *G. angustifolia* in each nine (9) farms, planted during 1995 to 2000 (72 months or 2190 days) were harvested.

Sampling and measurement of the response variables

In clumps from 6 to 36 months old (1080 days) were measured fresh weight, dry weight, leaf area, culm number and height. Rhizome fresh weight was determined using a digital balance (Mettler 16E), material was chopped and dried down to constant weight in a DIES-640 oven with an air recycling system at 80°C.

In clumps older than 1080 days only 3 new shoots, 3 young culms and 5 mature culms of each clump with its rhizomes were sampled. Culms were cut in two or three segments of the same length, depending on total height, and the diameter in the middle of each segment was measured, weighed and a 10 cm long sample from the middle was weighed and dried to constant weight.

Fresh weight of branches, cauline leaves and culm leaves were measured using a 200 g sample of both branches and leaves dried to constant weight. Leaf area was measured with a Delta T- Device. Fresh weight, dry weight and leaf area were measured on three early shooting culms, three young culms and five mature culms. Rhizome dry weight was measured on 200 g samples.

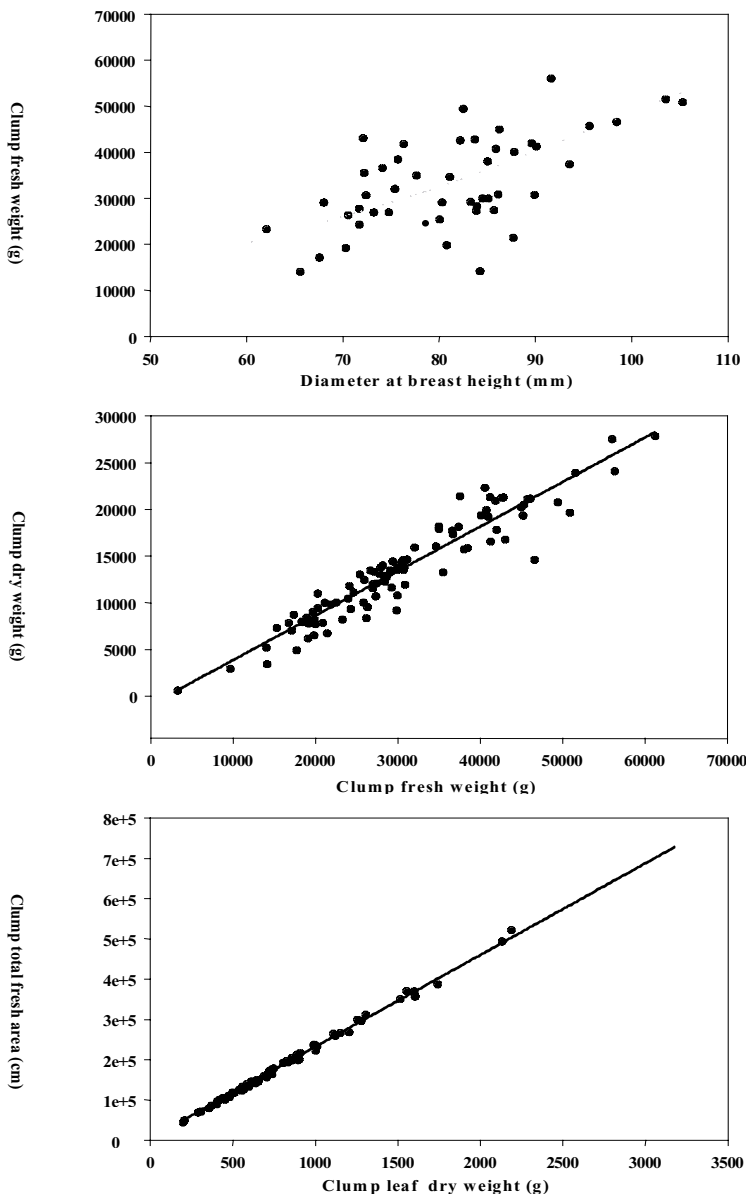Data processing was carried out using MS Excel, Sigma Plot 5.0 and SAS.

Figure 1. Linear relationships between: a. DBH/ clump fresh weight; b. whole plant fresh weight / whole plant dry weight; c. clump leaf dry weight / clump total leaf area. Fitted model (center line) and confidence limits (0.95).

RESULTS AND DISCUSSION

Although the correlation coefficient (r) between total fresh weight and the diameter at 1.5 m height (DBH) seems to be high (0.67), only 45% of the whole fresh weight can be explained by the variation of DBH in *G. angustifolia*.

Nonetheless, the magnitude of r is statistically significant (p<0.0001). This result seems to indicate a rough tendency to linearity of total fresh weight as a function of DBH although it is not sufficient to estimate accurately total fresh weight on DBH basis. This can be illustrated because most of the points fall outside of
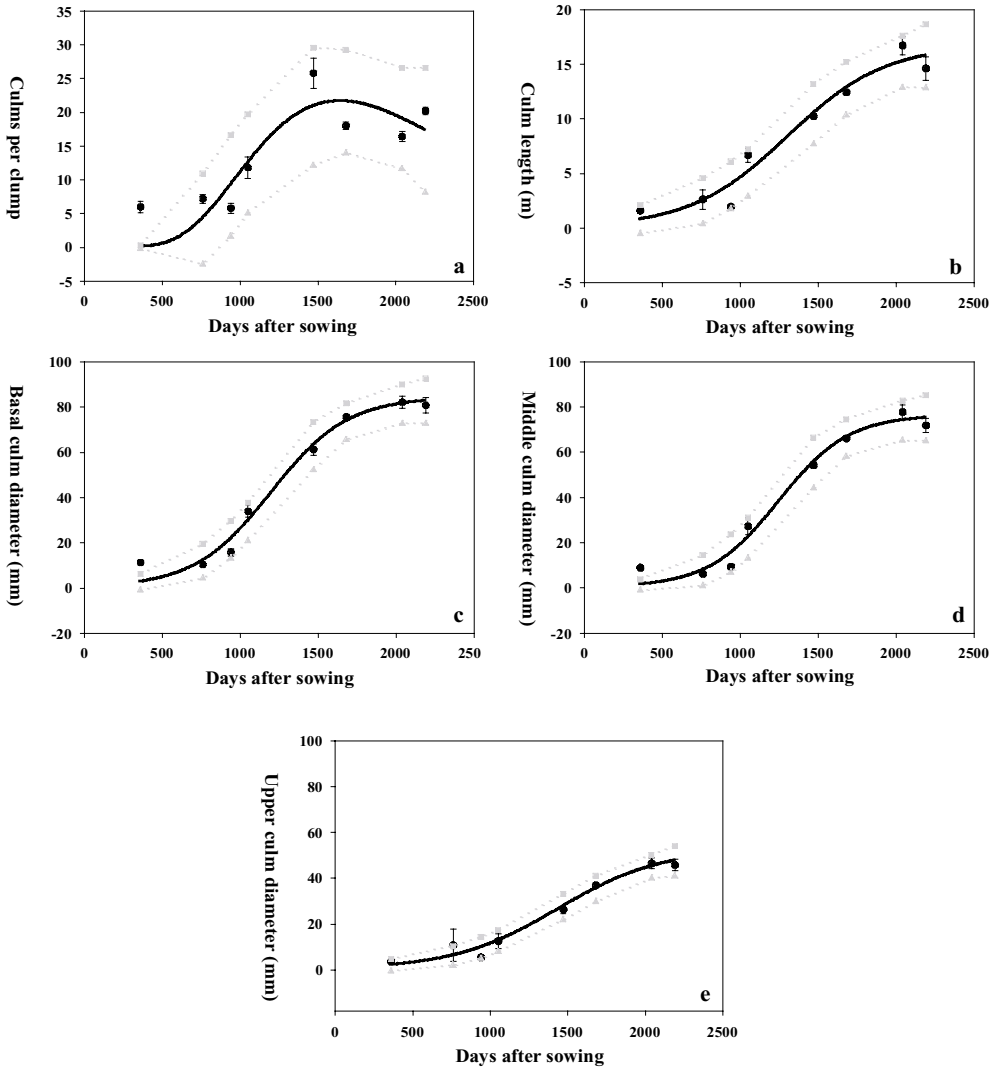
Figure 2. Growth models for: a. culms per plants; b. culm length; c. lower culm diameter; d. middle culm diameter; e. upper culm diameter. Vertical bars standard error. Fitted model (center line) and confidence limits (0.95)

confidence limits at p<0.05 (Fig.1a). The behavior of clump fresh weight as a function of DBH can be described as follows:

$$CFW = -25474.8 + 705.09 \ DBH$$

Where: CFW = clump fresh weight in g
DBH = Diameter in mm at breast height (1.5 m)

A closer relationship was found between clump total dry weight and clump total fresh weight (Fig. 1b). The observed dispersion of points can be explained by the effect of the water content variability inside the plant tissues. The linear tendency of clump total dry weight as a function of total clump fresh weight is described by

$$CDW = -1.007 + 0.476 \ CFW$$

Where: CDW = clump dry weight (g)

Table 2. Characteristics of the exponential functions describing length and diameter of the culm (at lower, middle and upper third) as function of time (days after sowing).

F	a	b	Xo	r^2	p
Culm length (m)	16.7	313.27	1310.4	0.96	0.0004
Lower diameter (mm)	84.07	245.83	1203.55	0.98	< 0.0001
Meddle diameter (mm)	76.17	224.73	1252.01	0.98	< 0.0001
Upper diameter (mm)	52.49	334.4	1438.32	0.98	< 0.0001

The correlation coefficient (r = 0.95) and determination coefficient (r^2 = 0.90) indicate that the biomass accumulation in the total clump of *G. angustifolia* can be estimated on the basis of total fresh weight of the clump.

Regression analysis of clump total leaf area as a function of total leaf dry weight (r = 0.99 and r^2 = 0.99) indicate that clump leaf dry weight is a useful variable to estimate clump total leaf area. Linearity of the clump leaf area is described by:

CLA = 2337.70 + 227.73 CLDW

Where: CLA = Clump total leaf area (cm²)
CLDW = Clump leaf dry weight (g)

Growth analysis

Culms per clump

This variable is described by:

$$C = ae^{\left[\frac{0.5(\ln(x-x_0))}{b}\right]^2}$$

Where: *C* = culms per clump; *a* = 21,6; *b* = 0,4; *Xo* = 1648.9; *X*= Time after planted.

The determination coefficient is r^2 = 0.72 (r = 0.85). Although r^2 can explain 72% of the variability due to the effect of time on culm

number, the confidence limits (p<0.05) seems to be very broad (Fig. 2a). This result can be explained because samplings were affected by different climate conditions registered along time. The maximum number of culms per clump is achieved around 1650 days after planting, and then it diminishes because the culms reached maturity and were harvested.

Culm length and basal, middle and upper diameter

The behavior of these variables can be described following the logistic function

$$F = \frac{a}{1+ e^{-\left(\frac{x-x_0}{b}\right)}}$$

The statistics for the different variables are registered in Table 2.

The culm length and similarity of the different culm diameter sections along time, seems to indicate that the different plantations from which the samples were chosen are originated from the same clone, and indicate that this clone has a high genetic stability at different localities. These results suggest also that the culm diameter at different heights can be estimated as a function of time for the different localities

Table 3. Characteristics of exponential models describing accumulation of dry matter in rhizomes, new shoots, whole leaf area and whole clump as function of time (days after planting).

Variable F	Xo	r^2	p
Rhizome (g)	1672.96	0.97	0.0002
Culm (g)	1662.90	0.98	< 0.0001
Branch (g)	1063.06	0.97	0.0001
Culm Leaves (g)	1092.48	0.99	< 0.0001
Foliage Leaves (g)	1055.96	0.91	0.0023
New Shoot (g)	1614.29	0.97	0.0001
Whole Clump (g)	1611.90	0.97	0.0001
Clump total leaf area (m²)	1070.20	0.93	0.0012

Table 4. Weight organs ratio and leaf area ratio for *Guadua angustifolia* through time.

Days after planting	Rhizome ratio g g⁻¹	Culm ratio g g⁻¹	Branch ratio g g⁻¹	Culm leaves ratio g g⁻¹	Foliage leaves ratio g g⁻¹	Leaf area cm² g⁻¹
360	0.18	0.29	0.26	0.003	0.26	49.02
760	0.20	0.36	0.24	0.020	0.20	46.54
940	0.24	0.36	0.27	0.002	0.14	37.92
1050	0.57	0.51	0.28	0.010	0.20	36.91
1470	0.18	0.67	0.09	0.008	0.06	13.29
1680	0.19	0.69	0.07	0.007	0.04	9.90
2040	0.18	0.73	0.06	0.005	0.03	7.55
2190	0.19	0.72	0.05	0.004	0.04	8.35
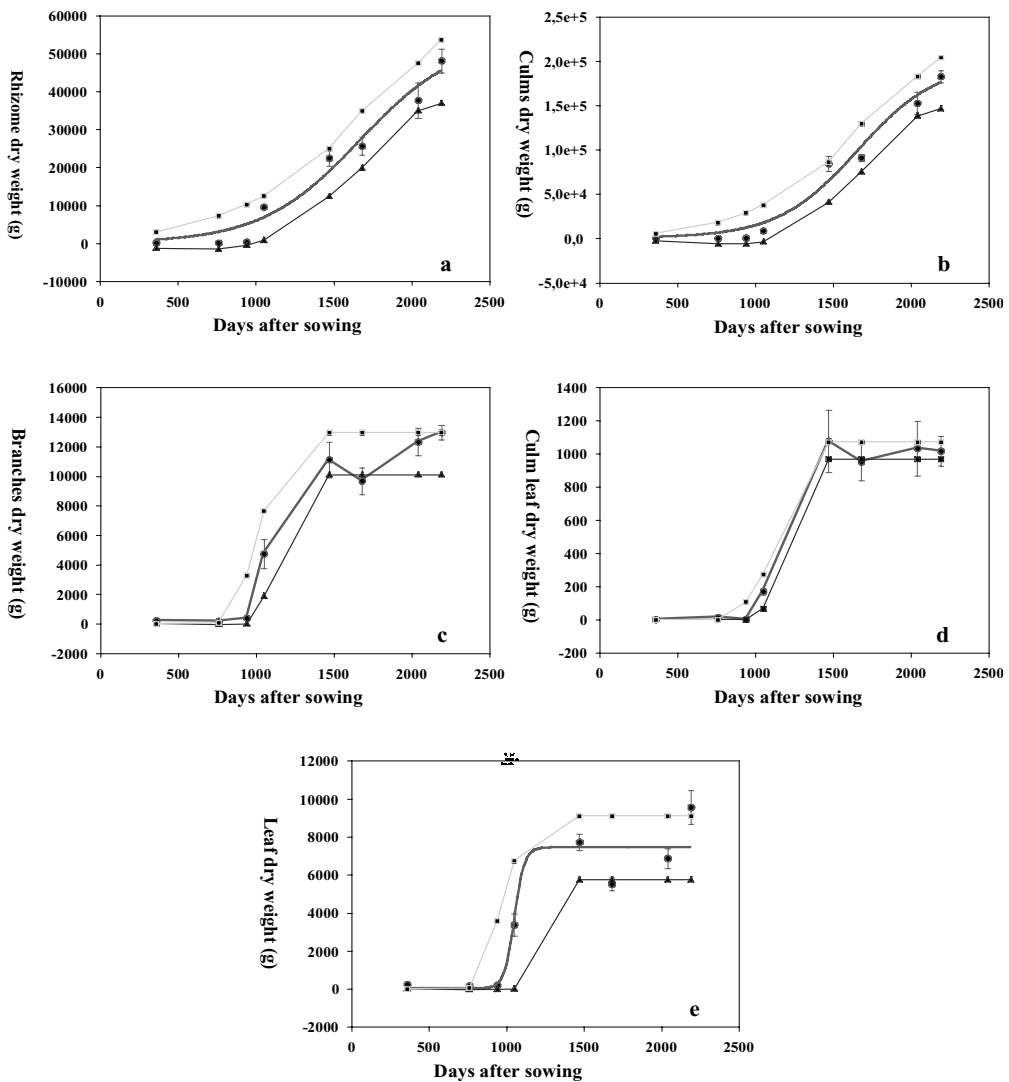
Figure 3. Dry weight growth models for: a. rhizome; b. culm; c. branches; d. culm leaves; e. leaves. Vertical bars standard error. Fitted model (center line) and confidence limits (0.95)

of the Cauca River Valley with a high degree of accuracy (Fig. 2b, 2c, 2d, 2e).

The same logistic function can be used to describe dry matter accumulation in rhizome, new shoots, culm, branches, caulinar leaves, foliage leaves and the whole clumps of *G. angustifolia* as a function of time. For all the measured variables the values of r^2 are higher than 0.91. The minimum value (0.91) was registered for leaves dry weight and it can be explained by a high rate of leaf exchange as was reported by De Wilde (1993) and Shanmughavel & Francis (1996) (Table 3).

Branches, caulinar leaves, foliage leaves and leaf area achieved its maximum growth rate to 1050 days after planting. Meanwhile, rhizome, culm, new shoot and whole clump reached the inflexion point at 1600 days after planting. This delay can be explained because rhizomes and culms are the major organs for dry matter accumulation in the clump and the filling time goes beyond the peak of maximum growth of the assimilates sources. The highest rhizome ratio (0.57) (Table 4) was registered at 1050 days after planting. This result is in accord with the maximum growth rate obtained for branches, caulinar leaves, foliage leaves and leaf area.

During the growth period, the rhizome maintains its relative contribution to clump at an almost constant value of 0.2. Meanwhile, the culm increases its relative contribution from 0.3 to 0.7. These data suggest that in the early stages of growth, the contribution of rhizome and culm reach 50 % of the total biomass and six years later the contribution of rhizome and the culm to the total biomass reaches 90%. From a production and environmental point of view, this value is desirable because at the harvesting time the 90% of the carbon fixed can remain long term as fixed biomass (culms as durable products and the rhizome as underground biomass with slow degradation) (Table 4). Only 10% of fixed carbon is quickly turned over in the biosphere (IPCC 2001).

Branch and leaves weight ratio exponentially decreases up to 0.05, while the contribution of the caulinar leaves does not surpass 0.02

independently of age. Leaf area ratio decreases exponentially from 50 to 8 cm^2 (leaf area) g^{-1} (clump dry weight). These results indicate that at the early stages of growth the plant depends completely upon the leaf area to build the carbon chains and metabolites, which allow the growth of each organ. However, the distribution of these assimilates are more efficient with increase of the ageing because the morphological characteristics of the rhizome system guaranteeing a better supply of assimilates from mature culms (with optimum photosynthetic activity) to the new shoots and young culms (which have a limited active photosynthetic area).

Further research could be focused on culm, cauline leaves and leaf photosynthetic activity in order to explain their contribution to biomass accumulation. There is evidence (López *et al*, non published data) of active stomata and significative activity of PEPC, NAD-ME and PPDK in culm epidermis tissues. Presence of C_4 syndrome in tissues different to those of Kranz anatomy is reported by Hibberd (2002) in tobacco and celery.

Following our methodology, the carbon fixation estimated for 400 clumps ha^{-1} of *Guadua angustifolia*, for a growth period of 2190 days (6 years), is 54.3 ton, where 10.8 ton (19.9%) of CO_2 fixation corresponds to the rhizome and 43.5 ton (80.1%) to the aerial part of the clump (Table 5). The 0.5 international coefficient assumed for timber (Brown 2001) was used in this study.

ACKNOWLEDGEMENTS

Table 5. Dry weight and carbon fixed into different organs of *Guadua angustifolia* (400 clumps ha-1).

Organ	Dry weight (ton ha-1)	Carbon Fixated (ton ha-1)
Rhizome	21.6	10.8
Culm	79.11	39.5
Branches	4.6	2.3
Caulinar leaves	0.4	0.2
Foliage leaves	2.96	1.48
Aerial part	87.07	43.5
400 clumps ha-1	108.67	54.3

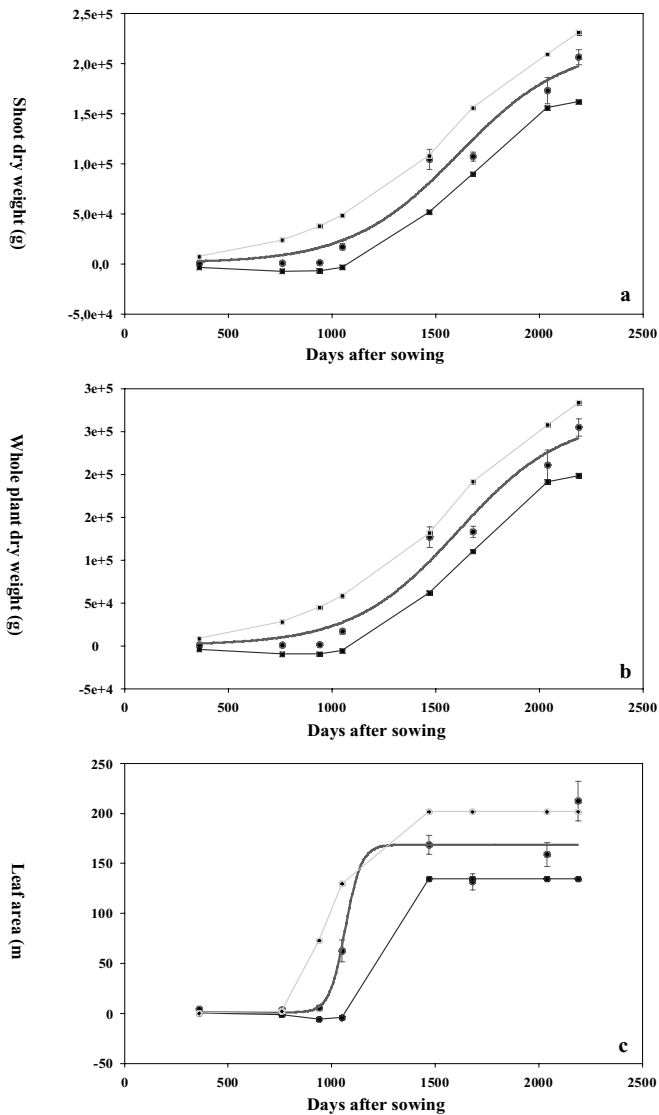Number of culms = 8640; clump total leaf area =67152 m2; leaf area index = 6.7

Figure 4. Dry weight and leaf area growth models for: a. shoots; b. whole plant; c. leaf area. Vertical bars standard error. Fitted model (center line) and confidence limits (0.95).

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Bamboo Notes[1]

Notes from TBGRI Bambusetum:
precocious flowering in *Dendrocalamus strictus* (Roxb.) Nees

K .C.Koshy

Tropical Botanic Garden and Research Institute (TBGRI)
Palode, Thiruvananthapuram-695562, Kerala, India

Native in India, Nepal, Bangladesh, Myanmar and Thailand *Dendrocalamus strictus* is widely used as a building material and as a principal source of paper pulp (Dransfield and Widjaja, 1995).The flowering cycle of this species is estimated to be 24 to 65 years (Banik 1981). A few instances of precocious flowering were also recorded. Sunder Lal Pathak (1897), supported by an excellent frontispiece photograph, reported the flowering of five 13 month old seedlings, three of which died within 3 to 4 months of flowering. Birbal (1899) reported the flowering of 5 year old seedlings, Sen Gupta (1939) in 2 to 4 year old ones, Ahmed (1969) in a good number of 2 to 3 year old seedlings and Ashok Kumar (1983) in a one year old seedling. A peculiar type of freak flowering observed in a clump in the Bambusetum of TBGRI is reported here.

One Kilogram seeds of *D. strictus*, procured from Forest Development Corporation Gujarat, India, was sown in the nursery on 24.6.1988. 14 day old seedlings were transplanted into poly-bags. From this lot, 30 seedlings were planted into a plot in the bambusetum on 24.8.1988. In March 1993, one clump (Acc.No.109) exhibited signs of flowering. As of March 1993 the clump, 4 years and 8 months of age, had a total of 20 culms of which 7 were produced in 1991. Out of these 7 culms one (21 months old) having 31 nodes started initiation of spikelet clusters from four basal nodes of the main branch complement at the 22nd node. Most of these spikelet clusters showed an arrested growth and only a few developed into mature florets. Two months later all the spikelet clusters dried up without producing any seeds. Since then no flowering was observed from any part of the said culm which dried completely by November 2002.The clump involved in flowering remains healthy and vigorous similar to other seedling clumps.

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[1]*Bamboo Notes* are one page communications of brief and generally self-evident data not requiring extensive discussion or explanation.

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http://www.virtualherbarium.org/ABS/ or through the American Bamboo Society web site at: **http://www.bamboo.org/**

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Contents

Gerald Guala, Ph.D.
Editor-in-Chief
Bamboo Science & Culture
Fairchild Tropical Garden Research Center
11935 Old Cutler Rd.
Coral Gables, FL 33156-4299