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An Updated Tribal and Subtribal Classification of the Bamboos (Poaceae: Bambusoideae)

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ABSTRACT

The Bambusoideae (bamboos), comprising 1,439 described species in 116 genera, is one of 12 subfamilies of Poaceae (grass family), and it is the only major lineage of the family to diversify in forests. In this paper, reprinted in part from the Proceedings of the 9th World Bamboo Congress, a compilation of described bamboo diversity by tribe and subtribe is presented and the basis for the revised classification presented here is discussed, with putative synapomorphies indicated in the text and descriptions. The taxonomic treatment includes descriptions of the subfamily, the three tribes, and all accepted subtribes, and included genera are listed as appropriate.

Keywords. Arundinarieae, bamboo classification, Bambuseae, Bambusoideae classification, Olyreae.

INTRODUCTION

The Bambusoideae (bamboos) is one of 12 currently recognized subfamilies of Poaceae (grasses), receiving strong bootstrap support in comprehensive molecular analyses of the family [Grass Phylogeny Working Group (GPWG) 2001; Duvall *et al*. 2007; Bouchenak-Khelladi *et al*. 2008; GPWG II 2012; Wu and Ge 2012]. A putative structural synapomorphy for the subfamily is the presence of strongly asymmetrically invaginated arm cells in the foliage leaf chlorophyll (Zhang and Clark 2000). The bamboos are notably the only major lineage of grasses to diversify in forests (Zhang and Clark 2000; Judziewicz and Clark 2007; Sungkaew *et al*. 2009) and the complex morphology and unusual flowering behavior of most bamboos are likely the result of adaptations to this habitat or the retention of ancestral states, as is the case with their broad, pseudopetiolate leaves with fusoid cells in the mesophyll (Clark 1997; Judziewicz *et al*. 1999).

Bambusoideae are worldwide in distribution (see Maps, Bamboo Biodiversity), occurring between 46° N and 47° S latitude, with an altitudinal range from sea level to 4,300 m (Judziewicz *et al*. 1999). Estimates of total diversity vary from source to source, but our compilation reveals 1,439 described species in 116 genera (Table 1). Three tribes reflecting the three main lineages of Bambusoideae are

currently recognized (Sungkaew *et al*. 2009): Arundinarieae (temperate woody bamboos, 533 species), Bambuseae (tropical woody bamboos, 784 species) and Olyreae (herbaceous bamboos, 122 species). New species and new genera in all of these tribes continue to be discovered and described and phylogenetic analyses in some cases support generic recircumscriptions (e.g., Fisher *et al*. 2009).

Table 1. Diversity of Bambusoideae by tribe and subtribe and by region for Bambuseae.

Taxon	Number of genera	Number of species
Arundinarieae	**28**	**533**
Bambuseae	**66**	**784**
Neotropical	19	377
Arthrostylidiinae	14	172
Chusqueinae	1	160
Guaduinae	5	45
Paleotropical	47	407
Bambusinae	28	264
Hickeliinae	8	33
Melocanninae	10	88
Racemobambosinae	1	22
Olyreae	**21**	**122**
Buergersiochloinae	1	1
Parianinae	2	36
Olyrinae	18	85
Total for subfamily	**116**	**1,439**

Woody bamboos possess culm leaves (leaves modified for the protection and support of the tender young shoots), complex vegetative branching, an outer ligule (contraligule) on the foliage leaves, usually gregarious monocarpy (with flowering cycles ranging from a few years to 120 years), and bisexual flowers (Judziewicz *et al*. 1999; GPWG 2001; Judziewicz and Clark 2007). Herbaceous bamboos usually lack differentiated culm leaves and outer ligules and have restricted vegetative branching, usually nearly continuous or seasonal flowering, and unisexual spikelets (Judziewicz *et al*. 1999; Judziewicz and Clark 2007). All Olyreae, except for the New Guinea endemic *Buergersiochloa*, also have crenate (olyroid) silica bodies (Soderstrom and Ellis 1987; Zhang and Clark 2000; Clark *et al*. 2007).

The Bamboo Phylogeny Group was formed in 2005 to address the need for a robust, global phylogeny of the Bambusoideae and an updated tribal, subtribal, and generic classification based on the phylogenetic results (BPG 2006). We here present a revised and updated tribal and subtribal classification of the Bambusoideae based on a synthesis of the phylogenetic results summarized in BPG (2012) and reprinted in part from that work. A separate manuscript by the BPG is in preparation, in which a rigorously tested phylogenetic analysis of plastid sequences is presented for representatives of all tribes and subtribes of Bambusoideae.

BASIS FOR AN UPDATED CLASSIFICATION

The recognition of three tribes within the Bambusoideae is clearly supported by the molecular phylogenetic results (Bouchenak-Khelladi *et al*. 2008; Sungkaew *et al*. 2009; BPG, in prep.). Although a formal morphological analysis is not yet available, putative synapomorphies have now been identified for the three tribes. These need to be further tested, but for now, Arundinarieae is diagnosed by basipetal branch development and a chromosome number of $2n = 48$, Bambuseae by acropetal or bidirectional branch development, and Olyreae by unisexual, often strongly dimorphic, 1-flowered spikelets with no rachilla extension, although all but the earliest diverging lineage *(Buergersiochloa)* also share cross-shaped silica bodies in the costal zone and crenate (olyroid) silica bodies in the intercostal zone.

Members of what is now recognized as the Arundinarieae were traditionally classified in up to three subtribes, the Arundinariinae, Shibateinae and Thamnocalaminae, based on the presence or absence of pseudospikelets and rhizome structure. The evident polyphyly of all three subtribes has caused them to be abandoned in favor of the recognition of numbered lineages (Triplett and Clark 2010; Zeng and Zhang *et al*. 2010). Branching order among the 10 current lineages is largely unresolved, so until more data are available, we simply list the genera for the tribe without reference to subtribes or lineages.

Within the Bambuseae, the three neotropical subtribes as delimited by Judziewicz *et al*. (1999) are supported by molecular phylogenetic analyses, and each has at least one morphological synapormophy, so we continue to recognize these three. Among the paleotropical subtribes, the Melocanninae, Hickeliinae and Bambusinae remain largely as circumscribed by Soderstrom and Ellis (1987), with the addition of a number of more recently described genera mainly in the Bambusinae and Hickeliinae and the placement of Greslania in the Bambusinae. The Racemobambosinae here is restricted to *Racemobambos*, based on recent molecular results indicating that *Neomicrocalamus* and *Vietnamosasa* fall within the Bambusinae (Yang *et al*. 2008; Sungkaew *et al*. 2009). Morphological synapomorphies have not yet been identified for either the Bambusinae or the Racemobambosinae. As Goh *et al*. (in prep. and pers. comm.) suggest, it may be necessary to recognize one or more additional subtribes segregated from the Bambusinae once the major lineages of paleotropical woody bamboos are more fully understood.

Our subtribal treatment of Olyreae is consistent with Judziewicz and Clark (2007) and the few phylogenetic analyses including sampling across the diversity of this tribe (BPG, in prep.). A more comprehensive phylogenetic analysis is in progress (de Oliveira *et al*., in prep.) and will provide more insight into the evolution of the herbaceous bamboos.

TAXONOMIC TREATMENT

The subfamily description is modified from GPWG (2001). Potential synapomorphies for tribes or subtribes are underlined within the descriptions. Genera are listed alphabetically within each tribe or subtribe, and the number of species for each genus is given in parentheses after the genus name. Two electronic databases are available with more detailed information on bamboo genera: GrassBase (www.kew.org/data/grasses-db/) and Grass Genera of the World. (www.delta-intkey.com/grass).

Bambusoideae Luerss., Grundz. Bot., ed. 5: 451. 1893. Type: *Bambusa* Schreb.

Plants perennial (possibly rarely annual in Olyreae), rhizomes (leptomorph) present or absent, herbaceous or woody, of temperate and tropical forests, tropical high montane grasslands, riverbanks, and sometimes savannas or swamps. Culms hollow or solid; aerial branching often present. Leaves distichous; outer (abaxial) ligule absent (Olyreae) or present (Arundinarieae, Bambuseae); adaxial ligule membranous or chartaceous, fringed or unfringed; sheaths often auriculate or fimbriate or both; blades usually relatively broad, pseudopetiolate, venation parallel; mesophyll non-radiate, an adaxial palisade layer absent, fusoid cells large and well developed in at least shade leaves, arm cells usually well developed and strongly asymmetrically invaginated; Kranz anatomy absent, photosynthetic pathway C3; midrib complex or simple; adaxial bulliform cells present; stomates with dome-shaped, triangular or parallel-sided subsidiary cells; bicellular microhairs present, panicoid-type; papillae common and abundent. Synflorescences spicate, racemose or paniculate, completing development of all spikelets in one period of growth and subtending bracts and prophylls usually absent, or pseudospikelets with basal bud-bearing bracts producing two or more orders of spikelets with different phases of maturity and subtending bracts and prophylls usually present. Spikelets (or spikelets proper of the pseudospikelets) bisexual (Arundinarieae, Bambuseae) or unisexual (Olyreae), consisting of 0, 1, 2 or several glumes and 1 to many florets; lemma lacking uncinate macrohairs, if awned, the awns single; palea well developed; lodicules usually 3 (rarely 0 to 6 or many), membranous, vascularized, often ciliate; stamens usually 2, 3 or 6 (2 to 40 in *Pariana*, 6 to 120 in *Ochlandra*); ovary glabrous or hairy, sometimes with an apical appendage, haustorial synergids absent, styles 2 or 3, sometimes very short but close, stigmas 2 or 3. Caryopsis with hilum linear (rarely punctate), extending its full length (rarely less than full length); endosperm hard, without lipid, containing compound starch grains; embryo small, epiblast present, scutellar cleft present, mesocotyl internode absent, embryonic leaf margins overlapping. First seedling leaf blade absent. Base chromosome numbers: x = 7, 9, 10, 11, and 12.

Included Tribes: Arundinarieae, Bambuseae, Olyreae.

Arundinarieae Nees ex Asch. & Graebn., Syn. Mitteleurop. Fl. 2, 1: 770. 1902.
Type: *Arundinaria* Michx.

Rhizomes (leptomorph) and culm bases well developed, some taxa lacking leptomorph rhizomes. Culms woody, usually hollow; culm development occurring in two phases, first, new, unbranched shoots bearing culm leaves elongate to full height, second, culm lignification and branch development with production of foliage leaves occur; branch development basipetal; aerial vegetative branching complex, usually derived from a single bud per node (multiple, subequal buds per node in *Chimonocalamus* and *Chimonobambusa*). Culm leaves usually well developed with expanded sheaths and well developed to reduced blades. Foliage leaves with an outer ligule; sheaths often bearing fimbriae and/or auricular appendages at the summit; blades pseudopetiolate, articulated, deciduous; epidermal silica cells lacking cross-shaped or crenate silica bodies. Flowering usually cyclical, gregarious and monocarpic. Synflorescences bracteate or not, determinate (spikelets) or indeterminate (pseudospikelets). Spikelets (or spikelets proper of the pseudospikelets) bisexual with 1 to many bisexual florets; glumes (0-1) 2-4; lemmas multinerved, similar in texture to the glumes; paleas several-nerved with an even number of nerves, bicarinate. Caryopsis basic, uncommonly baccate (e.g., *Ferrocalamus*); hilum linear. Base chromosome number x = 12; $2n = 48$.

Included genera: *Acidosasa* C. D. Chu & C. S. Chao ex P. C. Keng (11) (including *Metasasa* W. T. Lin), *Ampelocalamus* S. L. Chen, T. H. Wen & G. Y. Sheng (13), *Arundinaria* Michx. (3), *Bashania* P. C. Keng & Yi (2), *Chimonobambusa* Makino (37) (including *Menstruocalamus* T. P. Yi, *Oreocalamus* Keng, *Qiongzhuea* Hsueh & Yi), *Chimonocalamus* Hsueh & Yi (11), *Drepanostachyum* P. C. Keng (10), *Fargesia* Franchet (90) (including *Borinda* Stapleton, *Sinarundinaria* Nakai), *Ferrocalamus* Hsueh & P. C. Keng (2), *Gaoligongshania* D. Z. Li, Hsueh & N. H. Xia (1), *Gelidocalamus* T. H. Wen (9), *Himalayacalamus* P. C. Keng (8), *Indocalamus* Nakai (23), *Indosasa* McClure (15), *Oligostachyum* Z. P. Wang & G. H. Ye (15) (including *Clavinodum* T. H. Wen), X*Phyllosasa* Demoly (1), *Phyllostachys* Sieb. & Zucc. (51), *Pleioblastus* Nakai (40) (including *Nipponocalamus* Nakai, *Polyanthus* C. H. Hu), *Pseudosasa* Makino ex Nakai (19), *Sarocalamus* Stapleton (3), *Sasa* Makino & Shibata (40), *Sasaella* Makino (13), *Sasamorpha* Nakai (5), *Semiarundinaria* Makino ex Nakai (10) (including *Brachystachyum* Keng), *Shibataea* Makino ex Nakai (7), *Sinobambusa* Makino ex Nakai (10), *Thamnocalamus* Munro (4), *Yushania* P. C. Keng (80) (including *Burmabambus* P. C. Keng, *Butania* P. C. Keng, *Monospatha* W. T. Lin).

Bambuseae Kunth ex Dumort., Anal. Fam. Pl.: 63. 1829. Type: Bambusa Schreb.

Rhizomes (leptomorph) and culm bases well developed, leptomorph rhizomes occurring only within *Chusquea*. Culms woody, usually hollow (solid in most *Chusquea* and a few species of other genera); culm development occurring in two phases, first, new, unbranched shoots bearing culm leaves elongate to full height, second, culm lignification and branch development with production of foliage leaves occur; branch development acropetal or bidirectional; aerial vegetative branching complex (but absent in *Glaziophyton, Greslania* and two clades within *Chusquea*), usually derived from a single bud per node (multiple, subequal buds per node in *Apoclada, Filgueirasia, Holttumochloa;* multiple, dimorphic buds in most of *Chusquea*). Culm leaves usually well developed with expanded sheaths and well developed to reduced blades, sometimes poorly differentiated from foliage leaves (e.g., *Aulonemia*, two clades within *Chusquea*) or absent. Foliage leaves with an outer ligule; sheaths often bearing fimbriae and/or auricular appendages at the summit; blades usually pseudopetiolate, articulate, deciduous; epidermal silica cells lacking cross-shaped or crenate silica bodies. Flowering usually cyclical, gregarious and monocarpic. Synflorescences bracteate or not, determinate (spikelets) or indeterminate (pseudospikelets). Spikelets (or spikelets proper of the pseudospikelets) bisexual with 1 to many bisexual florets; glumes (0-)(-6), sometimes very reduced; lemmas multinerved, similar in texture to the glumes; paleas several-nerved with an even number of nerves, bicarinate. Caryopsis usually basic,

sometimes baccate (e.g., A*lvimia, Dinochloa, Melocanna, Ochlandra, Olmeca*, at least one species of *Guadua*) or nucoid (e.g., *Actinocladum, Merostachys*); hilum linear. Base chromosome numbers x = 10, (11), and 12; 2*n* = (20) 40, (44), 46, 48, 70, 72.

Neotropical Woody Bamboo Subtribes

Arthrostylidiinae Bews, World's Grasses: 96. 1929. Type: *Arthrostylidium* Rupr.

Rhizomes (leptomorph) absent. Culm bases sympodial, pachymorph, necks short to somewhat elongated; internodes of the aerial culms usually hollow, sometimes thick-walled, rarely septate (*Glaziophyton*), all subequal or sometimes very short internodes alternating in various combinations with elongated internodes; nodes of aerial culms without a patella. Aerial branching usually well developed and derived from a single bud per node; thorns absent. Culm leaves usually well developed (absent in *Glaziophyton*); margins of the sheath and blade more or less continuous or distinct; sheaths usually bearing fimbriae or fimbriate auricles; oral setae absent; blades erect or reflexed. Foliage leaf sheaths usually bearing fimbriae or fimbriate auricles at the summit, oral setae absent; blades with a simple, abaxially projecting midrib; intercostal sclerenchyma usually present; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually with a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences usually ebracteate, indeterminate (pseudospikelets) or determinate (spikelets), paniculate or racemose; prophylls present or absent. Spikelets (or spikelets proper of the pseudospikelets) consisting of 2-3 glumes, 1 to many female-fertile florets, and a rachilla extension bearing a rudimentary floret; palea keels wingless. Stamens (2) 3 (6), filaments free. Ovary glabrous, with a short style; stigmas 2 (3). Caryopsis basic, uncommonly baccate (*Alvimia*) or nucoid (*Actinocladum, Merostachys*). Base chromosome number x = 10; *2n* = 40 (but only 2 counts available for the subtribe).

Included genera: *Actinocladum* Soderstr. (1), *Alvimia* Soderstr. & Londoño (3), *Arthrostylidium* Rupr. (32), *Athroostachys* Benth (1), *Atractantha* McClure (6), *Aulonemia* Goudot (40) (including *Matudacalamus* F. Maekawa), *Colanthelia* McClure & E. W. Sm. (7), *Didymogonyx* (L.G. Clark & Londoño) C.D. Tyrrell, L.G. Clark & Londoño (2), *Elytrostachys* McClure (2), *Filgueirasia* Guala (2), *Glaziophyton* Franch. (1), *Merostachys* Spreng. (48), *Myriocladus* Swallen (12), *Rhipidocladum* McClure (15).

Chusqueinae Bews, World's Grasses: 96. 1929. Type: *Chusquea* Kunth.

Neurolepidinae Soderstr. & R. P. Ellis in Soderstr. *et al*., Grass Syst. Evol.: 238. 1987. Type: *Neurolepis* Meisner.

Rhizomes (leptomorph) sometimes present. Culm bases sympodial, pachymorph, necks short; internodes of the aerial culms usually solid, all subequal; nodes of the aerial culms without a patella. Aerial branching usually well developed and derived from a multiple, dimorphic bud complement, absent in two clades (= *Neurolepis*) but a single bud per node often present in these taxa; thorns absent. Culm leaves usually well developed (sometimes not well differentiated in the *Neurolepis* clades); margins of the sheath and blade usually distinct; fimbriae or fimbriate auricles absent; oral setae absent; blades usually erect, rarely reflexed. Foliage leaf sheaths usually bearing cilia at the summit, rarely well developed fimbriae present, oral setae absent, auricles absent; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus bearing two or more papillae per subsidiary cell and also often overarched by papillae from adjacent long cells. Synflorescences usually ebracteate, determinate (spikelets), paniculate or rarely racemose; prophylls absent. Spikelets consisting of 4 glumes and 1 female-fertile floret, rachilla extension absent; palea keels lacking wings. Stamens (2) 3, filaments free. Ovary glabrous, with a short style; stigmas

2. Caryopsis basic. Base chromosome number x = 10 (11, 12); 2*n* = (20) 40 (44, 48).

Included genus: *Chusquea* Kunth (160) (including *Neurolepis* Meisn., *Rettbergia* Raddi, *Swallenochloa* McClure).

Guaduinae Soderstr. & R. P. Ellis in Soderstr. *et al*., Grass Syst. Evol.: 238. 1987. Type: *Guadua* Kunth.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to elongated; internodes of the aerial culms hollow to solid, all subequal; nodes of the aerial culms without a patella. Aerial branching well developed and derived from a single bud per node (1-4 subequal buds per node in Apoclada); thorns absent or present (*Guadua*). Culm leaves well developed; margins of the sheath and blade continuous or nearly so, uncommonly distinct; sheaths often bearing fimbriae or fimbriate auricles at the sheath summit; oral setae usually present (absent in *Guadua*); blades erect or reflexed. Foliage leaf sheaths often with fimbriae or fimbriate auricles at the summit; oral setae present; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis usually with abundant stomates and well developed papillae, rarely these lacking or infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates present and abundant (absent in *Apoclada*) and papillae absent to well developed; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences bracteate or not, indeterminate (pseudospikelets) or determinate (spikelets), paniculate; prophylls present or absent. Spikelets (or spikelets proper of the pseudospikelets) consisting of (0-) 1 to 4 (-7) glumes, 1 to many female-fertile florets, and a rachilla extension bearing a rudimentary floret; palea keels wingless to prominently winged. Stamens 3 or 6, filaments free. Ovary glabrous or hairy, with a short style; stigmas 2 or 3. Caryopsis basic, uncommonly baccate (*Olmeca* and *Guadua sarcocarpa*). Base chromosome number x =12; 2n = 46 or 48.

Included genera: *Apoclada* McClure (1), *Eremocaulon* Soderstr. & Londoño (4) (including *Criciuma* Soderstr. & Londoño), *Guadua* Kunth (27), *Olmeca* Soderstr. (5), *Otatea* (McClure & E. W. Sm.) C. E. Calderón & Soderstr. (8)

Paleotropical Woody Bamboo Subtribes

Bambusinae J. S. Presl in K. B. Presl, Rel. Haenk. 1: 256. 1830. Type: *Bambusa* Schreb.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to slightly elongated; internodes of the aerial culms hollow or solid, all subequal; nodes of the aerial culms with or without a patella. Aerial branching well developed and derived from a single bud per node (multiple buds in *Holttumochloa*); thorns usually absent, sometimes present (*Bambusa*). Culm leaves well developed; margins of the sheath and blade continuous or distinct; sheaths bearing fimbriae or fimbriate auricles at the summit or neither; oral setae present or absent; blades erect or reflexed. Foliage leaf sheaths often with fimbriae or fimbriate auricles at the summit; oral setae present or absent; blades with a complex or simple, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis with or without stomates, with or without papillae; abaxial epidermis usually lacking a green stripe along the narrow-side margin, usually with abundant stomates and well developed papillae; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences bracteate or not, indeterminate (pseudospikelets) or less commonly determinate (spikelets), paniculate; prophylls present or absent. Spikelets or spikelets proper of the pseudospikelets consisting of (0-) 1 to several glumes, 1-10 or more female-fertile florets and sometimes a rachilla extension bearing 1-3 rudimentary florets; palea keels wingless to prominently winged. Stamens 6, filaments free or fused. Ovary glabrous or hairy, usually with a short style; stigmas 1, 2 or 3. Caryopsis basic or baccate (*Cyrtochloa, Dinochloa, Melocalamus, Sphaerobambos*). Base chromosome number x = 10 or 12; 2*n* = 48, 70, 72.

Included genera: *Bambusa* Schreber (100) (including *Dendrocalamopsis* Q. H. Dai & X. L.. Tao, *Isurochloa* Buse, *Leleba* Rumphius ex Nakai, *Lingnania* McClure, *Neosinocalamus*

P.C. Keng, *Tetragonocalamus* Nakai), *Bonia* Balansa (5) (including *Monocladus* Chia, H. L. Fung & Y. L. Yang), *Cyrtochloa* S. Dransf. (5), *Dendrocalamus* Nees (41) (including *Klemachloa* R. N. Parker, *Sinocalamus* McClure), *Dinochloa* Buse (31), *Fimbribambusa* Widjaja (2), *Gigantochloa* Kurz ex Munro (30), *Greslania* Balansa (4), *Holttumochloa K. M. Wong (3), Kinabaluchloa* K. M. Wong (2), *Maclurochloa* K. M. Wong (1), *Melocalamus* Benth. (5), *Mullerochloa* K. M. Wong (1), *Neololeba* Widjaja (5), *Neomicrocalamus* P. C. Keng (5) (including *Microcalamus* Gamble), *Oreobambos* K. Schumann (1), *Oxytenanthera* Munro (1), *Parabambusa* Widjaja (1), *Phuphanochloa* Sungkaew & Teerawat. (1), *Pinga* Widjaja (1), *Pseudobambusa* Nguyen (1), *Pseudoxytenanthera* Soderstr. & Ellis (12), *Soejatmia* K. M. Wong (1), *Sphaerobambos* S. Dransf. (3), *Temochloa* S. Dransf. (1), *Temburongia* S. Dransf. & K. M. Wong (1), *Thyrsostachys* Gamble (2), *Vietnamosasa* Nguyen (3).

Hickeliinae A. Camus, Compt. Rend. Acad. Sci. 179: 480. 1924. Type: *Hickelia* A. Camus.

Nastinae Soderstr. & R. P. Ellis in Soderstr. *et al*., Grass Syst. Evol.: 238. 1987. Type: *Nastus* A. L. Juss.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short to elongated; internodes of the aerial culms usually hollow or rarely solid, all subequal along the aerial culms. Aerial branching well developed and derived from a single bud per node (multiple buds in Nastus productus), central branch dominant; thorns absent. Culm leaves well developed; margins of sheath and blade usually discontinuous; sheaths bearing fimbriae or fimbriate auricles or neither; oral setae absent; blades erect or reflexed. Foliage leaf sheaths with fimbriae or fimbriate auricles present or absent; oral setae absent; blades with a complex, adaxially projecting midrib; intercostal sclerenchyma and fiber-like epidermal cells sometimes present; adaxial epidermis lacking stomates and papillae or these infrequent and poorly developed; abaxial epidermis usually lacking a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long cells; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences determinate (spikelets), bracteate or ebracteate, paniculate, racemose or capitate; prophylls usually absent. Spikelets consisting of 4-6 glumes and 1 female-fertile floret; rachilla extension present or absent, if present well developed or much reduced bearing a rudimentary or reduced floret; palea usually 2-keeled (without keels when rachilla extension absent), keels wingless. Stamens 6, filaments usually free. Ovary glabrous or hairy, with long or short style; stigmas 3. Caryopsis basic, sessile or stalked (*Cathariostachys*). Base chromosome number and ploidy level unknown.

Included genera: *Cathariostachys* S. Dransf. (2), *Decaryochloa* A. Camus (1), *Hickelia* A. Camus (4) (including *Pseudocoix* A. Camus), *Hitchcockella* A. Camus (1), *Nastus* Juss. (20) (including *Chloothamnus* Büse, *Oreiostachys* Gamble), Perrierbambus A. Camus (2), *Sirochloa* S. Dransf. (1), *Valiha* S. Dransf. (2).

Melocanninae Benth., J. Linn. Soc. London 19: 31. 1881. Type: *Melocanna* Trin.

Schizostachyidinae Soderstr. & R. P. Ellis in Soderstr. *et al*., Grass Syst. Evol.: 238. 1987. Type: *Schizostachyum* Nees.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short or elongated; internodes of the aerial culms moderately long or very long, hollow, with thin walls; nodes of the aerial culms lacking a patella. Aerial branching well developed and derived from a single bud per node; thorns absent. Culm leaves well developed; margins of the sheath and blade distinct; sheaths bearing fimbriae or fimbriate auricles at the summit or neither; oral setae usually absent; blades often reflexed. Foliage leaf sheaths bearing fimbriae or small fimbriate auricles or neither; oral setae present or absent; blades with a complex, abaxially projecting midrib; intercostal sclerenchyma absent; adaxial epidermis lacking stomates or these infrequent and poorly developed, papillae often present; abaxial epidermis with (usually) or without a green stripe along the narrow-side margin, with stomates common and papillae usually well developed on at least some long

cells; stomatal apparatus with papillae absent from the subsidiary cells but usually overarched by papillae from adjacent long cells. Synflorescences indeterminate (pseudospikelets), spicate or capitate, prophylls present. Spikelets proper consisting of (0) 2 (or 4) glumes, one female-fertile floret (3 in *Schizostachyum grande*), with or without rachilla extension, if present bearing a rudimentary floret; palea keels wingless or winged. Stamens 6 (15-120 in *Ochlandra*), filaments free or fused. Ovary glabrous, with a long, slender, hollow style; stigmas (2-) 3. Caryopsis basic or baccate (*Melocanna, Ochlandra, Stapletonia*) or nucoid (*Pseudostachyum*). Base chromosome number x = 12; 2*n* = 72.

Included genera: *Cephalostachyum* Munro (14), *Davidsea* Soderstr. & Ellis (1), *Dendrochloa* C. E. Parkinson (1), *Melocanna* Trin. (2), *Neohouzeaua* A. Camus (7), *Ochlandra* Thwaites (9), *Pseudostachyum* Munro (1), *Schizostachyum* Nees (50) (including *Leptocanna* L. C. Chia & H. L. Fung), *Stapletonia* Singh, Dash & Kumari (1), *Teinostachyum* Munro (2).

Racemobambosinae Stapleton, Edinburgh J. Bot. 51: 323-324. 1994. Type: *Racemobambos* Holttum.

Rhizomes (leptomorph) lacking. Culm bases sympodial, pachymorph, necks short or elongated; internodes of the aerial culms hollow, all subequal; nodes of the aerial culms without a patella. Aerial branching well developed and derived from a single bud per node; thorns absent. Culm leaves well developed; margins of the sheath and blade more or less continuous or distinct; sheaths usually bearing small fimbriate auricles at the summit or rarely efimbriate and exauriculate; oral setae absent; blades erect or reflexed. Foliage leaf sheaths usually bearing small fimbriate auricles at the summit or rarely efimbriate and eauriculate; oral setae absent; blades with an abaxially projecting midrib; blade anatomy and micromorphology unknown. Synflorescences bracteate, determinate (spikelets), racemose; prophylls absent. Spikelets consisting of 2-3 glumes, 3-8 female-fertile florets and a rachilla extension bearing 1 rudimentary floret; palea keels wingless. Stamens 6, filaments free. Ovary usually hairy toward the apex, usually with a short style; stigmas 3. Caryopsis basic. Base chromosome number unknown.

Included genus: *Racemobambos* Holttum (16).

Olyreae Kunth ex Spenn., Fl. Friburg. 1: 172. 1825. Type: Olyra L.

Rhizomes (leptomorph) weakly or sometimes strongly developed (*Olyra, Pariana*). Culms herbaceous to subwoody, vegetative branching restricted and only one phase of culm development observed. Culm leaves usually absent, sometimes differentiated in taxa with larger culms. Foliage leaves with the outer ligule absent; sheaths sometimes bearing fimbriae (*Eremitis, Pariana*) and/or blister-like swellings at or near the summit (*Pariana*), more often fimbriae, swellings, and auriculate appendages absent; blades pseudopetiolate, not articulated, persistent or sometimes deciduous, exhibiting nocturnal folding (nyctinasty) in some genera (e.g., *Eremitis, Lithachne, Raddia, Raddiella*); epidermal silica cells usually with cross-shaped silica bodies in the costal zone and crenate (olyroid) silica bodies in the intercostal zone (these absent in *Buergersiochloa*). Flowering usually annual or seasonal for extended periods, very rarely gregarious and monocarpic. Synflorescences ebracteate or rarely enclosed by a spathaceous leaf sheath (*Eremitis*), apparently determinate. Spikelets unisexual, dimorphic and 1-flowered with no rachilla extension, the plants monoecious; pistillodes or staminodes sometimes present in male or female spikelets respectively. Female spikelets with 2 glumes; lemma chartaceous to more commonly coriaceous, several-nerved, usually non-aristate except in *Agnesia, Buergersiochloa* and *Ekmanochloa*; palea with few to several nerves. Male spikelets usually smaller than the females, glumes usually absent or rarely 2 and well developed; lemmas membranous, 3-nerved. Caryopsis basic; hilum usually linear, sometimes punctate. Base chromosome number *x* = 7, 9, 10, 11, and (12).

Buergersiochloinae (S. T. Blake) L. G. Clark & Judz., Aliso 23: 311. 2007.

Foliage leaf sheaths bearing fimbriae at the apex; blades lacking cross-shaped and crenate (olyroid) silica bodies in both epidermises. Synflorescences paniculate. Female lemmas

awned. Stamens 2-3.

Included genus: *Buergersiochloa* Pilg. (1).

Parianinae Hack. in Engler & Prantl, Naturl. Pflanzenfam. 2, 2: 88. 1887. Type: *Pariana* Aubl.

Foliage leaf sheaths bearing fimbriae at the apex; blades with cross-shaped and crenate (olyroid) silica bodies in the epidermises. Synflorescences spicate. Female lemmas unawned. Stamens 2 or 6 (to 36-40).

Included genera: *Eremitis* Döll (1), *Pariana* Aubl. (35) (Generic and species delimitations in this subtribe are uncertain, so these numbers represent estimates; de Oliveira and Moreira, pers. comm.)

Olyrinae Kromb., Fl. Luxembourg 496. 1875. Type: *Olyra* L.

Foliage leaf sheaths lacking fimbriae at the apex; blade with cross-shaped and crenate (olyroid) silica bodies in the epidermises. Synflorescences paniculate or racemose. Female lemmas usually unawned (awned only in *Agnesia, Ekmanochloa*). Stamens 2-3.

Included genera: *Agnesia* Zuloaga & Judz. (1), *Arberella* Soderstr. & C. E. Calderón (7), *Cryptochloa* Swallen (8), *Diandrolyra* Stapf (3), *Ekmanochloa* Swallen (2), *Froesiochloa* G. A. Black (1), *Lithachne* P. Beauv. (4), *Maclurolyra* C. E. Calderón & Soderstr. (1), *Mniochloa* Chase (1), *Olyra* L. (24), *Parodiolyra* Soderstr. & Zuloaga (5), *Piresia* Swallen (5), *Piresiella* Judz., Zuloaga & Morrone (1), *Raddia* Bertol. (9), *Raddiella* Swallen (8), *Rehia* Fijten (1), *Reitzia* Swallen (1), *Sucrea* Soderstr. (3).

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Visualisation of endophytic bacteria in *Phyllostachys* sp. and *Fargesia* sp.

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ABSTRACT

The identification of cultivated and uncultivated microorganisms via microbiological and molecular tools is a traditional way to study plant associated micro-communities. However, the functional activity of microorganisms in a micro-community associated with a plant is comprised of a multitude of complex cellular interactions in a specific niche. Therefore, it is not enough to know the composition of a microbial community if one wishes to understand the cytoecology that grows out of all these fine interactions. Even worse – in spite of the significant amount of data regarding the importance of endophytic micro-organisms for plants, little progress has been made with respect to the spatial architecture of the microbial communities and their interactions. Here we offer a new methodological approach to allow the combination of structural, microbiological and molecular techniques for direct observation of plant-microbial interactions under environmental conditions. In order to study plant-microbe relationships, a few effective modifications of the decades old methods of Cholodny were put forward, and these have been used to visualize the bacterial and fungal endoflora of the bamboo *Phyllostachys atrovaginata.* Different cell forms and community architectures have been observed along the culm. The method used here offers a lot of potential to complement the metagenomic approach to bacterial ecology which dominates the field today.

Keywords: endophytes, bamboo, 16S rDNA

INTRODUCTION

For decades, scientists have investigated the interactions between micro-organisms and plants. This has led to the discovery of many useful symbiotic relations, such as the nodules on the roots of *Fabaceae* or on the leaves of *Myrsinaceae* and *Rubiaceae*. Overall, the coexistence of plants and their associated microorganisms demonstrates a complex variety of interactions (Compant *et al.* 2005, 2009; Schulz and Boyle, 2006; Moshynets and Kosakivska, 2010), from the rather independent colonies of bacteria (such as in the rhizosphere or the phyllosphere) to the very specific interrelations between the host plant and its internal, endophytic bacterial flora. In the latter case, the plant provides a suitable habitat and nutrients, while endophytes directly and indirectly stimulate the growth and development of plants (Mastretta *et al.,* 2009). The main source of a plant's endoflora is the rhizosphere, although some endophytes are being transferred to the next generation through the seeds, and colonise the plant during germination (Weyens *et al.*, 2009).

Over the past twenty years, a significant amount of information has been gathered to describe the interactions between endophytic bacteria and plants. Endophytes play a special role in plant adaptation to stress (Kozyrovska, 1998; Thomas *et al.*, 2007, Pirtilla *et al.*, 2008;

Compant *et al.*, 2010), by influencing the plant's tolerance to environmental factors or by degrading the unhealthy components in the environment (Siciliano *et al.*, 2001, Van Aken *et al.*, 2004, Moore *et al.*, 2006).

However, when it comes to the cytological and (eco)physiological relation between a host plant and its endoflora, or between member species within the endoflora, there are still a lot of gaps in our understanding. Of course, one of the main problems in this line of research is the absence of good methodologies to investigate the endophytic bacterial communities. It is established that the functional activity of bacterial endophytes is determined by a variety of architectural properties and cellular interactions within the microbial community (Dworkin 1991; Caldwell *et al.* 1992; Costerton *et al.* 1994; Massol-Deya 1995).

In this paper, we used a modification of the classic technique of Cholodny (1934), who used glass microscopy slides to obtain patterns of bacterial soil communities, forming a micro-detailed landscape, which he later studied with different microscopic techniques. In our modification, plastic film strips made with polyethylene tetraphtalate were used as a matrix for the attachment and direct growth of the bacterial communities, maintaining the original *(in situ*) spatial shape and structure of these communities (Moshynets *et al.*, 2011). Closer observation of this substrate with confocal laser scanning microscopy (CLSM) as well as scanning electron microscopy (SEM) reveals a hitherto unexpected view of the microbial world inside the bamboo culm.

MATERIAL AND METHODS

Plant material

Six months old specimens of *Phyllostachys atrovaginata* CS Chou & HY Zou, obtained through micropropagation (Oprins Plant NV, Rijkevorsel, Belgium) but already rooted and acclimated to soil, were used as the basis for the experiments.

Modified Cholodny method for visualisation of bacterial communities

For the visualisation and further morphological and cytochemical analysis of the endophytic communities of bamboo, a modification of the classic method of Cholodny was used (Moshynets *et al.*, 2011). The original method used glass as a substrate for the attachment of bacterial communities, which was here replaced by slips of polyethylene terephthalate of 40 µm thickness. To avoid complete blocking of all water and solute transport through these slips, holes were provided with a diameter of on average 0.5 mm. The slips were then sterilized in 70% ethyl alcohol for 5 minutes. The surface of the bamboo culms was disinfected with 70% ethyl alcohol as well, and subsequently a longitudinal cut through the culm was made using a sterile scalpel blade. The plastic slip was pressed in the open culm and the wound with the plastic inside was covered with a sterile bandage. Every culm was outfitted with three slips at different heights, applied through the nodes or not. The exposure of the plastic strips lasted 4 months, while the plants were growing in the laboratory, under natural sunlight. Afterwards, the culms were cut in different pieces; the pieces with a plastic slip inside were stored at -80°C awaiting further treatment.

Morphological and cytological analysis

For further analysis, the plastic slips were fixed in 37% formalin vapours for 30 minutes. Nucleic acids were visualised using either ethidium bromide (EB) in a concentration of 2 mg mL^{-1} distilled water, applied for staining for 2-5 min at room temperature before CLSM (confocal laser scanning microscopy) (excitation: 488 nm, emission: 560 nm) or SYBR Green (SG), in a concentration 0.5 µg mL^{-1} distilled water, and again with an exposure of 2-5 minutes at room temperature before microscopic analyses (excitation: 497 nm, emission: 520 nm). Polypeptides were stained with thiazine red R (TRR) in a concentration of 0.5 µg mL^{-1} of distilled water, with an exposure time of 2-5 min before fluorescence microscopy (excitation: 510 nm, emission: 580 nm). Acridin orange (AO) was used for obtaining the total morphological pattern, and was applied in an aqueous solution of 5 mg mL^{-1} with a staining time of 5 min (excitation: 488 nm, emission: 560 nm). Anti-bleach reagent was used according to Johnson *et al.* (1982).

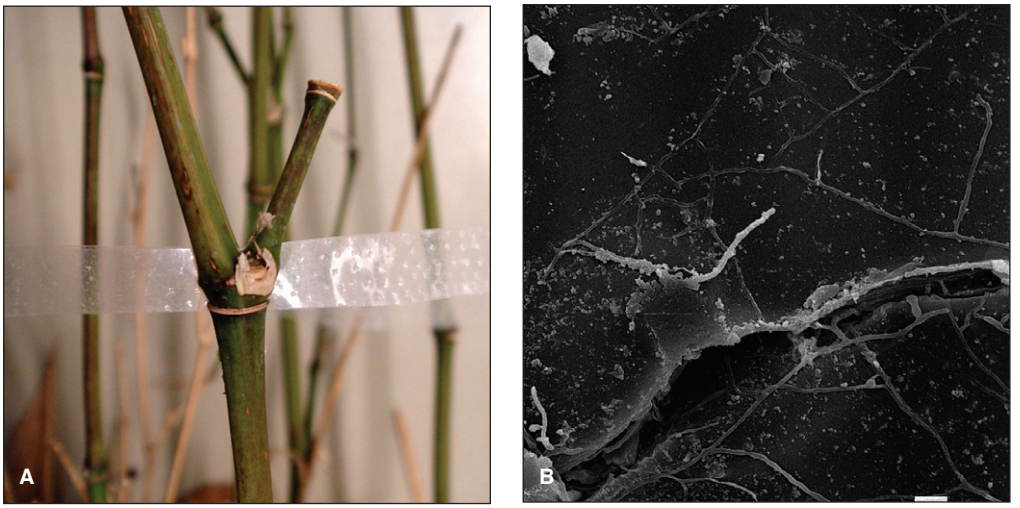

Figure 1A – A plastic slip inserted in the culm of *P. atrovaginata.*
B – Hyphal growth at the surface of the plastic slip. Bar corresponds with 10 μm.

The analyses were done using a confocal laser scanning microscope ZEISS AXIOSCOPE- 2 Plus and software LSM 5 PASCAL. Pre-efficiency staining was tested for fluorescence using a LM-2 microscope (LOMO, Russia). Morphological analysis of sections of the culm occurred using electron microscopy on tissue which were dried, fixed in 37% formalin vapor, and covered with gold. Scanning electron microscopy (SEM) occurred using SEMicroscopes Jeol JSM 35C and Jeol JSM 6060LA.

RESULTS AND DISCUSSION

Visualization of the bacterial communities

To obtain innovative data on the nature of the relation between plant cells and their endophytes, a modification of the method of Cholodny was used, based on plastic slips inserted in the culm of *P. atrovaginata* (Fig. 1A). This method has already been applied successfully in a study of the architecture of microbial communities in the soil and rhizosphere (Moshynets *et al.*, 2010; Moshynets *et al.*, 2011). To allow for gas and fluid exchange between the tissues separated by the plastic slips, a number of holes were made in the material. These openings had been overgrown by endophytic organisms (fungi and bacteria), indicating that this is a reasonable and functional precaution (Fig. 1B), needed for the normal functioning of the culm. To study the architecture of the microbial coenosis both confocal laser scanning and scanning electron microscopy were used. The use of one or two dyes simultaneously allowed a proper visualization of the internal arrangement of the bacterial and fungal clusters (Assmus *et al.*, 1995; Ghiorse *et al.*, 1996; Moller *et al.*, 1996; Lawrence *et al.*, 1998).

Distribution of fungi and bacteria over the culm

The maximal microbial growth was found on the level of the second and the third node of the culm. Characteristic features of microbial fouling on the whole were the absence of classical biofilm structures, such as those frequently found in microbial communities in soils (Costerton *et al.*, 2004; Hall-Stoodley *et al.*, 2004; Spiers *et al.*, 2006; Ude *et al.*, 2006). However, some areas were still covered with a layer of mucus-rich DNA, as evidenced by the cytochemical reaction after application of the DNA-specific dye SG (Fig. 2A).

In the lower part of the shoot, mostly fungal hyphae could be detected, mainly in association with bacteria, although there were also individual hyphae. At the bottom of the shoots, three hyphal morphotypes could be observed. Fig. 2A is an example of first morphotype: hyphae, up to 2 μm wide, covered with mucus containing relatively small amounts of DNA.

These hyphae were usually not associated with bacteria. The second morphotype shows up to 7 µm wide hyphae, in association with bacteria (Fig. 2B). Associated bacteria were small in size (up to 1 µm), with a coccoid form, were located along the hyphae, which is explained by the high humidity at the site of exposure. The third morphotype presented thick septate hyphae, in close association with bacilliform bacteria (Fig. 2C).

In contrast, in the upper part of the culms, the growth of microorganisms was rarely observed. Hyphae of the third morphotype were predominantly found, linked to colonies of small bacteria, ranging from 0.5 µm and smaller. These colonies were often associated with hyphae and had mucous sheaths.

Individual microcolonies were characterized by high metabolic activity, demonstrated by an intense coloration with TRR, due to the high concentration of protein in cells (Fig. 2D-F). The absence of staining with EB and low TRR coloration indicates a small quantity of nucleic acids in the cells and low metabolism level (Fig. 2G-I). Apparently, these bacteria can be in the low nucleic acid (LNA) ecological

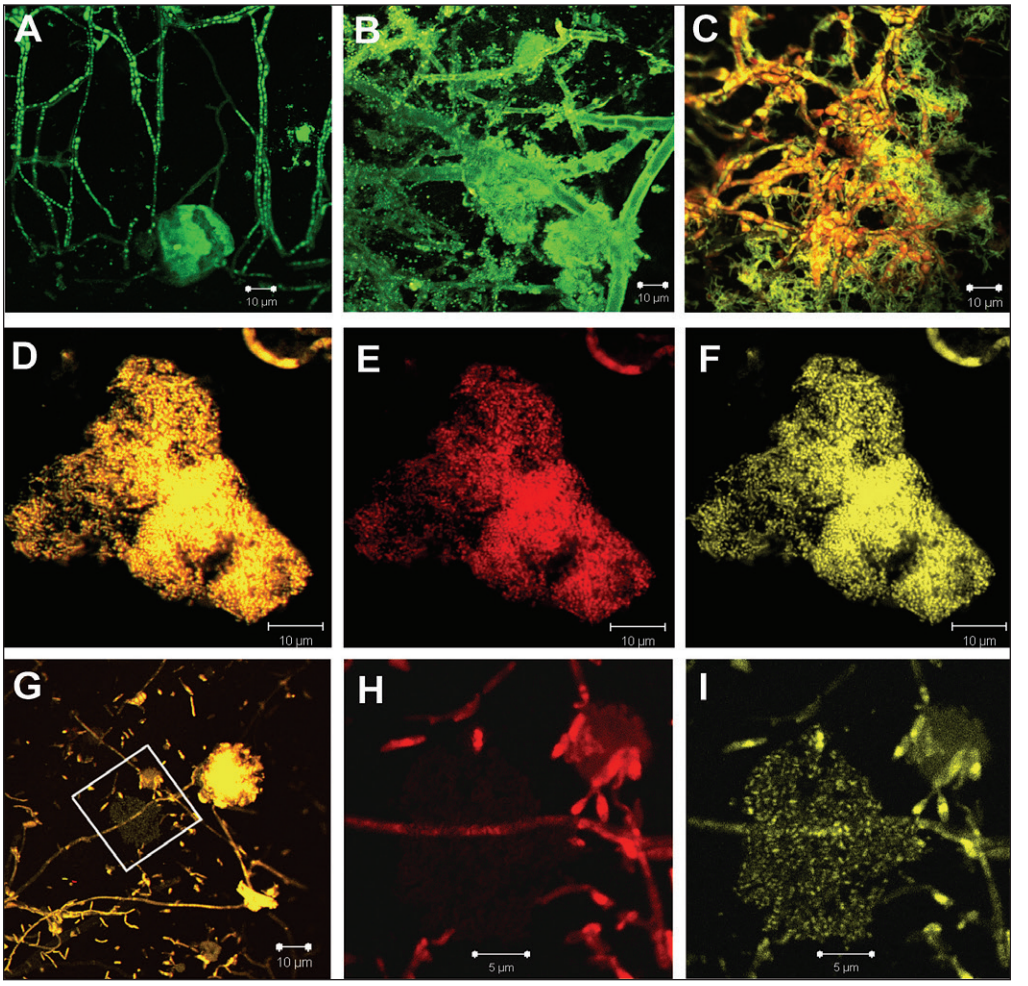

Figure 2. Endophytic microbial communities of *P. atrovaginata,* visualized using a confocal laser scanning microscope ZEISS AXIOSCOPE-2 Plus and software LSM 5 PASCAL. A, B, C – endophytes from the bottom part of the culm (the second - the third node), A, B – SYBR Green (SG) staining, C – acridin Orange (AO) staining; D, E, F - endophytes from the upper part of the culm (the fifth - the seventh node), D – ethidium bromide (EB) and thiazine red R (TRR) staining, E – EB staining, F – TRR staining; G, H, I – LNA bacteria associated with a hypha, G – EB and TRR staining, H – EB staining, I – TRR staining. Bars in A-G correspond with 10 µm; bars in H and I correspond with 5 µm.

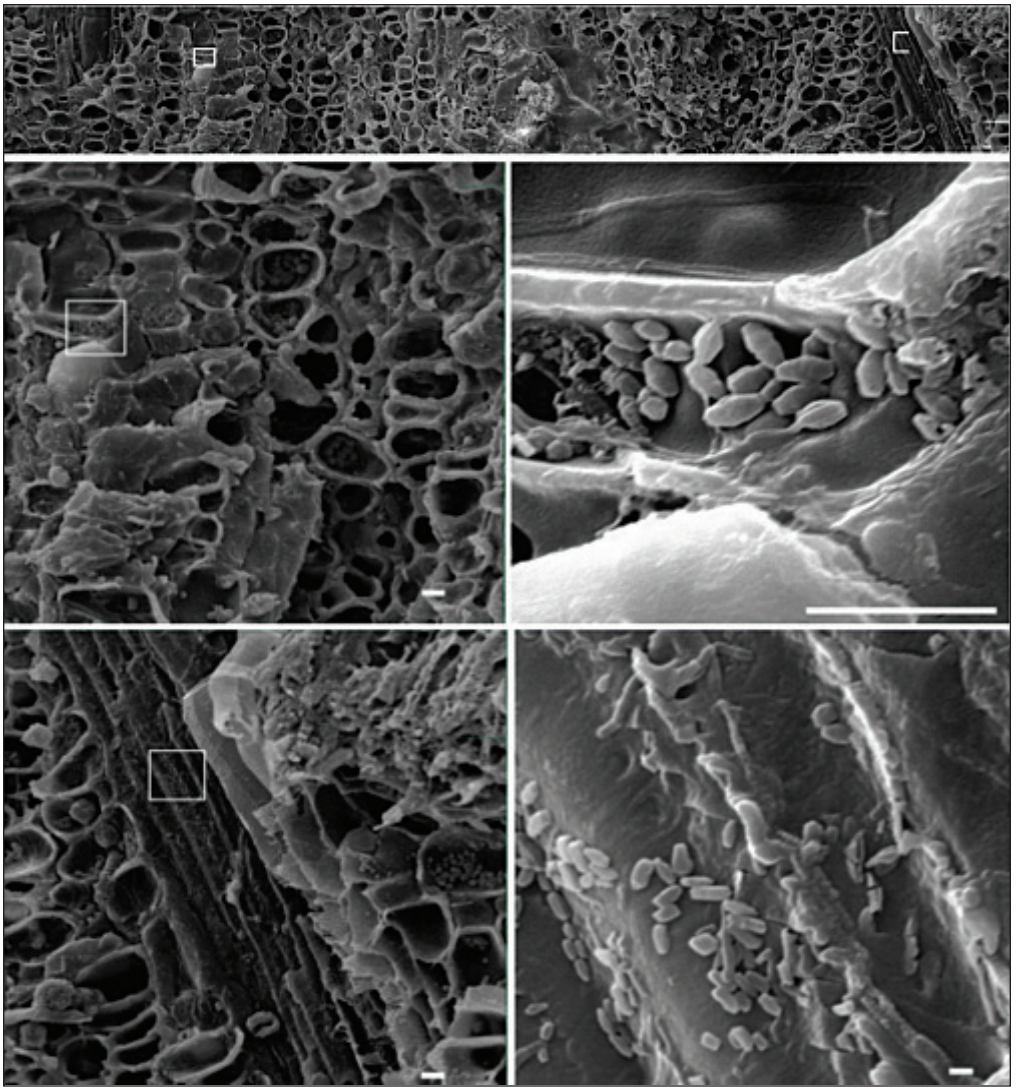

Figure 3. Endophytic bacteria in the bamboo culm tissues, visualized using SEMicroscopes Jeol JSM 35C and Jeol JSM 6060LA. A. General overview of the section, where bar corresponds with 10 μm. B, D. Detailed view of the plant tissue, as indicated in A. C, E. Detailed view of the bacterial colonies, as indicated in resp. B and D. Bar corresponds with 10 μm, except for E, where the bar corresponds with 1 μm.

form. This LNA form is an alternative to the high nucleic acid (HNA) form and can be characterized with low content of DNA and low metabolism level. LNA bacteria are viable and can even be cultivated under laboratory conditions (Servais *et al.*, 2003; Longnecker *et al.*, 2005).

In the tissues of bamboo, endophytic bacteria were less common than fungal spores. Bacteria with a bacillary form were found in intracellular spaces (Fig. 3A-C) and vessels (Fig. 3A, D-E) within the bamboo tissue.

Potential of the modified method of Cholodny within the frame of the metagenomics era

The classic approach towards the identification of bacteria starts off with the effort of obtaining a pure culture of the organisms under study, followed by a colony PCR amplifying the 16SrRNA gene (as exemplified by Moshynets *et al.*, BSC, this issue). However, while this approach has worked wonders in the past for the elucidation of the identity and mode of action of many human pathogens, less

than 1% of the bacteria in a random environmental sample seems amenable for cultivation in a petri dish. The cultivation step was circumvented by working directly on the genomic DNA that could be obtained from the whole sample, albeit a mixture of different organisms (Amann *et al.*, 1995). Amplifying all the copies of a suitable gene (such as the one coding for 16SrRNA or 5SrRNA) followed by separation of the fragments by subcloning, by PCR-DGGE or by capillary electrophoresis offered a way to visualise if not the cells, then at least the species diversity (Theron and Cloete 2000). The more recent approach goes further along that path. By relying upon the latest developments in sequencing technology, scientists now attempt to come up with complete genomic sequences. The related branch of systems biology, "metagenomics", aims to provide a sequence-based functional analysis of the whole set of microbial genomes obtained from an environmental sample.

However, neither of these methodological approaches is able to offer a complete picture of the microbial communities. Both cultivation and metagenomic analysis (analysis of the nucleotide composition of the pool sections 16S rRNA genes) are destructive, as they break up the natural communities, and therefore do not provide information about the spatial arrangement of the micro-organisms. Laboratory cultivation at best produces only a handful of species, and therefore does not permit us to define the size of the population (Ward *et al.*, 1992), nor obtain an adequate view of the morphological, physiological and biochemical variation within the endophytic population (Deretic *et al.*, 1994; Caldwell *et al.*, 1997). Metagenomic analysis can only relate micro-organisms to the nearest related strains which were cultivated before, by using familiar rRNA sequences of known species, that were entered into the database (Ovcharenko and Kozyrovska, 2008). And while each of these methods in itself provides us with pieces of the general puzzle, what is lacking is a method that provides us with the spatial arrangement – say, the picture on the puzzle box, in order to obtain a more coherent view of the bacteria inside the plant.

The modified Cholodny method may just as well what is needed to bridge the gap. On the one hand, it improves on the original design which worked with glass surfaces. These were well suited to traditional bright field microscopy, but did not allow for further analysis of the material attached to them. The plastic films presented here, on the contrary, allow DNA elution, and even PCR amplification directly on (pieces of) the films themselves. As such, the visualisation of the community structure can at least be complemented with a list of the species involved. But the reverse is possible as well. Preliminary results (not shown) in the hands of one of us (O.M.) have indicated that the use of fluorescent probes based upon specific 16SrRNA sequences are able to identify several bacteria on the plastics slips by way of fluorescent in situ hybridisation (FISH). Combination of all these methods, for structural insight as well as for identification of individual cell lines, might then very well provide the unified image of the endophytic community we are looking for.

CONCLUSION

In *P. atrovaginata*, we identified associations of fungal hyphae and bacterial colonies. The microflora of the lower part of the shoots turns out to be rather large and diverse, while the upper part of the microflora was much poorer. In the microflora of the upper part of the shoots we mainly discovered LNA bacterial forms, in the intercellular spaces and vascular tissues in shoots. These observations indicate that our modifications to the Cholodny method can be successfully applied for observation and analysis in situ and can be recommended for more profound studies of aspects of coexistence of endophytic microorganisms in plant tissues. In the future, the modified Cholodny method can be combined with modern molecular genetic methods, which will expand the range of usefulness of the method in the study of plant-microbe-relations.

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Identification of endophytic bacteria in *Phyllostachys* sp. and *Fargesia* sp.

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ABSTRACT

Given the importance of the relation between endophytic bacteria and vascular plants for a better understanding of the functional biology of the latter, more effort should be welcomed to show the diversity of bacterial inhabitants of plant tissues. In this manuscript, we focus on the endoflora of a set of temperate bamboos, implicated in the search for novel materials and sustainable energy sources. Two strategies were undertaken: isolation of bacterial pure cultures as well as direct DNA extraction, and this from aerial as well as subterranean parts of soil grown *Fargesia rufa* and *Phyllostachys humilis* and from *in vitro* plantlets of *Phyllostachys nigra, P. humilis* and *P. atrovaginata.* Eighteen species of bacteria, identified with the aid of the sequence of their 16SrDNA gene, were detected so far colonising the endosphere of bamboo. *Agrobacterium/ Rhizobium* sp., *Bacillus amyloliquefaciens, B. subtilis, B. mojavensis, Mycobacterium palustre, M. lentiflavum, M. avium complex, M. arosiense, Pseudomonas fuscovaginae, P. fluorescens, Paenibacillus chondroitinus, Microbacterium laevaniformans, Achromobacter* sp. and *A. calcoaceticus* have been now for the first time been identified as bamboo endophytes.

Keywords: 16SrDNA – Endophytic bacteria – *Fargesia* – *Phyllostachys* – Pure culture

INTRODUCTION

Many members of the group of the bamboos (family Poaceae, subfamily Bambusoideae) are important agricultural and industrial plants, all over the world. Their wood is important building and decorative material, the fibers can be used for paper, clothing and novel composite plastics, and in the end, bamboo biomass is a source of renewable energy (Scurlock *et al.*, 2000; Potters *et al.*, 2010). Given the possibility to cultivate temperate bamboos such as *Phyllostachys vivax, P. aureosulcata, P. praecox, P. humilis, P. decora, P. bissetti* and *P. aurea* in the fields of Ireland and Flanders (Belgium) (Potters *et al.*, 2009), it stands to reason that even the European continent, where the taxon has been extinct since the last Ice Age, may also benefit from the plant.

One specific advantage is that bamboo plants are able to grow on marginal land (Potters *et al.*, 2009; Aslibekian and Moles, 2003), thereby avoiding unnecessary and unwanted competition with food crop production. For example, some bamboo species can grow in acid soils with pH down to 3 (unpublished data), while others thrive in soils that are contaminated with different types of metals such as Zn, Pb or Al (publication in preparation). As such, bamboo is currently being considered as a candidate for sustainable biomass production in combination with metal decontamination under the climate conditions of Northwest-Europe.

One aspect of a plant's ecophysiology that has a great impact on both the tolerance of the plant against environmental stressors and its ability to contribute to phytoremediation, is its associated endomicroflora (Leigh *et al.*, 2002;

White *et al.*, 2003; Newman and Reynolds, 2005). Endophytes play a special role in plant adaptation to stressors due to their particularly close structural and functional partnership with a plant (Kozyrovska, 1998; Thomas *et al.*, 2007, Pirtilla *et al.*, 2008; Compant *et al.*, 2010). Endophytes are able to influence the plant's tolerance to environmental factors or to modify some environmental toxins. (Siciliano *et al.*, 2001). The recently discovered endophytic microorganism *Methylobacterium populum* sp. nov., strain BJ001, is involved in the degradation of some organic pollutants such as 2,4,6-trinitrotoluene (TNT), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Van Aken *et al.*, 2004). In the case of the endophytic populations of some poplar trees, bacteria were found with the ability to metabolise benzene, toluene, ethylbenzene, xylene (the so-called BTEX-toxins) as well as and trichloroethylene (TCE) (Moore et al, 2006).

It is therefore not unreasonable that a more intimate knowledge of the endoflora of a multifunctional plant such as bamboo, could enhance the already wide range of applications and possibilities the plants is being used for (Scurlock *et al.* 2000). However, in contrast with the possible benefits of the knowledge, the bamboo endophytic communities remain almost unstudied, with the sole exception of one study in *Phyllostachys edulis* (Han *et al.*, 2009). The purpose of this study was therefore to come up with a comprehensive list of both cultivated and uncultivated endophytic bacteria of some bamboo plants, as well to obtain a collection of these endophytic bacteria isolates.

MATERIAL AND METHODS

Plant material

In the current work bamboo plants *Phyllostachys humilis, P. atrovaginata, P. nigra* and *Fargesia rufa*, obtained via micropropagation approach by Oprins Plant NV, Rijkevorsel, Belgium, were used. Both *in vitro* plants and plants, obtained after hardening of the *in vitro* plantlets in soil for months, were used for the experiments, respectively for the extraction of DNA and bacteria and for the application of the plastic film strips. Sterilization of the above-ground and underground surfaces of plants, grown in the substrate under non-sterile conditions, was done in accordance with Han *et al.* (2009), in a laminar flow as follows: 3 minutes in 70% ethanol, 5 minutes in 12% sodium hypochlorite soltion, and 1 minute in 70% ethanol. Afterwards the tissues were washed five times with sterile distilled water. The whole sterilization procedure was done two times.

Isolation of endophytic bacteria from culms and rhizomes from bamboo

Endophytic bacteria from the above-ground parts of plants were collected by the following way, again according to Han *et al.* (2009). Surfaces of each piece of a plant were sterilized, and subsequently these parts were cut into small pieces and immersed in a volume with 5 mL of nutrient medium, either dextrose broth (5 g pepton C, 2 g proteose peptone No 3, 3 g peptone G, 3 g beef extract, 5 g dextrose, 5 g NaCl per liter) or Columbia broth (12 g pepton C, 5 g peptone A, 3 g east extract, 3 g beef extract, 1 g wheat starch, 5 g NaCl per liter). The material was incubated under shaking condition at 28 ºC for 48 hours. Microorganisms were plated out to the corresponding solid media containing 1.5% of agar and cultivated further at 28 ºC for 24 hours. Pure culture isolates selected for subsequent molecular analysis were additionally cultivated on the corresponding dextrose and Columbia broths.

Plants grown in soil were used to collect rhizosphere bacteria as well. The rhizomes were first washed in distilled water to remove all soil particles. The plant surfaces were sterilized according to Han *et al.* (2009). Plant tissue samples of 1 g were cut up and ground in 10 mL of phosphate buffer saline (PBS, pH 7.4). This suspension was diluted 10 and 100 times. 100 µL of each dilution was cultivated on LB and dextrose agar plates at 28 ºC from 24 till 48 hours. Each colony was cultivated in the corresponding liquid medium, and the obtained biomass was used for DNA extraction.

Endophytes from *in vitro* plants were obtained in the following way. The biomass (0.5 g) was ground in 5 mL of PBS. This suspension was diluted in 10 and 100 times. 100 µL of each dilution was cultivated on LB and dextrose agar plates under 28 ºC from 24

till 72 hours. Each colony was cultivated in the corresponding liquid medium, and the obtained biomass was used for DNA extraction.

All obtained colonies were stored as a stock culture by adding 20% glycerol to an overnight culture and kept at -80 ºC.

DNA extraction from isolated bacteria and bamboo tissue

DNA extraction from bacterial cells was conducted according to Khanuya *et al.* (1999). DNA from bamboo tissue was extracted by freezing each sample (0.25 g) in liquid nitrogen. Frozen bamboo tissue was then homogenised in 1 mL of ethanol using a Magnalyser (Roche, Germany). DNA extraction and deproteinisation were done according to the standard protocol of Khanuya *et al.* (1999). The concentration of DNA was measured by NanoDrop® (Thermo Fisher Scientific Inc., USA)

Identification of bacterial species

Fragments of the 16S rDNA gene were amplified using the universal forward primer 25f (5'-AAC TKA AGA GTT TGA TCC TGG CTC-3') and reverse primer 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Han *et al.* 2009), following the instructions of the High Fidelity PCR kit (Roche, Germany), either on the isolated DNA from bacteria or bamboo tissue, or, in some cases, directly on the bacterial cells during a colony PCR. The reaction regime was in all cases: denaturation 10 min under 94 ºC; 35 cycles of: 45 s under 94 ºC, 45 s under 62 ºC, 1 min under 72 ºC; 10 min under 72 ºC. The product was visualized by gel electrophoresis in 0.8% agarose gel according Sambrook *et al.* (1989).

After PCR on isolated strains, both after colony PCR or after PCR on extracted DNA from isolated strains, the PCR products were sent directly for sequencing. PCR product sequencing was done using specific primers T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') and SP6 (5'-GAT TTA GGT GAC ACT ATA G-3') on the sequenator Applied Biosystems 3730 (USA). The sequence of the 16S rDNA fragments was analyzed using BLAST (Basic local alignment search tool) and the NCBI database (USA) (http://www.ncbi.nlm.nih.gov/BLAST/; Altschula *et al.* 1990). Positive identification was obtained when maximal identities of 98-99% and E-values of 0 were returned.

RESULTS AND DISCUSSION

Isolation and identification of bamboo endophytes

Two approaches were followed for the isolation and the identification of the bamboo endophytes. The first one consists of the cultivation of endophytic microorganisms isolated directly from different bamboo tissues (and subsequent identification using the 16S ribosomal DNA sequence), while the second one is based on direct extraction of total DNA from plant tissues and its subsequent analysis of the 16S ribosomal DNA sequences found in the extracts. An overview can be found in Table 1.

Shallow white colonies were obtained after cultivation of bamboo tissues of *P. humilis* and *F. rufa* on dextrose agar. White and yellow colonies were obtained after cultivation of bamboo tissues of *P. humilis* on Columbia agar. DNA was extracted from 24-hours cultures; fragments of 16S rDNA were amplified and analysed. Identification of the strains isolated from *P. humilis* and *F. rufa* shows that both species contain the bacteria *Bacillus amyloliquefaciens* and *B. subtilis* as endophytic bacteria. *P. humilis* also contained the congeneric species *B. mojavensis*. In general, members of the genus *Bacillus* are widespread as endophytes (McInroy and Kloepper, 1995; Bai *et al.*, 2002; Melnick *et al.*, 2008). These bacteria play an important phytoprotective role, by increasing the resistance of the plant against fungi (Wilhelm *et al.*, 1998).

Other experiments were focused on total DNA extraction from culm tissues of young bamboo plants (grown in soil) of the species *P. atrovaginata, P. nigra* and *P. humilis*. Amplification and sequencing of the 16S rDNA fragments revealed several endophytic bacteria belonging to the *Mycobacteriaceae* family (phylum *Actinobacteria*): *Mycobacterium palustre* and *M. lentiflavum* were found in *P. atrovaginata; M. avium* complex and *M. arosiense* were detected in *P. nigra;* there were a few unculturable bacterial clones in *P. humilis*. Fragments of mitochondrion and

Table 1. Overview of the BLAST identification (including homology in nucleotide composition of 16S rDNA PCR fragments, given in %) of the clones obtained. For every clone, the best match was given.

Bamboo species	The closest microorganism	Homology %
Endophytic bacteria of the aerial parts obtained in pure culture		
F. rufa	*Bacillus amyloliquefaciens* (XE6676)	100%
	Bacillus subtilis (XB7767)	100%
P. humilis	*Bacillus amyloliquefaciens* (XE6676)	100%
	Bacillus subtilis (XB7767)	100%
	Bacillus amyloliquefaciens (XE6676)	99%
	Bacillus subtilis (XB7767)	99%
	Bacillus subtilis (XB7767)	100%
	Bacillus amyloliquefaciens (XE6676)	98%
	Bacillus subtilis (XB7767)	98%
	Bacillus mojavensis	98%
Endophytic bacteria of the aerial parts, found after DNA extraction		
P. atrovaginata	*Mycobacterium palustre* (E846)	99%
	Mycobacterium lentiflavum (UN-106)	99%
	Mycobacterium lentiflavum (GR-2466)	99%
P. nigra	*Mycobacterium avium complex* (5356591)	99%
	Mycobacterium arosiense (T1919)	99%
P. humilis	Uncultured bacterium clone nbt35d06	99%
	Uncultured bacterium clone nbt36d12	99%
	Uncultured bacterium clone p8k02ok	99%
Endophytic bacteria of the subterranean parts obtained in pure culture		
P. humilis	*Microbacterium laevaniformans* (EU545414)	99%
	Paenibacillus chondroitinus (EU290158)	99%
	Paenibacillus sp.	99%
	Leifsonia sp.	98%
	Burkholderia sp. (*B. fungorum* HM113360)	99% (98%)
	Burkholderia cepacia complex	99%
	Agrobacterium/Rhizobium	100%
	Pseudomonas fuscovaginae (FJ483524)	100%
	Pseudomonas fluorescens (EU159479)	99%

chloroplast DNA were evidently detected in all of the experiments as well (not shown). Uncultivable endophytes of the *Mycobacterium* genus were also found in other plants belonging to the order of the *Poales* (White 1987; Koskimaki *et al.* 2010). Generally, mycobacteria can be considered as saprophytes, commensals and symbionts of animals, humans and protozoa. A few endophytic *Mycobacterium* spp. were detected in rice root tissues (Mano *et al.*, 2007), wheat (Conn and Franco, 2004) and peat moss (Katila *et al.*, 1995). In some rare instances,

endophytic mycobacteria can be found as well (Laukkanen *et al.*, 2000; Koskimaki *et al.* 2010).

Besides identified uncultivable endophytes, unidentified uncultivable bacteria were also found. Unidentified uncultivable endophytes in bamboo plants have never been found before, which is most probably due to the fact that the microflora of bamboo is poorly studied. Uncultivable endophytes were detected in cereals (Tejesvi *et al.*, 2010) and potato (Podolich *et al.*, 2007).

The endophytic microbial communities of subterranean bamboo parts were studied via cultivation. There were 61 cultures obtained. The isolates were classified in function of cell morphology and colony colour. The molecular analysis of the fragment of 16S rDNA of some isolates is given in table 1. The cultivation of endophytes of the rhizome parts of the bamboo plants revealed a larger variety than in the culms (Tables 1 and 2). Among these were found: *Microbacterium laevaniformans* and *Leifsonia* sp. (family *Microbacteriaceae*, phylum *Actinobacteria*), *Paenibacillus* sp. and *Paenibacillus chondroitinus* (family *Paenibacillaceae*, phylum *Firmicutes*), and finally several isolates related to the phylum *Proteobacteria*: one isolate *Agrobacterium/ Rhizobium* belonging to the *Alphaproteobacteria*; two isolates, *Pseudomonas fuscovaginae* and *Pseudomonas fluorescens,* belonging to *Gammaproteobacteria*; and two isolates, *Burkholderia cepacia* complex and *Burkholderia* sp., belonging to the *Betaproteobacteria*.

Likewise, the endophytic population of *in vitro* tissues of *P. atrovaginata* and *P. humilis* was analysed using a cultivation step. This indicated the presence of *Acinetobacter calcoaceticus* in the tissues of *P. atrovaginata,* while isolates obtained from *P. humilis* corresponded to *Achromobacter* sp. Both bacteria belong to the phylum Proteobacteria, with A. calcoaceticus belonging to the Gammaproteobacteria, and *Achromobacter* sp. to the *Betaproteobacteria*. Such a reduction in the diversity of endophytic population of *in vitro* plants in comparison to plants grown in soil, perhaps, was the result of repeated cloning of plant material under sterile conditions (Podolich *et al.*, 2007).

The results obtained here coincide with the results of other authors. Endophyte communities of subterranean parts of *Phyllostachys edulis* and *Zea mays* L. were found to be more diverse than the communities of endophytes in the aerial parts of the plant (Han *et al.*, 2009; Bai *et al.*, 2002), while endophytes of plants grown in a substrate were more diverse, than endophytes of *in vitro* plants (Koskimaki *et al.*, 2010; Podolich *et al.*, 2007). Members of the genera *Burkholderia* and *Agrobacterium* were detected within endophytic populations of the subterranean parts of *P. edulis, Z. mays* L. (Han *et al.*, 2009; Bai *et al.*, 2002) and *P. humilis* Table 1), but also in the aerial parts of the plants. Bacteria belonging to the *Bacillaceae* were detected in aerial parts of *P. humilis* and *F. rufa*; members of this genus were also found as endophytes in other plants, including maize (McInroy and Kloepper, 1995). Members of *Mycobacteriaceae* were found in the aerial endophytic populations of bamboo plants, while *Mycobacterium* spp. were detected as aerial endophytes of other cereals (Koskimaki *et al.*, 2010). The endophyte *Acinetobacter calcoaceticus* was found in *in vitro* bamboo tissues, while stems of maize at least contain a congeneric *Acinetobacter* sp. (Han *et al.*, 2009).

Finally, one needs to remark that the present study is purely qualitative and does not pretend to provide an exhaustive overview of the bacteria living inside bamboo tissue. To say the least, the design of the experiments discussed in this paper fails to take such things into account, and moreover, this is a task for which the pre-metagenomic methodology applied here does not suffice. Combination of the Cholodny visualization method (Moshynets *et al.*, this issue) with the use of fluorescently labeled DNA probes (for fluorescent in situ hybridization), however, might form the future basis for a much more thorough and quantitative study of the endophyte populations in bamboo.

CONCLUSIONS

Cultivable microorganisms as well as uncultivable were found within the endophytic populations of bamboo plants. Analysis of the experimental and already published data shown

Table 2. Taxonomic rearrangement of the endophytic bacteria found in *Phyllostachys* sp. and *Fargesia* sp.

Phylum	Genera	Species	Bamboo species	Plant tissues
Firmicutes	*Bacillus*	*B. amyloliquefaciens*	*P. humilis* *F. rufa*	culm
		B. subtilis		
		B. mojavensis	*P. humilis*	
Actinobacteria	*Mycobacterium*	*M. palustre*	*P. atrovaginata*	
		M. lentiflavum		
		M. avium complex	*P. nigra*	
		M. arosiense		
Uncultured bacterium clones			*P. humilis*	
Alphaproteobacteria	*Agrobacterium/ Rhizobium*	sp.	*P. humilis*	Root, rhizomes *In vitro*
Betaproteobacteria	*Burkholderia*	sp.		
		B. cepacia complex		
Gammaproteobacteria	*Pseudomonas*	*P. fuscovaginae*		
		P. fluorescens		
Firmicutes	*Paenibacillus*	sp.		
		P. chondroitinus		
Actinobacteria	*Microbacterium*	*M. laevaniformans*		
	Leifsonia	sp.		
Betaproteobacteria	*Achromobacter*	sp.	*P. humilis*	
Gammaproteobacteria	*Acinetobacter*	*A. calcoaceticus*	*P. atrovaginata*	

that plant tissues of bamboo grown in different climate zones, specifically in the natural habitat in China and in the natural conditions of Belgium, were colonised by the same endophytic bacteria: *Paenibacillaceae* sp., *Bacillaceae* sp., *Pseudomonas* sp., *Microbacteriaceae* sp., *Acinetobacter* sp. and *B. cepacia* complex. Apparently, plant tissues of bamboo grown in soil as well as *in vitro* can be considered as a niche for endophytic bacteria, albeit that the endoflora of plants grown in soil is more diverse, than the endoflora of *in vitro* plants; also, the subterranean endophytic populations can be considered as more diverse than the aerial parts. The aerial parts of endophytic bamboo populations were found to contain exclusively a number of gram-positive bacteria, while of the bacteria in the subterranean parts only 44.4% belonged to the gram-positive group, and 55.6% to the gram-negative bacteria. The endophytic population of *in vitro* bamboo plants consisted mainly of gram-negative bacteria (Tables 1 and 2). Analyses such as these will help to attain some fundamental and applied goals in the ecology, cytology and plant biotechnology.

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Investigation on optimisation of kiln drying for the bamboo species *Bambusa stenostachya, Dendrocalamus asper* and *Thyrsostachys siamensis*

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ABSTRACT

Results on kiln drying of the bamboo species *Bambusa stenostachya, Dendrocalamus asper* and *Thyrsostachys siamensis* are presented. Samples of culm parts at basic and middle sections of the species were dried in a pilot kiln using three different schedules with grades of low, middle and high drying rate. The moisture loss, drying time and drying defects were determined.

Culms of the solid species *Thyrsostachys siamensis* are easier to dry than the cavity species *Bambusa stenostachya, Dendrocalamus asper.* For fresh culms of *Thyrsostachys siamensis* with initial moisture content of over 100 % the drying time to reach a final moisture content of 10% by applying a severe drying schedule was 7 days for the middle part and 9 days for the basic part. *Dendrocalamus aspe*r is the most difficult species to dry and severely susceptible to checks and splits, so that it needed a mild drying schedule and drying time of 13 days for middle and 16 days for basic part. *Bambusa stenostachya* dries moderately using a relative milder drying schedule with 10 days for the middle and 12 days for the basic part.

Keywords: Bamboo drying, *T. siamensis, B. stenostachya, D. asper*

INTRODUCTION

Bamboo is one of the important vegetative ligno–cellulose resources besides plantation wood. In many tropical countries it is a major raw material for the forest product industry. In recent years, bamboo has become a main mate- rial for the industrial manufacturing of round and laminated furniture, parquet and for the worldwide export of culms.

Drying is a key step in processing bamboo products and solving the drying problems will add further value to bamboo resource. Well-dried bamboo culms have the desired appearance, finish and structural properties for the successful export into high value markets. Dried culms are more easily and efficiently processed in steps such as cutting, machining and finishing during production of high quality products. Proper drying also reduces weight, preserves colour, improves the strength of the bamboo, inhibits infestations and minimizes shrinkage in service.

The traditional method of drying bamboo is simple air drying. It has been commonly used for a long time in rural areas and in bamboo factories with small capacities. With proper stacking for air circulation, culms can be dried with no further energy than contained in the ambient air. However, there are some disadvantages. One is the long drying time, which can range from several weeks to several months to obtain the required moisture content for end use. Furthermore, bamboo can be easily infected by fungi, especially moulds during drying. Air drying depends largely on climatic conditions and is undertaken under uncontrollable conditions.

Kiln drying provides means for overcoming these limitations. The significant advantages of kiln drying include higher throughput and better control of the required moisture content. Kiln drying enables bamboo to be dried to any moisture content regardless of weather conditions. For large–scale drying operations, kiln drying is more efficient than air drying and could ensure high level bamboo quality.

In Vietnam the demand for the export of large quantities of quality products has recently increased. Bamboo manufacturers recognized the disadvantages of air drying and have introduced dry kiln techniques. However, considerable problems with drying still exist because the development of bamboo kiln drying has rarely been supported by adequate research efforts.

To contribute to the development of bamboo kiln drying for the benefit of bamboo producers in Vietnam, the project ”Investigation on kiln drying of some commercial bamboo species of Vietnam” was initiated. It is supported by the Duy Quy Company of Mechanical Engineering, Ho Chi Minh City and the Bamboo Nature Company, Binh Duong Province, Vietnam. For this project, *Bambusa stenostachya* (Tre Gai), *Dendrocalamus asper* (Manh Tong) and *Thyrsostachys siamensis* (Tam Vong) were investigated, which are the most important bamboo species in South Vietnam for production of furniture and export. The goal is to develop suitable kiln dry schedules for culm parts of these species for furniture making.

MATERIALS AND METHODS

The experiments were carried out at the factory of the Bamboo Nature Company, Binh Duong province, South Vietnam during the rainy seasons from May to November in 2008, 2009 and 2010.

Bamboo samples

Mature 3 year old bamboo culms from *Bambusa stenostachya* Hackel (*Bambusa stenostachya* is a synonym of *Bambusa blumeana* J.H. Schultes. Flora of China 2006 Volume 22 Poaceae), *Dendrocalamus asper (*J.H. Schultes) Backer ex K. Heyne and *Thyrsostachys siamensis* Gamble were harvested from a bamboo plantation of the Bamboo Nature Company. Culms were cut about 25 centimetres from ground level and the basic, middle and top parts were marked. The material was transported the same day to the factory for further experiments.

Samples with a length of 140 cm were prepared from the basic and middle culm sections. The epidermis was removed by machine sanding as common for processing. Culm diameter and wall thickness were measured (see Table 1).

Lay out of the pilot dry–kiln

Dry–kiln

The experiments were performed in a pilot dry–kiln of 1.7m length, 1.5 m high and 1.2 m width. Its heating system was capable of generating temperatures up to 90°C by electrical heating coils located vertically near the kiln roof. The relative humidity was adjusted by hot water spraying and venting. The air circulation system consisted of two fans with 34 cm diameters. The air velocity was maintained at a constant speed of 3.5 m/s reflecting current industrial standards. The kiln was operated by means of a PLC-controller connected to a PC work station, ensuring control and monitoring of the drying protocol, temperature and relative humidity in the chamber in real time.

Kiln drying

Bamboo samples were dried in the dry–kiln. For the drying of *Thyrsostachys siamensis* 154 samples were stacked in 11 rows with 1 cm

Table 1. The dimensions of the samples tested

Species	Length (in mm)		Average diameter (in mm)		Average wall thickness (in mm)	
	Basic	Middle	Basic	Middle	Basic	Middle
T. siamensis	1400	1400	45	38	21	11
B. stenostachya	1400	1400	80	68	20	12
D. asper	1400	1400	88	72	22	13

Photo 1. Stacking basic parts of *T. siamensis* and middle parts of *D. asper*

distance. For *Bambusa stenostachya* and *Dendrocalamus asper*, 64 samples were stacked in 8 rows with 1.5 cm distance (see Photo 1). Five controls of the sample lot were used to estimate the average moisture content and moisture loss.

During the drying process, the conditions in the kiln were adapted to predefined set point values in the schedule according to the mois ture content of the samples at various times during the run. The controls were weighed daily to compute the moisture content.

Drying schedules

The moisture content schedules applied had four grades of drying intensity: mild, medium and severe and very severe. The design of the schedules was based on the studies on bamboo drying by Laxamana (1985), Yosias (2002), Montoya Argango (2006) and Pham (2006). The drying schedules of tropical wood species published by Boone (1988) were also considered. The applied schedules are presented in Table 2.

For each of the bamboo species, three different schedules were tested. Schedule no.1 with mild drying intensity was applied to the cavity species *Bambusa stenostachya* and *Dendrocalamus asper.* Schedule no. 2 with medium drying and schedule no. 3 with severe drying intensity were also applied to these cavity species and also to the solid species *Thyrsostachys siamensis.* Schedule no. 4 with very severe drying conditions was tested only on *Thyrsostachys siamensis.*

Moisture content

The initial moisture content of the control sample was determined from the moisture sections cut from both ends of the control sample (see Fig. 1). The average moisture content of these two sections and the weight of the control sample at the time of cutting were used to calculate the oven-dry weight of the

Table 2: The conditions (set–point values) of the four drying schedules

Step	Moisture content (%)	No.1		No.2		No.3		No.4	
		T(°C)	RH (%)	T(°C)	RH (%)	T(°C)	RH (%)	T(°C)	RH (%)
1	Over 90	45	80	50	80	55	80	65	80
2	90 – 70	45	70	50	70	55	75	65	60
3	70 – 50	50	60	60	60	60	65	70	45
4	50 – 40	50	50	60	50	65	50	70	35
5	40 – 30	50	40	60	30	65	35	70	30
6	30 – 20	55	40	65	30	70	25	75	25
7	20 –10	55	30	65	20	70	20	75	15
Conditioning with 50°C T and 70% RH									

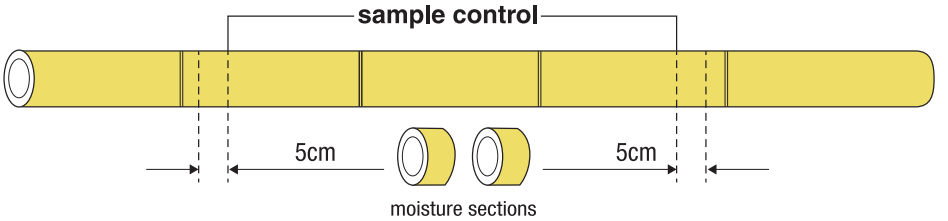

Fig.1: Method of cutting sample control and moisture content sections for initial MC

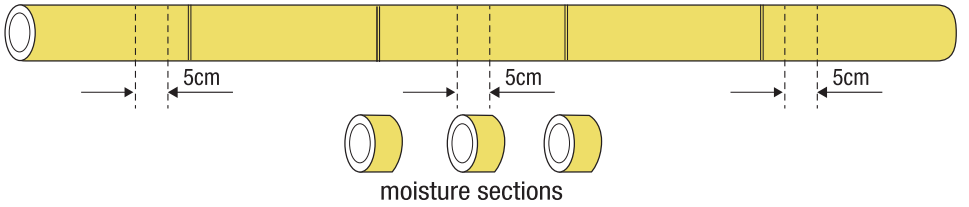

Fig.2: Method of cutting moisture content sections for final MC

control sample. The oven–dry weight and the subsequent weights of the sample obtained at intervals during drying, called current weights, were used to calculate the moisture content at those times. The moisture content (MC) of the moisture sections was determined by oven drying and calculated as

$$MC\ (\%) = 100(W_{or} - W_o)/\ W_o$$

with W_{or} as original weight of samples and W_o as oven dry weight. The ovendry weight of control sample (W_{oc}) was computed by using the following formula:

W_{oc}= (original weight of control /100 + average moisture content of two sections) x100

For determination of the average initial moisture content, sections of 5 cm were cut from both ends of the samples. Five controls and five further samples were used.

To evaluate the moisture gradient and the final moisture content, sections of 5 cm were taken from both ends and from the middle of 13 samples (see Fig. 2). The drying rate was determined by the relationship between moisture decreases with drying time.

Drying defects

All culms of the drying experiment were visually inspected for defects like collapse, cracking, and splitting that had occurred during drying. Drying defects were expressed as percentage of all samples in each kiln run.

RESULTS AND DISCUSSION

All the results for the experiments with three bamboo species are summarized on Table 3.

Table 3. Summary of the results for the experiments with three bamboo species

Schedule	Result	*T. siamensis*		*B. stenostachya*		*D. asper*	
		B	M	B	M	B	M
No. 1	IMC (in %)	–	–	100	92	102	89
	FMC (in %)	–	–	10	10	9	8
	Defect (in %)	–	–	3.7	1.9	4.9	3.5
	Time (in hours)	–	–	344	320	362	296
No. 2	IMC	120	110	101	98	104	92
	FMC	8	10	9	9	9	10
	Defect	2.5	1.6	5.1	2.9	17.8	12.5
	Time	294	224	272	249	303	253
No. 3	IMC	118	106	105	96	108	92
	FMC	9	10	9	8	10	9
	Defect	4.8	3.9	15.7	18.9	28.9	19.5
	Time	247	197	249	224	276	230
No. 4	IMC	120	108	–	–	–	–
	FMC	8	10	–	–	–	–
	Defect	5.5	4.2	–	–	–	–
	Time	243	195	–	–	–	–

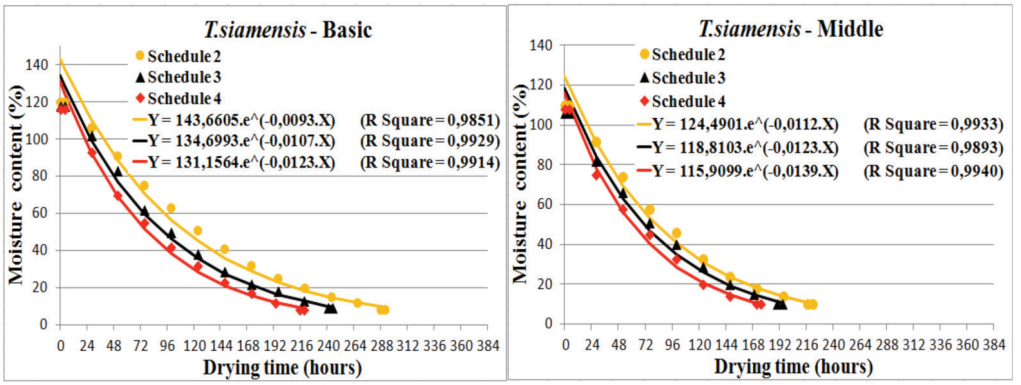

Fig.3. Relationship between drying time and moisture loss of *T. siamensis*

Drying rate and moisture loss

There is a notable difference in drying rate between the solid bamboo species *Thyrsostachys siamensis* and the cavity species *Bambusa stenostachya* and *Dendrocalamus asper.* The former showed a higher drying rate, whereas the other two dried more slowly.

This can be partly explained by the differences in specific gravity. In general, the heavier the wood is, the slower the drying rate and the greater the likelihood of defects will be (Simpson, 1992). The study on the physical and mechanical properties of the above mentioned bamboo species by Hoang el al. (2007) showed that the specific gravity of *Thyrsostachys siamensis* was 0.41 for the basic and 0.46 for middle part, whereas the species *Bambusa stenostachya* had specific gravity values of 0.69 and 0.74 and *Dendrocalamus asper* of 0.71 and 0.78 respectively.

A difference in drying rate was also measured for the culm section. The middle section showed a higher drying rate than the basic part. This result could be explained by the physical and structural variation of a culm. Though the specific gravity of the middle is slightly higher than the basic part, wall thickness and the diameter of the basic part of the culm are greater than the middle one. Moreover, the middle section contains more vascular bundles than the basic (Liese 1998).

The loss of moisture occurred at a regular rate during all four drying schedules and is presented in Fig. 3, 4 and 5.

Final moisture content

The average final moisture content of the three species is reported in Table 4.

In the first drying run of schedule no.1 for *Bambusa stenostachya*, the basic samples showed a great variation of 3 to 16% of moisture content

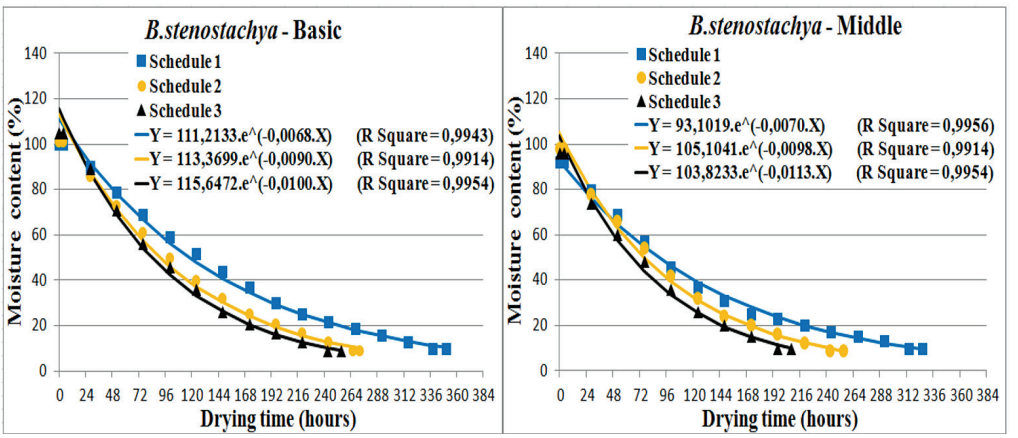

Fig. 4. Relationship between drying time and moisture loss of *B. stenostachya*

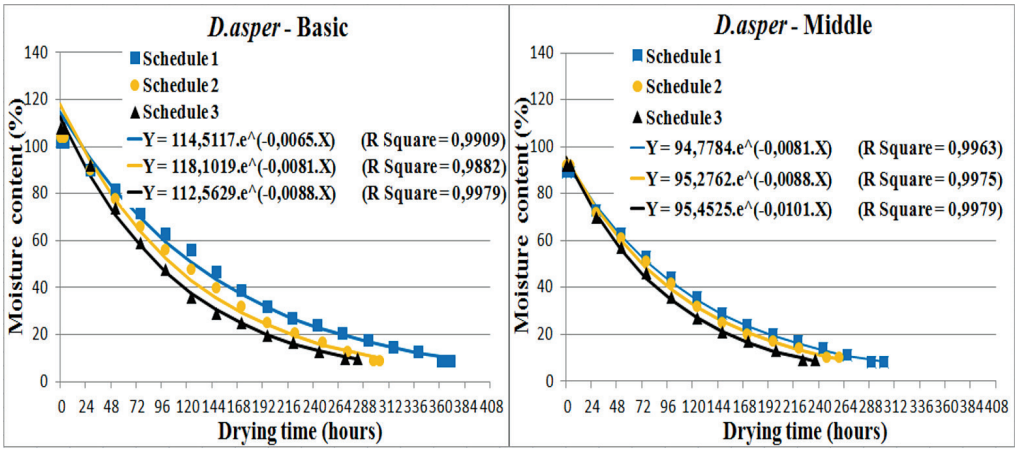

Fig. 5. Relationship between drying time and moisture loss of *D. asper*

among 13 tested samples. Variations in final moisture content can affect the machining and use of bamboo. To reduce the variation, the conditioning period was increased from 4 to 12 hours during the next drying runs of *Bambusa stenostachya* and *Dendrocalamus asper*. The conditioning of *Thyrsostachys siamensis* was kept short with 4 hours.

The average moisture content of the basic and middle parts after drying showed no pronounced differences. The final moisture content of the three species ranged from 6 to 12% for the basic and 7 to 11% for the middle with standard deviations of 1.2 to 1.4 for basic and 1.0 to 1.3 for middle parts.

Drying time

The time affected by the drying intensities from mild to severe is presented in Fig. 3, 4 and 5.

When using the milder schedule no. 1 with a final temperature of 50°C and RH of 30% on the species *Bambusa stenostachya,* the drying time for the basic sections was 350 hours for reducing the initial MC from higher than 100% to 9%. The middle parts dried in 326 hours with a reduction of MC from 98% to 9%.

By applying the medium schedule no. 2 with a temperature of 55°C and 20% RH, the time was reduced to 272 hours for the basic and 255 hours for the middle sections. The severe drying schedule no. 3 with a final temperature of 70°C and 25% RH procured drying time of 255 hours for the basic and 208 hours for middle sections. However, severe defects such as splits end and node checks developed in the both parts.

For *Dendrocalamus asper,* the severe drying schedule no. 3 had the shortest drying time of 282 hours for basic and 236 hours for the middle, but serious defects as splits developed. When applying the slightly milder schedule no. 2, the time increased to 303 hours for basic and 253 hours for middle sections, both with notable defects. The milder schedule no. 1 reduced defects, and the drying time was 396 hours for basic and 362 hours for the middle sections.

Schedule no. 3 and the very severe schedule no. 4 with higher temperature of 75°C and lower RH of 15% can be applied to reduce drying times for the solid species *Thyrsostachys siamensis.* For schedule no. 3, the time was 245 hours for basic and 195 hours for middle sections. The shortest time was achieved with schedule no. 4 with 219 hours for basic and 176 hours for middle sections.

In comparison to the kiln drying results by Laxamana (1985) for the species *Bambusa vulgaris, Dendrocalamus merillianus, Phyllostachys nigra* and *Schizostacbyum diffusum* and the studies on *Guadua angustifolia* by Montoya Arango (2006), the drying time was generally shorter than in these investigated species. Drying time for *Dendrocalamus merillianus* was 128 hours and for *Guadua angustifolia* 118 hours. The shortest time for *Thyrsostachys siamensis* was 176 hours, *Bambusa stenostachya* 208 hours and *Dendrocalamus asper* 236 hours.

Table 4: The average initial moisture content with samples n= 20 and the final moisture content with n = 39 of the four experiments

Schedule	Species		*T. siamensis*		*B. stenostachya*		*D. asper*	
	Moisture content		Basic	Middle	Basic	Middle	Basic	Middle
No. 1	Mean (in %)	initial	–	–	103	92	102	89
		final	–	–	10.4	10.1	9.3	8.2
	SD (in %)	initial	–	–	6.8	5	7.5	5.6
		final	–	–	1.4	1.2	1.3	1.1
	VC (in %)	final	–	–	13.8	12.1	14.4	13.6
	min	final	–	–	7.5	6.9	8.3	6.3
	max	final	–	–	12.1	12	12.4	12
No. 2	Mean (in %)	initial	120	110	102	99	105	93
		final	8.5	10.1	9.6	9.5	9.2	10.4
	SD (in %)	initial	8.8	7.2	6.1	5.9	6.9	4.8
		final	1.3	1.1	1.3	1.1	1.3	1
	VC (in %)	final	15.7	11.2	13.9	11.7	13.7	9.8
	min	final	6	6.9	6.9	5.9	6	5.9
	max	final	12.4	11.2	11.9	12.2	12.2	12
No. 3	Mean (in %)	initial	119	106	105	96	108	92
		final	9.7	10.3	9.6	8.3	10.2	9.2
	SD (in %)	initial	8.1	7.2	6.4	4.8	7.1	5.9
		final	1.2	1.3	1.4	1.1	1.4	1.1
	VC (in %)	final	12.8	12.5	14.9	13.1	14.1	11.5
	min	final	6.1	6.8	6	5.9	6.2	5.9
	max	final	12.4	11.6	11.9	12.4	11.8	12
No. 4	Mean (in %)	initial	120	108	–	–	–	–
		final	8.8	10.2	–	–	–	–
	SD (in %)	initial	8.2	7.1	–	–	–	–
		final	1.4	1.2	–	–	–	–
	VC (in %)	final	15.7	11.2	–	–	–	–
	min	final	6	7.3	–	–	–	–
	max	final	12.4	11.1	–	–	–	–

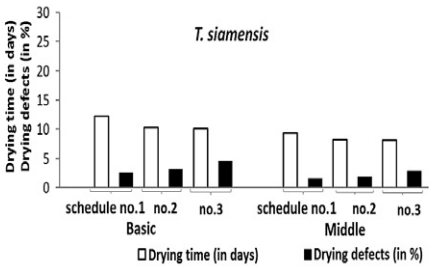

Fig. 6. Drying time and percentage of defects for *T. siamensis*

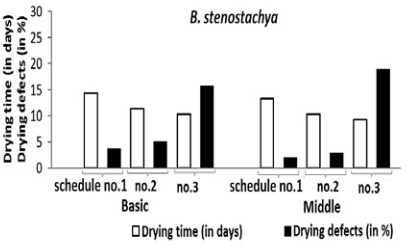

Fig. 7. Drying time and percentage of defects for *B. stenostachya*

The difference between the species is partly explained by their physical properties. *Bambusa stenostachya* has a specific gravity of 0.71 and a wall thickness of 20 mm which is less in comparison to *Dendrocalamus asper* (0.78 and 22mm, resp.) and more to *Dendrocalamus merillianus* (0.6 and 10 mm, resp.). *Guadua angustifolia* has a specific gravity of 0.6 and a wall thickness of 23 mm. The solid species *Thyrsostachys siamensis* has a low specific

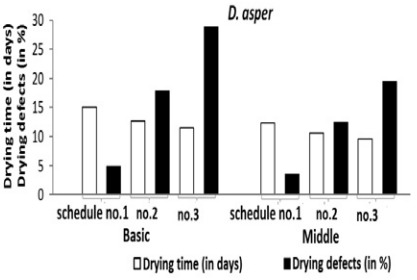

Fig. 8. Drying time and percentage of defects for *D.asper*

gravity 0.46 but its wall thickness is much thicker. In fact, both solid and cavity species have a wide range of structural features and physical properties (specific gravity, moisture diffusion and gas/liquid permeability) that influence the drying behavior.

Drying defects

In kiln drying of bamboo, defects may develop during and after drying. Some common defects are ruptures of culm tissue such as surface checks and splits. Uneven moisture content and discoloration such as mould, blue staining and water staining at the nodes also reduce to drying quality. Most physical defects were end checks, node checks and splits (see Photo 2).

The two cavity species, especially *Dendrocalamus asper* were susceptible to splits, end checks and node checks. The basic part of all species developed more severe defects in comparison to the middle part. The most severe defects in *D. asper* and *B. stenostachya* occurred with the drying schedule no. 3. End splits and node checks lead to 29% defects in basic sections for *D. asper* and to 19% for *Bambusa stenostachya.*

For *B. stenostachya* the slightly milder schedule no. 2 with a final temperature of 55°C and a 20% RH the defect percentage reduced to 6% for the basic and 4 % for the middle parts. Applying the milder schedule no.1 with a low temperature of 50°C and a high RH of 30% for *D. asper* minimized defects at the basic to 6% and at the middle 3.5%.

For the solid species *T. siamensis*, the very severe drying schedule no. 4 with high temperature of 75°C and a very low RH of 15% the defect percentage was 7% for the basic and 5% for the middle parts. End checks at internal layer occurred mainly with the basic samples. The solid species *T. siamensis* is easier to dry and less susceptible to defects than the cavity species *B. stenostachya* and *D. asper.*

In drying bamboo, discolourating fungi such as mould and sap staining can grow on green bamboo in kilns operating at a low temperature and high humidity regime (Tang *et al.* 2009). In the drying process using the mild schedule no. 1, mould developed on the basic parts of *D. asper* during the initial stage with a temperature of 40°C and a relative humidity of 85%. Mould was prevented by a high temperature treatment with 80°C and a relative humidity of 90% for 2 hours.

Photo 2: End checks of *T. siamensis* and *D. asper*

CONCLUSION

The initial experiments have shown that kiln drying of bamboo parts can be conducted successfully using proper schedules of temperature and relative humidity. Drying the solid species *Thyrsostachys siamensis* requires a severe drying schedule with high temperature of 65°C and low relative humidity of 60% at the initial stage and 75°C with 17% RH at the final step. The drying time was 10 days for the basic and 8 days for the middle sections. The cavity species *Dendrocalamus asper* is a difficult species to dry and susceptible to drying defects and therefore needs a mild schedule with initial temperature of 40°C and initial RH of 80% and a final temperature of 50°C and RH of 30 %; the required drying time was 17 days for the basic and 14 days for the middle sections. *Bambusa stenostachya* dried moderately fast using the relative milder schedule with 65°C temperature and 20% relative humidity and resulted in a drying time of 12 days for the basic and 10 days for the middle.

The dry–kiln industry in South Vietnam will apply these effective and feasible schedules for drying longer culms. Additionally, the drying schedules will be further developed for bamboo treated with preservatives based on boron compounds. Since drying is an essential step for processing bamboo into final products, the investigations should also include other commercial species.

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Flowering, reproductive biology and post flowering behaviour of *Dendrocalamus sikkimensis* Gamble, in Kerala, India

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ABSTRACT

Gregarious flowering of *Dendrocalamus sikkimensis* clumps located at Kerala Forest Research Institute campus and Field Research Centre, Veluppadam, Thrissur, Kerala initiated in August 2009. Observations were made on floral morphology, anthesis, pollination, pollen viability and germination and post flowering behaviour. The flowers were dichogamous, protogynous and closed. The inflorescence was a large leafy panicle, with stiff nodose branches bearing large globose heads. Pollens were numerous and monoporate. Ovary was sub globular, hairy with club shaped stigma. Anthesis occurred from 6.00 am onwards. High pollen viability (92 %) was observed in acetocarmine staining and *in vitro* pollen germination ranged from 3-74 per cent. The pollination was anemophilous. Flowering was followed by abundant seed production and subsequent drying and death of the culms.

Key words: *D. sikkimensis*, reproductive biology, seed production, pollen viability, post flowering behaviour.

INTRODUCTION

Bamboo flowering has been amazing to man from time immemorial. Most of the bamboo species are moncarpic and due to the long intermast period for mass flowering (Janzen 1976), only a very few reports are available on their reproductive biology. Detailed knowledge on reproductive biology is inevitable for successful cultivation and conservation of bamboos. Moreover, it helps in developing protocols to combat the problems that impede regeneration. *Dendrocalamus sikkimensis* Gamble is a large sized bamboo with caespitose clump growing upto an altitude of 2100 msl. In India, it is naturally distributed in North-Eastern India (Garo Hills) and West Bengal and it has been introduced to various parts of the country. *D. sikkimensis* has versatile uses like fencing, posts, huts, ropes, boxes, water pipes, animal fodder etc. It also can be used for pulp and paper (Holstrom, 1993). Flowering in *D. sikkimensis* is reported as early as 1916, 1932 and 1982 (Seethalakshmi and Kumar, 1998). It flowered gregariously in Arunachal Pradesh in 2004. Other than the reports on flowering and the floral descriptions, detailed account on reproductive biology is lacking in this species. *D. sikkimensis* was introduced to Kerala during 1992 and planted in the Kerala Forest Research Institute campus, Peechi and bambusetum at Field Research Centre, Velupadam, Thrissur. Clump and culm attributes during January 2011 are presented in the Table 1. It flowered in both locations during August, 2009. The present investigation was conducted to study the floral morphology, anthesis, mode of pollination, pollen germination and the post flowering behaviour of *D. sikkimensis*.

MATERIALS AND METHODS

D. sikkimensis clumps situated in the Kerala Forest Research Institute campus (N 10°31'819" E 076°20'855") and Field Research Centre at Veluppadam (N 10° 26’ 31” E 76° 21’36.9”)

Table 1- Clump and culm attributes of *D. sikkimensis*

Clump attributes	Mean
Diameter (m)	8.02
Number of culms	54.75
Flowering culms	All
Culm attributes	
Height (m)	21.01 ± 3.71
Girth at fifth node (cm)	25.66 ± 7.26
Number of nodes	34.33 ± 5.53
Internodal length (cm)	38.66 ± 10.11

initiated flowering during August, 2009. The gregarious flowering of the clumps occurred in second year after initiation of flowering (Fig 1A). We recorded the clump attributes viz. clump diameter and number of flowering and non-flowering culms and the height, girth (at fifth node), number of nodes and internodal length of the culms within the clump. Two noded stem cuttings were collected from the culms which were not flowered during 2009, treated with NAA 200 ppm and planted in standard nursery beds at KFRI nursery. Morphology and dimensions were studied from the freshly collected inflorescences as well as those fixed in FAA (Formalin Acetic acid Alcohol) under dissection microscope (10x). Entire observations were replicated twenty times and extra observations were done in the field for confirmation. Fifty flowers were randomly tagged and time of anthesis, anther dehiscence and stigma receptivity were observed. In order to determine the type of pollination, insect visitations to flowers were observed. Adhesive tapes fixed near the open flowers were also observed under microscope for pollen deposition. Periods of observation ranged from ten minutes to one hour and were done during daylight hours (6.00 am to 6.00 pm). Viability of pollen at the time of dehiscence was tested using 1% Acetocarmine, considering stained grains as viable and the shrivelled as non-viable (Radford *et al.* 1974, Koshy & Jee, 2001, Beena *et al*. 2007). The viable pollen in the microscopic field was counted and expressed as percentage of the total. *In vitro* germination of pollen was tested in five different germination media (Table 2). Fresh mature anthers were collected from the field at anthesis and pollen grains were carefully dusted in cavity slides containing germination media. One hour after inoculation, the number of pollen grains germinated and the number of grains per field of view was recorded. Pollen grains were considered to be germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra & Wedel, 2000). Pollen diameter and tube length was observed under image analyzer (Leica Q 500 MC) under 40 x magnifications.

Fig. 1. Flowering of *D. sikkimensis* A. Flowering clump B. Flowering in the sprouted cuttings C. Branch bearing heads *D. Spikelets* arranged in heads.

Post flowering behaviour of *D. sikkimensis* was keenly observed at frequent intervals. In order to collect viable seeds, a polythene sheet was spread below flowered clumps and culms were shaken well; fallen mass was collected periodically and fertile seeds were separated by filtering through a 2 mm sieve and winnowing.

RESULTS

During August, 2009, signs of flowering were observed at the tips of a few culms in the clumps located at Velupadam and only a few clumps flowered during that season. The flowering was confined to two to three culms per clump and it was completed by April, 2010. In 2010, gregarious flowering occurred in the entire clumps in the locality and all the culms in clumps were in bloom. The clumps located in the KFRI campus as well as the rooted stem

Table 2. Composition of the pollen germination media

	Media 1	Media 2	Media 3	Media 4	Media 5
Composition					
Sucrose (g)	10	10	10	0	10
Boric acid (g)	0.01	0.01	0	0.01	0
Calcium nitrate (g)	0.03	0	0.03	0.03	0
Distilled water (ml)	100	100	100	100	100
Pollen germination					
Germination %	68	59.5	70.3	3.25	74.13
Duration (min)	20	20	20	50	10-15

cutting also were in bloom during this period. Flowering was at its peak during December, 2010 to January, 2011. Flowering shoots were initiated from the nodes of culms and elongated up to 40-50 cm, ended with formation of spikelet at the tip. The whole process of flowering was completed with in three to four weeks (Fig 2A). Flowers were developed in acropetal succession on flowering shoots (Fig 2 B).

Flower morphology

Inflorescence in *D. sikkimensis* is a large leafy panicle, with stiff nodose branches bearing large globose heads; rachis dull brown, sparsely pubescent. The spikelets were several in numbers and arranged capitate wise on nodes (Fig. 1C). Spikelets were pale green when young and turned dark green on maturity and the exserted yellow anthers gave yellow appearance to heads. (Fig 1D). The dimensions of floral parts of *D. sikkimensis* are given in the Table 3. Spikelets were dichogamous, protogynous and closed. Maturation of pistil three to five days prior to anther emergence was observed in the field studies indicating the protogynous nature (Fig 2A). Presence of young anthers along with matured pistil in the FAA fixed flowers also confirmed this. Three to four empty glumes were seen per spikelet, which were basal broadly ovate rounded with ciliate keels. Fertile spikelets were two to three and two to three florets per spikelet were observed. Lemma was pale white in colour when young and turned pale green on maturity, broad at base ending as needle at top and glabrous. Palea was pale green to white many-nerved and shortly bifid. Stamens were six, pale green in colour and filaments were narrow, long, apiculate. Anthers and pollens were yellow; pollens were monoporate and 29.82 ± 1.00 µm in diameter. Ovary sub globular, hairy with club shaped stigma (Fig 2C-F). The present observations on floral morphology agree with that of the classical description (Gamble 1896).

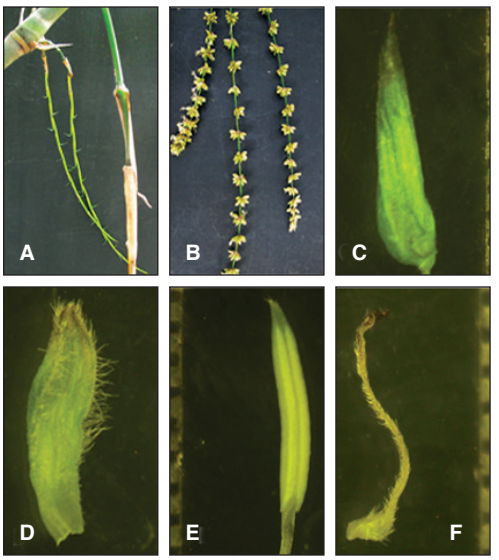

Fig. 2. A. Flowering shoot initiation B. Spikelets in different stages C. Lemma D. Palea E. Androecium F. Gynoecium

Anthesis and pollination

Time of anthesis varied from 6.00 am to 12 noon. Stigma of the flowers emerged out 4-5 days prior to anther emergence. The stigma became receptive by 7.30 am and the receptivity was indicated by the viscous fluid secretion. The yellow anthers emerged out from 7.30 am and they were in the peeping out stage at 8.00 am. They exserted out completely after 8.30 am and linearly dehisced to disperse yellow

Table 3. Details of spike, spikelets and florets in *D. sikkimensis*

Characters	Mean
Flowering period	August to May
Flower type	Large panicle
Flower colour	Pale green
The number of spikes per branch	25-30
Number of spikelets with exposed stigma per head	Most of the florets
Number of spikelet with exposed anthers per head	Most of the florets
Width of spike (cm)	1.83 ± 0.18
Number of spikelets per head	25.5±5.74
Length and width of the spikelets (cm)	0.67 ±0.06, 0.39 ±0.07
Number of florets per spikelets	2-3
Number of glumes	2-3
Length and width of lemma (cm)	0.36±0.04, 0.19 ± 0.04
Length and width of Palea (cm)	0.35±0.02, 0.15 ± 0.04
Number and Length of stamen (cm)	5 , 0.55± 0.03
Anther dehiscence mode	Longitudinal.
Length of the stigma + style (cm)	0.56 ± 0.05
Stigma type	Wet and papillate
Length of ovary (cm)	0.1
Number of stigma	One
Diameter of pollen grains (μm)	29.82 ± 1.00
Pollen shape	Round and circular/spherical

pollens between 8.30 am and 10.00 am. It was the peak time of pollen dispersal. Gentle breeze shook the anthers to liberate dusty pollen grains in the air. Majority of the anthers curled out in the afternoon.

Like many other bamboo species, *D. sikkimensis* is anemophilous (wind pollinated). Honey bees *Apis floria, Apis dorsata* and *Apis cerana* were seen visiting the flowers during morning hours. They foraged mainly on anthers of flowers. Peak insect visit was observed during 8-11 am. Presence of large number of pollen grains on the adhesive tapes fixed near flowers also indicated the anemophily. Moreover, the presence of large anthers producing abundant uniform pollen grains which is the characteristic of wind pollinated species also leads to conclusion of the occurrence of anemophily in this species.

Pollen viability and in vitro pollen germination

Freshly collected pollen grains of this species showed 90 to 92 % viability when stained with 1% Acetocarmine. *In vitro* pollen germination was found to be moderate (3.25-74.13 per cent) when the pollens grains dusted in different germination media was observed under microscope after one hour of incubation (Table 2). The highest pollen germination was observed in the medium 5 (74.13 per cent). Duration for pollen germination also was low in that medium (10- 15 min). Lowest germination was obtained in the medium 4 (3.25 per cent) which does not contain sucrose and the duration of germination was long (50 min).

Post flowering behaviour

The recurrent visits to the flowering locality confirmed that flowering of this species is followed by seed production. Although profuse flowering was observed in 2009, seed production was very low and no fertile seeds and wildlings were observed. During December to January 2011 only very few fertile seeds were present. However, after February the seed production was found to increase. Seeds obtained had a high germination percentage (90%) when sown in nursery beds. Flowering and seed production in *D. sikkimensis* was followed by the death of the culms. The flowered clumps in bambusetum started to dry up and one of the flowered clump in the KFRI campus has already died.

A simple technique for observations on reproductive biology in bamboos

In large sized bamboo clumps, it may not be easy to collect and observe flower very closely. As the reproductive biology studies require closer examination of flowers, the whole process become cumbersome. Hence, the culms which are about to flower or to flower in the next season are selected and stem cuttings are rooted in nursery beds using growth regulating substance like NAA and IBA. All the sprouts originated from these cuttings will be in bloom during the next growing season. This would help to provide inflorescence at convenient height for closer examination of the various stages of the flowering in taller bamboos. The observations can be compared with that in the natural condition. This methodology was practiced earlier in *Dendrocalamus stocksii* and *Ochlandra travancorica* (Beena *et al.* 2007) and in *D. sikkimensis* also (Fig 1B).

DISCUSSION

Previous reports on flowering of *D. sikkimensis* have been made in 1916 in Kalimpong, North Bengal (Indian Forester, 1917) and in SIkkim in 1982 (Lahiry, 1982). Recently, it flowered gregariously in Yingkiong, Upper Siang, Arunachal Pradesh in 2004. In 2006 it flowered in Australia and most of the seeds were viable. Flowering cycle of the species could not be ascertained from the flowering records available so far. Most of the historical records on bamboo flowering are fragmentary and not dependable since adequate verification is not possible (Campbell,1985). *D. sikkimensis* belongs to gregarious flowering group of bamboos in which whole clumps in a locality flowers in a period. Although the floral morphology has been described in this species, the present investigation provides detailed account of inflorescence and florets obtained from recent specimens.

Flowering of majority of the bamboo species occurs during August to December (Banik 1998, Jijeesh *et al.*, 2009, Seethalakshmi *et al.*, 2010) and flowering of *D. sikkimensis* also followed this season. Dichogamy is observed in most of the bamboo species and protogyny is common. It took four weeks to complete flowering. Opening of florets, time of stigma receptivity, type of pollination etc. agrees with the earlier reports from other bamboo species such as *Melocanna baccifera, Bambusa vulgaris, B. bambos, D. strictus, Pseudoxytenanthera monadelpha* etc. (Banik 1998, Nadguada *et al*. 1993, Jijeesh *et al*. 2009). All these species produced large number of monoporate pollen. Up to 92 per cent pollen viability was observed on staining with acetocarmine (1%). Pollen viability indicates the ability of pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination (Shivanna *et al*., 1991). Studies on *in vitro* pollen germination also showed a higher pollen viability (74 per cent in Media 5). Sucrose solution is generally considered as an effective medium for pollen germination and the results of the present investigation also agree with this. Lowest germination was obtained in the medium 4 which doesn't contain sucrose and the duration of germination also was high. A successful system of *in vitro* pollen germination is a prerequisite for pollen research (Williams *et al.* 1982) and is important for testing the capacity and viability of pollen for controlled pollinations (Griffin 1982; Heslop-Harrison 1979). *In vitro* pollen germination medium is needed to study the various aspects of pollen biology, pollen selection, pollen transformation and detection of cytoplasmic male sterility system. As many bamboos flower at long intervals and seed

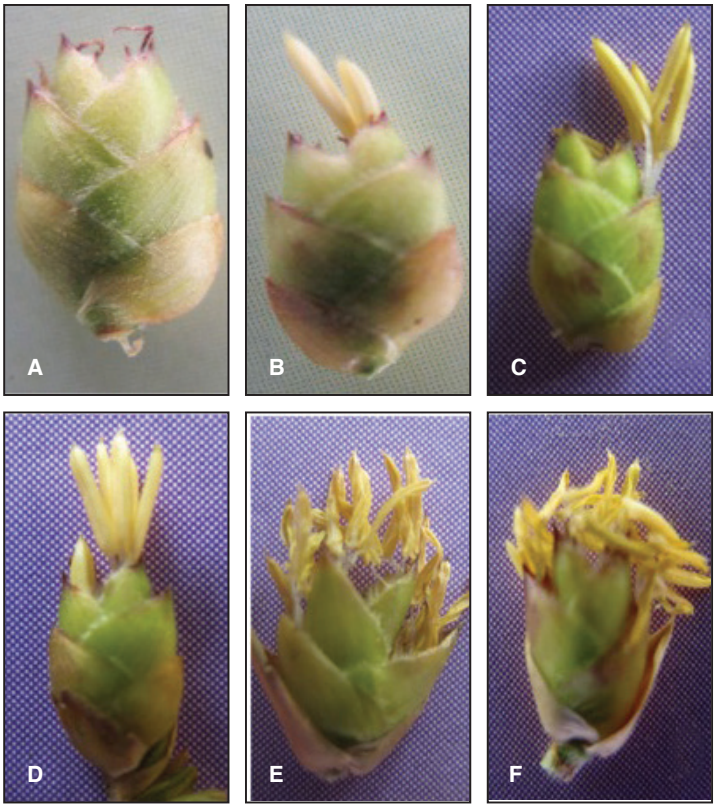

Fig. 3. Different stages of flower opening. A. Female phase. B-F Anther emergence and curling.

production is poor or completely absent in some species the studies on pollen viability and germination is inevitable. *In vitro* pollen germination rates are considered the best indicator of pollen viability (Shivanna *et al*., 1991). The seed production in this species can be attributed to the high pollen viability.

Although profuse flowering was initiated in 2009, the fallen mass contained more chaff than seeds. In 2010, the seed set was low during initial stages of flowering and only during January the seeds were obtained. It is observed that the seed lots obtained from some bamboo species during the initial stages contain more chaff than seeds. The flowers opened during initial stages didn't set seed. After anthesis the spikelets usually dried and fall off. In this period the female phase of the flowers rarely emerged out, when the flowers were cut open, the partially developed stigma could be seen without emerging out of the spikelet. The absence of the seed production may be attributed to the underdeveloped gynoecium.

Unpredictable nature of flowering as well as long flowering cycle of bamboos is still an intriguing concern for the bamboo researchers all over the world. The information on flowering and reproductive biology of most of the bamboo species are lacking. Hence, the present study can contribute to the information on bamboo reproductive biology. Since seed production is observed a seedling population with known flowering age can be raised. Raising plantations with planting stock of known flowering age will help to predict flowering in future and correct flowering cycle of each species can be determined.

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Traditional uses of bamboos among the Karbis, a hill tribe of India

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ABSTRACT

The present paper describes traditional uses of bamboos among the Karbis, a hill tribe of state of Assam in India and their significance in the social, cultural and religious life of the people. Field study was undertaken among the Karbis in different parts of Karbi Anglong district of Assam state, India during 2003-2010. Information was collected from elders of 24 randomly selected villages following unstructured interview because the informants were illiterate, and observations. Bamboo is the most extensively used plant resources among the Karbis, *Bambusa tulda* Roxb. and *B. balcooa* Roxb. (Sil borua) in the plains and *Dendrocalamus hamiltonii* Nees et Arn. ex Munro in the hills being the most commonly used species. There is a popular proverb prevalent among the people read as *"Karbi aso ke jintak cheripdongse pirthe kevang lapen jintak cherip pondongse chomarong kedam"* [a Karbi is born with *jintak* (bamboo split) in his hand and leaves the world holding *jintak*]. This phrase speaks a volume about the dependence of the Karbis on bamboo resources. Though metallic articles are available, the uses of certain bamboo crafts are mandatory during rituals and in social occasions. Han-up or bamboo shoots is a major source of food during scarcity of rice; a traditional festival is celebrated to mark the bamboo shoots harvesting season. Karbis also observe certain taboos on use of bamboo. Bamboo also is a potential resource for improving rural economy of the rural Karbis.

Key words: Karbis, *Jintak, Seh, Hor-heh, Mucham, Chuhu, Vo-um, Beleng,* culture, taboo

INTRODUCTION

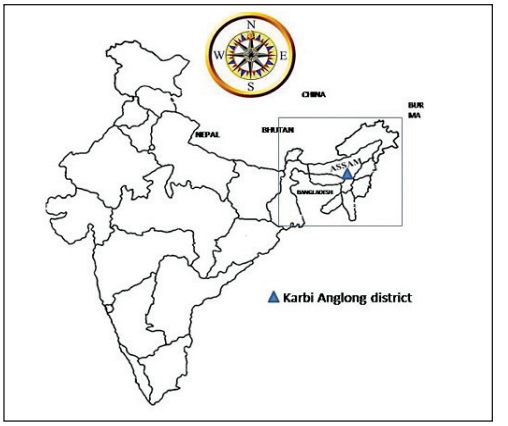
Bamboo also known as "poor man timber" is also stands true for countryside in Northeast India. It is probably the most extensively used plant resources and associated with all spheres of life (e.g., food, medicines, crafts, agricultural implements, house building material, cordage, etc). However, bamboo is commonly used in construction of houses in rural areas. The Northeastern region of India is rich in the diversity of this perennial arborescent grass (i.e., bamboos). The region has 58 species of bamboo under 16 genera (Arora & Maurya 1988). Biswas (1998) however, reported the availability of 63 species of bamboo belonging to 15 genera in the region. Barooah and Borthakur (2003) have recorded the occurrence of 40 species, one variety and one forma under 10 genera from Assam. Ethnic tribes of the region are largely dependent on bamboos for varied uses in their day to day life. The works of Laha (2000) on traditional uses of bamboo for housing by different tribes of Northeast India, Sundriyal et al. (2002) on bamboo utilization in Apatani Plateau of Arunachal Pradesh, Sarkar and Sundriyal (2002) on indigenous uses and management of bamboo in Arunachal Pradesh, Bhatt et al. (2003) on indigenous uses and commercial edible bamboos of Northeast India, Handique *et al.* (2010) on socio economic significance of bamboo resources in Arunachal Pradesh, and Nath et al. (2011) on traditional uses of bamboo among the tea tribes of Barak Valley in Assam have thrown light on the importance of

bamboo in life and culture of the ethnic groups of Northeast India. In the present paper traditional uses of bamboo among the Karbis, one of the hill tribes of Assam in Northeast India has been discussed.

Karbis as well as other hill tribes of Northeast India have inherited a rich treasure of knowledge on art and crafts which are reflected in their material culture such as motifs on garments, metal works, pottery, wood, cane and bamboo works, blacksmith, musical instruments. "Necessity" may have compelled the hill Karbis to utilize forest resources for meeting their daily needs. Due long usage and emotional relationship many plants and plant products have been incorporated in their social and cultural life. One such example is use of handicrafts whose development may be attributed to the needs of day to day life, but today are religious and social requirements. Because use of certain crafts is mandatory during religious as well as social occasions among the Karbis.

MATERIAL AND METHODS

Karbis represents one the major tribes of Northeastern region of India with their unique tradition and culture. Though scattered in states of Arunachal Pradesh, Meghalaya and Assam they are largely concentrated in Karbi Anglong district of Assam. The district lies between latitudes 25°30'-26°36' N and longitudes 92°90'-93°54' E covering a geographical area of 10,434 sq km (Maps 1A and 1B). Ethnically Karbis are Mongoloids and speak a dialect belonging to Tibeto-Burmese particularly Kuki-Chin subgroup of languages (Lyall 1908; Bhattacharjee 1986; Phangcho 2001). They call themselves *Arleng* which literally means *man*. In features the men resemble Assemese people of the lower classes more than Tibeto-Burman races. Their color is light brown, and the girls are often fair. The men are as tall as majority of the hill races of Assam averaging 1633 mm in height. The nose is broad at the base, and often flat, giving a nasal index of 85.1 and an orbito-nasal of 107.7. The facial hair is scanty, and only a thin moustache is worn. The hair is gathered into a knot behind, which hangs over the nape of the neck. The body is muscular and the men are capable of

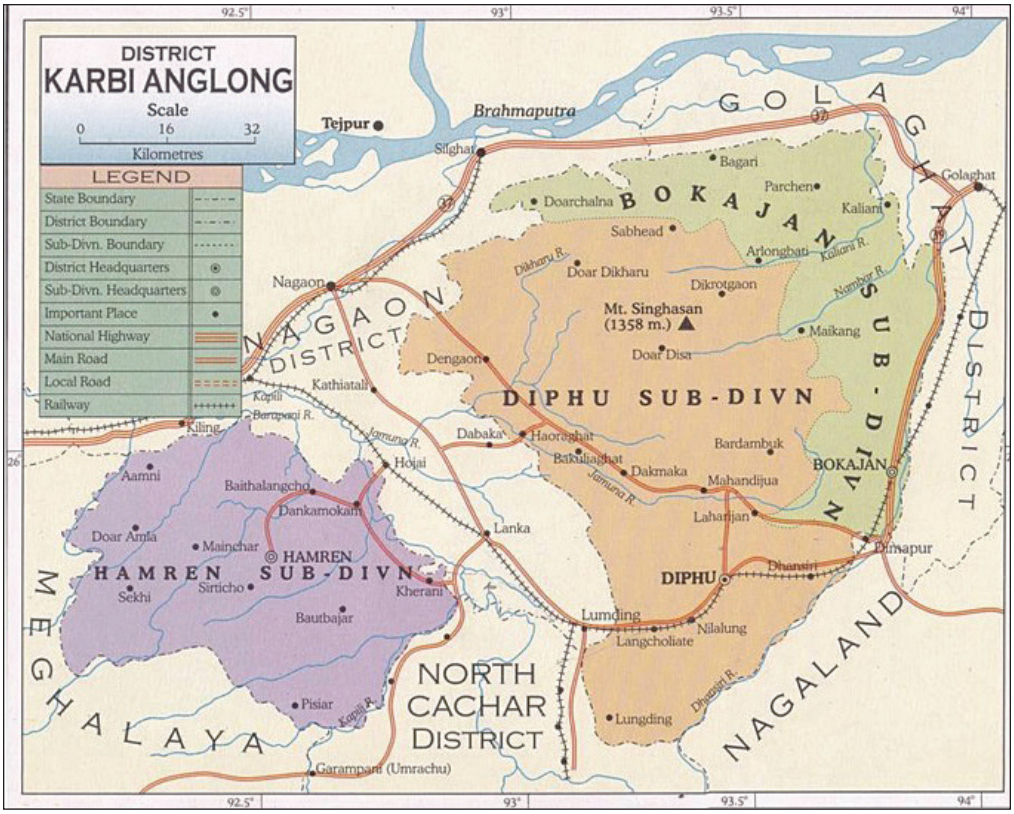


Map1A. Map of India showing Northeast region (box).

prolonged exertion (Lyall 1908). Karbis are mainly agriculturists and practice shifting cultivation where they grow multiple crops. Rice is the staple food supplemented by wild vegetables while locally prepared rice beer is the common drink. Karbis practice patriarchal system of family and follow traditional animistic religion that preaches rebirth and immortality of souls. In the hills houses are built on raised platforms probably to protect themselves from wild animals. Marriage is strictly clan exogamy and violation of this social rule leads to excommunication.

Like other hill tribes of northeastern region of India and elsewhere in the world, use of bamboo is prevalent among the Karbis. Bamboos are used as raw material in day to day life such as for house construction, as cordage, as fuel, for crafts, in rituals, as food and medicine. During the scarcity of staple food rice it also served as famine food. The use of certain bamboo crafts have become part and parcel of Karbi culture and the practice is still vibrant today. A festival is celebrated to mark the harvesting of bamboo shoots which is accompanied by a traditional dance called *Hacha Kekan.* Now a days bamboo is also considered as a potential plant resource and play an important role in rural economy as its demand is increasing day by day in paper industries, as construction material, for handicrafts and for its edible shoots.

Field study was undertaken among the Karbis in different parts of Karbi Anglong district of Assam state during 2003-2010. Information was collected from elders following unstructured interview because the informants were illiterate,



Map 1B. Map of Karbi Anglong district where field study was undertaken (Map not to scale).

and observations. Specific questions asked during field study include a) history of use of bamboo crafts, b) origin of bamboo and c) cultural significance of bamboo. A total of 24 villages were randomly selected for field study and 75 (48 male and 27 female) respondents from the villages were interviewed. In addition to record the uses through personal observations, social as well as religious rituals were also attended to study the actual uses of bamboo or its products. Visits to local markets were undertaken to gather information on bamboo and bamboo products being sold.

Studies on the art and crafts of the tribes of Northeast India including the Karbis started during the British colonial rule. The earlier workers were by the British officers who collected certain information on crafts besides their official duties. Elwin (1959) and Alemchiba (1968) have made special study on art of Arunachal Pradesh and Nagaland respectively. Several monographs on the tribes of Northeast India written by British Administrators and contemporary anthropologists also made profuse references about the rich artistic accomplishment of the people of the region. These reports, however, is silent about the rich artistic crafts of the Karbis. And hence the present study.

RESULTS

There is a popular proverb among the Karbis which reads *"Karbi aso ke jintak cheripdongse pirthe kevang lapen jintak cherip pondongse chomarong kedam"* [a Karbi is born with *jintak* (bamboo split) in his hand and leaves the world holding *jintak*). This phrase speaks a volume about the dependence of the Karbis on bamboo resources. Bamboo is indispensable part of Karbi society – when a mother conceives, the family perform certain rituals in honor of the unborn baby using rice grains wrapped in banana leaves and tied with *jintak*; during life time of the person he/she use bamboo for all purposes; after death the corpse is

laid on a bamboo mat, tied to a bamboo *culm* (i.e., stem) with *jintak* and taken to cremation ground. Therefore, for the Karbis life begins with bamboo and ends with bamboo.

Karbis are highly acclaimed for their expertise not only in bamboo crafts but also for their cane and wood works. These crafts which are often decorated with unique designs include totem, baskets, utensils, mats, containers, sieves, fans, granary, weaving implements, mortar and pestle, musical instruments, weapons, etc. Bamboo is the most versatile of all forest resources among the Karbis as almost all articles are made and/or can be made from bamboo. More than five species of bamboo are used by the Karbis for various purposes and of which, *Kaipho (Dendrocalamus hamiltonii* Nees *et* Arn. ex Munro) is the most frequently used species in the hills while in the plains *Bambusa tulda* Roxb. and *B. balcooa* Roxb. (Sil borua) are the commonly used species. Other species include *Bambusa affinis* Munro., *Melocanna baccifera* (Roxb.) Kurz. and *Schizostachyum dullooa* (Gamble) R. Majunder. Bamboo is emotionally associated with the social, culture and religious life and also with traditional institutions of the Karbis. Crafts made from bamboo form part and parcel of their culture and in certain occasions cannot be substituted with crafts made from other resources. *Kaipho* comes handy for house building as well as for other day to day requirements including vegetables. *Han-up* (bamboo shoots) is one of the common sources of food in absence of rice. In the days of yore rice and vegetables were cooked or steamed in bamboo stem called *Lankpong*. Eatables prepared in *Lankpong* impart pleasant aroma and taste and even today such items are considered revered and highly sought after.

The history of use of bamboo, for whatsoever purpose is based on legends that also include traditional belief of origin of bamboo. A popular dance called *Hacha Kekan* is performed to mark the harvesting season of *Han-up* (bamboo shoots). On the occasion elders narrate the origin of bamboo and its use among the Karbis through folk songs while young boys dance to the tune of the song. Certain taboos are also observed on bamboo – bamboo once used in funeral rites, other bamboos from the same grove are not used for household purposes, and bamboo shoots of new season are not consumed until *Rongker*, an annual community festival is performed.

Crafts occupy indispensable parts in the social, cultural and religious life of the Karbis. Though metallic articles are available, the uses of certain bamboo crafts are mandatory during rituals and in social occasions. In a Karbi society irrespective of economic status, bamboo crafts are part of belonging of a family and considered as assets. Certain crafts become part of cultural heritage and therefore, their use is continued even today in the face of modernity. Further, knowledge of crafts is considered as qualification for boys for marriage just as knowledge of weaving is for girls. Traditional use of bamboo and its cultural significance is enumerated below. Only those crafts which cannot be substituted with other items have been discussed.

Enumeration

1. ***Chohu:*** Chohu is made from *jintak* or bamboo splits and used as cushion for earthen pots. This craft is made to mark the beginning of the religious festival Chojun (Fig. 1A) or invoking of *Arnam Kethe,* the supreme deity of the Karbis. *Hor kangthir* or holy rice beer for the occasion is stored in earthen pot which in turn is customarily placed on *chohu*.
2. ***Mucham:*** This craft which is loosely woven with *jintak* or bamboo splits is also made to mark the arrival of the religious ritual *Chojun* (Fig. 1B); on the occasion it is customary to store some meat of the sacrificed pig in mucham (Fig. 1B inset).

Fig. 1A. *Chohu*, bamboo craft used during the ritual *Chojun*. Fig. 1B. *Mucham*, bamboo craft used during the ritual *Chojun*. Inset: Cooked meat of pig kept in *mucham*.

Fig. 2. *Hor-heh,* bamboo basket made on the occasion of the ritual *chojun* for fermenting rice.

3. ***Hor-heh:*** This is a cylindrical craft made from a single piece of bamboo and leaving a node at the base. During religious festivals like *Chojun* and *Chomangkan*, it is customary to keep fermented malt in the *hor-heh* (Fig. 2).
4. ***Seh:*** It is a conical sieve used during brewing of rice beer. During major religions festivals such as *Chomkan, Peng* and *Chojun*, it is traditional to process rice beer with *seh* to produce a milk-like suspension called *horpo*. This practice is called *hor kebeh* (Fig. 3). A woman is specially appointed for the purpose.
5. ***Hak:*** These are cylindrical basket of various sizes but falling into two groups – with or without akeng or foot. Both types are primarily used for carrying *jhum* products

Fig. 3. woman processing malt with *Seh*, bamboo craft to prepare local alcoholic drink.

Fig. 4. Woman carries *hor* or rice beer in *Hak* or bamboo basket during traditional marriage ceremony.

though it is also used for other purposes. It is customary to carry *horlank* (rice beer), filled in gourd shells in *Hak* during marriage and religious festivals (Fig. 4).

6. ***Batheli:*** This is a crude traditional catapult of the Karbis. The handle is made of bamboo while the string is made from home grown cotton and sometimes with bark of a local plant called *thehoi* (*Oreognide* sp.; family Euphorbiaceae). It is considered as cultural heritage of the Kabis and its symbol as been adopted in the logo of Karbi Cultural Society (KCS), the highest cultural organization of the Karbis.
7. ***Langthe:*** *Langthe* is a bamboo tube closed at the base and consisting of three internodes. During the religious festival *Chojun* traditional agricultural practice is performed and it is customary to carry drinking water in *langthe* (Fig. 5) as it is practiced during *jhum* cultivation (slash and burn).
8. ***Beleng:*** This is a large flat circular mat with the periphery stitched with cane splits. *Beleng* is predominantly used for winnowing particularly rice and paddy among other items. The act of cleaning items with *beleng* is called *kangrap* and this knowledge is considered a qualification for women to become brides; the other qualification is weaving. During the religious festival *Chojun* it is customary to receive *Arnam aton* (the divine basket) with *beleng*.
9. ***Ingtong:*** These are large footless *hak* often used for carrying paddy from the *jhum* field to Mandu or hut.

Fig. 5. A scene of people performing *jhum* cultivation on the occasion of the ritual *chojun*; two persons is seen distributing drinking water with *Langthe* or bamboo tubes.

10. ***Khangra:*** *Khangra* differs from *Ingtong* in having large mouth (opening) and is loosely woven; it is mainly used for carrying fire-wood, and other larger items. However, during the *Chomkan* festival it is customary to carry *khangra* for collecting the head of pigs sacrificed on the occasion. This practice is called *Rongsam dam.*
11. ***Vo-um:*** This is cage for domestic fowls. The pattern of making is similar with *Khangra* but *vo-um* differs in shape and uses. On the occasion of *Rongker*, a community festival each family has to contribute one fowl in *vo-um* (Fig. 6).
12. ***Hengru:*** This is a cylindrical sieve of flat bottom used for separating rice beer from fermented malt.

Fig. 6. Fowls kept in *Vo-um,* cage made from bamboo splits, during *Rongker* or community festival.

13. ***Ingkro:*** It is a granary made from bamboo splits or bark of *jintekong* (*Sterculia villosa* Roxb.; family Sterculiaceae). First it is made into mats of appropriate size and then rolled into a cylinder. Both ends are connected while the whole body is fixed with bamboo.
14. ***Lumphlak*** (spoon): For day to day use spoons are usually is made from bamboo. But when it is specially made along with the other two crafts *chohu* and *mucham*, it marks the celebration of the religious festival *chojun*.
15. ***Nopak abe*** (Knife handle): Solid rhizomatous basal part of bamboo is selected and the knife is fixed to the solid part. No ring is used to fix the knife and this is considered as a trademark of Karbi knife.

DISCUSSION

Karbis possess rich treasures of knowledge on crafts. This knowledge is passed orally from father to son, to grandson and so on but with high probability of dilution at each level of transfer. Bamboo is a valuable plant resource and has the potential for improving rural economy. Bamboo mats have high demand in bamboo ply industry and also for making ceiling. Many families in Karbi Anglong district are earning their living by making and selling mats. Many bamboo articles of day to day use reach local markets from where people from communities outside the Karbis procure it. Beside these, ever increasing requirement of bamboo for construction and paper industries opens up opportunity to address unemployment in rural areas. Value addition of bamboo articles and market accessibility will go a long way in generating employment to local youths. As of now indigenous crafts of the ethnic tribes of Northeast in general and Karbis in particular is a promising option for entrepreneurship development.

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The IXthWorld Bamboo Congress 2012

Johan Gielis

A SCIENTIFIC CONGRESS ON BAMBOO

In the bamboo world we do have many outstanding researchers. But as far as I know, there has only been one meeting in the past decades, entirely devoted to science in bamboo, and that was in 1997, the meeting at the Linnean Society in London. In all other conferences there was a mix of research, architecture and design, culture and very often also landscaping and gardening, for a mix of scientists, architects and Bambuseros. It is important to keep this wide communication, but in 2012 it was high time that focus was back on science.

Through EFRO (European Fund for Regional Development) we obtained funding to establish IKEBANA (International Knowledge Center for Bamboo. The goal of IKEBANA is to promote the use of bamboo in agriculture, in a broad sense.

The final goal of the EFRO-IKEBANA project however was the organisation of the IXth World Bamboo Congress, with focus on research. It is, to my knowledge, the first time that WBC is organized at a university and this important for two reasons. First to increase the level of the scientific contributions, and second for outreach: bamboo science should be taken seriously. In some sense, bamboo research is not cutting edge research. The genome is more complicated than *Arabidopsis*, controlled hybridization and genetic transformation is not yet possible, and so on, so there is not much glory to be gained with bamboo research. On the other hand, bamboo is a REAL plant, and a wild, natural resource, with many possible uses. Both from a biological point of view, and from a technological point of view, bamboo has in the past led to some great advances. Square bamboo even entered into mathematics through my own work.

Domes by BeBamboo

Previous WBC's have been held at private estates (Linda Garland, Bali & Prafrance for example) or in hotels or resorts. It is the first time that it is really organized at a university (University of Antwerp) with the explicit aim and hope that future WBC's will follow the same path. The reason to choose Belgium is a logical one: Belgium (esp. Flanders, northern part of Belgium), has become a real hothouse of bamboo research in the last decade Bamboo research today is done in almost every university and every university college today in Flanders and Belgium.

Geert Potters was appointed chair of the national organizing committee and Frances Schutte (program manager of IKEBANA) was appointed for organizational & financial work, with great help from Susanne Lucas of the World Bamboo Organisation (www.worldbamboo.net) on the promotional side (www.worldbamboo-congress.org). We built a strong scientific committee with Walter Liese, Lynn Clark, Azmy Mohammed, Rajani Nadgauda, Amita Pal, Tesfaye Hunde, David Midmore, Shozo Shibata, Chris Stapleton, Jinhe Fu, Ximena Londono, Pablo Vanderlugt, Geert Potters, Johan Gielis (chair). This gave top expertise in bamboo with a good geographical representation and gender balance.

All scientific papers were reviewed by two scientists and every reviewer got at most two papers (exceptions to the rule were Walter Liese and Geert Potters). More than 2/3 of the reviewers came from within the field of bamboo, and about 20% had no prior research in this field. 45% of the reviewers were Belgian, 55% from outside Belgium. Here we could use our own extensive academic network

Unlocking by Carla Feyen and Chiel Duran

for specific papers or topics including special statistics or molecular biology.

It has been an open review process, and the final level of papers is good in general, with some really excellent papers of young researchers, although initially we had hoped to receive more papers on molecular biology and on taxonomy. The proceedings were published before the congress on CD (Gielis and Potters, 2012) and we hope to have the best papers published in a book. One of the key aspects of the congress was the allocation of time. All presenters had 25 minutes or more. Our goal was not merely to bring people together to report, but to have in-depth interactions between scientists.

50 YEARS AGO, 50 YEARS AHEAD

The key theme of the congress was Bamboo – a 50 year perspective. Apart from the excellent level of the papers and presentations the idea was to position the current research in a long-term perspective. The congress opened with a great presentation of Prof. Marc Van Montagu on the future developments in genomics, which will transform agriculture and forestry in the next century. Goals to achieve for bamboo are, amongst others: breaking the code of flowering, and methods for genetic improvement of bamboo, including genetic transformation methods. Following a brilliant presentation of Lynn Clark on phylogeny and taxonomy (see also this issue) the opening of the congress also included a Bamboo Pioneers session. Profs. W. Liese, Wu and Watanabe started their career in the 1950's and their work underlines the value of fundamental and applied science. Without the seminal work of Prof. Liese on electron microscopical analysis of bamboo, for example, product development with bamboo could not have reached its current levels.

Going back 50 years and looking ahead another 50 years, is a humbling experience. I was born in 1962, exactly 50 years ago. My bamboo career is half my life: 25 years ago I started to work with Oprins Plant. The seminal book of Ueda on Bamboo Physiology was published in 1960 and I was five years old when McClure's book, Bamboos – a fresh perspective was published in 1967.

In the regular presentations we could witness the application of state-of-the-art technologies in plant physiology, much different compared to 20 years ago. We can now study biogenic volatiles from bamboo (Melnychenko and Rosenstiel, 2012), study kinetic profiles of phytohormones at picomole levels (Van den Akker *et al.*, 2012) and study potentially active components in bamboo (Van Hoyweghen *et al.*, 2012), with modern technology. We witnessed true advances in the application of molecular biology (BPG, 2012, and this issue), in bamboo transformation (Sood *et al.*, 2012), in material science (Van Vuure, 2012) and much more.

Speaking of modern technology: all keynotes and most presentations were live streamed. This allows researchers worldwide to experience the WBC presentations anywhere in the world, since many could not attend due to financial restrictions. Also, all presentations are still available for download (www.worldbamboo.net).

Still, there is a very long way to go, in a rapidly changing world where we have to face many challenges: climate change, food security, health, environment. With a rapidly growing world population, bamboo could be one of the plants that can become extremely valuable.

In this respect it is our duty as scientists – and perhaps the most important one – to deliver results and data that are reliable. All too often we hear about bamboo as the miracle plant, the golden bullet, the magical solution. We hear about fantastic yields of hundreds of tons of carbon sequestration by bamboo. As scientists we know that it is not possible for

Bamboo bridge by Frans Demedts

investments in bamboo to get returns of 500%. To go from science to applications in the field, to convince farmers and so on, we need to have reliable data, towards sustainable agriculture.

SCIENCE VALORISATION

The IXth World Bamboo Congress however, was not only about science, but also about valorization. We had a special session of South Asian Bamboo Foundation SABF (Kamesh Salam) and the INBAR session, organized by Jinhe Fu.

Secondly, we also organized a Part B of the WBC, with focus on Architecture, Design and Development. In part B, organized From 12-15 April 1012 at De Kolonie, Merksplas, many papers were presented on applications and aspects of development. And we not only look to the past, with the bamboo pioneers, and to the present, but also towards the future, both with respect to research as well as valorization in the 21st century. The Blue Economy session, with Gunter Pauli, founder of ZERI Zero Emission Research and Initiatives was superb.

John Neptune (Take Dake)

At this event BeBamboo (www.bebamboo.eu) built some great bamboo domes and we had some marvellous bamboo-art works of Georges Cuvillier (www.bamboost.be), Francesco Fransera, Carla Feijen and Luk Vermeerbergen. On Saturday we had music night, with Take Dake, an Indonesian Anklung Orchestra and Dr. H.S. Anasuya Kulkarni from South India on anklun. Spectacular fireworks were organized by Pierre Dhaenens (www.showflamme.be).

We can look back at a successful congress. Not only the level of the contributions made the congress into a success, but we had over 250 participants from a 38 different countries. Several international organisations supported the event. Apart from SABF and the World Bamboo Organisation, International Network for Bamboo and Rattan (with the organization of a special INBAR session), also the Food and Agricultural Organization FAO, and the International Union of Forestry Research Organizations IUFRO supported our congress.

The Congress was funded by the European Union co-sponsored by the Flemish government (Agentschap Ondernemen / Vlaanderen in Actie VIA), the University of Antwerp, the province of Antwerp, Oprins Plant, Ikebana and Genicap. We should also mention the presence of many bamboo pioneers, made possible by Susanne Lucas and Ecoplanet. Also Kamesh Salam helped with travel costs for Indian scientists. The American Bamboo Society awarded two travel grants of 1000$ to Francisca Ely and Andrea Melnychenko. Many volunteers assisted in making this conference into a success.

REFERENCES:

For references see Gielis and Potters (Eds) IXth World Bamboo Congress Proceedings. ISSN 2150-1165 (www.worldbamboo.net).

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