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EXPERIMENTAL TAXONOMY OF THE GIGANTOCHLOA ATTER-GIGANTOCHLOA PSEUDOARUNDINACEA COMPLEX

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ABSTRACT

Gigantochloa pseudoarundinacea, G. robusta, G. atter, G. atroviolacea and 14 other related Malesian species of bamboos were studied from preserved and living materials. Characters discerned by morphology, anatomy, chromatography and electrophoresis were analysed by numerical taxonomy. Most of the data indicated that the four species named above were distinct although closely related. The relationships of the remaining species were more complex, but the integrity of each species was established.

ABSTRAK

Gigantochloa pseudoarundinacea, G. robusta, G. atter, G. atroviolacea dan 14 jenis kerabatnya yang ada di Malesia dipelajari dari spesimen herbarium dan koleksi hidupnya. Ciri-ciri yang diambil dari morfologi, anatomi, kromatografi dan elektroforesis dianalisis dengan pendekatan taksonomi numeris. Data yang diperoleh menunjukkan bahwa keempat jenis tersebut di atas berbeda walaupun sangat erat berhubungan. Hubungan antar jenis lainnya ternyata lebih kompleks, tetapi integritas setiap jenis dimapankan.

INTRODUCTION

There has been much taxonomic confusion of species of the economically important bamboo genus *Gigantochloa* (Holttum 1958, Monod de Froideville 1968, Widjaja 1984). This has been partly due to inadequate herbarium specimens, since not only are these woody bamboos difficult to collect, but also vegetative structures are no longer available when the groves flowered. Furthermore only limited samples have been studied under cultivation and even less has been seen in the field by bamboo taxonomists themselves.

The taxonomic problems are well exemplified by *Gigantochloa atter* (Hassk.) Kurz and the three allied species, all of which are known only as

cultivated plants. Backer (1928), followed by Monod de Froideville (1968) concluded that *G. maxima* Kurz, *G. robusta* Kurz, *G. atter* and the so **called** its black variety *(G. atroviolacea* Widjaja of the present paper), were too closely related so that they treated them as one species, *G. verticillata* (Willd.) Munro (hereafter referred to as *G. pseudoarundinacea* (Steud.) Widjaja).

Experimental studies have been made of all available materials of **Malesian** species of *Gigantochloa,* using multi-disciplinary approaches as advocated by modern taxonomists, to support and extend the revisionary taxonomic study which is presented in a separate paper (Widjaja **1987).** Although cytogenetic investigations were not undertaken, studies of morphological, anatomical and biochemical characters, utilizing numerical taxonomy, have been accomplished.

MATERIALS AND METHODS

Observations were made on both living plants and preserved specimens. Field work was conducted in Malaysia, Sumatra, Kalimantan, Java, Bali and Sulawesi. Laboratory studies of 18 species were conducted in the herbaria at Bogor, Kew, Leiden, Singapore and Kepong, using specimens deposited at BO, K, L, SING, BM, DD, CAL, and KEP, as well as living plants of seven species growing in Bogor Botanic Gardens.

The nomenclature for all species mentioned in this paper followed that **adopted by** Widjaja (1987).

Morphological observations

Fifty-two characters of 177 accessions of 18 species were analysed **from** herbarium specimens. Characters of culm sheath, leaf blade, and inflorescence were measured. Four more culm characters were measured from living plants of other accessions of the same species.

Anatomical observations

A total of 37 characters of 33 accessions of 15 species were measured, based on leaf lamina epidermis, leaf lamina transverse section and culm epidermis. Since living plants were not always available, the anatomical characters were also taken from herbarium specimens. Specimens were observed by light microscope or by scanning electron microscope as **appropriate.**

Chromatography of **phenolic compounds**

Fully mature top leaves were collected from 24 plants (7 species) in Bogor Botanic Gardens. Each leaf was dried in a forced ventilation oven at 60°C for 2 — 3 days. Samples of 0.2 g were ground to a powder and washed in hexane to remove the chlorophylls, and dried. The powder was extracted in 2.5 cm 3 of *1%* v/v hydrochloric acid in methanol overnight in darkness.

Aliquots of 0.05 cm^3 of leaf extracts were separated by ascending two dimensional thin layer chromatography on cellulose. Chromatography was conducted in the first solvent (butan-1-ol: ethanoic acid: water = 3:1:1) for about 3 hours, then in the second solvent (ethanoic acid in water, 2% v/v) for 1 hour. The chromatograms were examined under UV light and UV light with ammonia fumes.

Electrophoresis of **proteins**

Electrophoresis of proteins from young shoots was done by polyacrylamide gel methods in acid gel rods (Davis 1964). About 10 g of the tips of fresh young shoots 7 cm high (about 7 days emergence) were sampled from 24 plants (6 species) in Bogor Botanic Gardens. After freezing and pounding the juices were collected and centrifuged. 1 ml samples of extract were analysed and after electrophoresis the gels were stained with naphthalene black. The distributions of the protein bands were examined over fluorescent light and recorded on a four-point density scale.

Data analyses

All data were analysed by numerical taxonomy using Principal Components Analysis, and clustering techniques by Euclidean Distance and Ward's method using Clustan 2.1 programmes (Wishart 1982). The chromotagraphic data were also binarily coded and analysed using Jaccard's coefficient and UPGMA.

RESULTS

Morphology

When all the 56 morphological characters were analysed by cluster analysis all accessions of each species had very great similarity, $d < 4.0$. The similarity between species was generally $d > 7.5$. At the level $d > 11.5$ the accessions clustered into 9 phenons : 1. *G. apus;* 2. *G. latifolia* was clustered closely to *G. manggong* and also to *G. pruriens,* which was closely clustered to *G. achmadii;* 3. *G. wrayi;* 4. *G. scortechinii* and *G.scortechinii* **var.** *albovestita;* 5. *G. rostrata* was clustered closely to *G. hasskarliana* and then G. *nigrociliata;* 6. G. *ligulata;* 7. G. *atter* was clustered closely to G. *atroviolacea;* 8. *G. levis* was clustered closely to G. *holttumiana;* 9. G. *robusta* **was** clustered together with G. *pseudoarundinacea.*

Cluster analysis using only the 26 inflorescence characters showed that two main clusters were clearly distinct, the one containing *G. apus*, G. *hasskarliana, G. manggong, G. wrayi, G. nigrociliata* and *G. rostrata,* and the other containing G. *atter, G. robusta, G. levis, G. atroviolacea, G. achmadii, G. latifolia, G. ligulata, G. pruriens, G. pseudoarundinacea,* and *G. scortechinii.* These two main groups are quite distinct in the width of the spikelets, and the colour of their anthers.

Principal Components Analysis using all morphological data (Fig. 1) dispersed the several species, but placed all members of each species together. *G. rostrata* was separated clearly by factor 1 which was mainly due to flower length characters. The second factor, which was due to culm size, flower shape and other characters separated *G. apus* on one side and *G. scortechinii* and *G. holttumiana* on the other. Further analyses, after removal of these four outliers, distinguished *G. pseudoarundinacea, G. atter, G. atroviolacea* and *G. robusta* at one end by the first factor and *G. wrayi, G. nigrociliata* and *G. hasskarlianai* at the other. By the second factor, *G. levis* was placed on one side and *G. ligulata* and *G. manggong* on the other. *G. latifolia, G. pruriens* and *G. achrnadii* remained in the middle.

The two main clusters of species shown by the cluster analyses were also separated in Brincipal Components Analysis, the one ranging from G. *apus* through *G. wrayi* to G. *rostrata,* the other ranging from G. *pseudoarundinacea* through G. *pruriens* to *G. scortechinii.*

The four taxa which have been confused under G. *verticillata* by many taxonomists working with herbarium specimens, in the present studies utilising data from both preserved and living specimens are all easily distinguished although these four taxa are similar. G. *pseudoarundinacea* and G. *robusta* are very distinct species; G. *atter* and G. *atroviolacea* are more similar, but still distinguishable by the palea apex shape and culm sheath ligule size as well as culm colour.

Likewise, although G. *latifolia* and G. *ligulata* are similar, they were shown as two distinct taxa by cluster analysis using all morphological characters or only using inflorescence characters. This contradicts Holttum's suggestion (1958) that these are merely parts of a variable complex: nevertheless observations in Thailand and Burma, where those species are originated, are still needed.

Anatomy

Analysis of the anatomical data, both by clustering and by Principal Components Analysis distinguished each species but indicated two main groups: the one included G. *apus, G. hasskarliana, G. atter, G. robusta, G. atroviolacea, G. ridleyi, G. manggong* and also G. *pseudoarundinacea,* the other included G. *levis* and G. *nigrociliata, G. ligulata, G. wrayi, G. scortechinii* and *G. pruriens* and also G. *achmadii.* There was partial concordance

between these two groups and those indicated by gross morphological studies.

The four species which have previously been confused were easily distinguished. *G. pseudoarundinacea* had lower values than the other three on both factors. Further Principal Components Analysis showed that G. *robusta* was distinguished by lower values than *G. atroviolacea* on factor 1, whereas G. *after* had lower values on factor 2.

Chromatography

In total, 30 spots were identified. The spots were characterized by their Rf values and colours. Only spot 6 (Rfi = 0.86, Rf£ = 0.37) was detected from all species, and may be a tricin flavonoid, as found by Harborne & Williams (1976) in *G. pseudoarundinacea.* The spot 8 was found in all species except *G. levis,* hence this absence may be considered as characteristic for that species.

Principal Components Analysis showed the distinctness of each species, except for G. *robusta* which overlapped with *G. atter* and G. *pseudoarundinacea.* This was confirmed by the cluster analysis using Jaccard's coefficient, which avoids the distortion caused by conjoint absences of spots, and UPGMA, where each species appeared as a distinct phenon, except for *G. robusta* being intermingled with G. *pseudoarundinacea* and G. *hasskarliana. G. atroviolacea* and G. *atter* were distinct but linked together.

Electrophoresis

Clear electrophoretograms with some strong bands and several weaker bands were obtained from almost all shoot tip protein samples; only a few were too faint or dark to allow easy analysis. Altogether 16 different bands were detected and recorded objectively, of which 6 occured in all taxa, although at different intensities.

Principal Components Analysis and cluster analysis, whether by Euclidean Distance on numeric data or Jaccard's coefficient on binary coded data, all produced similar results (Fig. 2). G. *hasskarliana* was clearly distinct from the other species; G. *atroviolacea* and *G. atter* were distinct but closely linked, and likewise G. *apus, G. robusta* and G. *pseudoarundinacea* were distinct but fairly closely linked.

Subjective assessment of the electrophoretograms, considering the general pattern of the stronger bands, indicated that both G. *apus* and G. *hasskarliana* were distinct. However, G. *pseudoarundinacea, G. atroviolacea, G. atter* and *G. robusta* were more similar, especially some individuals of the last two species, which again indicates that these are four closely related, though still distinct species.

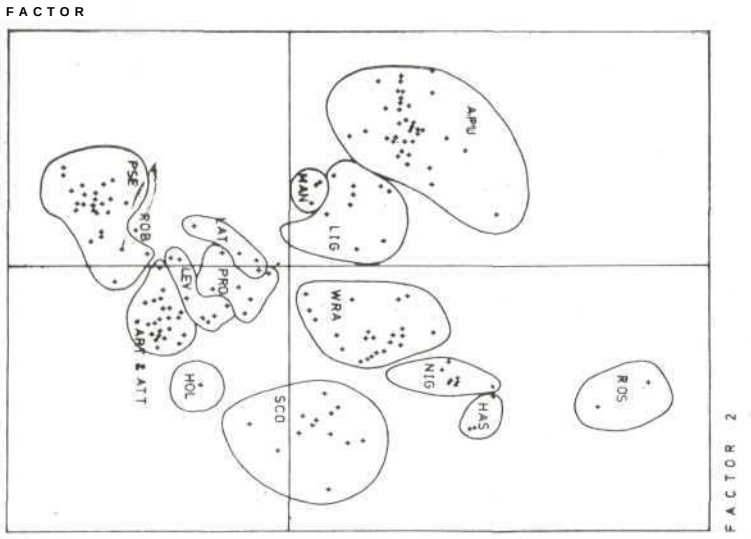

Fig. 1. Principal Components Analysis on morphology of *Gigantochloa* (17 species).

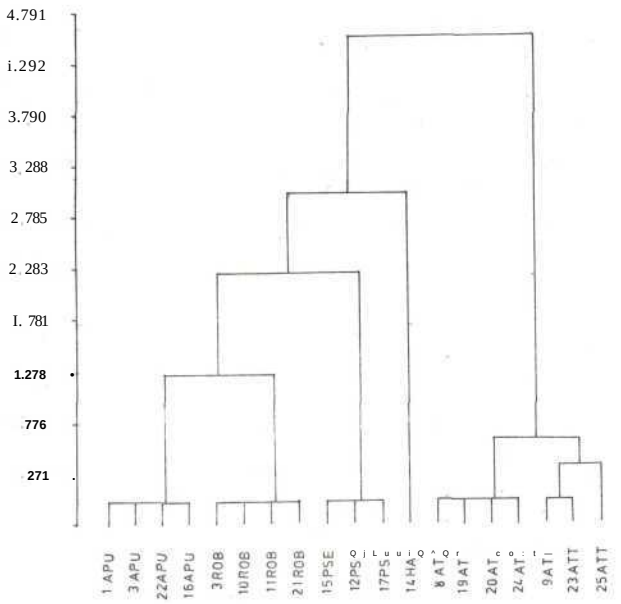

Fig. 2. Cluster analysis on *Gigantochloa* shoot electrophoresis.

DISCUSSION

All species of *Gigantochloa* included in the present study are distinct. This was clearly demonstrated after analysis of morphological data by numerical taxonomy, and also indicated by some of the other evidence from electrophoresis, chromatography, anatomy, morphology and inflorescence. The results of all these studies suggest more or less indicative phenetic relationships, but little can be deduced of the phyletic relationships or evolutionary trends within the genus *Gigantochloa.*

G. pseudoarundinacea was studied by all the methods used in this work, and in each case similarity between all accessions was high and distinction from almost all other species was shown. The morphological study suggested that *G. robusta* was close to *G. pseudoarundinacea.* This was supported by chromatography, and also slightly by electrophoresis but not by inflorescence or anatomy data. This similarity of these two species is mainly on anther colour, widely ovate spikelets, green with yellow striped culms, brownish hairs on upper parts of the internodes, and in chromatography. Close study of these species show that they are easily separated by differences of the lodicules, palea apex, hairy or glabrous spikelets, filament tubes, culm sheath auricle, bristle, leaf surfaces, culm sheath ligules and leaf sheath auricles. On anatomy and inflorescence these species were close to *G. atter* in having the same number of epidermis cells, papillae, fusoid cells, widely ovate spikelets, yellow anthers, long hairy anther tips and no lodicules. If one looks at the morphology of those species, both have very distinct characters such as palea apex, bristle of culm sheath auricle, bristle of leaf sheath auricle, leaf hairiness, culm colour and also some anatomical characters such as long cells on culm epidermis and waviness of the epidermis wall of the long cells. Great similarity was shown between *G. atter* and *G. atroviolacea* in morphology, inflorescence, chromatography and electrophoresis observa'tions but not in the anatomy investigation. The differences in anatomy were seen in epidermis files, the length of epidermis cells on culm epidermis and the sinuousity of the epidermis wall. Moreover the differences are also shown by the apices of their paleas, the tips of their anthers, the culm sheath auricles and the culm pigmentation. This evidence showed that these two species are distinct.

Although by Principal Components Analysis of the morphological data *G. rostrata* was well separated from the swarm of species composed of *G. hasskarliana, G. nigrociliata* and *G. wrayi,* all of these were clustered together on the dendrogram. On the dendrogram from the inflorescence data, the latter three species were intermingled, but G. *rostrata* was not linked so clearly. *G. rostrata* and *G. hasskarliana* are very similar in some features of general morphology and inflorescence characters but they are both different in some other characters such as leaf sheath auricle and dark brown hairs on their lemma. Based on the similarity of leaf sheath auricle, spikelet charac-

ters and hairy leaf, *G. rostrata* is similar to *G. nigrociliata.* This view disagrees with the evidence of the other morphological observations which showed that G. *rostrata* was closer first to *G. hasskarliana* and then to *G. nigrociliata.* Holttum (1958) did not find any *G. nigrociliata* in the Malay Peninsula, so he indicated the similarity of *G. rostrata* (which was referred to as *G. maxima* var. *minor)* to *G. hasskarliana.* On anatomy and inflorescence, *G. hasskarliana* was very distinct from *G. nigrociliata* due to the hairiness of the lower leaf surface, papillae, epidermis files, number of vascular bundles on adaxial side, leaf sheath auricle and hairiness of lemmas. *G. scortechinii* formed a discrete cluster; it was placed near to but distinct from the species discussed above. Morphologically it can be distinguished by many characters.

When *G. apus* was studied by each method it proved to be a very distinct species. It showed some affinity to *G. hasskarliana* on inflorescence characters and anatomy but not in electrophoresis, morphology or chromatography. This similarity is due to their both having magenta coloured anthers, narrowly ovate spikelet, non-dark brown hair on lemma, auricle of culm sheath, leaf sheath auricle, glabrous leaf, number of fusoid cells and number of minor vascular bundles.

The similarity in anther colour, widely ovate spikelets, and long bristles on the culm sheath ligule suggested that *G. levis* was close to *G. holttumiana.* In fact, they are different because G. *levis* does not have lodicules, but has a notched palea, slightly hairy spikelet, hairy leaf on the lower surface, waxy culm and rounded culm sheath auricle, whereas G. *holttumiana* has lodicule, pointed palea, glabrous spikelets and leaf, no wax on culm, and rim-like culm sheath auricle. By chromatography, *G. levis* was close to G. *hasskarliana* due to their having spots 6, 10 and 11 in common, but other studies did not support a close relationship. After some species were taken away, *G. levis* appeared close to G. *robusta* on inflorescence and also fairly close on morphology. In anatomy, however, G. *levis* was very distinct from the other species.

G. latifolia, which clustered closely with G. *manggong* and G. *pruriens* on the basis of general morphology, was close to *G. ligulata* on inflorescence characters. Both species have yellow anthers and widely ovate spikelets which might place them closely together. This view supports Holttum's work on bamboo of the Malay Peninsula (1958). G. *latifolia* is very distinct from G. *manggong* in several characters such as the number of spikelets (2 for G. *latifolia* and 3 for G. *manggong),* anther colour (yellow in G. *latifolia* and maroon to dark magenta in G. *manggong)* and culm sheath ligule (more than 10 mm in G. *latifolia* but less than 5 mm in G. *manggong).* In the morphological observations *G. latifolia* is close to G. *manggong* due to their having erect and deltoid blades so that the junction line between sheath and blade becomes inconspicuous.

G. pruriens was linked to *G. achmadii* on the morphology, but this, species was linked to *G. ligulata* and then to G. *latifolia* on the inflorescence study. On morphology, the similarity of G. *achmadii* and *G. pruriens* was very high, which was based mainly on spikelet, culm sheath and leaf sheath characters. However, in the inflorescence study these two species were very distinct and G. *pruriens* was slightly closer to G. *ligulata* and G. *latifolia*, rather than to G. *achmadii,* due to palea apex differences. The inflorescence characters put G. *pruriens* close together with *G. ligulata* and G. *latifolia.* On anatomy, G. *pruriens* has a hairy leaf lower surface as does G. *scortechinii,* and also papillae patterns surrounding the stoma. Because of this evidence, it appears that these two species are close to one another; but they would not be close on morphology due to their having different morphological characters such as waxiness of culm, hairiness of the culm, culm sheath, leaf sheath and spikelet characters.

Numerical taxonomy of morphological, anatomical as well as chemotaxonomic characters proved the integrity of most species and also showed the distinctness of some species *(G. pseudoarundinacea, G. apus, G. scortechinii, G. ligulata, G. rostrata, G. levis* and G. *wrayi).* This study showed affinities within complex groups such as G. *atter* and G. *atroviolacea; G. manggong, G. latifolia, G. pruriens* and G. *achmadii;* and G. *rostrata, G. hasskarliana* and *G. nigrociliata.*

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REFERENCES

BACKER, C.A. 1928. *Handboek voorde Flora van Java* 2 : 273-277.

DAVIS, B.J. 1964. Disc electrophoresis II. Method and application to human proteins. *In* Ann. New York Acad. Sci. 121 : 404-442.

HARBONE, J.B. & WILLIAMS, C.A. 1976. *In* Biochemical Systematics and Ecology 4 : 267

HOLTTUM, R.E. 1958. The bamboos of the Malay Peninsula, in Gard. Bull. Sing. 16 : 104-401.

MONOD DE FROIDEVILLE, CH. 1968. Poaceae. *In* BACKER, C.A. & BAKHUIZEN V.D. BRINK Jr., R.C. *Flora of Java.* 3 : 634-637.

WIDJAJA, E.A. 1984. *Gigantochloa (Bambusoideae, Poaceae) in Malesia.* Ph.D. Thesis, Department of Plant Biology, University of Birmingham, Birmingham, England.

WIDJAJA, E.A. 1987. A revision of Malesian *Gigantockloa* (Poaceae — Bambusoideae) *In* Reinwardtia 10 (3) : 291 — 380.

WBHART, D. 1982. *CLUSTAN 2.1. Supplement.* Program Library Unit. University of Edinburgh, Edinburgh, England.

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