The *Vigna vexillata* (L.) A. Rich. gene pool

M. Garba[1] and R.S. Pasquet[2]

[1] Ecole Normale Supérieure, Université Abdou Moumouni, BP 10963 Niamey, NIGER
[2] ORSTOM, 213 rue La Fayette, 75480 Paris, FRANCE.

ABSTRACT

Vigna vexillata (L.) A.Rich. is a tuberous legume which is interesting for several reasons. *V. vexillata* is the species closest to the cowpea *Vigna unguiculata*. Amongst other agronomic characters of interest, *V. vexillata* seeds are resistant to cowpea weevil, one of the main pests of cowpea. So, the first research topic is to transfer, by hybridization or genetic transformation these resistance genes to the cowpea. But *V. vexillata* is also a species cultivated for its tuberous root-stock, mainly in Asia, and this should be another research topic.

Though the gene pool of cowpea is now well-known, the *V. vexillata* gene pool has not been the focus of any specific study yet. The current taxonomic treatments show that *V. vexillata* includes about ten varieties mainly separated by their leaf shape and pilosity, and *V. vexillata* is surrounded by several closely related species (*V. nuda* in Zambia, *V. lobatifolia* in Namibia).

In the present study, 128 accessions were characterized through their morphology and their isoenzymatic polymorphism. This was completed by an exhaustive herbarium survey.

Morphological study shows five groups among *V. vexillata*:

- var. *angustifolia* - var. *linearis* with narrow leaflets,
- coastal south-african var. *ovata* with small round leaflets,
- Zimbabwe accessions with short peduncles,
- American and other African accessions,
- cultivated accessions with larger organs (leaves, pods, seeds).

Otherwise, isoenzymatic study shows that the most remote accessions are Asian accessions. A clear and presumed old cut occured between East Asian and African *V. vexillata*.

Closely related species *Vigna lobatifolia* is also genetically remote from remaining *V. vexillata*, but close enough to be included in *V. vexillata*.

On the other hand, based on isoenzymatic profiles, Australian accessions are more closely related to some African forms than to other Asian forms. Although they both share close narrow leaflets, Sudanian / West African var. *angustifolia* is clearly separated from Australian var. *linearis*. American accessions are homogenous and appear similar to some Central African accessions, suggesting a recent introduction in the New World.

Geographic groups, though not morphologically obvious, appeared distinct through isoenzymatic analysis, e.g. a Kivu-Rwanda group, a Guinean / West African group. Thus, for African V. vexillata, there is a strong similarity between the organization of the *V. vexillata* and the *V. unguiculata* gene pools. Though current data suggests that *V. unguiculata* and *V. vexillata* have shared a common evolutionary pathway, *V. vexillata* gene pool appears to be less hierarchically organized and structured than *V. unguiculata* gene pool.

RESUMEN

Vigna vexillata (L.) A. Rich. es una legumbre tuberosa interesante por dos razones:

a) *V. vexillata* es la especie más relacionada al caupí, *Vigna unguiculata* (L.) Walp. Entre otras características agronómicas interesantes hay que mencionar que las semillas de *V. vexillata* son capaces de resistir el gorgojo del caupí, una de las principales plagas de dicho cultiva. Algunos equipos de investigación están tratando de transferir estos genes resistentes al caupí por hibridación o transformación genética; y

b) *V. vexillata* es cultivado también por el stock de raíces tuberosas, principalmente en Asia.

Aunque la organización de pool de genes del caupí está bien entendida, con base en trabajos biosistemáticos recientes, *V. vexillata* no ha sido enfocada en estudios específicos. En el tratamiento taxonómico actual, *V. vexillata* incluye aproximadamente diez variedades clasificadas primeramente con base en la forma de hoja y la pilosidad. *V. vexillata* está también emparentada con varias especies muy relacionadas (*V. nuda* N.E. Br. en Zambia, *V. hundtii* Rossberg en Angola, *V. lobatifolia* Bak. en Namibia).

En la presente investigación 128 accesiones fueron caracterizadas en base a morfología y polimorfismo isoenzimático. Esta trabajo es el resultado de una investigación exhaustiva en herbario.

Los estudios morfológicos mostraron cinco grupos de *V. vexillata*:

1. - var. *angustifolia* (Schumach. & Thonn.) Bak. y var. *linearis* Craib con hojas angostas,

2. - var. *ovata* (E. Mey) Pienaar de la costa de Africa del Sur, con pequeñas hojas circulares,
3. - accesiones de Zimbabwe, con pedúnculos cortos,
4. - accesiones cultivadas con órganos más grandes (hojas, vainas, semillas).
5. - accesiones americanas y africanas.

Los estudios isoenzimáticos mostraron que las accesiones menos emparentadas fueron las asiáticas. Parece haber existido en el pasado una separación clara entre *V. vexillata* del este de Asia y la *V. vexillata* africana.

Las especies cercanas de *Vigna lobatifolia* están también genéticamente alejadas de la *V. vexillata* que aún persiste; sin embargo, puede ser incluida dentro *V. vexillata.*

Por otra parte, las accesiones australianas están más relacionadas a algunos tipos africanos que a los tipos asiáticos. Pero la var. *angustifolia* del Africa del Occidente está claramente separada de la var. *linearis* de Australia, ambas de hojas angostas. Las accesiones americanas son homogéneas y relacionadas a algunas accesiones de Africa Central, sugeriendo una introducción al nuevo mundo.

Grupos geográficos morfológicamente similares parecieron distintos a través de análisis iso-enzimáticos, pero similares a los grupos encontrados en el pool de genes del caupí; por ejemplo, un grupo de Kivu-Rwanda, un grupo de Guinea y de Africa Occidental. Esto sugiere que para *V. vexillata* africana hay una correlación considerable entre la organización de pool de genes de *V. vexillata* y *V. unguiculata. V. unguiculata* subsp. *dekindtiana* (Harms) Verdc. (*sensu stricto*) corresponde a la *V. vexillata* pirofítico de Zambia y Angola, subsp. *tenuis* (E. Mey) M.M. & S. corresponde a *V. vexillata* de Zimbabwe, y subsp. *baoulensis* corresponde a la *V. vexillata* de Guinea del Africa Occidental.

Parece que las dos especies han tenido el mismo camino evolutivo, aunque los tipos africanos de *V. vexillata* son menos diferenciados en el nivel molecular y morfológico que *V. unguiculata* silvestre.

INTRODUCTION

Vigna vexillata (L.) A.Rich. is a twining vine or prostrate herb, widely distributed in tropical Africa, Asia and Australia, which produces large, thickened edible tubers. *Vigna vexillata* is also regarded as a pasture cover crop, green manure and erosion control plant.

Another interesting aspect of *V. vexillata* lies in its proximity with *V. unguiculata.* (Vaillancourt *et al.*, 1993). Both flowers and pods are similar. Differences are in stipule and in keel shapes. The keel shapes (Figure 1) lead to a nototribic pollination in *V. vexillata* and to a sternotribic pollination in *V. unguiculata* (Hedström & Thulin, 1986).

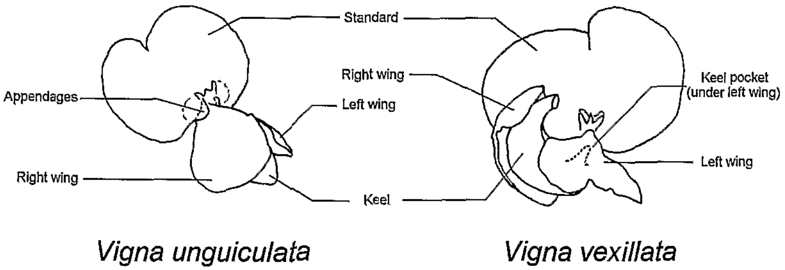

Figure 1. *Vigna unguiculata* and *V. vexillata* flowers.

Amongst other agronomic characters of interest, *V. vexillata* seeds are resistant to cowpea weevil, one of the main pests of cowpea (Birch *et al.*, 1985; Birch *et al.*, 1986). So, the main research topic is to transfer, by hybridization or genetic transformation, these resistance genes to cowpea, even if successful crossing of both species has not yet been done (Fatokun, 1991; Barone *et al.*, 1992).

Unfortunately, both complex species are poorly characterized. Until Pasquet's work (1993a, 1993b), knowledge of the gene pool of *V. unguiculata* came from Verdcourt's (1970) herbarium work. In the same way, *V. vexillata* classification is still primarily that of Verdcourt (1970) (Maréchal *et al.*, 1978; Babu *et al.*, 1987; Pienaar & Kok, 1991)

In order to understand *V. vexillata* gene pool organization, we undertook an herbarium survey and a morphological and isoenzymatic study of available *V. vexillata* accessions.

All pink flowered Vigna species with a pocketed keel and a long beak were pooled in the subgenus *Plectrotropis* (Schum.) Verdc. by Verdcourt (1970) and Maréchal *et al.* (1978).

In this subgenus, there are additional taxa besides *V. vexillata*: *V. nuda* N.E.Br., *V. wightii* Bedom. (unfortunatly both without available accessions for this study) and *V. lobatifolia* Bak.

On the other hand, inside *V. vexillata*, the taxonomic classification is much more confusing than in *V. unguiculata*. Nearly forty taxa relevant to *V. vexillata* were described by botanists, which can be explained by the wider geographic distribution of *V. vexillata*. Many taxa were described from Asian or American material.

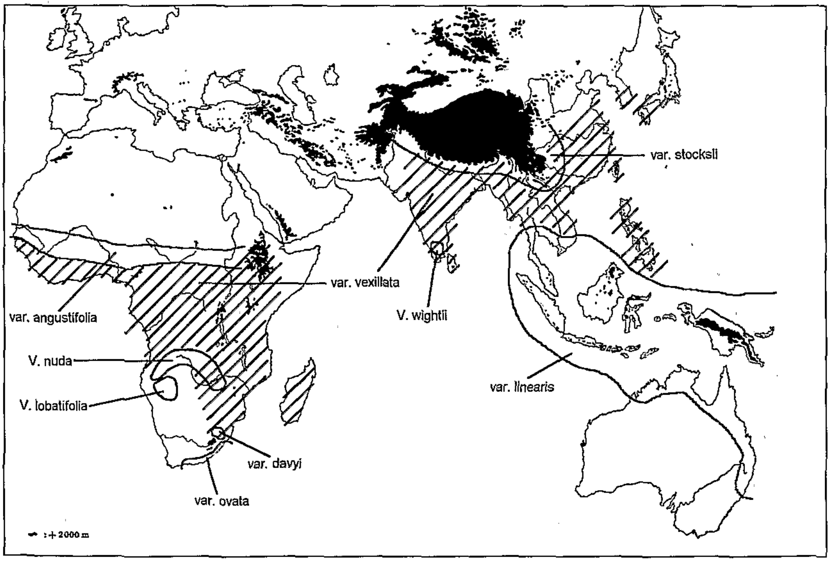

Figure 2. Geographic distribution of subgenus *Plectrotropis* taxa.

For the purpose of this study, we will list a few taxa well characterized by their leaf morphology (Figure 2):

- var. *stocksii* Benth. ex Bak. (Himalayan forms with small leaflets),
- var. *davyi* (Bolus) Pienaar (unifoliolate forms from Swaziland and Transvaal),

- var. *ovata* (E. Mey.) Pienaar (coastal forms from South Africa),
- var. *wightii* (Benth. ex Bak.) Babu & Sharma (South Indian forms with thick leaflets),
- var. *linearis* Craib. (coastal forms from South East Asia and Australia, morphologically close to var. *angustifolia* (Schumach. & Thonn.) Bak. from West Africa).

Maréchal *et al.* (1978) also distinguish:

- var. *macrosperma* M.M.S. for cultivated forms,
- var. *angustifolia* (glabrous forms with short calyx-lobes and narrow leaflets, in dry savannas from Sudanian / West and East Africa),
- var. *vexillata* (pubescent forms with longer calyx lobes),
- var. *dolichonema* (Harms) Verdc. (extreme forms of the previous variety, in east African highlands).

MATERIAL AND METHODS

Herbarium specimens from more than 20 institutes (BM, BR, CAL, COI, E, EA, FI, G, GH, K, L, LISC, M, NY, P, PE, PRE, SRGH, US, W, WU, Z) were surveyed.

Living accessions (128) were also studied. Accessions were issued from the IPGRI base collection of Phaseoleae maintained at the National Botanic Garden of Belgium, Meise or from the ORSTOM collection which is now duplicated at Meise. Each accession is made of one to three autogamous lines, and maintained as such, each of these lines coming from one seed of the original stock.

The morphological study was conducted with field grown plants cultivated in Niamey, Niger. Characters analyzed were recorded from leaves (size and shape), inflorescences (peduncle dimensions, number of nodes), flowers (size, color, insect guide pattern), pods (size and number of ovules) and seed (dimensions).

Isozyme studies were conducted with 19 enzyme systems, representing 32 loci (Pasquet, 1993b).

RESULTS

Herbarium survey - There were fewer usable characters in *V. vexillata* than in *V. unguiculata*, as keel shape is not variable in *V. vexillata*. Measurements included the number of ovules, calyx-lobes length, leaf shape and pubescence.

As observed by the previous authors, var. *angustifolia* is easily characterized by glabrous and short calyx lobes and narrow leaflets. Overall, ovule number separates East

Asian specimens (with low ovule number) from African, Australian and American specimens (with high ovule number). Most Indian specimens display high ovule number. This was not noted by previous authors.

Morphology - Morphological studies of living accessions are not yet finished, but five groups are already highlighted:

- var. *angustifolia* and var. *linearis* (from Australia)
- var. *ovata*
- Zimbabwe forms with short peduncles
- cultivated var. *macrosperma* with large seeds, pods and leaflets
- remaining African and American forms

As with dried specimens, we observed less variability in living accessions from *V. vexillata* than from *V. unguiculata. V. vexillata* does not show variability in keel shape and displays lower levels of polymorphism in standard insect color guides and standard appendages.

Isozyme polymorphism - Nei's distances UPGMA (Figure 3) shows a gene pool less hierarchically organized and structured than wild *V. unguiculata*'s one. With the exception of the Indian accessions, which are markedly separated from the other accessions, the genetic distances are much smaller than those encountered in *V. unguiculata*.

After the Indian accessions, UPGMA analysis separates American and some Guinean West African accessions (from Togo to Cameroon). Next, we find a unique Ethiopian accession characterized by an arillate seed (exceptional in *V. vexillata* gene pool), *V. lobatifolia* from Namibia, cultivated var. *macrosperma*, some southern African accessions, a group including West African var. *angustifolia* and some accessions from southern Zaire's savannas. Among accessions in the top of the tree, var. *linearis* from Australia is clearly distinguishable but distant from Sudanian / West African var. *angustifolia*. Both groups are homogeneous but clearly distinct. At the top of the tree, the var. *ovata* accession does not display a unique isozyme pattern.

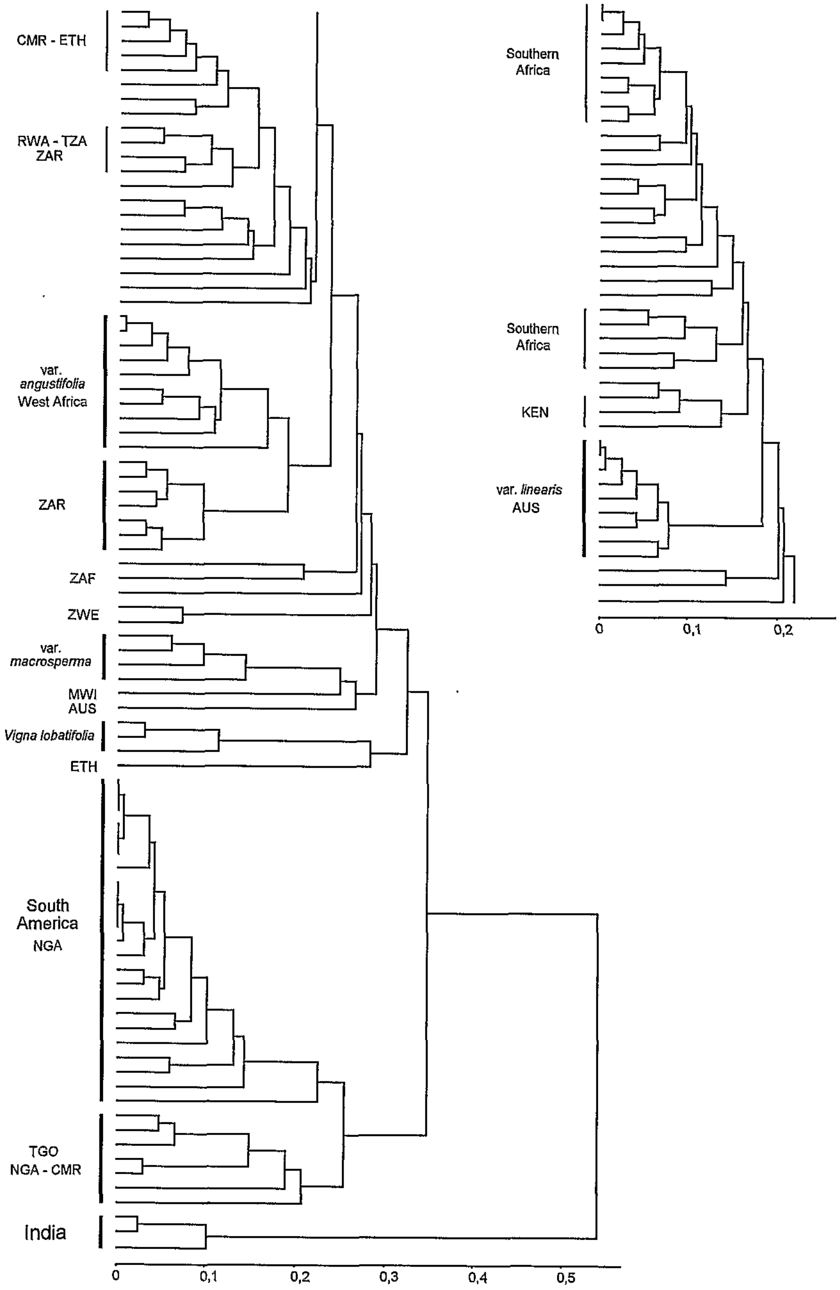

Figure 3. Dendrogram obtained after isozyme polymorphism analysis using Nei's distance UPGMA method.

DISCUSSION

These results lead to several taxonomic conclusions. Asian *V. vexillata* should be considered as a distinct subspecies, even if our three accessions are poorly representative of the whole Asian *V. vexillata* (they show an ovule number intermediate between African *V. vexillata* and *V. vexillata* specimens from China). But, *V. lobatifolia* should be merged with *V. vexillata*.

There are also some biogeographical considerations. American *V. vexillata* materials are strongly linked with Guinean / West African *V. vexillata* accessions, especially the ones from Nigeria. American *V. vexillata* is obviously an historical introduction from the Slave Coast. A similar situation could account for Australian *V. vexillata*, but East African var. *angustifolia* (which could link var. *linearis* and West African var. *angustifolia*) is too poorly represented in this study.

In Africa, there are strong similarities in the organization of the *V. unguiculata* and *V. vexillata* gene pools.

- *V. nuda* and *V. unguiculata* subsp. *dekindtiana* (Harms) Verdc., both pyrophytic taxa, display exactly the same crescent shaped geographic distribution, from Angola to Zimbabwe through Zambia.
- Similarly, *V. vexillata* var. *ovata* and coastal pubescent *V. unguiculata* subsp. *stenophylla* (Harv.) M.M.S. share the same territory in Southern Africa.
- The group of *V. vexillata* with short inflorescence and *V. unguiculata* subsp. *tenuis* (E.Mey.) M.M.S. share the same territory in Zimbabwe and Zambia.
- One accession from Tanzania displays a unique type of pilosity similar to *V. unguiculata* subsp. *pubescens* (R.Wilczek) Pasquet, encountered in the same area.
- In both *V. unguiculata* and *V. vexillata* studies, the first African group separated by the Nei's distance UPGMA is a group from Guinean / West African distribution.
- Accessions with unique isozyme patterns come from highland areas (Ethiopia, Malawi, Transvaal) where perennial *V. unguiculata* is encountered.
- Some poorly separated *V. vexillata* groups could be linked with other perennial *V. unguiculata* subspecies, for instance: *V. vexillata* from southern Zaire with *V. unguiculata* subsp. *letouzeyi* Pasquet, the *V. vexillata* from Kivu and Rwanda with *V. unguiculata* subsp. *burundiensis* Pasquet.

It seems that, as the supposed recent extension of the annual and often weedy *V. unguiculata* var. *spontanea* (which hybridizes with all perennial taxa) made the different perennial taxa evolution scheme less clear, the extension of possibly weedy *V. vexillata* could have blurred the organization of the *V. vexillata* complex.

After this study a major enigma still remains. Many lowland Asian *V. vexillata* herbarium specimens come from cultivated plants. All these specimens show large pods and seeds, such as the accessions from India. Nevertheless, the var. *macrosperma* accessions studied here are isozymatically related to African *V. vexillata* and not to Indian accessions. The var. *macrosperma* accessions studied display round seeds and pods with high ovule number, instead of longer than wide seeds and low ovule number of most Asian herbarium specimens and the three Indian accessions.

This suggests that a domestication process occured in Asia and another domestication process occured in Africa; or as a result of in breeding seasons, where *V. vexillata* was bred as a fodder or cover crop, because all these var. *macrosperma* accessions have poorly characterized origins.

CONCLUSION

Unfortunately, this study highlights the lack of available accessions from important parts of the *V. vexillata* gene pool: *V. nuda,* peri-himalayan var. *stocksii* and Asian var. *vexillata* from China, Korea and Japan.

However, this study allows a first glimpse into the organization of the *V. vexillata* gene pool. It shows many similarities between the *V. vexillata* and the *V. unguiculata* gene pool organizations, but *V. vexillata* also displays an interesting variability in Asia.

The utilization of this variability could lead to important improvement in *V. vexillata* as a root crop or perhaps as a seed crop. The utilization of this variability could also be interesting for the purpose of interspecific crosses between *V. unguiculata* and *V. vexillata.* Both species display remote accessions that could lead to some highly heterozygous and disequilibrated progenies which could be crossed more successfully.

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REFERENCES

Babu, C.R., Sharma, S.K. & Johri, B.M., 1987. Leguminosae-Papilionoideae: tribe Phaseoleae. Bull. Bot. Surv. India 27-1/4: 1-28.

Barone, A., Delgiudice, A. & Ng, N.Q., 1992. Barriers to interspecific hybridization between *Vigna unguiculata* and *Vigna vexillata*. Sex. Plant Reprod. 5-3: 195-200.

Birch, N., Southgate, B.J. & Fellows, L.E., 1985. Wild and semi-cultivated legumes as potential sources of resistance to bruchid beetles for crop breeders: a study of *Vigna/Phaseolus*. In: Wickers, G.B., Goodie, J.D. & Field, D.A. (eds) Plants for arid lands. George Allen and Unwin, London. Pp. 303-320.

Birch, A.N.E., Fellows, L.E., Evans, S.V. & Doharty, K., 1986. Para-aminophenylalanine in *Vigna*: possible taxonomic and ecological significance as a seed defence against bruchids. Phytochemistry 25 (12): 2745-2749.

Fatokun, C.A., 1991. Wide hybridization in cowpea: problems and prospects. Euphytica 54 (2): 137-140.

Hedström, I. & Thulin, M., 1986. Pollination by a hugging mechanism in *Vigna vexillata* (Leguminosae-Papilionoideae). Pl. Syst. Evol. 154: 275-283.

Maréchal, R., Masherpa, J.M. & Stainier, F., 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28: 1-273.

Pasquet, R.S., 1993a. Classification infraspecifique des formes spontanées de *Vigna unguiculata* (L.) Walp. à partir de données morphologiques. Bull. Jard. Bot. Nat. Belg. 62 (1/4): 127-173.

Pasquet, R.S., 1993b. Variation at isozyme loci in wild *Vigna unguiculata* (L.) Walp. (Fabaceae, Phaseoleae). Plant System. Evol. 186 (3/4): 157-173.

Pienaar, B.J. & Kok, P.D.F., 1991. The *Vigna vexillata* complex (Fabaceae) in Southern Africa. S. Afr. J. Bot. 57 (5): 236-245.

Vaillancourt, R.E., Weeden, N.F., Bruneau, A. & Doyle, J.J., 1993. Chloroplast DNA phylogeny of Old World *Vigna* (Leguminosae). Syst. Bot. 18 (4): 642-651.

Verdcourt, B., 1970. Studies in the Leguminosae-Papilionoideae for the Flora of Tropical East Africa. IV. Kew Bull. 24: 507-569.