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# Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis

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**Summary.** The taxonomy of the genus *Vigna* has been primarily based on morphological attributes. We have used 27 genomic clones from soybean, common bean, mungbean and cowpea to examine restriction fragment length polymorphism (RFLP) among 44 accessions of different species belonging to four subgenera of the genus *Vigna*. One accession each of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) was included in the study. Total DNA from the various genotypes was digested with one restriction enzyme (*Eco*RV). Results of a numerical taxonomic analysis showed a high level of genetic variation within the genus with a remarkably higher amount of variation associated with *Vigna* sp. from Africa relative to those from Asia. The distinctness of the Asiatic grams in subgenus *Ceratotropis*, cowpea in section *Catiang*, bambara groundnut (*V. subterranea*) and members of the subgenus *Plectotropis* was elucidated by this study. Members of the subgenus *Plectotropis* were closer in genome homology to those of subgenus *Vigna* section *Catiang* than to those of subgenus *Ceratotropis*. The relative positions of some genotypes to one another on the dendrogram and minimum spanning tree were discussed in regard to hybridisations aimed generating well-saturated genomic maps and interspecies transfer of desirable genes.

**Key words:** *Vigna* – Numerical taxonomy – RFLP – Asiatic grams – Cowpea – Bambara groundnut

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## Introduction

The genus *Vigna* Savi has been subdivided into eight subgenera (Verdcourt 1970), two of which contain the important tropical grain legumes, cowpea [*Vigna unguiculata* (L.) Walp.], bambara groundnut [*V. subterranea* (L.) Verdc.] and mungbean [*V. radiata* (L.) Wilczek]. Cowpea and bambara groundnut belong to subgenus *Vigna*, with cowpea in section *Catiang* (DC) Verdc. and mungbean and other Asiatic *Vigna* species in subgenus *Ceratotropis* (Piper) Verdc. Until recently, current numbers of the genus *Vigna* were classified into three different genera: *Phaseolus* L., *Vigna* and *Voandzei*. However, the revised and enlarged taxonomy by Verdcourt (1970) and Marechal et al. (1978) as it is presently constituted is not entirely satisfactory, especially since it contains both ancient and more recently evolved phylogenetic lineages (Smartt 1990). The placement of individuals into the various subgenera, sections, species and subspecies has been primarily based on morphological attributes, but additional parameters are required to supplement the morphological traits so far used in order for the present controversy on the taxonomic status of this genus to be resolved.

Evidence for genome relationships in *Vigna* has not been described. Cytologically, most members of the genus have $2n = 22$ chromosomes, except for *V. ambacensis* Bak., *V. heterophylla*, *V. reticulata* Hook. f., and *V. wittei* Bak. f., which have $2n = 20$ each (Verdcourt 1970). *Vigna glabrescens* ($2n = 4x = 44$) is the only known natural amphiploid in the subtribe *Phaseolinae* (Marechal et al. 1978). Interspecies hybridizations have been attempted in the genus, but with limited success being reported only among some members of the subgenus *Ceratotropis* (Dana and Karmakar 1990). No viable hybrids have been obtained following crosses

between members of different subgenera of the genus *Vigna*.

Molecular taxonomic procedures can give unambiguous interpretations of relationships between species that would not have been possible with morphological attributes and crossability studies (Palmer et al. 1988). In *Vigna*, however, there is a dearth of reports on molecular taxonomic studies. According to Verdcourt (1970), studies conducted by Dr. E. A. Bell on free amino acids along with some related compounds in the seeds of several members of the genus revealed electrophoretic patterns that were distinctive enough to characterise individual species. D'Urzo et al. (1990) studied the seed globulin-1 fraction (G1) among 13 different *Vigna* species using SDS-polyacrylamide gel electrophoresis and detected variations in band patterns among some of them. These studies on protein band patterns were restricted to a few genotypes that did not cover a wide range of the variability within the genus. Moreover, data obtained were not analysed to show taxonomic relationships between them. Jaaska and Jaaska (1988) reported isozyme variations between the genera *Phaseolus* and *Vigna* of Phaseolineae.

Restriction fragment length polymorphisms (RFLPs) have become useful tools in determining relationships among members of various taxa. RFLPs have been used successfully to study genomic relationships in *Arachis* (Kochert et al. 1991), *Brassica* (Song et al. 1988), *Lycopersicon* (Miller and Tanksley 1990) and *Solanum* species (Debener et al. 1990). Nuclear RFLPs, in particular, are powerful in elucidating phylogenetic relationships because they have the added advantage of being a virtually unlimited source of characters that can effectively discriminate between genotypes, subspecies and species (Debener et al. 1990). On the basis of RFLP data subjected to phylogenetic analysis, it was possible for Song et al. (1988) to assert that an ascending order of chromosome numbers played a major role in the evolution of *Brassica* and related species.

We report here the results of a study using RFLPs to analyse members of the genus *Vigna* and the molecular numerical taxonomic relationships among them. The study clearly separated the Asiatic *Vigna* species of subgenus *Ceratotropis* from the African *Vigna* species distributed in several subgenera, three of which are occupied by materials used in this study.

**Table 1.** List of accessions from genus *Vigna*, *Phaseolus* and *Glycine* used in the numerical taxonomic study

| Plant no. | Identification no. | Species | ssp | var | Plant no. | Identification no. | Species | ssp | var |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PI 164725 | *V. radiata* | | | 30 | TVsu 136 | *V. subterranea* | | |
| 2 | PI 164725 | *V. radiata* | | | 31 | TVsu 334 | *V. subterranea* | | |
| 3 | PI 164725 | *V. radiata* | | | 32 | TVsu 1033 | *V. subterranea* | | |
| 4 | PI 164725 | *V. radiata* | | | 33 | TVu 14314 | *V. unguiculata* | | |
| 5 | PI 164725 | *V. radiata* | | | 34 | TVu 14314 | *V. unguiculata* | | |
| 6 | PI 4718 | *V. radiata* | | | 35 | TVu 14314 | *V. unguiculata* | | |
| 7 | PI 298913 | *V. radiata* | | | 36 | TVu 14314 | *V. unguiculata* | | |
| 8 | Texsprout | *V. radiata* | | | 37 | TVu 14314 | *V. unguiculata* | | |
| 9 | TC 1966 | *V. radiata* | *sublobata* | | 38 | PI 354799 | *V. unguiculata* | | |
| 10 | PI 157625 | *V. angularis* | | | 39 | PI 184953 | *V. unguiculata* | | |
| 11 | PI 416739 | *V. angularis* | | | 40 | IT 2246-4 | *V. unguiculata* | | |
| 12 | PI 223521 | *V. aconitifolia* | | | 41 | CA #5 | *V. unguiculata* | | |
| 13 | PI 269522 | *V. mungo* | | | 42 | TVu 10280 | *V. unguiculata* | *unguiculata* | textilis |
| 14 | PI 220249 | *V. umbellata* | | | 43 | TVNu 1963 | *V. unguiculata* | *dekindtiana* | dekindtiana |
| 15 | TVNu 1043 | *V. umbellata* | | | 44 | TVNu 271 | *V. unguiculata* | *dekindtiana* | protracta |
| 16 | TVNu 3 | *V. ambacensis* | | ambacensis | 45 | TVNu 110-3A | *V. unguiculata* | *dekindtiana* | pubescens |
| 17 | TVNu 187 | *V. ambacensis* | | pubigera | 46 | TVNu 1411 | *V. unguiculata* | *dekindtiana* | mensensis |
| 18 | TVNu 1461 | *V. davyi* | | | 47 | TVNu 266 | *V. unguiculata* | *dekindtiana* | stenophylla |
| 19 | TVNu 534 | *V. frutescens* | *frutescens* | frutescens | 48 | PI 406352 | *V. unguiculata* | *oblongifolia* | |
| 20 | TVNu 528 | *V. kirkii* | | | 49 | PI 286439 | *V. unguiculata* | *sesquipedalis* | |
| 21 | PI 365093 | *V. luteola* | | | 50 | TVNu 888 | *V. unguiculata* | *tenuis* | |
| 22 | TVNu 95 | *V. racemosa* | | | 51 | TVNu 1351 | *V. unguiculata* | *tenuis* | ovata |
| 23 | TVNu 149 | *V. reticulata* | | | 52 | TVNu 73 | *V. vexillata* | | vexillata |
| 24 | TVsu 736 | *V. subterranea* | | | 53 | TVNu 594 | *V. vexillata* | | vexillata |
| 25 | TVsu 736 | *V. subterranea* | | | 54 | TVNu 93 | *V. vexillata* | | angustifolia |
| 26 | TVsu 736 | *V. subterranea* | | | 55 | TVNu 64 | *V. vexillata* | | macrosperma |
| 27 | TVsu 736 | *V. subterranea* | | | 56 | TVNu 319 | *V. venulosa* | | |
| 28 | TVsu 736 | *V. subterranea* | | | 57 | Pinto 111 | *Phaseolus* | | |
| 29 | TVsu 6 | *V. subterranea* | | | 58 | Glenwood | *Glycine* | | |

## Materials and methods

*Plant materials*

Seeds of 44 different genotypes representing subgenera *Vigna*, *Ceratotropis*, *Plectotropis* (Schum.) Bak. and *Haydonia* (Wllczek) Verdc. of the genus *Vigna* and one genotype each of soybean [*Glycine max* (L.) Merr.] and common bean (*Phaseolus vulgaris* L.) were sown in pots containing a soil/perlite mixture. Each genotype was sown separately in a pot. In order to evaluate the extent of variability within field-collected genotypes of mungbean, cowpea and bambara groundnut, an accession of each of these crops was selected and 5 plants of each of the selected genotypes were established to give a total of 58 plants, subsequently referred to as operational taxonomic units (OTUs; Table 1) for the numerical taxonomic study. The seeds of different *Vigna* genotypes were obtained from the Southern Regional Plant Introduction Station, Experiment Station, Georgia, and the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. At the seedling stage plants were thinned to 1 per pot. The leaves were harvested from plants at 6 weeks after sowing and quickly frozen and ground before the DNA was extracted using a modification of the technique of Dellaporta et al. (1983).

*Restriction digests, electrophoresis and Southern blotting*

One restriction enzyme, *Eco*RV, was used to digest genomic DNA obtained from all of the OTUs. The digested DNA was electrophoresed on 1% agarose gel and transferred onto a Hybond $N^+$ membrane (Amersham Corporation) using a modification of Southern (1976).

*DNA clones and hybridizations*

Three different genomic libraries were the sources of RFLP markers (Table 2). These markers were selected because they represent identified loci on the cowpea and mungbean genomic maps. Hybridization procedures were the same as those described earlier (Fatokun et al. 1992).

*Data analysis*

Each autoradiogram was scored for the presence (1) or absence (0) of a specific restriction fragment in the OTUs. A data matrix was generated and entered into an IBM PC-AT for analysis using sequential, hierarchical and nested (SAHN) clustering methods of the NTSYS-PC programme (Rohlf 1990). This programme works by first producing a similarity matrix from the data matrix and subsequently constructing a dendrogram via the unweighted pair-group average method with arithmetic averages (UPGMA). A minimum spanning tree was also constructed from the nearest neighbour values derived from the similarity matrix.

**Table 2.** List of genomic clones used to detect RFLP variation in the genus *Vigna*

| Probe | Plant source | Probe | Plant source |
|---|---|---|---|
| *pA77* | Soybean | *pK411* | Soybean |
| *pA235* | Soybean | *pK443* | Soybean |
| *pA262* | Soybean | *pK472* | Soybean |
| *pA333* | Soybean | *pK644* | Soybean |
| *pA386* | Soybean | *p4D9* | Commonbean |
| *pA407* | Soybean | *pM182* | Mungbean |
| *pA816* | Soybean | *pM208* | Mungbean |
| *pA841* | Soybean | *pM209* | Mungbean |
| *pA878* | Soybean | *pM211* | Mungbean |
| *pB39* | Soybean | *pM228* | Mungbean |
| *pK69* | Soybean | *pO29* | Cowpea |
| *pK227* | Soybean | *pO103* | Cowpea |
| *pK265* | Soybean | *pQ22* | Mungbean |
| *pK266* | Soybean | | |

## Results

*Band patterns*

The 40 genomic clones that were used in this study hybridized to the DNA of all the 44 accessions belonging to genus *Vigna* and the one genotype each of *Phaseolus vulgaris* and *Glycine max*. These clones are located on 10 linkage groups already identified in cowpea and 9 in mungbean. For the numerical taxonomic analysis, only 27 of the clones (Table 1) were scored because these showed single or very few bands per genotype tested. Some clones showed single-copy bands with some genotypes but low-copy repeats in others (Fig. 1). Such clones, however, were not analysed in this study.

There were 369 individual bands observed from the 27 markers scored, of which 113 (31%) were unique, while the remaining 256 (69%) were shared by at least 2 different OTUs. Of the unique bands 20 (18%) were characteristic of soybean (*G. max*). The single genotypes which represented *P. vulgaris*, *V. mungo* (L.) Hepper, *V. venulosa*, *V. oblongifolia* A. Rich., *V. frutescens*, *V. davyi* Bolus, *V. racemosa*, *V. kirkii* Bak. and *V. reticulata* Hook. f. each had between 5% and 8% of the unique bands. No single band was shared by all of the 58 OTUs involved in this study.

*Numerical analysis*

*Dendrogram.* The NT-SYS clustering programme produced a dendrogram (Fig. 2) on which 27 steps could be identified. At the highest rank on the dendrogram, which coincided with a 62% similarity level, soybean separated from the other OTUs. This observation is an indication of the relatively low level of similarity between the genomes of soybean, a member of the subtribe Glycineae, and the '*Phaseolus-Vigna*' complex of the subtribe Phaseolineae. Palmer et al. (1988) reported the presence of an entire large single-copy region of cpDNA that separated mungbean and common bean from soybean. This single-copy region is inverted in mungbean and common bean relative to soybean, an event which occurred after the '*Phaseolus-Vigna*' complex and soybean diverged from a common ancestor.

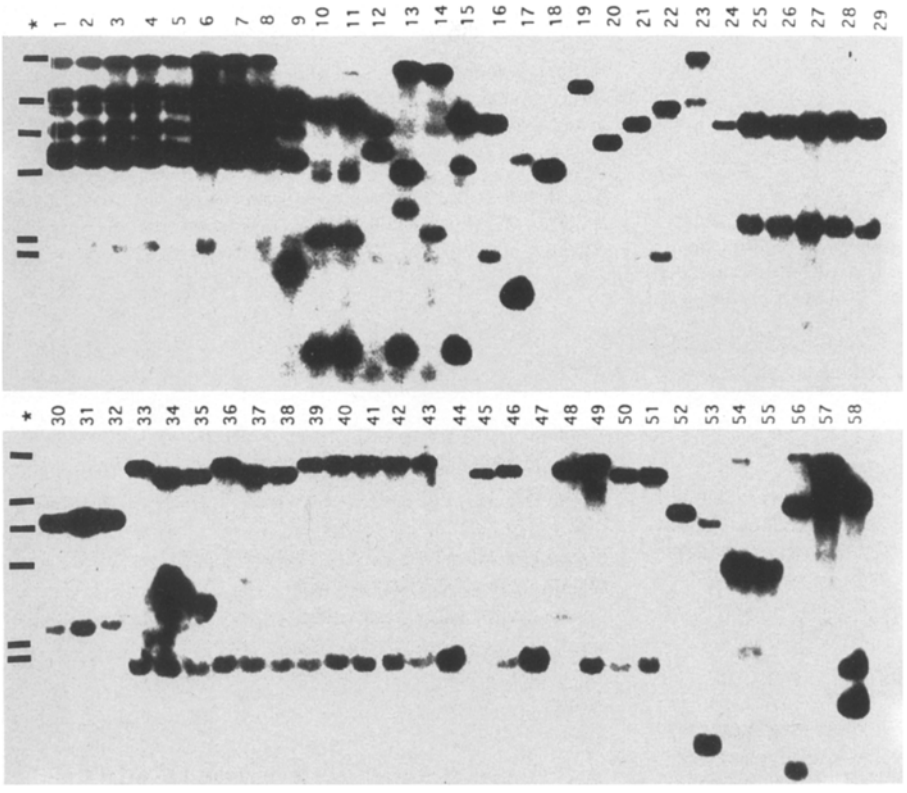

**Fig. 1.** Autoradiogram of *Eco*-RV-digested DNA from different genotypes belonging to genus *Vigna* (*lanes 1–56*) and from one genotype each of common bean (*lane 57*) and soybean (*lane 58*) hybridized to probe *p4D10* (not scored for numerical taxonomic study). *Lanes with *Hin*dIII-digested lambda DNA

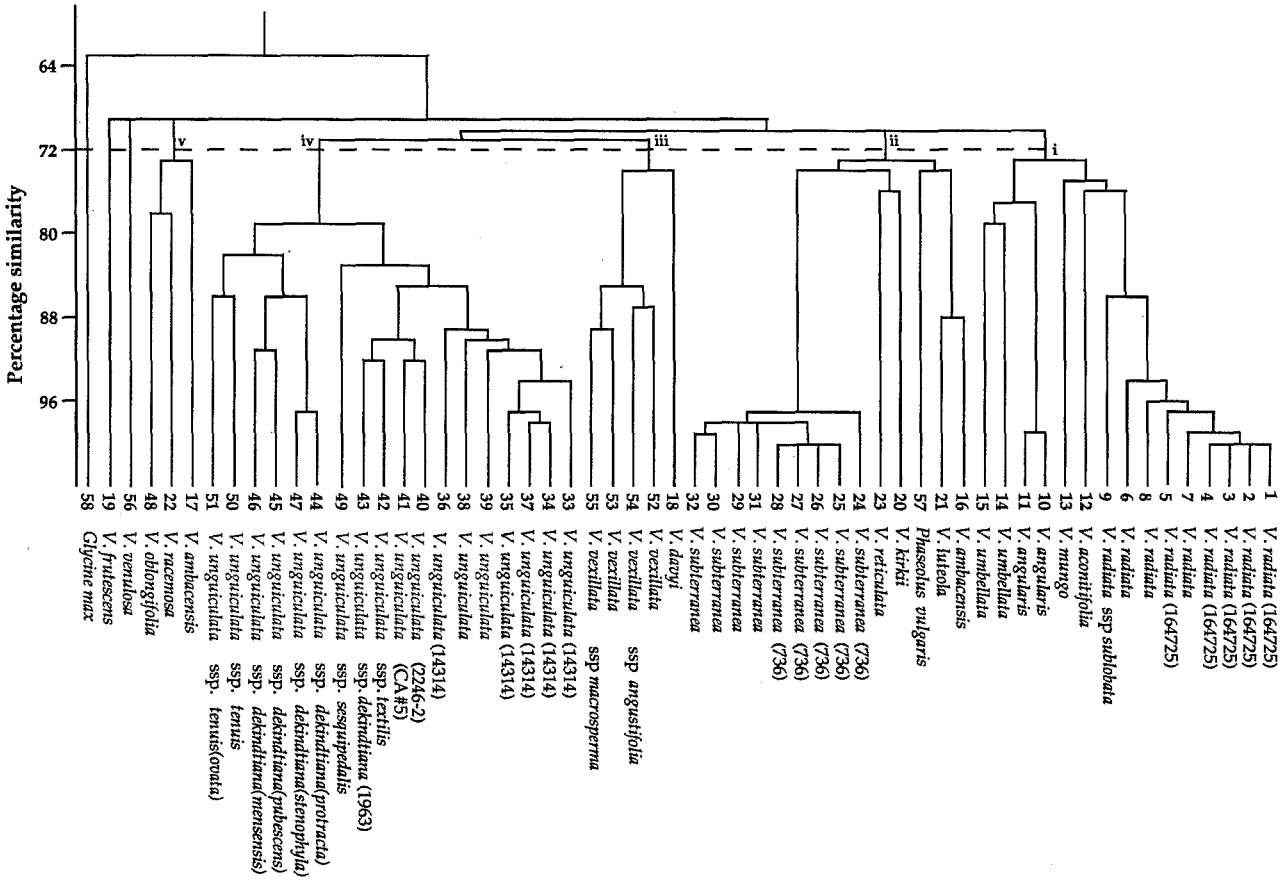


**Fig. 2.** A dendrogram of 58 OTUs comprising 44 accessions of genus *Vigna* and one each of *Phaseolus vulgaris* and *Glycine max*

At the next lower rank on the dendrogram, i.e. the 72% similarity level, 7 additional lineages could be recognised (5 are labelled on the dendrogram): 2 of these had 1 OTU each (*V. frutescens* and *V. venulosa*), 1 had 3 OTUs and the remaining 4 contained between 5 and 18 OTUs each. The separation of the single representatives of *V. venulosa* and *V. frutescens* from other *Vigna* species at such high rankings on the tree can be explained by the fact that the former belongs to subgenus *Haydonia* section *Glossostylus* Verdc. while *V. frutescens* belongs to subgenus *Vigna* section *Liebrechtsia* (De Wild.) Bak. f. on the basis of the established taxonomy of the genus, none of the other 42 *Vigna* accessions used in this study belong in these sections. The existence of some small, related subgenera in the genus had been recognised earlier by taxonomists. According to Marechal et al. (1981) the subgenus *Haydonia* appers to be a relatively recent evolutionary trend expressed by the loss of some *Vigna* characteristics.

When the dendrogram was truncated at the 72% similarity level, distribution of the OTUs into the five clusters containing 3 or more members showed interesting trends as described below:

*Cluster 1.* All of the 11 accessions representing the Asiatic *Vigna* species, which are also members of subgenus *Ceratotropis*, clustered to form this group. Two sub-clusters were discernible on the dendrogram: one consisted of *V. angularis* L. and *V. umbellata* (Thunb.) Ohwi and Ohashi, while the other had *V. radiata*, *V. radiata* ssp. *sublobata*, *V. mungo* and *V. aconitifolia* (Jacq.) as members (Fig. 2). Following crossability, hybrid fertility and chromosome pairing studies, Dana (1980) also identified these same two groups in subgenus *Ceratotropis*. The 4 accessions of *V. radiata* used in this study showed close taxonomic relationships. Among the 5 plants established from seeds of 1 of these four mungbean accessions (PI 164725), in order to evaluate within-accession variation, 4 showed 100% similarity between them, while the 5th differed slightly by showing 98% similarity with the others. This accession is an unimproved collection from a field in India, and since mungbean is highly self-pollinating, it is, therefore, not surprising to find such a high level of homology among these plants. Closest to the mungbean (*V. radiata*) accessions on the dendrogram was their wild relative, *V. radiata* ssp. *sublobata*, while *V. aconitifolia* and *V. mungo* were next in proximity.

An accession of *V. umbellata* (TVNu 1043) collected from somewhere in southern Africa clustered with the *V. umbellata* (PI 220249) from Asia.

*Cluster 2.* This comprised 10 accessions including all of the geocarpic *V. subterranea*. This cluster is the least uniform because of low levels of similarities between some of its members. It contained genotypes which belong to different subgenera and even included the only *P. vulgaris* accession considered in this study.

The 5 accessions representing *V. subterranea* showed a high level of taxonomic relationship among themselves as they formed a neat group that separated from the other members of this cluster at a high rank on the dendrogram (Fig. 2). A very high level of relationship (100% similarity) was observed in 4 of 5 plants grown from 1 of these accessions; the 5th plant, however, differed slightly from the others.

*Vigna luteola* and *V. ambacensis* var 'ambacensis', both members of subgenus *Vigna*, section *Vigna*, were closely located on the dendrogram and formed the bridge linking *P. vulgaris* with *Vigna*. This association of *P. vulgaris* with these lesser *Vigna* species on the dendrogram is not unusual. In fact, after studies on the morphological parameters of South American *Phaseolus* and *Vigna* species, Marechal et al. (1978) stated that the interpenetration of characters in the species of these subgenera is remarkable. The *Phaseolus* accession we used is 'Pinto', which is a land race of South America (J. V. Groth, personal communication). The notion of a '*Phaseolus-Vigna*' complex, suggested by earlier workers on the taxonomy of these genera, is thus further strengthened by the results of our study.

*Cluster 3.* This comprised 5 genotypes, all belonging to the subgenus *Plectotropis*. The 4 accessions of *V. vexillata* (L.) A. Rich. studied were placed together in this group, which also included the only accession of *V. davyi* Bolus. *Vigna kirkii* Bak., taxonomically regarded as belonging to subgenus *Plectotropis*, was however, placed on the dendrogram near *V. reticulata* (in the second cluster mentioned above).

*Cluster 4.* This consisted of all of the 14 accessions of subgenus *Vigna*, section *Catiang* that were included in this study. A high level of genetic variability among the accessions in this cluster was detected on the phylogenetic tree (Fig. 2). *Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc. was the first to be isolated from other members of this group. Two subgroups could be identified among the genotypes in this cluster. The cultivated cowpea (*V. unguiculata*) formed one of these two groups along with *V. unguiculata* ssp. *textilis* (E. Westphal) and an accession of *V. unguiculata* ssp. *dekindtiana* (Harms) Verdc. The second subgroup was made up of 4 different varieties of *V. unguiculata* ssp. *dekindtiana* and 2 varieties of *V. unguiculata* ssp. *tenuis* (E. May) M.M.&S.

The 5 plants shown from one cowpea accession (TVul14314) showed a high level of variability, especially when compared with the 5 plants from the single mungbean or bambara groundnut accession. It is worth noting that no 2 individuals among the 5 plants showed 100% similarity (Fig. 2), although morphologically, they could not be differentiated from one

another. The differences between these plants were detected at the DNA level and pointed to one of the unique advantages of RFLP in distinguishing among cultivars. Some of the probes which detected these differences should be potentially useful in cowpea germ plasm characterisation and management as well as in DNA fingerprinting for patenting and protecting improved cultivars. Cowpea, like mungbean and bambara groundnut, is highly self pollinating. Differences observed within accessions can be attributed to a mixture of seeds or variations caused by mutations or cross pollination. However, because none of the 5 plants showed any unique bands, which is an indication of their heterozygousity, it could be inferred that the observed differences are a result of natural variation. That there exists a wide range of genetic diversity in cowpea in tropical Africa, its centre of origin, is supported by this observation since the accession TVu14314 was collected from a site in West Africa.

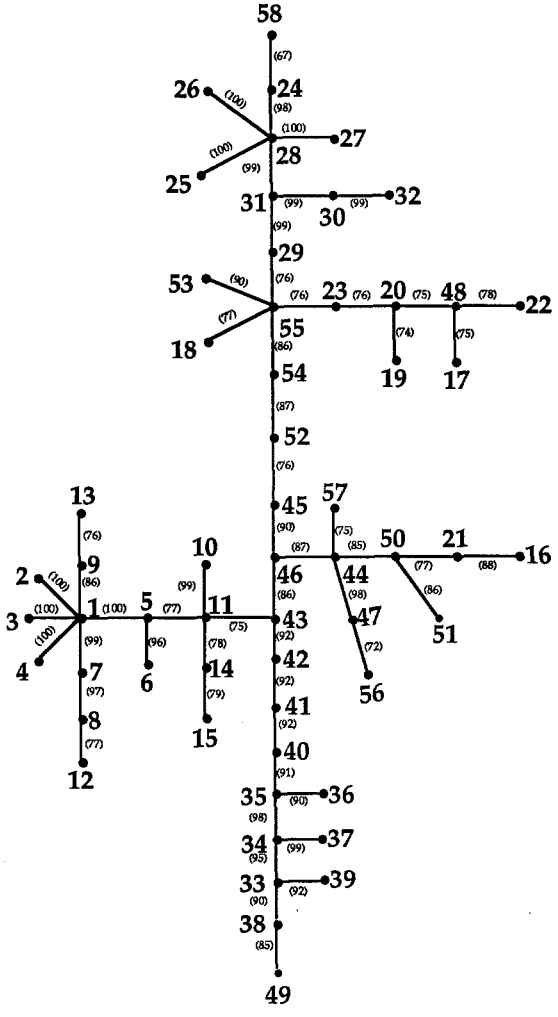


**Fig. 3.** A minimum spanning tree (MST) displaying the 58 OTUs in relation to their nearest neighbours

*Cluster 5.* This comprised 3 genotypes (*V. ambacensis* var '*pubigera*' Bak., *V. oblongifolia* and *V. racemosa*), all belonging to subgenus *Vigna* section *Vigna*. A low level of genome homology was, however, associated with these 3 genotypes.

*Minimum spanning tree*

The degree of similarity, based on RFLPs, among the genotypes used in this study as indicated by the dendrogram and the MST is similar. The minimum spanning tree (MST; Fig. 3) also separated the Asiatic *Vigna* species from the African *Vigna* species, and groups delineated by the dendrogram could be identified on the MST. In addition, the MST showed the extent of relationships within each subcluster and between adjacent OTUs, i.e. nearest neighbours. For example, among members of subgenus *Ceratotropis*, similarity values between nearest neighbours ranged from 96% to 100% and from 98% to 100% among *V. subterranea* genotypes. A comparatively higher level of variability among members of section *Catiang* was also confirmed by the MST (Fig. 3), which showed that similarity between nearest neighbours within the subcluster ranged from 85% (OTUs 38 and 49; OTUs 44 and 50) to 99% (OTUs 34 and 37). On the MST, *V. kirkii* was close to other members of the subgenus *Plectotropis*. It was separated from the nearest *V. vexillata* by 1 OTU, *V. reticulata*.

## Discussion

Our RFLP analysis of members of the genus *Vigna* followed by numerical taxonomic procedures gives an independent assessment of the phylogenetic hypotheses suggested by Verdcourt (1970) and Marechal et al. (1978). The present study provides further evidence in support of the separation of Asiatic and African members of the genus *Vigna* as proposed by Verdcourt (1970) and Marechal et al. (1978) on the basis of morphological attributes. A remarkably narrower range of genetic variability was detected among the Asiatic *Vigna* species of subgenus *Ceratotropis* than among the African *Vigna* species, the latter being distributed into several subgenera and sections. This observation represents additional evidence in support of the generally held view that Africa is the centre of diversity of *Vigna*. The Asiatic Vignas, comprising 11 species of which 7 are domesticated, therefore, evolved more recently (Smartt 1990). In general, the results of our study showed taxonomic relationships in *Vigna* that are consistent with recently established classifications.

It has been suggested that *V. mungo* and *V. radiata* have a common ancestor in *V. radiata* ssp. *sublobata* (Purseglove 1968). The locations of these 3 genotypes relative to one another on the dendrogram and MST

show that they are closely related but do not seem to support an ancestral (basal) position for ssp. *sublobata*. Further, both the dendrogram and MST demonstrate that *V. radiata* ssp. *sublobata* is closer to *V. radiata* than to *V. mungo*. The progenitors of *V. angularis* and *V. umbellata* are not known, but both were domesticated in the Far East: in particular in Japan for *V. angularis* and in Indo-China for *V. umbellata* (Lukoki et al. 1980). Wild forms of these species do exist.

*Vigna unguiculata* ssp. *sesquipedalis* showed high phyletic relationships with cultivated cowpea and its wild relative ssp *dekindtiana*. Both cowpea (*V. unguiculata*) and ssp. *sesquipedalis* are products of a post-domestication evolution of *V. unguiculata* in different parts of the world. Whereas the African use of cowpea as pulse remained unchanged over time, ssp. *sesquipedalis* became established as a long-podded vegetable in Asia (Smartt 1985). Selection practised for succulent and fleshy pod types among *V. unguiculata* introduced to Asia, especially in India, gave rise to the present-day yard-long bean (*V. unguiculata* ssp. *sesquipedalis*). The strong selection pressure that has been exerted on this crop in India would have modified it, thus explaining why it was the first to be isolated from other members of subgenus *Vigna* section *Catiang*. For example, it was an outlier on the MST and showed 85% similarity with its nearest neighbour.

*Gene pools*

Mungbean (*V. radiata*) is perhaps the most commonly grown of the Asiatic *Vigna* species. Because of this, attempts have been made in various laboratories to enhance its productivity through the introgression of desirable genes from other Asiatic *Vigna* species. Successes have been achieved when interspecies' hybridizations were carried out between mungbean and members of various other species in *Ceratotropis*. On the basis of reproductive affinity between these different species, it was possible to classify them into gene pool categories. For mungbean (*V. radiata*), *V. radiata* ssp. *sublobata* and *V. mungo* constitute the primary and secondary gene pools, respectively, while *V. umbellata* and *V. angularis* make up the teritary gene pool (Smartt 1990). The positions of these *Vigna* species relative to mungbean on the numerical taxonomic trees produced in this study lend support to this classification. *Vigna angularis* and *V. umbellata*, both of which make up one of two subclusters identified in subgenus *Ceratotropis*, have been reported to hybridise successfully and produce $F_1$ plants that are characterised by high pollen fertility (Ahn and Hartmann 1978).

In cowpea, however, no secondary or tertiary gene pool has been identified. The cultivated cowpea is cross-compatible with the other members of the section *Catiang* that constitute its primary gene pool (Baudoin and Marechal 1985). Taxonomically, members of the subgenus *Plectotropis* are close to subgenus *Vigna* section *Catiang*. Baudoin and Marechal (1988) have suggested that *V. vexillata* in subgenus *Plectotropis* is the intermediate species between African and Asiatic *Vigna* species. The results of our study, however, show that members of subgenus *Plectotropis* are closer to those of subgenus *Vigna* section *Catiang* than to the Asiatic *Vigna* species. Cowpea is particularly susceptible to several insect pests that cause extensive yield losses. Genes for resistances to some of these pests have been identified in various accessions of the highly pubescent *V. vexillata*. Attempts at transferring these genes to cowpea using conventional crossing procedures have been made, but with no success. At best, when crosses are made between the two species using *V. vexillata* as the seed parent, embryos which do not develop beyond the globular stage result (Fatokun 1991). On the MST, however, the slightly pubescent *V. unguiculata* ssp. *dekindtiana* var 'pubescens' serves as the link between members of subgenus *Vigna* section *Catiang* (cowpea and members of its primary gene pool) and those of subgenus *Plectotropis*. This observation has prompted us to grow several accessions of *V. unguiculata* ssp. *dekindtiana* var 'pubescens' and *V. vexillata* for the purpose of making crosses aimed at transferring genes for insect resistance from the latter to the former. If this cross is successful, it should then be possible to transfer the desirable genes to cowpea since it is compatible with *V. unguiculata* ssp. *dekindtiana* var 'pubescens' (Fatokun and Singh 1987).

*Taxonomic relationship and polymorphism*

Taxonomic or phylogenetic relationships that are based on RFLP analysis could be quite useful in selecting parents to be crossed for generating appropriate populations intended for genome mapping. The more distantly related two sexually compatible individuals are taxonomically, the higher the frequency of polymorphism detected between them. In essence, a genomic map well-saturated with markers could be assured within a relatively short period of time. An RFLP map for cowpea is being developed in out laboratory using an $F_2$ population derived from a cross between IT2246-2 and TVN1 963 (both are present on the dendrogram and MST). Four hundred genomic DNA clones have been hybridised to DNA from these 2 genotypes, and approximately 22% have shown polymorphisms between them. On the basis of the relative positions of these 2 genotypes on the trees, this low level of polymorphism is no surprise. A higher level of polymorphism will be detected, for example, between IT2246-2 and TVNu 110-3A (*V. unguiculata*

ssp. *dekindtiana* var 'pubescens'). An $F_2$ mapping population derived from a cross between these 2 genotypes should, therefore, make the identification of RFLP marker loci easier.

The taxonomic relationships among genotypes of the genus *Vigna* based on RFLP, as revealed by this study, should be a clear indication of evolutionary relationships between them because only one restriction enzyme (*Eco*RV) was used to digest total DNA from all of the OTUs. Genetic variations detected by RFLPs are known to be due mostly to point mutations and different types of DNA rearrangements. These can be detected by enzymes whose recognition sites are affected or which bind the sites where rearrangements have occurred. RFLPs uncovered by same probe but more than one restriction enzyme may not necessarily represent independent mutational events (Miller and Tanksley 1990). In the view of Miller and Tanksley (1990) the ideal in phylogenetic or taxonomic studies is that all detected mutations should be independent.

## Conclusion

The results of our study provide further evidence in support of the present taxonomic status of the genus *Vigna*, although the number of genotypes considered did not cover the entire range of variation in the genus. The distinctness of cowpea section *Catiang*, bambara groundnut (*V. subterranea*), Asiatic grams in subgenus *Ceratotropis* and members of the subgenus *Plectotropis* was particularly brought to light by this numerical taxonomy based on RFLP analysis.

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