

Wide crossing in African *Vigna* species

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Abstract

The genus *Vigna* comprises seven subgenera and sixteen sections. Cowpea, *Vigna unguiculata* (L.) Walp., is an important grain legume crop in sub-Saharan Africa and belongs to the subgenus *Vigna*, section *Catiang*. Morphologically, cowpea genotypes appear very variable. However, a high level of genome homology exists among the varieties and this, probably, is because genomes of cowpea's wild relatives have not been exploited while these varieties were being developed. Cowpea varieties are susceptible to some insect pests, especially the pod borer, *Maruca vitrata*, and a pod-sucking bug complex, both of which can cause high seed yield losses. Accessions of some wild *Vigna* species, e.g. *V. oblongifolia* and *V. vexillata*, are known to be resistant to these pests, and efforts continue to be made, through wide crossing, to transfer the resistance genes from these species to cultivated cowpea. So far cowpea has been successfully crossed only to genotypes belonging in section *Catiang*. Among the noncultivated African *Vigna* species, there have been successful crosses between *V. oblongifolia* and two others, *V. ambacensis* and *V. luteola*, although the hybrids are only partially fertile. Embryo rescue was used to recover an F₁ interspecific hybrid between *V. oblongifolia* and *V. luteola*. All three species belong to section *Vigna* of the subgenus *Vigna*. A successful cross has also been carried out between *V. davyi* and *V. vexillata*, both members of the subgenus *Plectotropis*.

Introduction

The genus *Vigna* comprises some important pulse crops that are commonly grown in the tropics. Among these is cowpea (*Vigna unguiculata* [L.] Walp.), which is grown mainly in the drier parts of sub-Saharan Africa for various uses: its grains are used as food, fresh pods of some varieties as a vegetable, leaves as spinach, and haulms as fodder. Cowpea belongs to section *Catiang*, subgenus *Vigna*. Among the several subspecies and varieties in the section *Catiang*, four cultigroups, *biflora*, *sesquipedalis*, *textilis*, and *unguiculata*, have been identified in the cultigen *unguiculata* (Baudoin and Marechal 1985).

Several cowpea varieties have been developed and adopted by farmers for planting. The progenitors of these improved varieties appear to be mainly members of the cultigroup *unguiculata*. Exploitation of the genetic potential of wild and close relatives of cowpea for enhancing cowpea productivity has not been well documented. A high level of relationship has been detected among several cowpea genotypes following the evaluation of variability in seed proteins among them (D'Urzo et al. 1990), and this may be partly attributable to the low level or nonexploitation of the crop's wild relatives. The high level of relationship reported among cowpea varieties may also be due to its being a self-pollinated crop.

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The germplasm of wild cowpea relatives distributed in southern Africa, a region now known to contain a high diversity of wild cowpea, began to be collected only recently, and many of the accessions are yet to be evaluated for their potential usefulness. These recent collections have added to the variability in the gene bank of the different subspecies and varieties of the section *Catiang*. Some of the freshly collected germplasm appear to be new taxa, which had not been previously characterized. These include *V. unguiculata* ssp. *rhomboidea* and *V. unguiculata* ssp. *protracta* var. *kgalagadiensis*, among others. Many of these new additions may share the same primary gene pool with cowpea, and it should not be difficult, therefore, to transfer any desirable traits found in any of them to cultivated cowpea. The closest relative of cowpea (*V. unguiculata*) is *V. nervosa* Markotter, which also belongs to the section *Catiang* (Baudoin and Maréchal 1985). Crossability studies between *V. unguiculata* and *V. nervosa* have yet to be reported.

A large proportion of the available germplasm lines in the primary gene pool of cowpea have been tested for their reactions to the major insect pests, and these have been mostly susceptible, especially to the *Maruca* pod borer and pod-sucking bugs. A few accessions with low resistance to these insect pests have also been identified, especially among cowpea wild relatives (*V. unguiculata* ssp. *dekindtiana*). However, all tested accessions of *Vigna vexillata*, and some of *V. luteola* and *V. oblongifolia*, show high resistance to all insect pests of cowpea. These three *Vigna* species are members of different sections and/or subgenera from cowpea. While *V. vexillata* belongs to section *Plectotropis*, *V. oblongifolia* and *V. luteola* belong to subgenus *Vigna* (Maréchal et al. 1978). As high resistance to insect pests has been identified among some other *Vigna* species outside of section *Catiang*, a resort to wide hybridization between cowpea and other *Vigna* species is justified.

Therefore, attempts continue to be made to transfer the genes conferring resistance to the insect pests in these *Vigna* species to cowpea.

Wide hybridization

Crosses between species or genera are usually made when it is necessary to transfer one or a few genes controlling desirable traits from one species to another, and also when a new trait absent in either parent is needed. For example, Dana and Karmakar (1990) cited the work of Rangaswamy, in which a bruchid-resistant genotype was detected among the segregating population following a cross between *V. radiata* and *V. mungo*. This trait was not expressed in either of the parents used in the cross.

Interspecific crosses are usually difficult to make because of a number of attendant problems, such as incompatibility and hybrid sterility or failure/breakdown (Table 1). Linkage drag, a situation wherein a transferred gene is flanked by the introgressed segment of DNA from the donor parent, is another problem associated with making wide crosses in crops. The DNA segment may differ in length and affect traits other than the one transferred. With the conventional backcross method of breeding, many backcrosses are required to significantly reduce the amount of linkage drag. Breaking such tight linkages may also require the growing of very large plant populations or, where feasible, DNA markers can be used to effectively select against such undesirable linkage drag (Young and Tanksley 1989). These authors suggested that monitoring recombination around the gene(s) under transfer with DNA markers would quickly and efficiently reduce the amount of linkage drag.

In view of the problems associated with making wide crosses, it is necessary that breeders should first explore variation within the species, i.e., within the crop's primary gene pool, before initiating interspecific hybridization. In grain legumes, varying levels of success have been obtained from crosses between species.

Wide crossing in the genus *Vigna*

Interspecific crosses have been attempted in the genus, but with limited success. There are several causes of failure in wide crosses between *Vigna* species: pollen may be unable to germinate on stigma or pollen tubes may be unable to penetrate stigma and style (Chowdhury and Chowdhury 1977); pollen tubes may be distorted and grow slowly in the style (Barone et al. 1992); young embryos may abort (Ahn and Hartmann 1978a; Fatokun 1991); and F_1 interspecific hybrids may be completely sterile (Chen et al. 1983) (Table 1).

Recent advances in tissue culture techniques, such as embryo rescue and protoplast fusion, have increased the chances of successful interspecific crosses in plants. The embryo rescue technique has been used to enhance successful interspecific hybridization between *V. radiata* and *V. angularis* (Ahn and Hartmann 1978a), and between *V. mungo* and *V. umbellata* (Chen et al. 1983). However, regeneration of plants from protoplasts of grain legumes has not been generally successful; hence, protoplast fusion as a means of bringing about interspecific hybridization is not yet routine in these crops.

Within the genus *Vigna*, some successful crosses have been reported between species, especially those in the same subgenus or section. Crosses among some species within the following subgenera have been successful: *Ceratotropis*, *Vigna*, and *Plectotropis*.

Interspecific crosses in the subgenus *Ceratotropis*

F_1 interspecific hybrids have been obtained from crosses between some of the Asiatic grams: *V. radiata* (mung bean), *V. mungo* (black gram), *V. umbellata* (rice bean), *V. angularis* (adzuki bean), and *V. aconitifolia* (moth bean) (Table 2). Mung bean (*V. radiata*) is

Table 1. Problems identified with wide crosses among *Vigna* species.

Cross	Description of barrier	Reference
<i>Vigna umbellata</i> × <i>V. radiata</i>	Failure of pollen tube to penetrate stigma of other species	Chen et al. (1983)
<i>V. vexillata</i> × <i>V. unguiculata</i>	Low frequency of pollen germination on stigma of the other species	Barone et al. (1992)
<i>V. vexillata</i> × <i>V. unguiculata</i> <i>V. umbellata</i> × <i>V. radiata</i>	Slow rate of pollen tube elongation in the sylar tissue and/or distorted pollen tubes	Barone et al. (1992) Chowdhury and Chowdhury (1983)
<i>V. vexillata</i> × <i>V. unguiculata</i> <i>V. mungo</i> × <i>V. radiata</i> <i>V. aconitifolia</i> × <i>V. trilobata</i>	Hybrid embryo fails to develop fully/or complete seed sterility	Fatokun (1991) Barone et al. (1992) Chen et al. (1983) Biswas and Dana (1976)
<i>V. radiata</i> × <i>V. angularis</i>	Complete sterility of F_1 plants	Ahn and Hartmann (1978a)

Table 2. Interspecific hybridization among Asiatic *Vigna* species.

Cross	11 bivalents	Pollen fertility (%)	Reference
Interspecific hybridization			
<i>V. angularis</i> × <i>V. umbellata</i>	100	76	Ahn and Hartmann (1978b)
<i>V. mungo</i> × <i>V. radiata</i>	61	46	Gosal and Bajaj (1983)
<i>V. radiata</i> × <i>V. trilobata</i>	42	31	Dana (1966b)
<i>V. radiata</i> × <i>V. glabrescens</i>	51	19	Biswas (1973)
<i>V. glabrescens</i> × <i>V. umbellata</i>	82	8	Dana (1964)
<i>V. radiata</i> × <i>V. umbellata</i>	73	3	Chowdhury and Chowdhury (1983)
Intergeneric hybridization			
<i>Vigna mungo</i> × <i>Phaseolus calcaratus</i>			Chowdhury and Chowdhury (1977)

probably the most widely grown Asiatic gram; successful crosses have been made among several of its accessions and some other species (*V. mungo*, *V. glabrescens*, *V. macroptilium*, *V. umbellata*, and *V. trilobata*). The F_1 interspecific hybrids resulting from crossing *V. radiata* with *V. mungo*, *V. macroptilium lathyroides*, and *V. trilobata* were partially fertile, indicating their close relationship and the possibility of gene exchange among them (Dana 1966a; Biswas and Dana 1975). On the other hand, F_1 interspecific hybrids from crossing *V. radiata* with *V. angularis*, *V. umbellata*, and *V. glabrescens* were completely sterile (Dana and Karmakar 1990). Crosses between other species such as *V. aconitifolia* × *V. trilobata*, *V. mungo* × *V. trilobata*, *V. mungo* × *V. umbellata*, and *V. mungo* × *V. angularis* resulted in completely seed sterile hybrids. The most successful interspecific hybridization in subgenus *Ceratotropis* is the *V. angularis* × *V. umbellata* cross (Ahn and Hartmann 1978b), as well as *V. radiata* × *V. mungo*, as F_1 hybrids, in both cases, were highly fertile.

A numerical taxonomy of 44 accessions belonging to several *Vigna* species and subspecies, using data from restriction fragment length polymorphism (RFLP) analysis, revealed that *V. radiata* has the closest relationship with *V. mungo*, and *V. angularis* was closest to *V. umbellata* (Fatokun et al. 1993). Also, taxonomy based on the gene pool concept showed that *V. radiata* and *V. mungo* are closely related, since they share the same primary gene pool (Dana 1980). The successful cross between both species is, therefore, to be expected, although the vigorous F_1 hybrid plants were partially fertile. Also, *V. angularis* and *V. umbellata* share the same primary gene pool.

Failures of interspecific hybridization involving members of the subgenus *Ceratotropis* are due mainly to postfertilization events. The isolating barriers reported from interspecific hybridization within this subgenus are the delay or absence of divisions in endosperm and/or failure of embryo to divide (Dana and Karmakar 1990). The consequences of these events are the formation of empty shriveled hybrid seeds, with reduced germination. The death of F_1 interspecific hybrid plants at critical stages of development has also been observed. According to Dana and Karmakar (1990), unidirectional success is a common occurrence in interspecific hybridization in the subgenus *Ceratotropis*. While the cross between *V. radiata* as female and *V. umbellata* as male was successful, the reciprocal cross was not (Chen et al. 1983; Chowdhury and Chowdhury 1983). This unidirectional success was attributed to differential nucleocytoplasmic interactions in reciprocal combinations. In the cross between *V. radiata* and *V. angularis*,

weak F₁ plants were produced, and these were also characterized by irregular meiosis (Ahn and Hartmann 1978a), causing them to be sterile. Chromosome pairing at metaphase I in the hybrid plants ranged from 0 to 4 bivalents, with a mean of 2.39II + 17.22I.

An intergeneric cross between *Vigna mungo* and *Phaseolus calcaratus* was attempted by Chowdhury and Chowdhury (1977). Pods were formed and remained for only 12 days before drying and dropping when *V. mungo* was used as female parent. Endosperm tissue around the embryos soon degenerated, and the nondevelopment of the embryos led to the pods collapsing. In the reciprocal cross, pollen tubes could not penetrate the stigma.

Interspecific crosses in the subgenus Plectotropis

There are four species in this subgenus: *V. vexillata* with six identified varieties, *V. davyi*, *V. kirkii*, and *V. hundtii* (Maréchal et al. 1978). None of these species is cultivated. Accessions of *V. vexillata* have been evaluated for potentially useful genes lacking in cowpea and its wild relatives, and this has led to the identification of *V. vexillata* accessions with high resistance to insects. Crosses have been attempted between cowpea and *V. vexillata*, with the aim of transferring to the former the gene(s) for insect resistance.

A numerical taxonomic study, based on RFLP analysis, showed that *V. vexillata* is intermediate between the Asiatic grams and African *Vigna* species. When some data from RFLP analysis were subjected to an algorithm that determines nearest neighbor, an accession of *V. unguiculata* ssp. *dekindtiana* var. *pubescens* was closest to the outlier among the *V. vexillata* accessions. Interestingly, both *V. vexillata* and *V. unguiculata* var. *pubescens* are hairy, although the hairs are long and bristly in the former, but short and velvety in the latter. No hybrid has been obtained from crosses between accessions of both *V. vexillata* and *V. unguiculata*. There is no report of a successful cross between *V. vexillata* and any other *Vigna* species. The high resistance to insect pests exhibited by *V. vexillata* accessions calls for concerted efforts at identifying possible species that can be used as bridges for moving resistance genes from *V. vexillata* to *V. unguiculata*.

***Vigna vexillata* × *V. davyi*:** A cross has been made between *V. vexillata* and *V. davyi*, both of which are members of the subgenus *Plectotropis*. The F₁ interspecific hybrid is partially fertile and produces few viable seeds. The degree of fertility of the F₁ hybrid depends on the parents that are crossed. Pollen fertility, as measured by acetocarmine staining, was 47% when the cross involved TVNu 1335 (*V. davyi*) and TVNu 381 (*V. vexillata* var. *angustifolia*), and 59% when TVNu 1335 was crossed to TVNu 72 (*V. vexillata* var. *vexillata*). Pollen fertility of the parents was > 95% (C.A. Fatokun, unpublished data). In the former cross, pod set by F₁ plants was very low and the pods contained fewer seeds. Chromosome pairing in the hybrids at metaphase I of meiosis was generally normal, i.e., there were 11 bivalents in most pollen mother cells observed. At a very low frequency, however, there was precocious separation of chromosomes during anaphase I. Abnormal chromosome behavior was more frequently detected in the second stage of meiosis such that, at telophase II, chromosomes were not uniformly distributed to the tetrads. Micro-nuclei were formed and consequently pollen grains of variable sizes characterized the hybrids. In view of the partial fertility that was observed in the hybrid between *V. davyi* and *V. vexillata*, gene exchange is feasible between them. Genome relationship between the two species at DNA level was found to be ~ 76% (Fatokun et al. 1993). The relationship

between *V. vexillata* and *V. davyi* at the genome level is similar to that between *V. radiata* and *V. mungo*. Crosses between the latter pair result in hybrids that are also partially fertile. Pollen fertility of the F₁ *V. radiata* × *V. mungo* hybrid was 31–46%, and the abnormality observed during meiosis was a reduced frequency of bivalents (~ 70%) in metaphase I (Dana and Karmakar 1990). The F₁ interspecific hybrids between *V. davyi* and *V. vexillata* are now being crossed with cowpea accessions, but no hybrid has been obtained.

Interspecific hybridization in the subgenus *Vigna*

This subgenus is divided into six sections (Maréchal et al. 1978), and it is perhaps the most complex because it contains very diverse types. This subgenus contains the geocarpic bambara groundnut (*V. subterranea*, formerly known as *Voandzeia subterranea*), cowpea (*V. unguiculata*, along with the several subspecies in section *Catiang*), and many other noncultivated species; these include *V. luteola*, *V. ambacensis*, *V. frutescens*, *V. oblongifolia*, *V. venulosa*, *V. marina*, and *V. reticulata*. Crosses have been attempted between some of these species with the primary aim of identifying any that could serve as a bridge for transferring some useful genes from noncultivated to cultivated cowpea.

***Vigna oblongifolia* × *V. luteola*.** Some accessions of these species have been identified as resistant to insect pests, especially the *Maruca* pod borer and pod-sucking bugs, both of which cause high yield losses in cowpea. A cross was successfully made between these two species with the aid of in vitro culture of the hybrid embryo (S.R. Schnapp, Purdue University, USA, personal communication). The F₁ interspecific hybrid grew vigorously, though it was only partially fertile. It produced viable seeds, which were advanced to the F₂ generation. Attempts were made to cross the more fertile F₂ plants with *V. unguiculata* accessions, but this has not yet resulted in any hybrid. It has not been possible, therefore, to transfer the resistance genes from any of these *Vigna* species to cowpea.

Crosses among members of the section *Catiang*. It has been reported by many cowpea researchers that members of section *Catiang* are cross compatible and gene exchange should, therefore, not be difficult to accomplish. Experience has shown, however, that crosses between some members of this section are not easy to make and at times may result in hybrids which show partial fertility. For example, in crosses between an improved cowpea variety (IT84S-2246-4) and a genotype of *V. unguiculata* ssp. *dekindtiana* var. *pubescens* (TVNu 110-3A), using the latter as pollen parent, pods along with seeds in them collapsed after ~ 12 days; to recover most of the hybrids embryo rescue was needed (Fatokun and Singh 1987). The F₁ plants grew vigorously, but were only partially fertile. The purpose of the cross was to transfer hairiness, a characteristic of var. *pubescens*, to cowpea, in the hope that this trait may confer some degree of insect resistance on cowpea.

The F₁ hybrids of a cross between *V. unguiculata* and *V. unguiculata* ssp. *rhomboidea* were partially fertile, with pollen stainability of ~ 70%. Under greenhouse conditions, the F₁ plants flowered profusely but these flowers dropped after anthesis, thus producing no pods. During August and September, when ambient humidity was high and temperature low due to cloud overcast, the F₁ plants produced pods at a higher frequency. The few pods produced contained, on average, three seeds each. Among the F₂ plants, up to 30% set no pods, although all plants flowered (C.A. Fatokun, unpublished).

Table 3. Morphological attributes of *Vigna unguiculata* ssp. *sesquipedalis*, *V. unguiculata* ssp. *tenuis*, and their F₁ hybrid.

	<i>V. unguiculata</i> ssp. <i>sesquipedalis</i>	F ₁ hybrid	<i>V. unguiculata</i> ssp. <i>tenuis</i>
Petiole length (cm)	8	9	5
Terminal leaf length (cm)	12	10	3
Terminal leaf width (cm)	8	7	2
Standard petal width (cm)	3	3	3
Pod length (cm)	38	9	6
Seed number/pod	13	4	11
100-seed weight (g)	17	3	1
Pollen stainability (%)	95	60	96
Peduncle length (cm)	21	36	17

These observations suggest the existence of some barrier to gene flow between cowpea and *V. unguiculata* ssp. *rhomboidea*. An examination of pollen mother cells of F₁ plants showed a high level of homology between chromosomes of both parents as 11 bivalents were commonly observed. Unequal distribution of chromosomes to the microspores at late telophase II was observed (C.A. Fatokun, unpublished), and this probably explains the presence of small, unstained pollen grains. *Vigna unguiculata* ssp. *rhomboidea* plants do not grow vigorously at Ibadan, where they flower, however, but produce few pods. As the plants are pubescent, they may be useful in developing insect-resistant cowpea varieties.

A cross between yard-long bean (*V. unguiculata* ssp. *sesquipedalis*) and *V. unguiculata* ssp. *tenuis* resulted in F₁ plants that were vigorous in growth. They were intermediate in several characters between the two parents. They showed hybrid vigor for petiole and peduncle length. Pollen stainability in the F₁ plants was 60.4%, and the pods had very few seeds (Table 3). The fertility level of hybrid plants between these two genotypes was less than that of the F₁ interspecific hybrid between *V. umbellata* and *V. angularis* (Ahn and Hartmann 1978b). Causes of the relatively low fertility of F₁ plants from a cross between ssp. *sesquipedalis* and ssp. *tenuis* are being investigated.

Chromosomal behavior during meiosis is normal when there is complete homology between the parents that were crossed to obtain the hybrid. In such cases, pollen grains are normal in size and shape and generally highly fertile. The fertility levels of some of the F₁ hybrids from crosses among members of section *Catiang* suggest a lack of complete homology. These observations call for a closer examination of the classification of accessions in the section *Catiang*. Apart from *V. nervosa* Markotter, all other members of this section belong to *V. unguiculata* and its various subspecies. Already, Smartt (1985) had opined that the present genus *Vigna* does not seem to constitute a natural group, and he further indicated that some genus as known now might be dismembered in the near future, while some subgenera might be raised to the generic rank. When this happens, many members of the section *Catiang* will be likely distributed into more species. Crosses between some members of section *Catiang* will then be regarded as true wide crosses. Crossability studies and DNA analysis will help in placing the different members of the section into their respective genomic groups. Such a grouping should have a positive impact on the exploitation of genetic potential available among the different genotypes.

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