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Cytological Studies of Some *Vigna* (L.) Walp. Species

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ABSTRACT Cytological studies were carried out on twenty-five accessions of six species of *Vigna* in order to assess their genetic relationships. The accessions composed of 6 accessions of *V. unguiculata,* ten accessions of *V. vexillata,* four accessions of *V. luteola,* one accession of *V. ambacensis,* two accessions of *V. oblongifolia,* and two accessions *of V. racemosa.* Somatic chromosome counts of 2n=22 were made for all the *Vigna* accessions studied and meiosis was observed to be regular in all the accessions with formation of eleven bivalents. The pollen studies revealed that all the accessions produced highly fertile pollen grains, which is the direct effect of regular meiosis observed in all the accessions. The mitotic metaphase chromosomes are very small and are largely metacentrics or submetacentrics. The study indicated that the karyotype number would not itself contribute to genetic incompatibility in interspecific crosses.

INTRODUCTION

Most *Vigna* (L.) Walp species including cowpea are diploid with a relatively short life cycle, though some of the wild species are perennial. Many genes of *Vigna* species have been identified and characterized (Fery 2002; Schafleitner et al. 2016; Shree Pareek et al. 2015) but karyological data on *Vigna* species are very limited due to the extremely small (1.6–3.7 µm) of their chromosomes (She et al. 2015). Chromosome counts of 2n=22 have been reported for all the *Vigna* species examined (Adetulo 2006; Gaafar et al. 2016; Sen and Bhowal 1960; Srivastava and Naithani 1964; Willie and Aikpokpodion 2015). It was noted that 2n=22 chromosome counts as the main chromosome number because ten percent of the cells examined exhibited a different chromosome number of 2n-24 (Frahm-Leliveld 1965). However, it was not reported whether those cells with deviating chromosome counts were scored from different plants or from the same plants that showed 2n=2x=22 chromosome counts (Rama and Raina 2005).

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To overcome the limitation of small chromosome size at metaphase pachytene chromosome of cowpea were examined (Guerra et al. 1996; Kumar et al. 2011; Mukherjee 1968). It was found that the pachytene chromosomes possessed highly differentiated and densely stained heterochromatic zones (Knobs), which permitted better chromosome recognition. Barone and Saccardo (1990) karyotyped cowpea using pachytene bivalents. Galasso et al. (1998) used mitotic prometaphase chromosomes to develop a banded karyotype while Saccardo Barone and Saccardo (1990) used both convectional techniques and an Automatic Image Analyzing System to study cowpea pachytene, mitotic prometaphase and metaphase chromosomes.

Crossability among *Vigna* species is poor and reasons for the poor crossing ability have been studied from a historical and cytological point of view (Kumar et al. 2011; Ojomo and Chheda 1971; Sehrawat and Yadav 2014; Smartt 1985). The interspecific crossing barriers, which limit the use of genetic resource present in wild relatives of the cowpea, could be overcome through the knowledge of cytogenetics of cowpea (Baudoin and Maréchal 1985; Iwata et al. 2013; Menancio-Hautea et al. 1993). Further, such knowledge is unquestionably needed for adequate utilization of beneficial genes that are usually stocked in the wild relatives of cowpea (Harlan and de Wet 1971; Martin and Sauerborn 2013) and serve as a prerequisite for cytotaxonomic knowledge of the

genus *Vigna*, which would provide a better understanding of the phylogenetic relationships among the species and subspecies belonging to different sections and subgenus of the genus (Fery and Singh 1997; Galasso et al. 1998; Kumar et al. 2011; Pignone et al. 1990; Pignone et al. 1995). Thus, need for a paper in this regard becomes vital to offer information on the rudimentary biology and cytology of the crop.

Objectives

The objective of this research was to provide cytological descriptions of some *Vigna* species comprising cowpea and some of its wild relatives to reveal their similarities and differences as a way of detecting their occurrence of genetic incompatibility between cowpea and its wild relatives.

MATERIAL AND METHODS

Sources of Materials Used

The specimen used for this studies were collected from Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan IITA and local farmers from Abeokuta and Ogbomoso in the southern part of Nigeria shown in Table 1.

Cytological Analysis

The mitotic, meiotic and pollen studies were carried out on all the accessions.

Mitotic Studies

The root tips from sprouted seeds plated on moistened filter paper in petri dishes were used for the mitotic studies. The young roots (radicles) of about 1cm long were treated in 0.04 percent colchicine solution for 3 hours between the hours of 9:00 am and 12:00 pm. After pre-treatment, the roots were fixed in 1:3 acetic acid/ethanol (v/v) for twenty-four hours before used. The fixed roots were preserved in the refrigerator when they were not used immediately after fixation. The roots were hydrolyzed in 1N HCl for five minutes and rinsed with clean tap water. The hydrolyzed roots tips were squashed and

Table 1: Seed characters and accession numbers of *Vigna* species studied

Species	*Accession number*	*Sources of seeds*	*Seed colour*	*Seed texture*	*Seed size*
V. ambacensis	TVnu 456	IITA	Brown	Smooth	Small
V. luteola	TVnu 28	IITA	Brown	Smooth	Small
V. luteola	TVnu 172	IITA	Brown	Smooth	Small
V. luteola	TVnu 243	IITA	Brown	Smooth	Small
V. luteola	TVnu 986	IITA	Brown	Smooth	Small
V. oblongifolia	TVnu 42	IITA	Brownish black	Smooth	Small
V. oblongifolia	TVnu 95	IITA	Brownish black	Smooth	Small
V. racemosa	TVnu 45	IITA	Brown	Smooth	Small
V. racemosa	TVnu 1820	IITA	Brown	Smooth	Small
V. vexillata	TVnu 64	IITA	Greenish black	Smooth	Small
V. vexillata	TVnu 72	IITA	Greenish brown	Smooth	Small
V. vexillata	TVnu 73	IITA	Black	Smooth	very small
V. vexillata	TVnu 75	IITA	Greenish brown	smooth	very small
V. vexillata	TVnu 84	IITA	Brownish black	smooth	Small
V. vexillata	TVnu 93	IITA	Grayish brown	smooth	Small
V. vexillata	TVnu 97	IITA	Black	smooth	Small
V. vexillata	TVnu 143	IITA	Black	smooth	Small
V. vexillata	TVnu 160	IITA	Grayish black	smooth	Small
V. vexillata	TVnu 201	IITA	Black	Smooth	Small
V. unguiculata	TVu 1509	IITA	Cream with [c]BP	Rough	Small
V. unguiculata	TVu 8461	IITA	Brown	Smooth	Medium
V. unguiculata	TVu 14476	IITA	Tan	Smooth	Medium
V. unguiculata	BK-2K3-01N	[a]FO	White	Rough	Medium
V. unguiculata	AD-2K- 01AB	[b]FAA	Tan	Smooth	Medium
V. unguiculata	OR- NG-03-01	[b]FAA	Black	Smooth	Medium

[a]FO: Farmers in Ogbomosho; [b]FAA: Farmers in Abeokuta; [c]BP: Brown Patches

stained with FLP-Orcein using (Adegbite and Olorode 2002) squashing techniques. The squashes were observed under the microscope and photomicrographs of good mitotic stages were taken at X1000 magnification under oil immersion using Leica 2000 phase contrast microscope.

Meiotic Studies

The pollen mother cells from young flower buds fixed directly in 1:3 acetic acid/ethanol (v/v) between the hours of 9:00 am and 12:00 pm were used for the mitotic studies. The fixed flower buds were dissected to extract the young anthers. Two of the young anthers were squashed at a time on a clean slide using a mounted needle and irrigating with the fixative until homogenous solution was obtained. A drop of FLP-Orcein was applied on the slide before covering with a cover slip. The prepared slides were left for hours before tapping the cover slip to remove excess stains. Photomicrographs of good mitotic stages were taken at X1000 magnification under oil immersion, using a Leica 2000 phase contrast microscope.

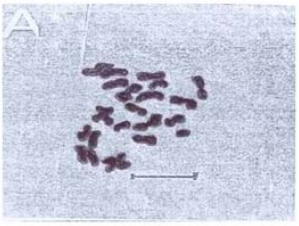
Pollen Studies

Slides of pollen were prepared by dusting pollen grains from the opened flowers in a drop of cotton blue in lacto phenol on a clean slide and applying a cover slip or by squashing mature anthers from unopened flowers in the stain. Five slides from five different flowers were prepared for each of the accession. Pollen fertility was established by counting pollen grains from at least ten fields on each of the five slides prepared for each accession at X100 magnification. The pollen grains with cytoplasmic content stained deep blue were considered fertile while those unstained or only partially stained or with collapsed outlined were considered as sterile. The percentage pollen fertility was estimated by expressing the number of fertile pollen grains as a percentage of the total pollen grains counted. Pollen size was determined by measuring the diameter of thirty, full and deeply stained, randomly selected pollen grains on the five slides prepared for each accession at X400 magnification using ocular micrometer. These were later converted to microns using a stage micrometer. Photomicrographs of pollen grains stained with FLP-Orcein were taken at X400 magnification to show the pollen structure.

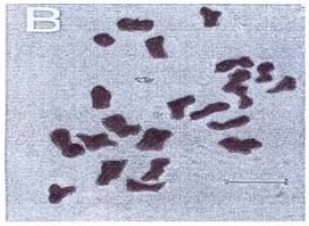
RESULTS AND DISCUSSION

Cytological Studies

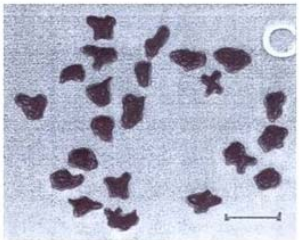
All the *Vigna* species studied showed somatic chromosome counts of 2n=22 and meiotic counts of n=11 depicted in Figures 1 and 2.



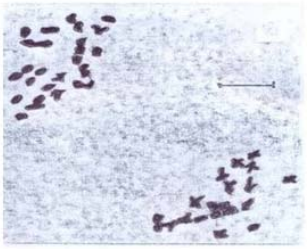
Metaphase in *V. unguiculata*



Metaphase *in V. cexillata*



Metaphase in *V. racemosa*



Metaphase *in V. hiteola*

Fig.1. Mitotic chromosomes in some *Vigna* species: (A). Metaphase in *V. unguiculata* (OR-ENG-03-01) (2n=22), (B). Metaphase in *V. vexillata* (TVnu 72) (2n=22), (C). Metaphase in *V. racemosa* (TVnu 45) (2n=22), (D). Telophase in *V. luteola* (TVnu 172) (2n=22). Bar 3.50µm

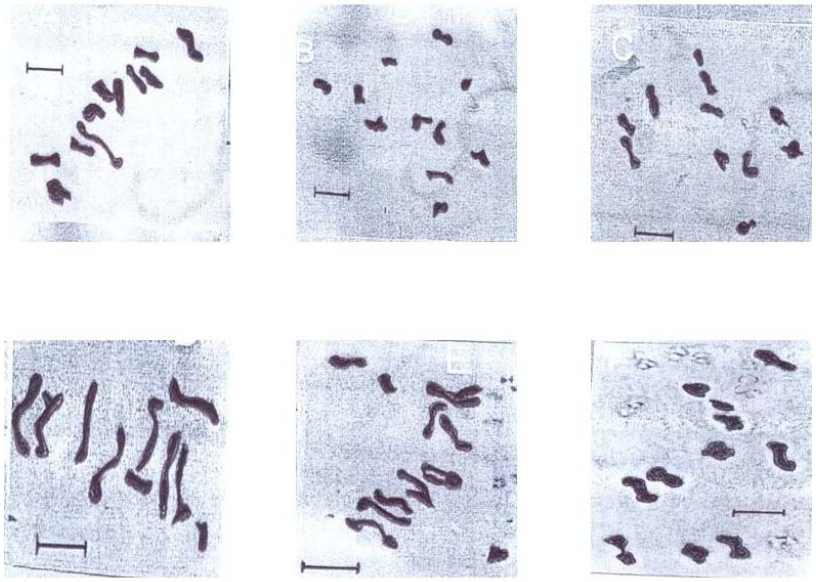


Fig. 2. Meiotic chromosomes of some *Vigna* species: (A). Metaphase I in *V. luteola* (TVnu 172) (n=11). (B). Metaphase I in *V. racemosa* (TVnu 45) (n=11). (C). Metaphase I in *V. vexillata* (TVnu 72) (n=11). (D). Metaphase I in *V. unguiculata* (OR-ENG-03-01) (n=11). (E). Metaphase I in *V. oblongifolia* (TVnu 42) (n=11). (F). Metaphase I in *V. ambacensis* (TVnu 456) (n=11). Bar 3.50µm

Meiosis was normal in all the species with the regular formation of 11 bivalents and normal separation and movement of chromosomes to the poles. These counts and observations are similar to previous chromosome count reported (Adetulo 2006; Faris 1965; Sen and Bhowal 1960; Srivastava and Naithani 1964). The mitotic metaphase chromosomes are very small and mostly metacentrics and submetacentrics similar to She et al. (2015). Although, all the species possessed the same chromosome number of 2n=22, there were some slight variations observed in sizes of their mitotic metaphase. Due to the small sizes of the metaphase chromosomes observed in this study, the karyotypic differences among the species could not be ascertained.

Pollen Studies

Fenestrate pollen grains were observed in all the species of *Vigna* studied. These are single spherical reticulate pollen grains with spinous cover that are interrupted by three protuberances (germ pores) in a fixed geometrical pattern (De Leonardis et al. 1993). Pollen fertility percentages were observed to be high in all the *Vigna* species studied. *V. luteola* (TVnu 243) recorded the higher pollen fertility percentage value of 97.9 while *V. vexillata* (TVnu 73) had the lowest pollen fertility value of 84.0 expressed in Table 2.

This observation was not unexpected given the regularity of the meiosis in the species and their ability for self-fertilization. The pollen studies revealed that all the accessions produced highly fertile pollen grains, which are the direct effect of the regular meiotic pairing observed in all the accessions (Damayanti et al. 2010).

Further, the accessions also showed wide inter-specific and intra-specific variations in their mean pollen size, which may be a reflection of the differences in the volume of their genomic contents. The similarities observed in the pollen, ligule and stigma colors in the different accessions suggested that the same gene might be responsible for the color expression in the different species. This result showed the correlation between meiotic chromosome regularities and pollen fertility. Hence, revealed that these species may undergo interspecific crossing as a result of their cytological closeness and evolu-

Table 2: Pollen data of the *Vigna* species studied

Species	*Accession number*	*N**	*Pollen fertility %*	*** x±s.d (µm)*	*CV (%)*	*Pollen colour*	*Ligule colour*	*Stigma colour*
V. ambacensis	Tvnu 456	2499	88.3	46.0± 9.1	19.6	Yellow	Yellow	Yellow
V. luteola	Tvnu 28	2859	95.3	50.3± 5.6	11.1	Yellow	Yellow	Yellow
V. luteola	Tvnu 172	2928	97.6	49.9± 4.8	9.6	Yellow	Yellow	Yellow
V. luteola	Tvnu 243	2936	97.9	51.6± 4.5	8.8	Yellow	Yellow	Yellow
V. luteola	Tvnu 986	2736	91.2	50.3± 5.3	10.6	Yellow	Yellow	Yellow
V. oblongifolia	Tvnu 42	2901	96.7	39.0± 3.1	7.9	Yellow	Yellow	Yellow
V. oblongifolia	Tvnu 95	2718	90.6	39.1± 3.9	9.9	Yellow	Yellow	Yellow
V. racemosa	Tvnu 45	2642	88.1	39.8± 3.7	9.4	Violet	Violet	Violet
V. racemosa	Tvnu 1820	2554	88.1	36.0± 3.1	8.7	Violet	Violet	Violet
V. vexillata	Tvnu 64	2928	97.6	93.3± 2.7	2.9	Purple	Purple	Purple
V. vexillata	Tvnu 72	2534	84.5	89.6± 6.2	6.9	Purple	Purple	Purple
V. vexillata	Tvnu 73	2521	84.0	92.5± 4.9	5.3	Purple	Purple	Purple
V. vexillata	Tvnu 75	2903	96.8	91.3± 10.6	11.6	Purple	Purple	Purple
V. vexillata	Tvnu 84	2702	90.1	94.2± 8.2	8.7	Purple	Purple	Purple
V. vexillata	Tvnu 97	2854	95.1	86.7± 10.9	12.5	Purple	Purple	Purple
V. vexillata	Tvnu 143	2812	93.7	93.4± 5.1	5.4	Purple	Purple	Purple
V. vexillata	Tvnu 160	2732	91.0	97.3± 7.2	7.4	Purple	Purple	Purple
V. unguiculata	Tvu 1509	2704	90.1	99.7± 1.6	1.7	White	White	White
V. unguiculata	Tvu 8461	2739	91.3	106.4± 2.6	2.5	Purple	Purple	Purple
V. unguiculata	Tvu 14476	2708	90.3	105.4± 2.8	2.7	Purple	Purple	Purple
V. unguiculata	BK-2K3-01N	2643	88.1	103.9± 1.0	0.9	Violet	Violet	Violet
V. unguiculata	AD-2K- 01AB	2669	89.0	106.8± 3.8	3.6	Purple	Purple	Purple
V. unguiculata	OR- NG-03-01	2588	86.25	103.6± 1.6	1.5	Violet	Violet	Violet

CV (%): Coefficient of variation; ** x±s.d (µm): Mean Pollen Size with Standard Deviation in micrometre; N*: Number of Total Pollen Grains Counted

tionary relatedness through the recent molecular techniques that are likely to circumvent the karyotypic and crossability constraints. Thus, small size of the chromosome makes it difficult to use karyotypic number to conclude on the genetic incompatibility within these accessions.

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