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ORIGINAL ARTICLE

Further observations on chromosome diversity analysis in wild species of *Vigna* from India

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Abstract Ten wild species of *Vigna* from the Indian gene centre have been karyo-morphologically analysed. All the studied taxa had somatic chromosome number of 2n = 22 except *V. glabrescens* which had 2n = 44. The chromosomes were found to be either metacentric, submetacentric and subtelocentric in type with their respective chromosome complements, and the complete absence of telocentric chromosomes in all of the taxa studied. The karyotypes of the species were mostly symmetrical. The significance of karyotypic variation among wild species in comparison with the cultivated ones of the genus *Vigna* has been discussed in detail. Such information has value in understanding cytogenetical relationship amongst these wild species reported to comprise of genes for resistance to insect, pests and diseases.

Keywords *Vigna* · Wild species · Karyotype · Asymmetry index · NOR-chromosomes

Abbreviations

NOR	Nuclear Organizing Region
AI	Asymmetry index
SC	The shortest chromosome length
LC	The longest chromosome length
CL	Mean length of chromosome
CI	Mean centromeric index
SD	Standard Deviation
CVCL	Component expressing the relative variation in chromosome length
CVCI	Component expressing the relative variation in centromeric index

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Introduction

The genus *Vigna* Savi belongs to the family Fabaceae that includes 104 described species [8] and is grown in the tropical and subtropical areas of the world. Some of the *Vigna* species are cultivated worldwide and are adapted to a wide range of extreme environmental conditions. They grow in poor soil without supplementary nitrogen and some of them are also cultivated as cover crop, green manure and for the control of soil erosion etc. [3]. Many of the species produce multiple edible products, which provide subsistence to farmers with a food supply throughout the growing season as well as dry seeds in off season since the seeds are easy to store and transport. Tender shoots/leaves, and unripe pods/seeds of cowpea are consumed as vegetables, and the harvested dry seeds of all of the *Vigna* species are consumed directly/or as sprouts, but the dry seeds are often used to make flour. The residues of various plant parts can be used as fodder for farm animals. *Vigna* food products exhibit many excellent nutritional attributes and these products provide much needed complements in human diet [15].

Vigna constitute an important group of cultivated and wild species for which rich diversity occurs in India. Several wild collections of the genus *Vigna* were reported from natural habitats [1]. These wild accessions are reportedly the

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reservoirs of large number of allelic variants of useful genes which could not pass through the domestication bottleneck and hence are not found in the cultivated gene pool. In order to facilitate their use in routine plant genetic improvement programmes, there is a need to transfer these useful genes to agronomically important cultivars to allow transfer of desirable traits associated with wild collections. A better understanding of phylogenetic interrelationship among the wild species could facilitate of transfer of desirable characters [7].

Despite the presence of a rich diversity, the wild relative complexes have not been the subject of intense studies [17], which is essential for identification, classification and management of the genetic resources. The information related to chromosome number and comparative karyotype is fundamental to overall understanding of the genome in different species or in morphologically diverse populations within a species [20]. Karyo-morphological analysis is a useful method for characterizing plant chromosomes and genome organization [12]. The structure and morphology of the chromosome is of vital importance when studying the origin, evolution and classification of taxa [22]. The differences and similarities in the karyotype are regarded as basis of genetic variation, as well as distance or relatedness among diverse genomes [6]. Chromosome studies are important when attempting to evaluate relationships and deduce phylogenetic distances [4], and have played a major role in creation of the genome concept of wild and cultivated species [9]. In an earlier attempt, the present authors have reported chromosome diversity analysis in various species of *Vigna* Savi from India. In the present investigation, however, an attempt was made to further analyze a few wild species from India for their chromosome diversity. Hence, it is proposed to evaluate the comprehensive cytogenetical studies of the Indian representative of wild *Vigna* species.

Materials and methods

Karyomorphological studies were undertaken in nine *Vigna* species and one sub-species all belonging to wild taxa of *Vigna*. The germplasm along with corresponding accession numbers has been obtained from National Bureau of Plant Genetic Resources, New Delhi and also from their regional station at Thrissur, Kerala. Actively growing root tips of about 1–2 cm long were excised from germinating seeds on moist filter paper at 25 ± 2 °C, pre-treated with 0.025% colchicine (Himedia) for 3 h at room temperature (20 ± 2 °C). The root tips after pre-treatment were fixed in freshly prepared ethanol-acetic acid (*v*/*v*, 3:1) and subsequently stored at 4 °C until required. For slide preparation, the root tips were washed twice in distilled water, hydrolyzed in 1 N.HCl at 60 °C for 8 min and stained in Feulgen stain (Leuco basic fuchsin) for 45 min. The stained root tips were thoroughly washed and subsequently squashed in 1 % acetocarmine. The microphotographs of the metaphase plates were taken from both temporary and permanent preparations. At least 10–15 clear preparations of chromosome complements of each species were analyzed. Photo-idiograms were prepared from photomicrographs by cutting out individual chromosome and arranging them in descending order of their length. On the basis of gross morphology, the chromosomes were resolved into homologous pairs. To determine the exact position of centromere; Battaglia's classification [2] of metacentric /median (V), submetacentric/submedian (L), subtelocentric (J) and telocentric (I) based on the arm ratio of 1:1; > 1:1- < 1.3 or > 1:3 < 1.0 and 0.1 respectively was employed. The degree of asymmetry was estimated as per the scheme proposed by Paszko [10].

Results

Mitotic data on chromosome complements of nine species and one sub-species of *Vigna* are summarized in Tables 1 and 2 and illustrated in Figs. 1 and 2. The somatic chromosome number of all the taxa studied was 2n = 2× = 22 except for *V. glabrescens* which reported 2n = 4× =44. The chromosome complements of all the taxa were resolved into 11 pairs except in *V. glabrescens* which was resolved into 22 pairs and formed a graded series from the longest to shortest pairs within the complements. A noticeable difference in length between the longest and the shortest chromosomes within the complement was recorded in all the taxa (Table 1). These values ranged from 1.75 to 3.6. It was further observed that irrespective of the small size, the longest chromosome (*V. khandalensis*) of the haploid complement was almost 2.06 times longer than the shortest one (*V. minima*).

Further the various species of *Vigna*, had either metacentric, submetacentric and subtelocentric chromosomes in their respective chromosome complements, with complete absence of telocentric chromosome in all of the taxa studied. There is no report available on karyotypic details of, *V. khandalensis*, *V. minima*, *V. pilosa*, *V. sahyadriana* and *V. subramaniana* in the literature and the same is being reported here for the first time. The number of metacentric and submetacentric chromosomes differed significantly in all the taxa studied. In five taxa viz. *V. glabrescens*, *V. khandalensis*, *V. radiata* var. *radiata*, *V. pilosa* and *V. vexillata* metacentric type of chromosomes outnumber all the other types. However in the remaining five taxa, the more frequently observed chromosomes were submetacentric. The karyotype formula of ten taxa viz. *V. dalzelliana*, *V. khandalensis*, *V. minima*, *V. mungo*, *V. pilosa*, *V. radiata* var. *radiata*, *V. sahyadriana*, *V. subramaniana*, *V. vexillata* and *V. glabrescens* were resolved into 6 V + 16 L, 10 V + 8 L + 4 J, 6 V + 16 L, 10 V + 12 L, 14 V + 8 L, 12 V + 6 L + 4 J, 6 V + 16 L, 10 V + 12 L, 20 V + 2 L and 30 V + 14 L.

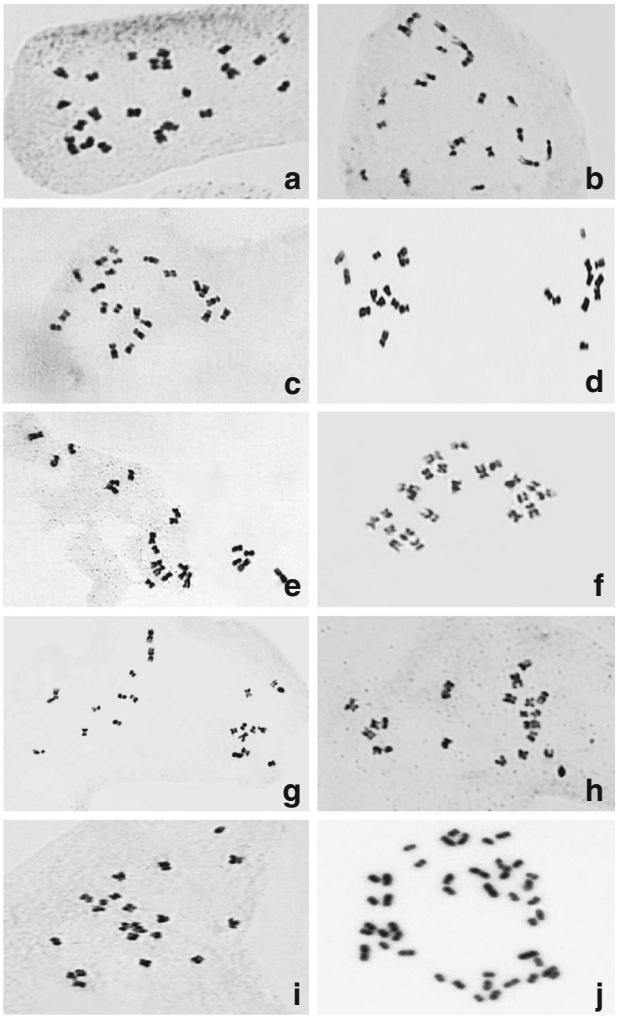

Fig. 1 **a-j** Somatic metaphase complements of *Vigna* spp.: **a** *V. dalzelliana* **2** *V. khandalensis* **c** *V. minima* **d** *V. mungo* **e** *V. pilosa* **f** *V. radiata* var. *radiata* **g** *V. sahyadriana* **h** *V. subramania* **i** *V. vexillata* **j** *V. glabrescens*. Scale bar =5 μm

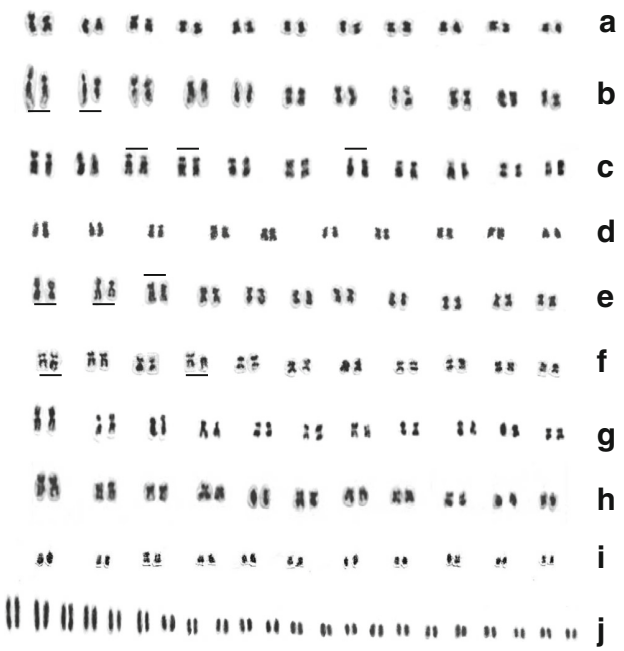

Fig. 2 **a-j** Photo-idiograms of 10 *Vigna* spp.: **a** *V. dalzelliana* **b** *V. khandalensis* **c** *V. minima* **d** *V. mungo* **e** *V. pilosa* **f** *V. radiata* var. *radiata* **g** *V.sahyadriana* **h** *V. subramania* **i** *V. vexillata* **j** *V. glabrescens*. Heteromorphic pairs are underlined and nucleolar pairs are lined above the value

Various taxa belonging to the genus *Vigna* have shown significant variation in the karyotype with respect to the number of metacentric, submetacentric and subtelocentric chromosomes, presence or absence of heteromorphic chromosome pairs and nucleolar chromosomes within the complements (Table 1). One notable feature of the present observation was the complete absence of telocentric chromosomes in all the taxa studied. Another unique feature is the presence of heteromorphic chromosome pair in some of the *Vigna* species viz. *V. khandalensis* (1st and 2nd pairs), *V. pilosa* (1st and 2nd pairs), *V. radiata* var. *radiata* (1st and 4th pairs), Nucleolar organizing region (NOR) was observed in the form of secondary constriction/satellites in *V. minima* (3rd, 4th and 7th pairs) and *V. pilosa* (3rd pair).

In the present investigation, two taxa *V. khandalensis* and *V. radiata* var. *radiata* were characterized by the presence of both metacentric and submetacentric chromosomes with two pairs of distinct subtelocentric chromosomes in the complement. The remaining taxa are signified by having both submetacentric and/or metacentic chromosome pairs in karyotype without any subtelocentric chromosomes.

The asymmetry index (AI) value which has been derived from the data related to chromosome length (CL) and centromeric index had shown significant gap among the various taxa. *V. dalzelliana* (5.97), *V. khandalensis* (12.55), *V. radiata* var. *radiata* (5.27), *V. sahyadriana* (4.97), had high asymmetry index value and revealed high level of karyotypic heterogeneity. *V. minima* (3.42), *V. mungo* (3.70), *V. subramaniana* (3.91) had intermediate asymmetry index while *V. glabrescens* (2.52), *V. pilosa* (2.36) and *V. vexillata* (2.25) shows lower asymmetry index indicating greater karyotypes symmetry and stabilised genome (Table 2).

Discussion

Cytogenetical data, especially the karyomorphological details of wild species of *Vigna* have been a matter of great importance owing to the scanty cytogenetical information of the wild relatives available for Indian representative *Vigna* species [12, 13, 16]. Therefore, comprehensive investigations dealing with nine *Vigna* species and one sub-species all belonging to wild taxa of *Vigna* were attempted for detailed karyomorphology. The

Table 1 Karyotype and arm ratio in various taxa of *Vigna*

Sl. no.	Species	Accessions no.	2n	Chromosome arm length (L/S ratio)											Ratio of longest and shortest chromosome	Karyotype formula
				I	II	III	IV	V	VI	VII	VIII	IX	X	XI		
1	*V. dalzelliana*	IC 583684	22	1.57	2.25	2.3	1.45	2.15	1	1	1.5	1.5	1.5	1	3	6 V + 16 L
2	*V. khandalensis*	SUV 13	22	2.14 3.5	3.5 1.5	5.5	2.8	2.6	1	1	1.6	1	1	1	3.6	10 V + 8 L + 4 J
3	*V. minima*	BBYD 2724	22	1.8	1.18	2.12	2.8	2.3	1	2	2	1.6	1	1	1.75	6 V + 16 L
4	*V. mungo*	BBD 8 01 B	22	1.6	2.5	2	1.25	1.5	1	1.5	1.25	1	1	1	2	10 V + 12 L
5	*V. pilosa*	TCR 131 136	22	1.35 1.2	2.25 1.5	1.3	1.75	1	1	1	1	1	1	1	2.3	14 V + 8 L
6	*V. radiata* var. *radiata*	IC 251431	22	3.5 4	2	1.6	3 7	1	1.15	1	1	1	1	1	2	12 V + 6 L + 4 J
7	*V. sahyadriana*	SUK 2	22	1.4	2.3	2.65	2.05	1.3	1.3	1.3	1	1	1	1.5	2.4	6 V + 16 L
8	*V. subramaniana*	SUK 176	22	1.9	1.6	1.3	1.3	1	1.75	1	1	1.5	1	1	2.5	10 V + 12 L
9	*V. vexillata*	BBD 15 01 A	22	1.5 1	1 1	1 1.3	1 1.16	1 1	1 1	1 1.2	1 1.2	1 1	1 1	1 1.5	2	20 V + 2 L
10	*V. glabrescens*	IC 25137	44	XII 1	XIII 1.5	XIV 1	XV 1	XVI 1.5	XVII 1	XVIII 1	XIX 1	XX 1	XXI 1	XXII 1	3	30 V + 14 L

present investigations involving Indian wild representative taxa, did reveal occurrence of gametic chromosome number of $n = 11$, thereby suggesting somatic chromosome number as 2n = 22, except *V. glabrescens* 2n = 2× = 44, but no other polyploid taxa were encountered. So far, polyploidy has been encountered in only one species i.e. *V. glabrescens* based on the basic number x = 11. Thus the present studies support the view of true basic number of the genus *Vigna* as x = 11. These observations are supported by recent reports of *Vigna* species by earlier workers [12, 13, 16, 18].

In the present study, characteristic differences have been recorded in karyotype/chromosome morphology as reflected in karyotype at inter-specific level of the genus *Vigna*. In general, 5 taxa viz. *V. dalzelliana*, *V. minima*, *V. mungo*, *V. sahyadriana* and *V. subramaniana* have submetacentric chromosomes in large number while five taxa viz. *V. glabrescens*, *V. khandalensis*, *V. pilosa*, *V. radiata* var. *radiata*, and *V. vexillata* have metacentric chromosomes. Such variations with respect to V and L types and presence of two pairs of distinct subtelocentric chromosomes in *V. khandalensis* and *V. radiata* var. *radiata*, may result due to structural changes in chromosomes viz., duplication, deletions, interchanges and inversions [12, 21], that may have taken place in somatic chromosome complements as contemplated by earlier workers in various genera viz. *Vigna*, *Cymbidium*, *Michelia* and *Manglietia* [12, 16, 23].

Table 2 Karyomorphological characteristics in various taxa of *Vigna*

Sl. no.	Species	Accessions no.	2n	Range SC-LC (μm)	Ratio LC/SC	CL (μm) Mean (±SD)	CI Mean (±SD)	CV_{CL}	CV_{CI}	AI
1	*V. dalzelliana*	IC 583684	22	4–12	3	6.77 (±2.13)	40.62 (±7.72)	31.45	19	5.97
2	*V. khandalensis*	SUV 13	22	6–22	3.6	10.68 (±4.0)	37.68 (±12.63)	37.48	33.51	12.55
3	*V. minima*	BBYD 2724	22	8–14	1.75	10.22 (±2.02)	42.67 (±7.402)	19.76	17.34	3.42
4	*V. mungo*	BBD 8 01 B	22	4–8	2	5.40 (±1.15)	42.67 (±7.40)	21.34	17.34	3.70
5	*V. pilosa*	TCR 131 136	22	6–14	2.3	9.72 (±1.88)	46.66 (±5.69)	19.40	12.20	2.36
6	*V. radiata* var. *radiata*	IC 251431	22	5–10	2	7.27 (±1.35)	41.55 (±11.78)	18.62	28.34	5.27
7	*V. sahyadriana*	SUK 2	22	5–12	2.4	7.5 (±1.99)	40.97 (±7.67)	26.59	18.72	4.97
8	*V. subramaniana*	SUK 176	22	4–10	2.5	6.09 (±1.67)	44.27 (±6.29)	27.52	14.22	3.91
9	*V. vexillata*	BBD 15 01 A	22	3–6	2	3.86 (±0.86)	50 (±5.02)	22.47	10.05	2.25
10	*V. glabrescens*	IC 25137	44	6–18	3	10.54 (±3.0)	47.15 (±4.18)	28.48	8.87	2.52

A symmetrical karyotype is characterized by the predominance of metacentric and submetacentric chromosomes mostly in equal number. Increasing asymmetry can occur through fusion and fission of chromosome segments involving metacentric and submetacentric chromosomes and are generally believed to be the causative factors for the genesis of subtelocentric and telocentric chromosomes [19, 21]. Ironically, in the present investigation although submetacentric chromosomes were highest in number, they did not generate any telocentric chromosomes.

The observation related to heteromorphic pairs in *V. khandalensis*, *V. pilosa*, *V. radiata* var. *radiata* comparatively exhibit less genome integrity and thereby helping the species to resort to structural alterations as means of speciation [24]. The absence of any deviant chromosome numbers other than $n = 11$ and overall symmetry suggests that the diversification at inter-specific level has occurred through structural alteration of chromosomes rather than any numerical change.

A pair of nucleolar organizers region (NOR) observed in the form of secondary constriction/satellites were recorded in two of the 10 taxa studied. The complete absence of nucleolar organizer in remaining taxa may be attributed to technical difficulties associated with small genome size reflected by small size of chromosome.

Paszko [10] opined that Stebbins' classification [21] as a qualitatively less powerful method, less flexible in terms of the types of conclusions it can provide and proposed a new asymmetry (AI) index which gives a measure of the heterogeneity of chromosome length and centromeric position in a given karyotype. A significant inter-specific variation among *Citrus*, *Dipcadi* and *Vigna* [5, 14, 16], species using the asymmetric index value could be evaluated. From the karyological data presented in Table 2, it can be observed that the asymmetry index in different species of *Vigna* presently investigated had shown variation. High asymmetry index value suggested high level of karyotypic heterogeneity, whereas an intermediate and lower asymmetry index indicates a greater karyotypes symmetry and stabilised genome, as advocated by Hynniewta et.al.,2011 [5].

Understanding of cytogenetical relationship of the wild species is important since wild ancestors are resistance to insect pests and diseases and possibility of transfer of such characters depends on the phylogenetic distances among the species [7]. Cytological studies of wild *Vigna* species reported in the present investigation though appear to be preliminary in nature form a baseline data for future molecular genetics and cytogenetic studies such as FISH and GISH which are expected to characterize the intricacies of *Vigna* genome in more a authentic manner [11], not only for physical mapping (including identification of homologous pairs) and genome analyses but also as a tool for evolutionary studies in the genus *Vigna*.

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