Chapter 3

KARYOTYPE ANALYSIS IN *PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC.

3.1 INTRODUCTION:

The importance of cytological study has been widely accepted in the studies of evolution, phylogeny and classification of plants. The Russian school of cytologists headed by S. Navashin, developed the fundamentals of the karyotype concept from their observation that the most species of living organism show a distinct and constant individuality of their somatic chromosomes and that closely related species have more similar chromosomes than those of more distantly related ones. Levitzsky (1931, 1931a) indicates how karyotype may be applied to any systematic unit. Thus the karyotype expression is essential to conceive in inter- and intrataxonomic relationships and hence to throw light on the evolutionary trends and processes.

The birth of study of somatic chromosomes for its morphology took with the work of Delaunay (1926). Delaunay (loc.cit.) first defined karyotype as a group of species resembling each other in morphology and number of that chromosome. However, Levitzsky (1931) defined it as phenotypic appearance of the somatic chromosome set of a species and is further characterized to form and size of chromosomes as well as to their number. The term karyotype by definition implies morphological expression of somatic chromosomes. In order to demonstrate the characteristics of karyotype "Idiogoram is constructed as per visual appearance of the chromosomes. An **"Idiogram** " is a diagrammatic representation of the gametic chromosome set (n) of the given species while **karyogram** is karyotypic representation in which representative cell

is photographed, the chromosomes cut out from photograph and collected in pairs of homologue (Sybenga, 1992). The analysis of karyotype is known as karyosystematics (Sato, 1939). After revealing the importance of karyotypic analytical studies in both animals and plants as a powerful tool of characterization of a species, several workers have elaborated a method. Heitz (1927) is one of the first to do so. He developed an elaborated system of representing a karyotype using different symbols. However, his system was not accepted as it did not contribute to develop the concept of karyotype. Tjio and Levan (1950) suggested an expression for somatic chromosome merely on the position of centromere, secondary constriction and satellite. He used Roman letters to express karyotype but Battaglia (1955) an Italian cytologist, suggested another method of karyotype based on chromosome morphology and position of centromere. Levan *et al.* (1964) have suggested a method of karyotypic expression.

The number of chromosomes over which the genome is distributed and microscopically visible morphology of these chromosomes forms the karyotype. Chromosome morphology traditionally includes the length of the chromosomes, the location of the primary constriction and if present, the secondary constriction (Near the NOR). Tertiary constrictions in somatic metaphase chromosomes are occasionally visible and can then be used to specify chromosome segments. They tend to coincide with heterochromatin (Sybenga, 1992).

Presently, a karyotype description includes chromosome segments with staining characteristics that are consistently different from the remainder of the chromosome. The description of the karyotype can be further extended to include chromosomal characteristics that can not be made visible by mere staining. One is the total amount of DNA per somatic nucleus. (Sybenga, 1992).

Karyotype analysis found helpful to plant breeder, as it provides information which can be used for number of different purposes.

1) Karyotype descriptions are often used as a character in species description (Cytotaxonomy).

2) For understanding evolutionary process, karyotype information can be of considerable importance.

3) Differences in chromosome number (other than polyploidy) and in chromosome morphology between wild species and cultivars suggest chromosomal differences that may disturb meiosis in hybrids, endangering the proposed gene transfer.

4) It provides information regarding barriers to the introduction of gene from related or more distant species.

5) An extended Karyotype description including the location of known desired gene in relation to other marker (RFLPs, bands, recognizable rearrangements) contain useful information for planning of gene transfer or chromosome segment. The transfer can be conveniently monitored by following the RFLPs by

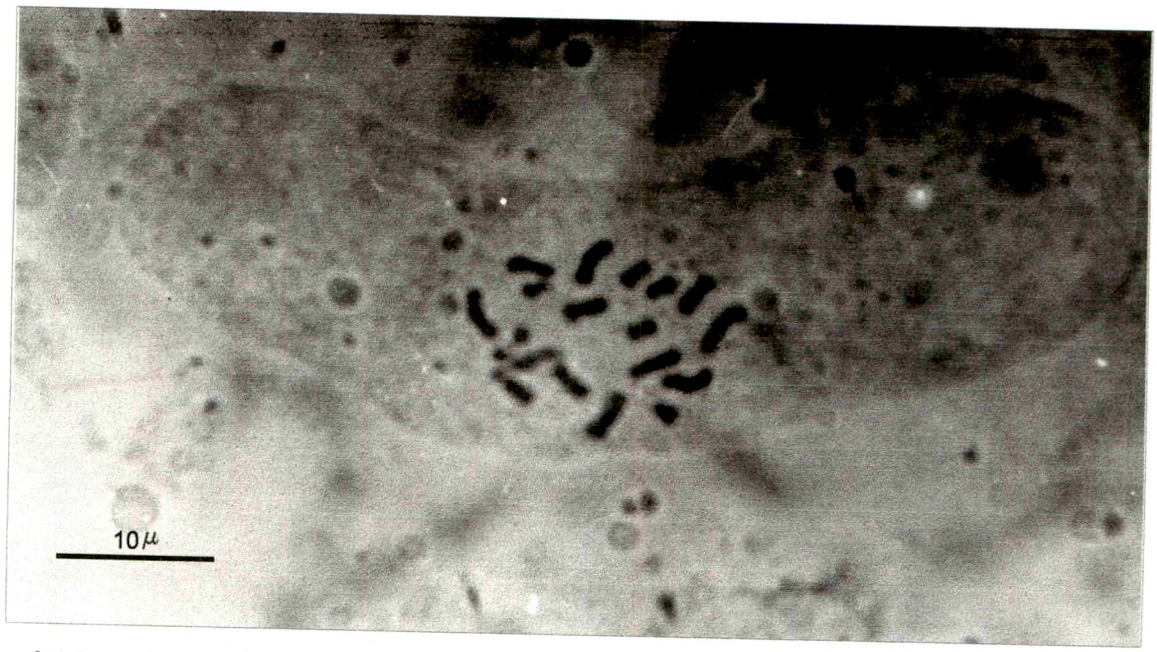
molecular methods, enzyme markers by biochemical meth and chromosome morphologically marked segment cytological methods.

6) Chromosomal aberrations and number variants within spec between cultivars or incidentally arising within cultivars, can ca unexpected undesired complications. They may disturb, prevent recombination and may cause practical sterility (Syber 1992).

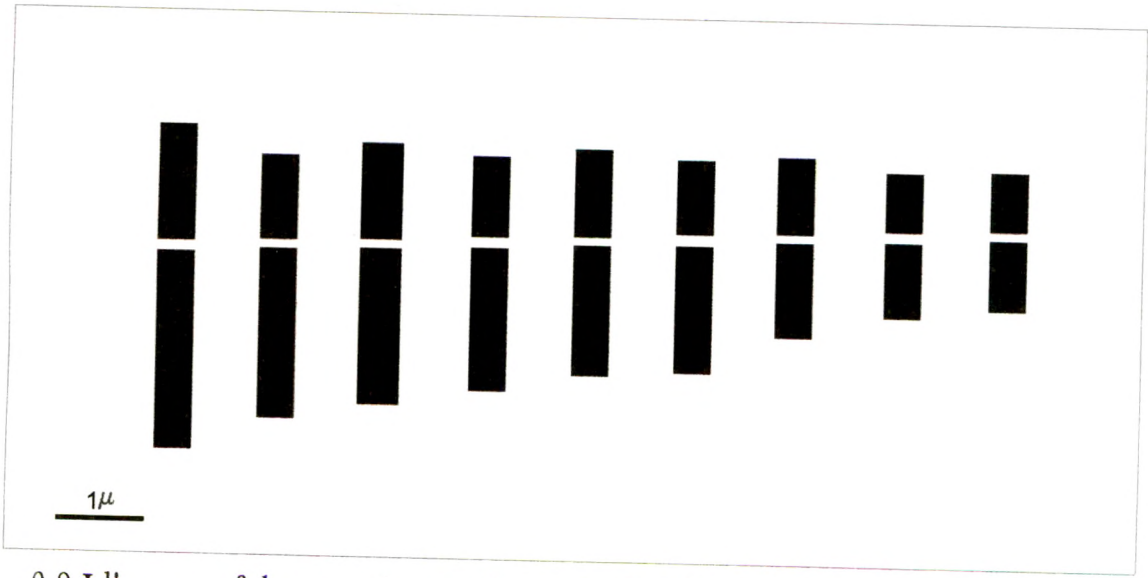
It reveals from the review of literature that there is no agreem regarding the chromosome number within **Psophocarpus tetragonolo** (L.) DC. and **Psophocarpus** species (Frahm Leliveld, 1960; Miege, 19 Ramirez, 1960; Cave, 1964; Tixer, 1965; Westphal, 1974; Thuan, 19 Zeven, and Zhukovsky, 1975; Khan,1976; Simmonds, 1976; Wark, 19 Haq and Smartt, 1977, 1978; Pickersgill, 1980; Liyanarachchi *et al.*, 19 Harder and Smartt, 1992).

It is also revealed from the literature that very meager atten have been made to study chromosome morphology and karyot analysis (Ramirez, 1960; Pickersgill, loc. cit.; Liyanarachchi *et al.*, 19 Harder and Smartt, loc. cit.).

The chromosome polymorphism has significant repercussior breeding depending on the nature of the structural differences betw chromosome races and other forms of genetic isolation. Hence present investigation attempts are made to study chromoso



3.1 Somatic metaphase chromosomes of **Psophocarpus tetragonolobus** (L.) DC (2n=18)



3.2 Idiogram of the somatic compliment in **Psophocarpus tetragonolobus** (L.) DC

To determine the length of the chromosomes, ten plates were selected (well-separated chromosomes with appropriate condensation), and the average length of each individual chromosome was determined from the data obtained. The plate photographs were taken from permanent preparations using MFAKS system of JENVAL Carl Zeiss microscope. The photographic film used was OROW 22 DN, 100 ASA, and 35mm negative. The sharp negatives were enlarged and chromosome measurements were made from selected photoprints.

In the karyotype analysis the nomenclature recommended by Levan *et al.* (1964) has been adopted. The karyotype symmetry has been determined by following Stebbins' (1958) system of classification.

For the karyotype analysis long arm and short arms of the chromosomes were denoted as 'l' and 's' respectively. The total length of the chromosomes was denoted as 'C'. The location of the centromere was expressed as a difference d = l-s. The ratio of long arm to short arm was denoted by 'r'. The centromeric index (i) was calculated as

$$i = \frac{100 \times s}{C}$$

Where, 's' = Length of short arm of chromosome.

C = Total length of the chromosome.

F% and TF% was calculated as given by Huziwara (1962), while relative length TCL% was determined by using formula,

morphology and karyotype analysis in winged bean [**Psophocarpus tetragonolobus** (L.) DC.]

3.2 MATERIALS AND METHODS:

The seeds of local variety SU-8-81 of winged bean [**P. tetragonolobus** (L.) DC.] were collected from farmers fields at Wai, Tal, Wai, Dist Satara (MS), India. These were multiplied and maintained in Botanical Garden of the Institute. The mature, dry and healthy seeds were selected and surface sterilized with 0.1% $HgCl_2$ solution for five minutes. These were thoroughly washed in distilled water and placed for germination in pertiplate containing moistened blotting paper.

The growing root tips of 1 cm. length were excised and washed thoroughly with distilled water (DW) pretreated with saturated aqueous para-dichlorobenzene (pDB) at 7-10°c for 2½ hours. After pretreatment the root tips were again thoroughly washed in DW and fixed in Carnoy's fluid for 12 hours, and again washed thoroughly in DW and transferred in 70% alcohol, and stored in refrigerator.

These fixed root tips were washed thoroughly in DW and hydrolyzed by gentle warming in 1N HCl over a spirit lamp flame for few minutes and squashed in 2% propionic-orcein. These squash preparations were made permanent following butyl alcohol: acetic acid series method and using DPX as a mounting medium.

$$\text{Relative length (TCL\%)} = \frac{\text{Chromosome length}}{\text{Absolute length}} \times 100$$

The gradient index (GI) and symmetry index (SI) were calculated by using following formulae (Pitchard, 1967).

$$GI = \frac{\text{Length of shortest chromosome}}{\text{Length of longest chromosome}} \times 100$$

$$SI = \frac{\text{Total length of short arms}}{\text{Total length of long arms}} \times 100$$

3.3 RESULTS:

The details of chromasome number, karyomorphology and karyotype parameters of **P. tetragonolobus** (L) DC are presented in Fig. 3.1 and Table 3.1 and 3.2. The "Idiogram" for the same is presented in Fig 3.2.

The somatic number of **P.tetragonolobus** accession SU-8-81 was determined to be 2n=18 (Fig. 3.1). For the analysis and comparison of the karyotype, the chromosomes were classified into following types on the basis of their length and centromeric position.

Type A: - Long chromosome (3.1597 ± 0.9581 to 3.6172 ± 1.3174 μm) with submedian (sm) centromere.

Type B: - Medium chromosome (2.8281 ± 0.7585 to 2.9939 ± 0.8782 μm) with submedian (sm) centromere.

Table 3.1 Karyomorphological details in **P. tetragonolobus** (L.) DC.

Chrom o-some Pair	Long arm length (l.) (µm)	Short arm length (s) (µm)	Total length of chromosome (C) (µm)	'd' value l-s	'r' value l÷s	'I' value s÷C x 100	Centro-meric position	Chromoso me type / category	Chromo-some size
I	2.4381	1.1792	3.6173	1.2589	2.0676	32.5998	sm	A	Long
	± 1.1969	± 0.9584	± 1.3174						
II	2.0880	1.0717	3.1597	1.0163	1.9483	33.9178	sm	A	Long
	± 1.0379	± 0.5990	± 0.9581						
III	1.8916	1.1024	2.9940	0.7892	1.7159	36.8215	sm	B	Medium
	± 0.7186	± 0.4790	± 0.8782						
IV	1.7902	1.0379	2.8281	0.7523	1.7248	36.6996	sm	B	Medium
	± 0.8777	± 0.5591	± 0.7585						
V	1.5998	1.1147	2.7145	0.4851	1.4352	41.0647	m	B	Medium
	± 0.8782	± 0.8782	± 0.5588						
VI	1.6120	0.9673	2.5793	0.6447	1.6665	37.5010	m	B	Medium
	± 0.4791	± 0.5193	± 0.5190						
VII	1.2252	0.9734	2.1986	0.2518	1.2587	44.2736	m	C	Short
	± 0.5988	± 0.4789	± 0.6780						
VIII	0.9396	0.8075	1.7471	0.1321	1.1636	46.2168	m	C	Short
	± 0.3593	± 0.3194	± 0.4790						
IX	0.8444	0.6939	1.5385	0.1505	1.2169	45.1024	m	C	Short
	± 0.3194	± 0.2395	± 0.3194						

Table 3.2 Karyotypic parameters in **P. tetragonolobus** (L.) DC.

Sr.No.	Name of Karyotypic parameter	Value
1.	TCL (μm)	1.5385 – 3.6172
2.	TCLH (μm) (Average Length)	11.68855
3.	TF %	38.27678
4.	Range of TCL %	6.5812 – 15.4733
5.	GI %	42.5329
6.	SI %	62.01443
7.	F % range	32 – 46.2168
8.	Chromosome number	2n = 18
9.	Karyotype symmetry	2 B
10.	Karyotype formula	K (2n) : 18 : $4A^{sm} + 4B^{sm} + 4B^{m} + 6C^{m}$

Type B_1: - Medium chromosome (2.5794 ± 0.5190 to 2.7145 ± 0.5588 μm) with median (m) centromere.

Type C:- Short chromosome (1.5385 ± 0.3194 to 2.1986 ± 0.6780 μm) with median centromere.

It was revealed from the present studies that the chromosomes of winged bean could be divided into three groups viz; long (3.1597 ± 0.9581 to 3.6172 ± 1.3174μm), medium (2.5794 ± 0.5190 to 2.9939 ± 0.8782 μm) and short (1.5385 ±0.3194 to 2.1986 ± 0.6780 μm). Further on the basis of primary constriction these were recognized as metacentric and submetacentric.

The karyotypic details of **P. tetragonolobus** are depicted in Table 3.1 and 3.2. It exhibits chromosome number 2n=18 with 9 homologous pairs and having absolute chromosome length of 23.3771 μm. with length ranging from 1.5385 ± 0.3194 to 3.6172 ± 1.3174 μm, and average chromosome length of 2.5975 ± 0.6310 μm. The ratio of shortest to longest chromosome of the complement was 0.4253. Range of TCL % 6.5812 to 15.4733, TF %-38.2768, SI %-62.0144, GI – 42.5329, TCLH -11.6886 μm, F% range-32 to 46.2168 and karyotype was of 2B type.

Out of nine homologous chromosome pairs, four pairs (from1st to 4th) were submetacentric and remaining five pairs (from 5th to 9th) were submetacentric. The long chromosomes were found submetacentric, medium chromosomes were submetacentric to metacentric and short chromosomes were metacentric types. No satellite was seen on any

chromosome of the winged bean accession studied. Karyotype formula was found as K (2n): 18: $4A^{sm} + 4B^{sm} + 4B_1 + 6C^m$.

Often it was very difficult to flatten the cells satisfactorily and orient the chromosomes in a single plane.

3.4. DISCUSSION:

The difficulties encountered in karyotypic analysis of winged bean have been reported include, close clumping of chromosomes (Ramirez, 1960); cells were very difficult to flatten satisfactorily and in most cells some chromosomes were not lying entirely in single plane and difficulties in determining homologue chromosome (Pickersgill, 1980); difficulties to identify chromosomes in monochromatic stain (Liyanarachchi *et al.* 1983). In present investigation it was observed that chromosomes could be identified in monochromatic stain (2% aceto-orcein/2% propionic orcein). It was difficult to flatten the cells satisfactorily and orient the chromosomes in a single plane. But with appropriate skill it was possible to flatten the cells and orient the chromosomes in single plane.

The result of present investigation showed that **P.tetragonolobus** (L) DC. has chromosome number 2n=18. This is in conformity with the earlier findings of Tixer (1965), Khan (1976), Haq and Smartt (1977,1978), Pickersgill (1980) and Liyanariachchi *et al.* (1989). However, it disaggrees with the findings made by Ramirez (1960), (2n=26), Zeven and Zhukovsky (1975) (2n=2), Wark (1976)(2n=20) and partially with that of Haq and Smartt (1977, 1978) (2n=16, 18 and 20).

The foregoing reports indicated that there is no consistency in reporting of chromosome number in **P. tetragouolobus.** It seemed possible at one time that a chromosome numerical polymorphism might exist in the genus and even in species; this possibility can now be discounted (Smartt, 1990). The present investigation confirms 2n=18 chromosome number in **P.tetragonolobus.**

The results of the present investigation indicated that the chromosomes of winged bean could be clearly distinguished into three classes viz., long (4 chromosomes), medium (8 chromosomes), and short (6 chromosomes). However, Pickersgill (1980) noted only two classes namely 6 short and 12 long chromosomes while Haq and Smartt (1977,1978) recognized three classes (long, medium and short) of chromosomes.

Pickersgill (1980) reported the presence of long chromosomes with centromere submetacentic to acrocentric while short chromosomes with metacentric or submetacentric one. He also noted the variation in position of the centromere. Further he observed that in one pair of short chromosomes the centromeric region frequently appears longer than in other chromosome which might be due to close juxtaposition of primary and secondary constriction (Personal communication to Pickersgill (1980) from Dr. J. P. Moss). However, Haq and Smartt (1977, 1978) observed chromosomes with median submedian or subterminal centromere.

Present study revealed that long chromosomes were with submedian, medium chromosomes with sumbmedian or median and

short chromosomes with median centromere. Variation in length was found in all these types of chromosomes while variation in centromeric position was observed in the medium chromosomes only. By and large results of present investigation coincides with the observation made by Pickersgill (1980) and Haq and Smartt (1977, 1978).

No satellite and acrocentric chromosome was observed in the present study. However, Pickersgill (1980) reported only acrocentric chromosomes while Haq and Smartt (1977, 1978) reported acrocentric chromosomes with satellite.

The inference of an accurate basic number in a genus exhibiting tremendous amount of variation in chromosome numbers, is an important factor in the study of evolutionary processes within a genus, tribe of a family. As far as the tribe phaseolae is concerned X = 11 is probably the primary basic number (Bir and Sidhu, 1967), though several genera of the tribe have more than one basic number.

Out of the 10 species of **Psophocarpus**, only three species **(P.Palmetothorum, P. Palustris, P scandens** and **P. tetragonoobus)** were studied cytologically and it is revealed from the literature that the somatic chromosome number of the genus ranges from 16 to 26. However present study has confirmed 2n = 18 in **P. tetragonolobus** which indicate X = 9 as the basic chromosome number. Apart from **Psophocarpus** only **Sphenostylis, Centrosema** and **Butea** have been reported to have X = 9 as the basic chromosome number and in these genera the chromosome count of X = 9 comes from a single species and

other basic numbers are reported for other species. Turner and Fearing (1959) have pointed out that x = 9 is rare in the Papilionoidae in general, outside the tribes Sophoreae and Podalyrieae, and is probably derived.

The karyotype of **P.tetragonolobus** is symmetrical, 2B type. It would be very interesting, from a taxonomic point of view, to study evolution in genus **Psophocarpus**, as karyotype of the studied taxa is showing primitive nature. However, aneuploidic reduction (2n = 18, x = 9) gives an idea of derived status of the taxa. However, it does show some size differences within the complement as in **Centrosema virginianum** (2n = 22) (Fritsch, 1972) but in both **Phaseolus** (Joseph and Bouwkamp, 1978) and **Vigna** (Frahm-Leliveld, 1965; Joseph and Bouwkamp, loc. cit.) there is a continuous gradation in size and the longest and shortest chromosomes in the complement do not differ very greatly in length. The cytology of **Psophocarpus** therefore supports the opinion of Lackey (1977) that this genus stands somewhat apart from the rest of phaseolinae.

However, Raven (1975) stressed that for inferring the original basic number of any group, a wide knowledge of its pylogeny is a prerequisite. From all this account, it is clear that the adequate data pertaining to chromosome number is a foremost necessity (Grant, 1982 a, b) and for deducing basic chromosome number of a genus, due attention has to be paid to the maximum number of species sharing the particular gametic number. From the foregoing account it is thus premature to deduce a basic chromosome number (x = 9) for **Psophocarpus.**