

Meiotic Chromosome Studies in the Diversity Diagnostics of *Cyamopsis tetragonoloba* Accessions – an Important Gum-Yielding Plant of Indian Thar Desert

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Cyamopsis tetragonoloba (Linn.) Taub (Fabaceae) is an important commercial crop of India. It is a multipurpose including gum-yielding, drought tolerant, kharif pulse crop grown on light textured soils of arid and semi arid regions. *C. tetragonoloba* is cytogenetically poorly understood. The 15 accessions originating from various regions of India, were investigated to ascertain the nature and extent of genotype differentiation, if any, in the meiotic details at diplotene, diakinesis, metaphase I, anaphase I and anaphase II. The accessions did show suitable variations with respect to meiotic pairing properties and recombination index. Eight accessions, for example, were well differentiated from the remaining seven accessions by the presence of quadrivalent(s) in otherwise diploid species and so on. The present input will have an important role in the improvement programme of the crop.

Key Words: Accessions, Chasima frequency, Chromosome associations, *Cyamopsis tetragonoloba*, Variation

Introduction

Cyamopsis (Fabaceae) comprises four species. *C. tetragonoloba*, commonly known as cluster bean or 'guar', is an important grain legume known for its diverse uses. The demand for the produce and products of this crop species is increasing by leaps and bounds (Bewal *et al.*, 2009). *Guar* is chiefly used for vegetable, animal feed, food additive, medicinal, gum/resin and other diverse purposes. It is also used as thickener and stabilizer in cheese and in cold- meat processing, and in salad dressings, ice-creams, lollipops, bakery products, meat and sausages. *Guar* Gum when used in oil well drilling muds gives a better colloid thereby reducing water losses. It regulates the viscosity of mud solution, stabilizes and regulates the flow properties of the drilling muds. The commercial quality *guar* gum can also be modified into derivatives of industrial importance (Sharma *et al.*, 2003). Despite the multipurpose importance of *Cyamopsis*, there is still paucity of information with regard to cytogenetic approach, such as meiotic behavior of germplasm resources. The details about degree of pairing and types of associations, recombination index and disjunction of chromosome/chromatids at anaphase I/II in various accessions can provide information on subtle differences within species, if any (Kumar *et al.*, 2002; Kumar and Rao, 2002; Kumar and Rao, 2003). It

is universally known that various stages of meiosis are genetically controlled (Zickler and Kleckner, 1998; 1999). Disturbance in the synaptic process due to various kinds of mutation, has been a subject of vast studies in many plant species (Bennett, 1984; John, 1990; Maguire and Reiss, 1996; Dave, 1998). The present work is concerned with species cytogenetics particularly with regard to the insight into extent and nature of genotype differentiation on the basis of meiotic behavior properties.

Materials and Methods

The seeds of different accessions (Table 1) of *Cyamopsis tetragonoloba* were obtained from the Division of Plant Improvement and Biotechnology, Central Arid Zone Research Institute, Jodhpur, Rajasthan. Flower buds of appropriate size were collected from mature plants. The buds were fixed on the spot in freshly prepared 1:3 glacial acetic acid: 95% ethanol mixture for at least 24 h at room temperature. These were subsequently transferred to 70% ethanol and stored at 10°C.

Anthers were squashed in 1% acetocarmine. On an average, 20-25 pollen mother cells (PMCs) were analyzed at diplotene/diakinesis and metaphase I. During compilation of the meiotic data, it was observed that only minor differences existed between diakinesis

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Table 1. Mean number and range of chromosome associations at diplotene/diakinesis/metaphase I in *Cyamopsis tetragonoloba* accessions

Accession number	No. of cells analyzed	2n	Chromosome associations												Univalents		
			Quadrivalents			Bivalents			Rod								
			Total			Ring			Rod			Univalents					
			No	Mean	Range	No	Mean	Range	No	Mean	Range	No	Mean	Range			
IC-370468	25	14	1	0.04±0.04	0-1	158	6.32 ±0.6	6-7	148	5.92±1.03	5-7	10	0.44±0.64	0-2	26.00	1.04±1.09	0-2
IC-373467	25	14	6	0.24±0.43	0-1	133	5.32±1.28	3-7	122	4.88±0.89	3-7	11.00	0.44±0.50	0-1	30.00	1.2±086	0-2
IC-40039	25	14	1	0.04±0.2	0-1	167	6.68±0.47	0-7	156	6.24±0.92	3-7	10.00	0.44±0.5	0-1	16.00	0.64±0.94	0-2
IC-40045	25	14	-	-	-	171	6.84±0.37	6-7	167	6-68±0.62	6-7	4.00	0.16±0.3	0-1	8.00	0.32±0.7	0-2
IC-368961	25	14	-	-	-	175	7.00 ± 0.31	-	171	6.84±0.46	5-7	4.00	0.16±0.46	0-2	0.00	0.00	0-0
SRV10JT14		14	-	-	-	172	6.88±0.32	6-7	164	6.56±0.57	5-7	8.00	0.32±0.46	0-1	6.00	0.24±0.6	0-2
SRV11JT15		14	-	-	-	172	6.88±0.32	6-7	170	6.80±0.4	6-7	2.00	0.80±0.27	0-1	6.00	0.24±0.64	0-2
SRV13JT17		14	6	0.24±0.43	0-1	133	5.32±1.28	3-7	122	4.88±0.89	3-7	11.00	0.44±0.50	0-1	30.00	1.2±086	0-2
SRV28JT32		14	6	0.24±0.59	0-2	147	6.12±1.05	4-7	137	5.48±1.63	2-7	12.00	0.48±0.77	0-2	36.00	1.401±2.04	0-6
SRV29JT32		14	8	0.32±0.47	0-1	133	5.32±1.78	2-7	129	5.16±1.15	3-7	12.00	0.48±0.71	0-2	34.00	1.36±1.60	0-4
SRV31JT35		14	-	-	-	175	7.00±0.00	-	170	6.80±0.4	6-7	5.00	0.20±0.4	0-2	0	0.00	0-0
PLG-520		14	1	0.04±0.2	0-1	167	6.68±0.47	5-7	156	6.24±0.92	3-7	10.00	0.44±0.5	0-1	16	0.64±0.94	0-2
PLG-600		14	6	0.24±0.43	0-1	171	6.84±0.37	6-7	167	6-68±0.62	6-7	4.00	0.16±0.3	0-1	8.00	0.32±0.7	0-2
PLG-747		14	-	-	-	175	7.00±0.00	-	157	6.28±0.72	4-7	18.00	0.72±0.72	0-3	0	0.00	0-0
PLG-752		14	-	-	-	133	5.32±1.78	5-7	129	5.16±1.15	3-7	12.00	0.48±0.71	0-2	34.00	1.36±1.60	0-4

and metaphase I stages with respect to associations and chiasma frequency. Therefore, the authors have analyzed the data for both these meiotic stages together, taking approximately equal number of cells from both the stages. About 15-20 PMCs were analyzed at anaphase I and/or II. For percentage pollen stainability, the pollen grains were stained in 1:1 glycerine: acetocarmine mixture. On an average, 10 slides were scored for stainable pollen. Photomicrographs of chromosome preparations were taken from temporary slides with Trinocular Research Microscope (Olympus, model BX60F).

Results and Discussion

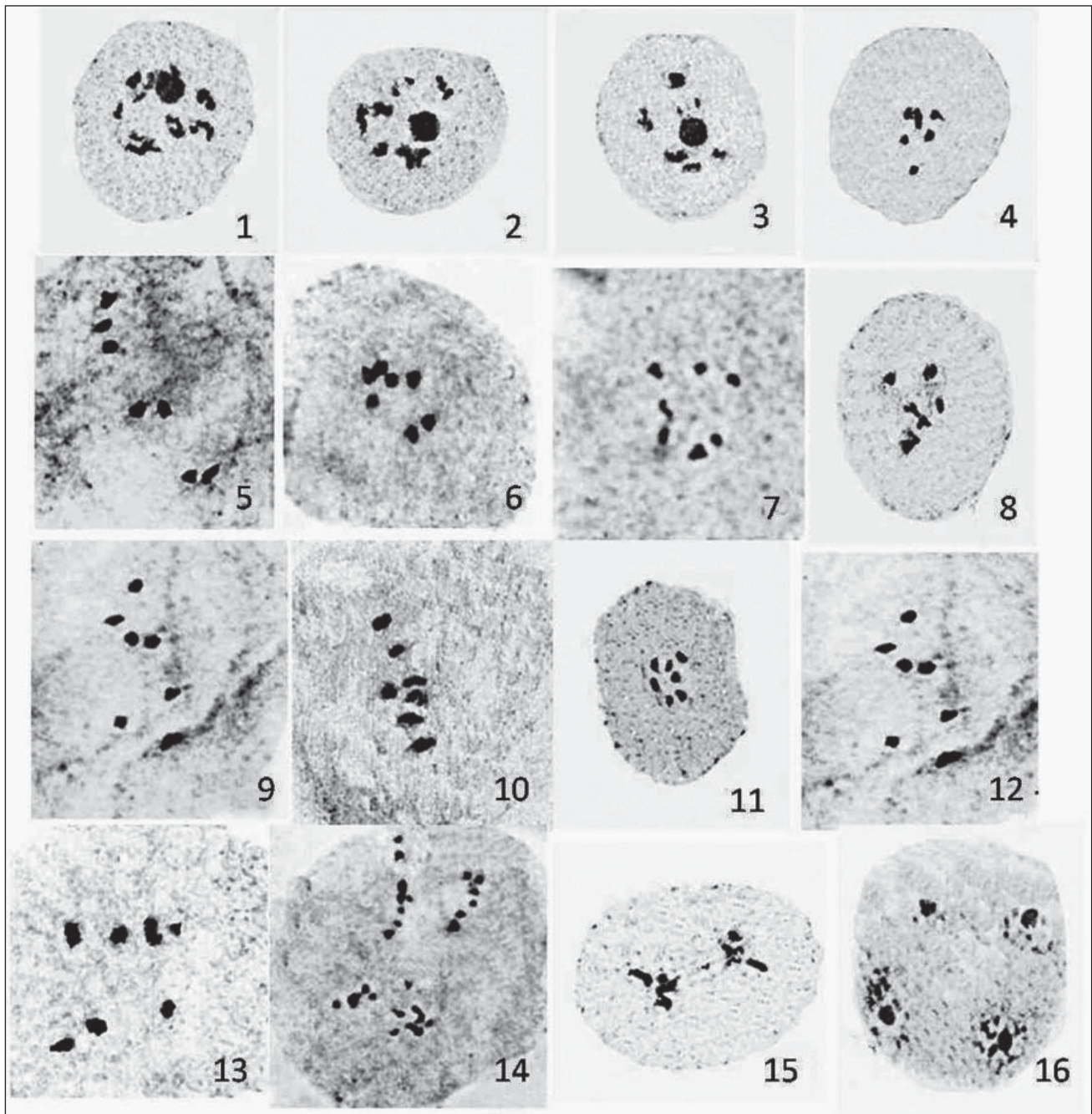
The details with regard to chromosome associations at diplotene/diakinesis/metaphase I, recombination index and distribution of chromosomes at respective poles and pollen stainability of the analyzed accessions are given in Tables 1-3 and illustrated in Figs 1-28.

From Table 1, it is amply clear that all the accessions presently investigated were characterized by gametic number of 7 ($n=7$). No deviation with respect to this number ($n=7$, $2n=14$) has been encountered in any of the 375 PMCs analyzed for 15 accessions. In ascertaining the true basic number of the genus, it is essential to know the chromosome number of all the species. The perusal of literature indicates that the chromosome number of only two (*C. tetragonoloba*, $2n=14$ and *C. psoroloides*, $2n=14$) out of the four species is known. Based on the chromosome number reported for the two species and the meiotic behavior of the presently investigated species, it can be stated that 7 could be the true basic number of the genus.

Barring three accessions (*C. tetragonoloba* IC-368961, SRV31JT35 and PLG-747), all the remaining accessions had a mixture of bivalents and univalents at diplotene/ diakinesis/ metaphase I. The three accessions have 7 bivalents in all PMCs analysed. The least (5.32) number of bivalents per PMC (76%) was observed in the four accessions, viz. IC-373467, SRV13JT17, SRV29JT32 and PLG-752. The highest number (1.40) of univalents per PMC was recorded in *C. tetragonoloba* SRV28JT32 followed by 1.361 in SRV29JT32 and PLG-752. Such variation of chromosome association across various accessions is not uncommon in the plant species from Thar desert (Kumar *et al.*, 2002; Rao and Kumar, 2003; Kumar and Rao, 2003; Rawat *et al.*, 2006, Rawat *et al.*, 2007). The presence of univalents in various PMCs, by and large, did not influence the distributional pattern of chromosomes at anaphase I in, 13 out of the 15 accessions, presently

Table 2. Mean number, range of chiasmata and terminalization coefficient and pollen stainability

Accession	No. of cell analysed	2n	Chiasmata												Terminilized coefficient	Pollen stainability percentage
			Total			Terminalised			Unterminalised							
			No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range		
IC-370468	25	14	317	12.68±1.5	0 – 14	231	9.24± 1.3	0 – 14	86	3.2± 1.7	7-1	0.73	94			
IC-373467	25	14	311	12.44± 1.40	11 – 14	246	9.84±3.17	4– 13	65	2.6± 1.80	0-7	0.79	92			
IC-40039	25	14	324	12.96± 1.05	11 – 14	253	10.00± 2.26	5 – 13	71	2.91± 1.90	0-6	0.78	88			
IC-40045	25	14	338	13.52± 0.90	12 – 17	265	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.78	90			
IC-368961	25	14	356	14.24± 0.90	12 – 17	196	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.81	92			
SRV10JT14	25	14	343	13.72± 0.87	11 – 15	201	8.04± 2.39	4 – 12	142	5.68± 2.34	1-11	0.70	94			
SRV11JT15	25	14	354	14.16± 1.40	12 – 16	200	8.00±2.17	3 – 11	154	6.16± 1.80	3-11	0.77	88			
SRV13JT17	25	14	317	12.68±1.5	0 – 14	231	9.24± 1.3	0 – 14	86	3.2± 1.7	7-1	0.72	93			
SRV28JT32	25	14	300	12.00± 2.46	7 – 14	217	8.68± 2.41	3 – 11	83	3.32± 1.84	1-7	0.73	96			
SRV29JT32	25	14	312	12.48±200	0 8-14	229	9.16 ±2.46	7-13	83	3.2±1.84	1-6	0.90	94			
SRV31JT35	25	14	324	12.96± 1.05	11 – 14	253	10.00± 2.26	5 – 13	71	2.91± 1.90	0-6	0.74	90			
PLG-520	25	14	343	13.72± 0.87	11 – 15	201	8.04± 2.39	4 – 12	142	5.68± 2.34	1-11	0.70	92			
PLG-600	25	14	351	14.04±0.87	13 – 15	184	7.36± 1.80	3 – 11	167	6.68± 2.07	2-11	0.90	89			
PLG-747	25	14	342	13.68± 0.88	12 – 15	146	5.84± 2.37	1 – 9	146	5.84± 2.32	1-9	0.74	98			
PLG-752	25	14	356	14.24± 0.90	12 – 17	196	7.84± 2.09	4 – 11	160	6.40± 1.89	3-9	0.81	96			



Figs. 1-28: Male meiosis in *Cyamopsis tetragonoloba* accessions

Figs. 1-3: Diakinesis 7II (IC-373467); Fig. 4: Metaphase I 7II (IC-373467); Fig. 5: Metaphase I 7II (IC-370468); Figs. 6-7: Metaphase I 7II (IC-40039); Fig. 8: Metaphase I 7II (SRV29JT32); Figs. 9 and 12: Metaphase I 7II (SRV10JT14); Fig. 10: Metaphase I 7II (SRV11JT15); Figs. 11 and 1: Metaphase I 7II (SRV28JT32); Fig. 13: Metaphase I 7II (SRV31JT35); Fig. 14: Anaphase II (SRV31JT35); Fig. 15: Anaphase I (SRV28JT32); Fig. 16: Anaphase II (SRV13JT32).

analyzed. However, in two accessions the distribution was affected leading to occurrence of lagging univalent/bivalents in a few PMCs. Precocious separation of rod bivalents or early separation of synapsed homologues is generally the reason for regular occurrence of univalents

in many of the tree species (Kumar, 2000; Singh, 1993). However, the present observations in *Cyamopsis tetragonoloba* indicate that the probability of existence of heterozygosity among few bivalents might be the reason for formation of univalents. In five accessions (IC 373467,

Table 3. Anaphase I distribution

Accession number	2n	No. of cell analysed	Chromosome distribution	No. of cells	Percentage
IC-370468	14	15	7:7	15	100
IC-373467	14	15	7:7	14	93.3
			7:1U:6	1	6.6
IC-40039	14	15	7:7	15	100
IC-40045	14	15	7:7	15	100
IC-368961	14	15	7:7	15	100
SRV10JT14	14	15	7:7	15	100
SRV11JT15	14	15	7:7	15	100
SRV13JT17	14	15	7:7	15	100
SRV28JT32	14	15	7:7	15	100
SRV29JT32	14	15	7:7	15	100
SRV31JT35	14	15	7:7	15	100
PLG-520	14	15	7:7	15	100
PLG-600	14	15	7:7	13	100
			6:1U:7	1	
			7:1U:6	1	
PLG-747	14	15	7:7	15	100
PLG-752	14	15	7:7	15	100

IC 373467, SRV29 JT32 and SRV28JT32), 1-2 bivalents at diplotene were closely associated with the nucleolus thereby indicating that 1-2 pairs of nucleolar chromosomes exist in the species.

The highest and lowest recombination index was recorded in four and one accessions, respectively. In general, two chiasma per bivalent was the rule. In most of the cases, the chiasmata was found to be located at distal ends. Interstitial chiasma were generally absent even at diplotene. Due to the fact that no bivalent had more than two chiasma makes it difficult to assume random distribution of chiasmata. Distal chiasmata prevents some chromosome segments in bivalents from undergoing recombinations resulting in preservation of specific gene combinations intact (John and Lewis, 1965; Sybenga, 1972; Singh, 1993; Gupta, 1995). Occurrence of distally localized chiasmata has been attributed to gene control, nature of chromosome pairing and/or interference pattern or even the availability of short segment for crossing over (Kumar, 2000; Stace, 2000; Kumar and Rao, 2002).

All but two accessions were characterized by the equal (7:7) distribution of chromosomes at anaphase I. Subsequent course of meiosis was normal resulting in good pollen stainability. Two accessions showed unequal

(7:6) distribution of chromosomes at anaphase I in one cell each. The existence of univalent(s) and (or) multivalents is considered to be main reason for abnormal distribution of chromosomes at anaphase I (Stebbins, 1971; Sybenga, 1972; Singh, 1993; Gupta, 1995; Rao and Chandel, 1991; Kesavacharyulu, 1988; Arya *et al.*, 1987; Rao and Kumar, 2003; Kumar *et al.*, 2002; Rawat *et al.*, 2007).

Three accessions were isolated from the remaining accessions by the presence of one quadrivalent in a few cells. The reason(s) for the formation of quadrivalents in the diploid cytotype is not clear.

Bewal *et al.* (2009) have induced colchitetraploidy in few accessions of *Cyamopsis tetragonoloba* and reported widespread occurrence of quadrivalent formation in PMCs analyzed. However their putative diploids were characteristic in not showing the presence of quadrivalents in them. Therefore, the sporadic occurrence of quadrivalent in diploids, may be attributed to plausible partial homology between otherwise non homologous chromosomes arising out of structural rearrangements (Stebbins, 1971).

Thus, it is concluded that chromosome structural changes rather than numerical changes have resulted in intra-specific diversity in *Cyamopsis tetragonoloba*.

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