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KARYOMORPHOLOGICAL AND NUCLEAR CHARACTERS IN SOME PULSE CROPS

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Karyotypic and nuclear DNA contents were estimated in six important pulse crops viz. *Cicer arietinum, Cajanus cajan, Lens culinaris, Pisum sativum, Vigna radiata* and *Vigna mungo*. There were 1-5 metacentrics, 3-8 sub-metacentrics and 0-3 acrocentrics. Except in *Cajanus* in all others, a pair of satellite chromosomes was noticed. TC1%, S%, F%, TF% values indicates, asymmetry is low in all the six pulses. Considerable variation was noticed in 2C nuclear DNA amounts among six genotypes. Both presence and absence of correlation between chromatin length and nuclear DNA was noticed. Variation in nuclear DNA contents was found due to quantitative variation in heterochromatin.

Keywords : Karyomorphology; Nuclear characters; Pulse crops.

Introduction

Karyotypic studies in various pulse crops made in the past were mainly aimed to study taxonomic relationships. No studies were made in nuclear DNA and heterochromatin in relation to karyomorphological studies. The present paper reports the standard karyotypes, and nuclear DNA and heterochromatin in six important Indian pulse crops.

Materials and Methods

Seeds of six important pulse crops used in the present investigation included C-235 (*Cicer arietinum* L.), ICP 7925 (*Cajanus cajan* (L.) Millsp), Rachna (*Pisum sativum* L.), L-4076 (*Lens culinaris* Medik.), PS-16 (*Vigna radiata* (L.) Wilczek), Pusa 105 (*Vigna mungo*).

For root tip mitosis, water soaked seeds were germinated on moist filter paper at 20°C in BOD incubator. Young and healthy root tips were pretreated with saturated solution of PDB (para-dichlorobenzene) for 3- $3\frac{1}{2}$ h at 15°C. The root tips were then fixed in 3:1 (alcoholacetic acid) for 24 h. Root tip squashes were made accorrding to standard procedures in 2% acetocarmine. Chromosome measurements were made by olympus micrometer on five well spread metaphase cells and average values were used to draw conclusion regarding the chromosome morphology. Somatic chromosome morphology based on, chromosome length, centromere position and other parameters like relative length, arm ratio, F% TC1%, S%, chromosome volume was recorded according to Reddy (1989a), Reddy and Annadurai (1992), Reddy and Thresiamma (1992). Chromosome types (A,B,C) based on Chromosome lengths (μ) were made using the following classification:

Type of chromosome/Crop variety	A	B	C
Cicer arietinum and *Cajanus cajan*	3.00	2.01-3.00	2.00
Lens culinaris	5.00	4.01-5.00	4.00
Pisum sativum	4.50	3.50-4.50	3.50
Vigna radiata and *Vigna mungo*	2.00	1.50-2.00	1.50

The nuclear DNA contents were estimated according to our own standard procedure (Reddy,1989b), while the heterochromatin was estimated according to the procedures of Banerjee and Sharma (1985).

Results and Discussion

The data on various chromosome morphological and nuclear characters in six different pulse crops are presented in Table 1 & 2 (Figs.1-6). In all the six genotypes under study, the diploid chromosome number was found to be same as reported in earlier studies. Chromosome number 2n = 16 was recorded in different species of *Cicer* (Ladizinsky and Adler, 1976; Ahmad and Godward, 1980); 2n = 22 in *Cajanus cajan* (Sinha and Kumar, 1979; Lavania and Lavania, 1983); 2n = 14 in the genus *Lens* (Reddy and Annadurai, 1992; Reddy and Thresiamma, 1992) and in *Pisum sativum* (Fouzdar and Tandon, 1976); 2n = 22 was observed in *Vigna radiata* (Sarbhoy, 1980) and in *Vigna mungo* (Lavania and Lavania, 1983). Similarly, the total haploid chromatin lengths also coincide with earlier reports, except for individual chromosome lengths and centromere positions. Among six pulse crops, considerable differences were noticed in chromatin length and chromatin volume. Highest chromatin length was noticed in *Lens* (31.91 μ) and the lowest was found in *Vigna mungo* (18.39 μ). It is also interesting to note from the present results that there was a reduction in chromatin length with increase in chromosome number. Reduced chromosome lengths in polyploids is, however, not uncommon in plant systems (Sharma, 1972). However, the mechanism by which the reduction is acheived is poorly understood. Factors like, chromosome condensation, inactivation of gene segments in polyploids, loss of heterochromatin segments of differential polynemy have been suggested to play a role in this regard.

In the present study, except in the genus *Cajanus* in all other five pulse crops, a pair of satellite chromosomes was noticed in the diploid complement. Presence of a pair of satellite chromosomes was also noticed in different varieties of *Cicer arietinum* (Ahmad and Godward, 1980), *Cajanus cajan* (Sinha and Kumar, 1979), *Lens culinaris* (Reddy and Annadurai, 1992; Reddy and Thresiamma, 1992), *Pisum sativum* (Lavania and Lavania, 1983), *Vigna radiata* (Sahai and Rana, 1980) and *V. mungo* (Goswami, 1979). However, varietal variation for presence or

TABLE 1 : KARYOMORPHOLOGICAL CHARACTERS IN SOME PULSE CROPS

Chromo-some pair	Type	Chromo-some length (μ)	Arm Length (μ)		Chromatid width (μ)	Chromo some volume (μ^3)	Arm length	Relative length (%)	F%	TCI%	Classification
			Long arm	Short arm							
I	A	3.63	2.12	1.51	0.94	2.51	1.40	100.00	41.59	18.75	M
II	A	3.24	2.09	1.15	0.94	2.24	1.81	89.25	35.49	16.74	Sm
III	B	2.58	1.48	1.10	0.95	1.82	1.34	71.07	42.63	13.33	M
IV	B	2.35	1.54	0.81	0.94	1.63	1.90	64.73	34.46	12.14	Sm
V	B	2.26	1.34	0.92	0.94	1.56	1.45	62.25	40.70	11.67	M
VI	B	2.04	1.54	0.50	0.93	1.38	3.08	56.19	24.50	10.54	Ac
VII	C	1.66	0.94	0.72	0.94	1.15	1.30	45.73	43.37	8.57	M
VIII	C	1.59	1.03	0.56	0.94	1.10	1.83	43.80	35.22	8.21	Sm
B. *Cajanus cajan* (L.) Mill sp. (2n=22)											
I	A	3.39	2.19	1.20	0.92	2.25	1.82	100.00	35.39	12.86	Sm
II	B	2.88	1.57	1.31	0.92	1.91	1.19	84.95	45.48	10.92	M
III	B	2.75	1.49	1.26	0.93	1.86	1.18	81.12	45.81	10.43	M
IV	B	2.54	1.62	0.92	0.91	1.65	1.76	74.92	36.22	9.63	Sm
V	B	2.33	1.30	1.03	0.91	1.51	1.26	68.73	44.20	8.83	M
VI	B	2.24	1.43	0.81	0.91	1.45	1.76	66.07	36.16	8.49	Sm
VII	B	2.13	1.37	0.76	0.92	1.41	1.80	62.83	35.68	8.08	Sm
VIII	B	2.08	1.33	0.75	0.92	1.38	1.77	61.35	36.05	7.89	Sm
IX	B	2.04	1.31	0.73	0.91	1.32	1.79	60.17	35.78	7.73	Sm
X	B	2.00	1.30	0.70	0.91	1.30	1.85	58.99	35.00	7.58	Sm
XI	C	1.98	1.29	0.69	0.92	1.31	1.86	58.40	34.84	7.51	Sm

Contd...

Chromosome pair	Type	Chromosome length (μ)	Arm Length (m)		Chromatid width (μ)	Chromosome volume (μ^3)	Arm length	Relative length (%)	F%	TCI%	Classification
			Long arm	Short arm							
C. *Lens culinaris* Medik. (2n=14)											
I	A	6.65	3.68	2.97	0.93	4.51	1.23	100.00	44.66	20.83	M
II	B	5.19	3.54	1.65	0.94	3.59	2.14	78.04	31.79	16.26	Sm
III	B	4.81	3.64	1.17	0.92	3.19	3.11	72.33	24.32	15.07	Ac
IV	B	4.56	3.04*	1.52	0.90	2.89	2.00	68.57	33.33	14.29	Sm
V	C	3.86	2.90	0.96	0.92	2.56	3.02	58.04	24.87	12.09	Ac
VI,	C	3.50	2.64	0.86	0.89	2.17	3.06	52.63	24.57	10.96	Ac
VII	C	3.34	2.27	1.07	0.88	2.03	2.12	50.22	32.03	10.46	Sm
D. *Pisum sativum* L. (2n=14)											
I	A	4.82	2.61	2.21	0.96	3.48	1.18	100.00	45.85	16.90	M
II	A	4.56	2.93	1.63	0.95	3.23	1.79	94.60	35.74	15.99	Sm
III	B	4.38	2.81	1.55	0.95	3.10	1.81	90.87	35.38	15.36	Sm
IV	B	4.12	2.64	1.48	0.96	2.98	1.78	85.47	35.92	14.45	Sm
V	B	3.88	2.92	0.96	0.96	2.80	3.04	80.49	24.74	13.60	Ac
VI	C	3.57	2.29	1.28	0.95	2.52	1.78	74.06	35.85	12.52	Sm
VII	C	3.18	2.06	1.12	0.96	2.30	1.83	65.97	35.22	11.15	Sm
E. *Vigna radiata* (L.) Wilczek (2n=22)											
I	A	2.34	1.29	1.06	0.89	1.45	1.21	100.00	45.29	12.50	M
II	A	2.13	1.11	1.02	0.87	1.26	1.08	91.02	47.88	11.38	M
III	B	1.84	1.06	0.78	0.88	1.11	1.35	78.63	42.39	9.83	M
IV	B	1.78	1.16	0.62	0.88	1.08	1.87	76.06	34.83	9.51	Sm

Contd...

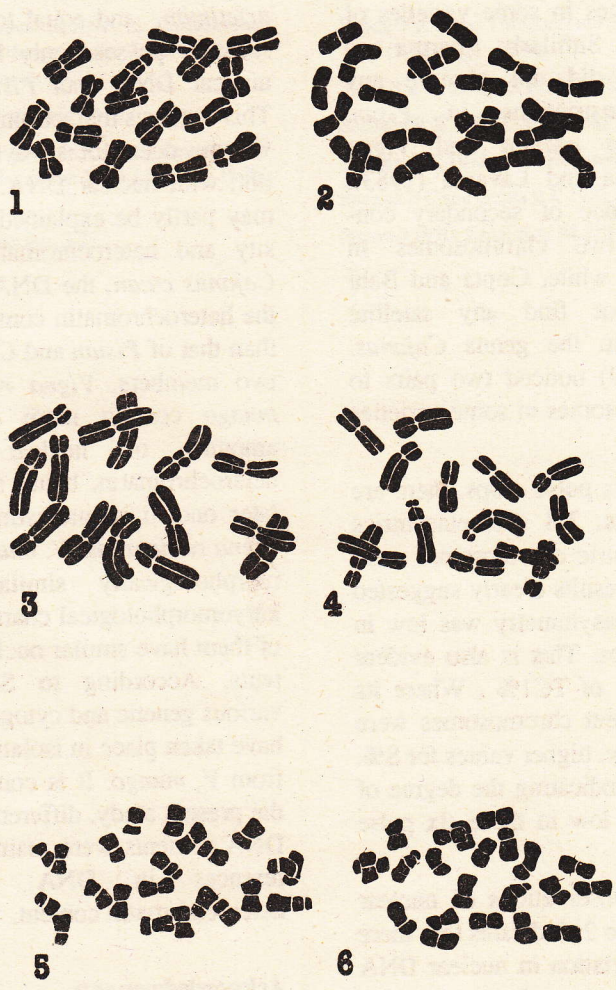
Table 1 Contd.....

Chromo-some pair	Type	Chromo-some length (μ)	Arm Length (m)		Chromatid width (μ)	Chromo some volume (μ^3)	Arm length	Relative length (%)	F%	TCI%	Classifica-tion
			Long arm	Short arm							
V	B	1.74	1.32	0.42	0.87	1.03	3.14	74.35	24.13	9.29	Ac
VI	B	1.63	1.24	0.39	0.87	0.96	3.17	69.65	23.92	8.71	Ac
VII	B	1.58	1.20	0.38	0.87	0.93	3.15	67.52	24.05	8.44	Ac
VIII	C	1.49	0.98	0.51	0.88	0.90	1.92	63.67	34.22	7.96	Sm
IX	C	1.44	0.96	0.48	0.87	0.85	2.00	61.53	33.33	7.69	Sm
X	C	1.39	0.93	0.46	0.87	0.82	2.02	59.40	33.09	7.42	Sm
XI	C	1.35	0.90	0.45	0.87	0.80	2.00	57.69	33.33	7.21	Sm
F. *Vigna mungo* (L.) Hepper (2n=22)											
I	A	2.38	1.29	1.09	0.86	1.38	1.18	100.00	45.79	12.94	M
II	A	2.22	1.17	1.05	0.85	1.31	1.11	93.27	45.25	12.07	M
III	B	1.78	1.14	0.64	0.86	1.03	1.78	74.78	35.95	9.67	Sm
IV	B	1.74	0.96	0.78	0.86	1.01	1.23	73.10	44.82	9.46	M
V	B	1.68	0.91	0.77	0.86	0.97	1.18	70.58	45.83	9.13	M
VI	B	1.62	0.89	0.73	0.86	0.94	1.21	68.06	45.06	8.80	M
VII	B	1.53	1.15	0.38	0.85	0.86	3.02	64.28	24.83	8.31	Ac
VIII	C	1.47	1.11	0.36	0.86	0.85	3.08	61.76	24.48	7.99	Ac
IX	C	1.35	0.87	0.48	0.86	0.78	1.81	56.72	35.55	7.34	Sm
X	C	1.33	0.86	0.47	0.85	0.75	1.82	55.88	35.33	7.23	Sm
XI	C	1.29	0.84	0.45	0.86	0.74	1.86	54.20	34.88	7.01	Sm

* = presence of satellite; M = median centromere; Sm = submedian centromere; Ac = acrocentric

TABLE 2 : SOMATIC CHROMOSOME MORPHOLOGY AND NUCLEAR CHARACTERSIN SOME PULSE CROPS

S.No.	Nuclear parameter	*Cicer arietinum*	*Cajanus cajan*	*Lens culinaris*	*Pisum sativum*	*Vigna radiata*	*Vigna mungo*
1.	Total chromatin length (μ)	19.35	26.36	31.91	28.51	18.71	18.39
2.	Average chromosome length (μ)	2.41	2.40	4.56	4.07	1.70	1.67
3.	Average chromatid width (μ)	0.94	0.91	0.91	0.95	0.87	0.85
4.	Total chromatin volume (μ^3)	13.39	17.35	20.94	20.41	11.19	10.62
5.	S%	43.80	58.40	50.22	65.97	57.69	54.20
6.	TF%	37.57	38.54	31.96	35.88	35.11	39.15
7.	Karyotypic formula	1A M+2A Sm+2B M+1B Sm+1B AC+1C M+1C Sm	1A Sm + 3BM +6B Sm+1C sm	1A M+2B SM+1B Ac+1C SM+2C Ac	1A M+1A Sm 2B+Sm+1B Ac+2C Sm	2A M+1B M+1B Sm+3B Ac+4C Sm	2A M+3B M+1B Sm+1B Ac 3C Sm+1C Ac
8.	Nuclear volume (μ^3)	347.08 ± 0.11 (333-354)	366.29± 0.06 (349- 378)	424.39 ± 0.12 (416-431)	389.51 ± 0.12 (371-396)	311.47 ± 0.09 (306- 318)	304.26 ± 0.12 (299-314)
9.	DNA density(2C) (arbitary Units)	1.90 ± 0.12 (1.79-1.98)	1.48 ± 0.18 (1.41-1.52)	3.29 ± 0.16(3.26-3.34)	3.18 ± 0.11 (3.11-3.24)	1.18 ± 0.6 (1.13-1.26)	1.09 ± 0.10 (1.04-1.23)
10.	DNA content (pg) (2C)	4.86 ± 0.13 (4.59-4.92)	3.42 ± 0.18 (3.28- 3.51)	10.29 ± 0.23 (10.01-10.89)	9.76 ± 0.18 (9.54-9.89)	2.78 ± 0.16 (2.64-2.97)	2.61 ± 0.14 (2.48-2.73)
11.	Heterochromatin (arbitary units)	0.932 ± 0.04 (0.872-0.944)	0.987 ± 0.06 (0.981-1.013)	0.513 ± 0.08 (0.503-0.596)	0.412 ± 0.06 (0.398-0.426)	0.118 ± 0.05 (0.112-0.129)	0.111 ± 0.04 (0.099-0.122)
12.	Heterochromatin (%) in total nuclear DNA	19.17	28.85	4.98	4.22	4.24	4.25



Figures : 1-6 Somatic metaphase chromosomes in different pulse crops (camera lucida drawings,1500 x) Fig 1. *Cicer arietinum* 2n= 16); Fig 2. *Cajanus cajan* (2n = 22); Fig 3. *Lens culinaris*, (2n = 14); Fig 4. *Pisum sativum* (2n = 14); Fig 5. *Vigna radiata* (2n = 22); Fig 6. *Vigna mungo* (2n = 22).

absence and number of satellite chromosomes were noticed in the above studies. For example, Iyengar (1939) reported presence of two satellite chromosomes in *Cicer arietinum*. Sinha and Kumar (1979) and Sharma and Gupta (1982) did not observe any satellite chromosomes in some varieties of *Cajanus cajan*. Similarly, Sharma and Gupta (1982) did not observe any secondary constrictions in *Pisum sativum, Vigna radiata* and *Vigna mungo*. Lavania and Lavania (1983), recorded presence of secondary constrictions in two chromosomes in *Cajanus cajan,* while, Gupta and Bahl (1983) did not find any satellite chromosomes in the genus *Cajanus*. Goswami (1979) noticed two pairs to satellite chromosomes in some varieties of *V. mungo*.

In all the six pulse crops, there are 1-5 metacentrics, 3-8 submetacentrics and 0-3 acrocentric chromosomes were noticed. These results clearly suggested that, karyotype asymmetry was low in these pulse crops. This is also evident from the values of TC1% , Where its range for different chromosomes were narrow. Similarly, higher values for S%, F%, TF% also indicating the degree of asymmetry was low in these six pulse crops.

A persual observations of nuclear DNA (2C) (Table 2) indicates that there is significant variation in nuclear DNA contents among six pulse crops. The genera *Lens* and *Pisum,* which have highest chromatin length and volume contain highest nuclear DNA. This confirms the generalized phenomena of existance of positive correlation between chromosome length and DNA content (Furuta and Nishikawa, 1991). However, the genus *Cajanus* having more chromatin length and volume than *Cicer arietinum,* and equal to that of *Pisum sativum,* possess only less amount of nuclear DNA than *Pisum* and *Cicer*. This suggests that, chromatin length and volume does not always have correlation with nuclear DNA. This variation may partly be explained by DNA density and heterochromatin content. In *Cajanus cajan,* the DNA is low, while the heterochromatin content was higher than that of *Pisum* and *Cicer*. The other two members, *Vigna radiata* and *V. mungo* contain more of less equal amounts of nuclear DNA and heterochromatin, being slightly low in later one. It is interesting to note that *Vigna radiata* and *V. mungo,* which are morphologically similar, differ in karyomorphological characters but both of them have similar nuclear DNA contents. According to Smartt (1980), various genetic and cytogenetic changes have taken place in isolating *V. radiata* from *V. mungo*. It is concluded that in the present study, differences in nuclear DNA contents were mainly due to differences in DNA density and heterochromatin content.

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