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Karyotype of the kabuli-type chickpea (*Cicer arietinum* L.) by image analysis system

GIANFRANCO VENORA, BRUNO OCAMPO * K.B. SINGH * and FRANCESCO SACCARDO **

Stazione Sperimentale di Granicoltura per la Sicilia (SSGS), Caltagirone, Italy; * International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria; ** Cattedra di Orticoltura, Università della Tuscia, Viterbo, Italy.

SUMMARY — Detailed information on karyotype of the kabuli-type chickpea (*Cicer arietinum* L.) has been lacking; hence this study was undertaken to produce the standard karyotype of kabuli chickpea and to understand the variation in karyotype within kabuli type. Mitotic analysis of four genotypes differing in morpho-agronomic traits and geographic origin were carried out. Little difference was observed among the four genotypes for total genome length, chromosome length and complement formula. All carry a distinguishable satellite on the longest chromosome pair. Small variation in karyological parameters within kabuli type might be due to its narrow adaptation. The karyotype of the variety Sultano has been chosen as the standard for kabuli-type chickpea on the base of its parameters being closest to the average of those genotypes that were investigated.

Key words: *Cicer arietinum*, chromosome, karyotype, image analysis, chickpea.

INTRODUCTION

Information on karyology in useful in the transfer of genes from wild to cultivated species. This knowledge has assumed a greater significance in recent years in chickpea (*Cicer arietinum* L.) after the discovery of wealth of genes for resistance to biotic and abiotic stresses in wild *Cicer* species (SINGH *et al.* 1989, 1990; SINGH and REDDY 1993; SINGH and WEIGAND 1993). The use of image analysis system has made it possible to study the karyotype in detail with precision (VENORA *et al.* 1991). This technique has already been used for the study of karyotypes of the eight annual wild *Cicer* species (OCAMPO *et al.* 1991).

This paper is a part of the collaborative project "Development of chickpea germplasm with combined resistance to ascochyta blight and fusarium wilt using wild and cultivated species", between Italian institutions and ICARDA, Syria.

DOMBROWSKY-SLUDSKY (1927) was the first to report the chromosome number ($2n = 14$) in chickpea. Over the next 30 years, researchers reported either $2n = 14$ or $2n = 16$ chromosome (RAO 1929; DIXIT 1932; FRAHM-LELIVELD 1957). This controversy has now been settled and the chromosome number in chickpea is accepted as $2n = 16$.

Most karyological investigations have been reported from desi-type chickpea (characterized by small, angular and coloured seeds) grown mainly in the Indian subcontinent and East Africa (PHANDIS 1970; AHAMD and GODWARD 1980; KUTAREKAR and WANJARI 1983; SHARMA and GUPTA 1986). Limited studies have been undertaken on the kabuli-type chickpea (characterized by large, ram-head shaped and beige coloured seeds) which is primarily grown in the Mediterranean region and the Americas. Hence in this study four kabuli-type chickpea and image analysis system was used with the objective of developing a karyotype of kabuli chickpea.

MATERIALS AND METHODS

Four kabuli-type lines and varieties, FLIP 82-150c, ILC 482, Calia and Sultano, were used in this study. These materials differ in geographical origin and morpho-physiologic traits (Table 1). Seeds were germinated on moist filter paper in Petri dishes kept in the dark at room temperature. Young and turgid primary roots 1.5 cm long were cut off and pretreated in a saturated aqueous solution of 1,4-dichlorobenzene for 2.5 hours at about 15° C, rinsed thoroughly in water and fixed in ethanol-acetic acid (3:1) for 24 hours. The staining process was performed following the Feulgen technique with hydrolysis in 5N HCl acid at room temperature for 55 minutes. The stained roots were then squashed in 45% acetic acid and mounted in Entellan (Merck).

The microscopic investigation was conducted by using an image analysis system, IBAS 2000, that enables more reliable results than the traditional hand-made karyotype (VENORA *et al.* 1991).

Metaphases were automatically detected by connecting a motorized scanning stage to the microscope and computer governing the scrutiny of the mounting. The system locates metaphases by simultaneously checking the shapes, dimensions, grey

TABLE 1 - Origin and morpho-agronomic traits of two lines and two varieties of kabuli-type chickpea.

Genotypes	Origin	Plant height (cm)	Growth habit	100-seed weight (g)	Seed surface	Seed shape	Maturity cycle	Sowing time
FLIP 82-150C	Syria	40	erect	28.5	rough	owl	medium	winter
ILC 482	Turkey	50	semi-erect	31.9	rough	owl	medium	winter
Calia	Italy	49	semi-erect	31.9	smooth	pea	medium	spring
Sultano	Russia	64	erect	27.7	smooth	pea	late	winter

levels (stain saturation) and number of chromosomes. At least ten well-spread out metaphase plates were used for karyotyping of each genotype. The total chromosome length and length of short arms, long arms and satellites were measured with the computer system. The short/long arm ratio does not include the satellite. The chromosomes were classified on the basis of their arm-ratio as metacentric (above 0.75) and submetacentric (between 0.75 and 0.51) according to KUTAREKAR and WANJARI (1983), and numbered in decreasing order of length, the longest being the first chromosome.

Cluster analysis (SCOTT and KNOTT 1974) was used to compare the chromosome parameters.

RESULTS AND DISCUSSION

A metaphase plate of chickpea with $2n = 16$ chromosomes is shown in Fig. 1.

The chromosome length ranged from 1.03 µm (FLIP 82-150c) to 3.45 µm (ILC 482) (Table 2). The satellite was always attached to the short arm of the

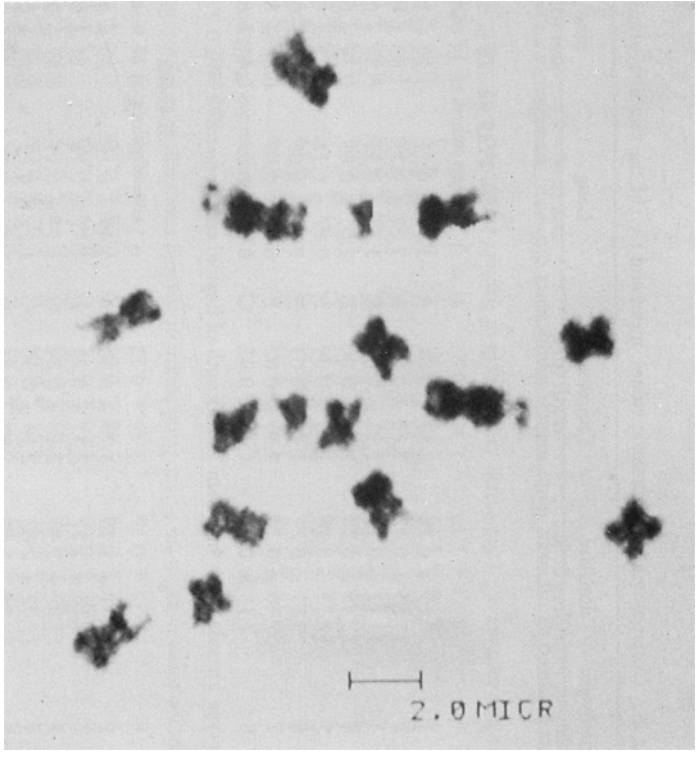

Fig. 1. — Metaphase plate $2n = 16$ chromosomes.

TABLE 2 - Chromosome morphometric values, mean and s.e., of the two lines and two varieties.

Chromosome No.	Relative length %	Chromosome length (μm)		Long arm (μm)	Short arm (μm)	Satellite (μm)	Arm Ratio short/long	Chromosome type
				FLIP 82-150 C mean values of 10 plates				
1	20.65 ± 0.28	2.97 ± 0.04	A	1.36 ± 0.11	1.10 ± 0.09	0.51 ± 0.07	0.81 ± 0.22	A metacentric
2	15.65 ± 0.42	2.25 ± 0.06	B	1.22 ± 0.11	1.03 ± 0.11		0.85 ± 0.01	A metacentric
3	13.77 ± 0.35	1.98 ± 0.05	C	1.15 ± 0.11	0.83 ± 0.11		0.73 ± 0.02	B submetacentric
4	12.45 ± 0.28	1.79 ± 0.04	D	1.05 ± 0.09	0.74 ± 0.09		0.70 ± 0.01	B submetacentric
5	11.13 ± 0.28	1.60 ± 0.04	E	0.87 ± 0.11	0.73 ± 0.07		0.85 ± 0.02	A metacentric
6	9.87 ± 0.21	1.42 ± 0.03	F	0.79 ± 0.09	0.63 ± 0.09		0.80 ± 0.02	A metacentric
7	9.46 ± 0.14	1.36 ± 0.02	F	0.76 ± 0.09	0.60 ± 0.07		0.79 ± 0.02	A metacentric
8	7.16 ± 0.14	1.03 ± 0.02	G	0.59 ± 0.07	0.44 ± 0.07		0.74 ± 0.01	B submetacentric
Karyotype formula $m^{sc} + 4m + 3sm$ Total length of haploid complement 14.38 ± 0.27 (μm).								
				ILC 482 mean values of 15 plates				
1	20.51 ± 1.78	3.29 ± 0.30	A	1.49 ± 0.16	1.29 ± 0.10	0.51 ± 0.14	0.87 ± 0.08	A metacentric
2	15.16 ± 1.37	2.55 ± 0.23	B	1.41 ± 0.16	1.14 ± 0.10		0.81 ± 0.08	A metacentric
3	13.02 ± 1.43	2.19 ± 0.24	C	1.34 ± 0.19	0.85 ± 0.11		0.63 ± 0.13	B submetacentric
4	13.02 ± 2.02	2.19 ± 0.34	C	1.21 ± 0.17	0.98 ± 0.12		0.81 ± 0.08	A metacentric
5	11.24 ± 1.43	1.89 ± 0.24	D	1.05 ± 0.16	0.84 ± 0.10		0.80 ± 0.09	A metacentric
6	10.11 ± 1.07	1.70 ± 0.18	E	0.99 ± 0.11	0.71 ± 0.11		0.72 ± 0.11	B submetacentric
7	9.69 ± 1.07	1.63 ± 0.18	E	0.59 ± 0.11	0.68 ± 0.14		0.72 ± 0.15	B submetacentric
8	7.25 ± 1.13	1.22 ± 0.19	F	0.74 ± 0.13	0.48 ± 0.08		0.65 ± 0.09	B submetacentric
Karyotype formula $m^{sc} + 3m + 4sm$ Total length of haploid complement 16.82 ± 1.55 (μm).								

Calia

mean values of 11 plates

1	20.79 ± 2.65	3.30 ± 0.42	A	1.34 ± 0.17	1.20 ± 0.16	0.76 ± 0.12	0.90 ± 0.05	A metacentric
2	15.88 ± 2.02	2.52 ± 0.32	B	1.34 ± 0.19	1.18 ± 0.14		0.88 ± 0.06	A metacentric
3	12.92 ± 0.69	2.05 ± 0.11	C	1.22 ± 0.05	0.83 ± 0.08		0.68 ± 0.07	B submetacentric
4	12.29 ± 0.57	1.95 ± 0.09	C	1.07 ± 0.06	0.88 ± 0.04		0.82 ± 0.05	A metacentric
5	11.47 ± 0.38	1.82 ± 0.06	C	0.98 ± 0.05	0.84 ± 0.05		0.86 ± 0.07	A metacentric
6	10.08 ± 0.69	1.60 ± 0.11	D	0.89 ± 0.05	0.71 ± 0.06		0.80 ± 0.04	A metacentric
7	9.33 ± 0.44	1.48 ± 0.07	D	0.88 ± 0.08	0.60 ± 0.09		0.68 ± 0.15	B submetacentric
8	7.25 ± 0.76	1.15 ± 0.12	E	0.71 ± 0.07	0.44 ± 0.05		0.62 ± 0.05	B submetacentric

Karyotype formula $m^{sc} + 4m + 3sm$ Total length of haploid complement 15.87 ± 0.88 (μm)

Sultano

mean values of 12 plates

1	20.24 ± 1.58	3.20 ± 0.25	A	1.37 ± 0.14	1.21 ± 0.17	0.62 ± 0.11	0.88 ± 0.06	A metacentric
2	15.56 ± 2.09	2.46 ± 0.33	B	1.34 ± 0.19	1.12 ± 0.11		0.84 ± 0.10	A metacentric
3	13.16 ± 1.58	2.08 ± 0.25	C	1.23 ± 0.11	0.85 ± 0.17		0.69 ± 0.12	B submetacentric
4	12.27 ± 0.95	1.94 ± 0.15	C	1.05 ± 0.09	0.89 ± 0.17		0.85 ± 0.04	A metacentric
5	11.13 ± 1.01	1.76 ± 0.16	D	0.96 ± 0.09	0.80 ± 0.09		0.83 ± 0.09	A metacentric
6	10.88 ± 0.89	1.72 ± 0.14	D	1.01 ± 0.09	0.71 ± 0.09		0.70 ± 0.10	B metacentric
7	8.60 ± 1.33	1.36 ± 0.21	E	0.73 ± 0.11	0.63 ± 0.11		0.86 ± 0.09	A submetacentric
8	8.22 ± 1.39	1.30 ± 0.22	E	0.76 ± 0.16	0.54 ± 0.09	±	0.71 ± 0.13	B submetacentric

Karyotype formula $m^{sc} + 4m + 3sm$ Total length of haploid complement 15.81 ± 1.38 (μm).

Values followed by the same letter are not significantly different, according to Cluster analysis of SCOTT and KNOTT (P = 0.01).

longest chromosome. Calia possess the longest satellite (0.76 μm), whereas ILC 482 and FLIP 82-150C had the shortest (0.51 μm). All genotypes have three submetacentric and five metacentric chromosome pairs with the exception of ILC 482 which had four submetacentric and four metacentric pairs.

Fig. 2 shows the ideograms of the haploid complement ($n = 8$) of each genotype.

Fig. 3 shows the comparisons of chromosome length and arm-ratios in eight coordinate systems, one for each size-level, by mean of cluster analysis

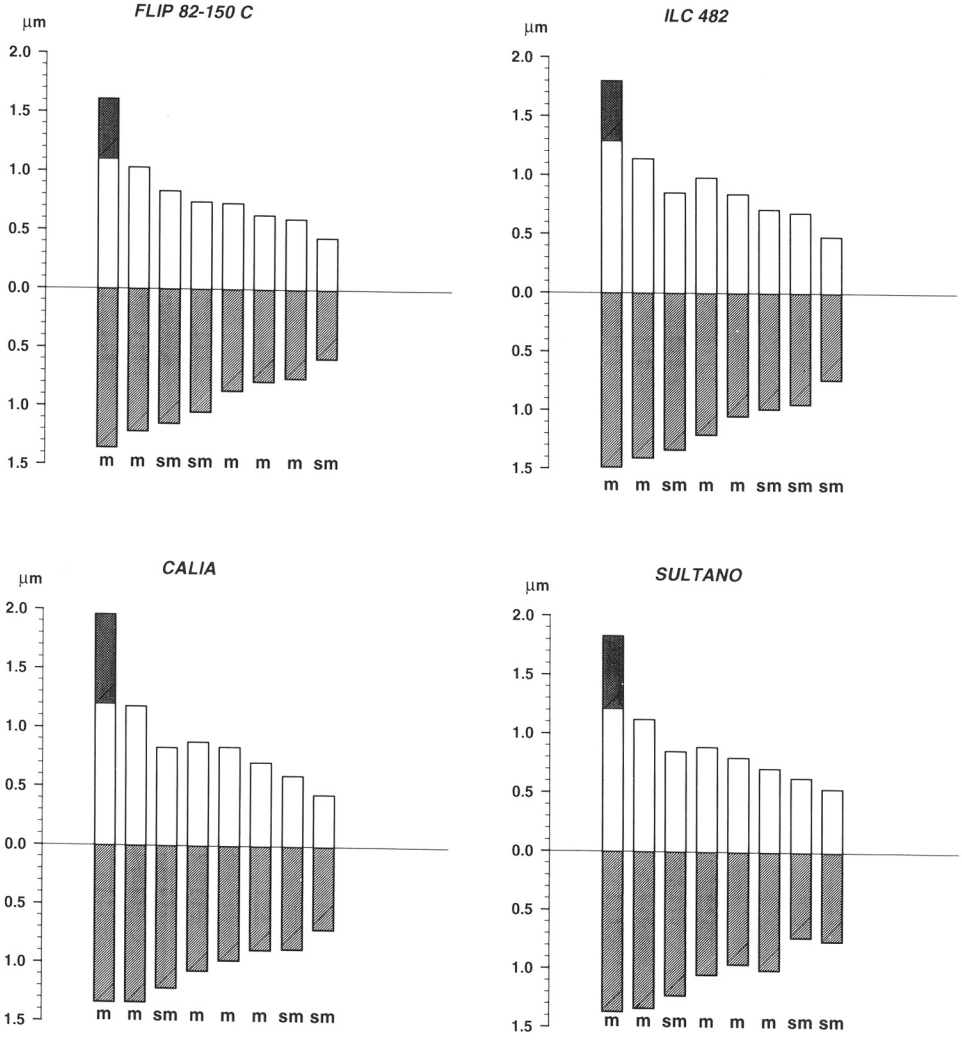

Fig. 2. — Idiogrammatic representation of the haploid complement of four genotypes.

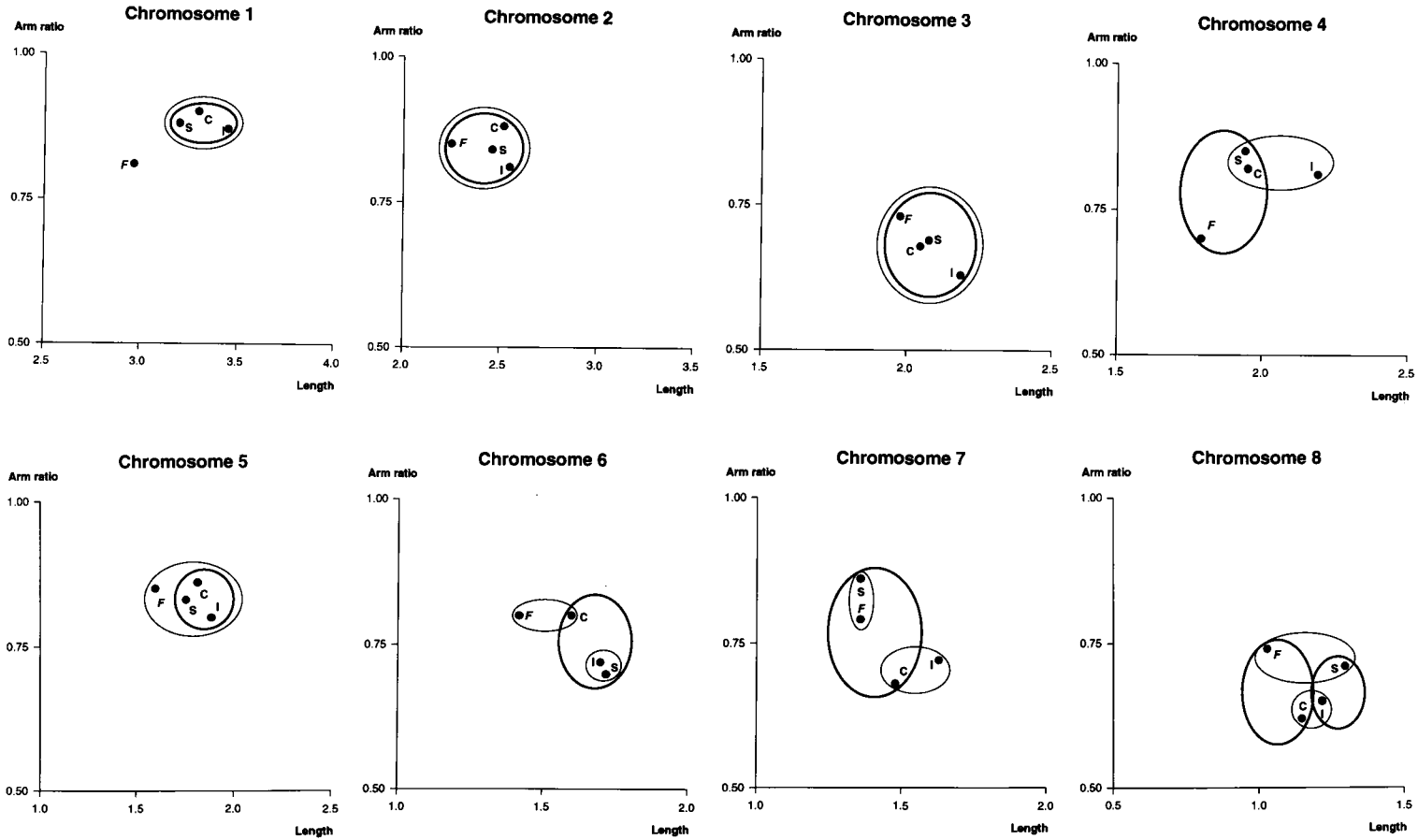

Fig. 3. — Graphical representation of arm/ratio vs length of the four genotypes for each chromosome. Inside each circle or ellipse there are no statistical differences at P = 0.01 level. ——— = length ——— = arm ratio s/l; F = FLIP 82-150C; I = ILC 482; C = Calia; S = Sultano.

significant differences were recorded among chromosomes of different genotypes. This enabled the identification of marker chromosomes for each genotype.

The total haploid genome length was the shortest in FLIP 82-150C (14.38 μm) and the longest in ILC 482 (16.82 μm), the difference indicating a little variation within the kabuli-type chickpea. MUKHERJEE and SHARMA (1987) used in their investigation also a kabuli line (L-550), this genotype is close to ours as far as the total haploid chromatin length (17.38 μm), the rang of chromosome length 3.9-1.3 μm and satellite location are concerned. In contrast, the total chromatin length in the desi-type is greater and the variability (number of satellited chromosomes, chromosome size and shape) among genotypes is wider than in the kabuli-type (PHANDIS 1970; AHMAD and GODWARD 1980; KUTAREKAR and WANJARI 1983; SHARMA and GUPTA 1986; MUKHERJEE and SHARMA 1987).

It is difficult to assign any special reason to this, but it could be due to narrow adaptation of the kabuli-type chickpea in contrast to wide adaptation of the desi-type. Desi type is believed to have originated first and kabuli-type developed from it through mutation. The spread of the desi-type is further and wider from their centre of origin compared to the kabuli type.

Although the variability for the total haploid genome length was narrow, it is possible to differentiate the lines and varieties by their karyotype using precise and detailed measurement of chromosomes by the image analysis system.

Comparing the four lines and varieties, FLIP 82-150C had chromosome pairs 1, 5 and 6 significantly shorter than other. Only this line had submetacentric chromosome 4. ILC 482 can be distinguished from pairs 4 and 7, which are longer than in others. Calia and Sultano can be differentiated by the length of chromosome 8, arm-ratio or pairs 6 and 7, satellite length. This confirms that the image analysis system is a powerful tool to differentiate plant chromosomes.

Differences between the lines and varieties in this study, particularly that regarding the centromere location, might be attributable to pericentric inversions. Similar rearrangements have been found by LADIZINSKY and ADLER (1976) in the metaphases of the F_1s of interspecific crosses between two lines of *C. arietinum* and accession of *C. reticulatum* and *C. echinospermum*.

There is no reference cultivar on standard karyotype for chickpea. We propose Sultano as the standard because it has the fewest extreme parameter values. Its karyotype formula m^{sc} + 4m + 3sm accounts for 75% of the plates analysed.

This is the first detailed study of the karyotype in kabuli type chickpea and it opens up new prospects for further cytogenetic investigation such as physical mapping of genes.

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