Theor Appl Genet (1993) 86:637–641

THEORETICAL AND APPLIED GENETICS

The fine structure of chickpea (*Cicer arietinum* L.) chromosomes as revealed by pachytene analysis

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Received: 10 October 1992 / Accepted: 30 November 1992

Abstract. A standard pachytene karyotype of chickpea (*Cicer arietinum* L.) is presented for the first time. Individual pachytene chromosomes were identified and described in detail. An idiogram was prepared on the basis of chromosome length, arm ratio, and distribution of heterochromatin and euchromatin. Chickpea pachytene chromosomes belong to the "differentiated" type with darker staining heterochromatin proximal to and lighter staining euchromatin distal to the centromeres. Chromosomes were numbered from 1 to 8 following a descending order of length. The total length of the chromosome complement at pachytene was 335.33 μ, and chromosome size ranged from 58.05 to 30.53 μ.

Key words: *Cicer arietinum* – Chickpea – Pachytene karyotype – Chromosomes – Meiosis

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated grain legume that belongs to the Leguminoseae family of the tribe Cicereae (Kupicha 1981). It is a diploid with $2n=2x=16$ chromosomes. While there is a large amount of cytogenetic information available on chickpea (see Bahl 1987), it deals mainly with chromosome number and somatic karyotype. In chickpea, mitotic chromosome squash preparations have been quite difficult to obtain because of the presence of globular structures in the cytoplasm that hinder the staining, spreading, and identification of chromosomes (Lather et al. 1990). Indeed, it is for this reason that chickpea cytology is plagued with an enormous number of contradictions and variations as far as somatic karyotype is concerned (see Sharma and Gupta 1986; Bahl 1987; Ahmad 1988, Gupta and Sharma 1991). A standard karyotype of chickpea is, therefore, urgently needed (Muehlbauer and Singh 1987).

In spite of certain limitations, pachytene chromosome analysis remains a very powerful cytogenetic tool. It has been widely applied in various plant species (McClintock 1929; Barton 1950; Gillies 1968; Ramanna and Wagenvoort 1976; Nakamura and Tsuchiya 1982; Dundas et al. 1983; Khush et al. 1984; Singh and Hymowitz 1988), but in chickpea only one brief mention has been made of pachytene chromosome morphology, and no photographs were provided (Sharma and Gupta 1986). This lack of information together with the above-mentioned absence of a reliable cytogenetic technique for identifying chromosomes prompted us to re-examine the chromosomes of chickpea at the pachytene stage of meiosis.

In this report, we define for the first time each pachytene chromosome by its length, arm ratio and differentiation of heterochromatic and euchromatic segments.

Materials and methods

Seeds of a "desi" type chickpea cv "Radhe" (originating from India) were obtained from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT). The plants were grown in the field (June-September) at Urbana, Ill., USA. Flower buds, at the appropriate stage of development, were collected and fixed in a freshly prepared solution of absolute ethanol (3 parts): propionic acid (1 part) with 1% ferric chloride. Staining of the anthers and preparation of slides were done as previously described for soybean (Singh and Hymowitz 1988). Individual chromosome arms and total lengths were measured. Chromo-

Communicated by G. S. Khush

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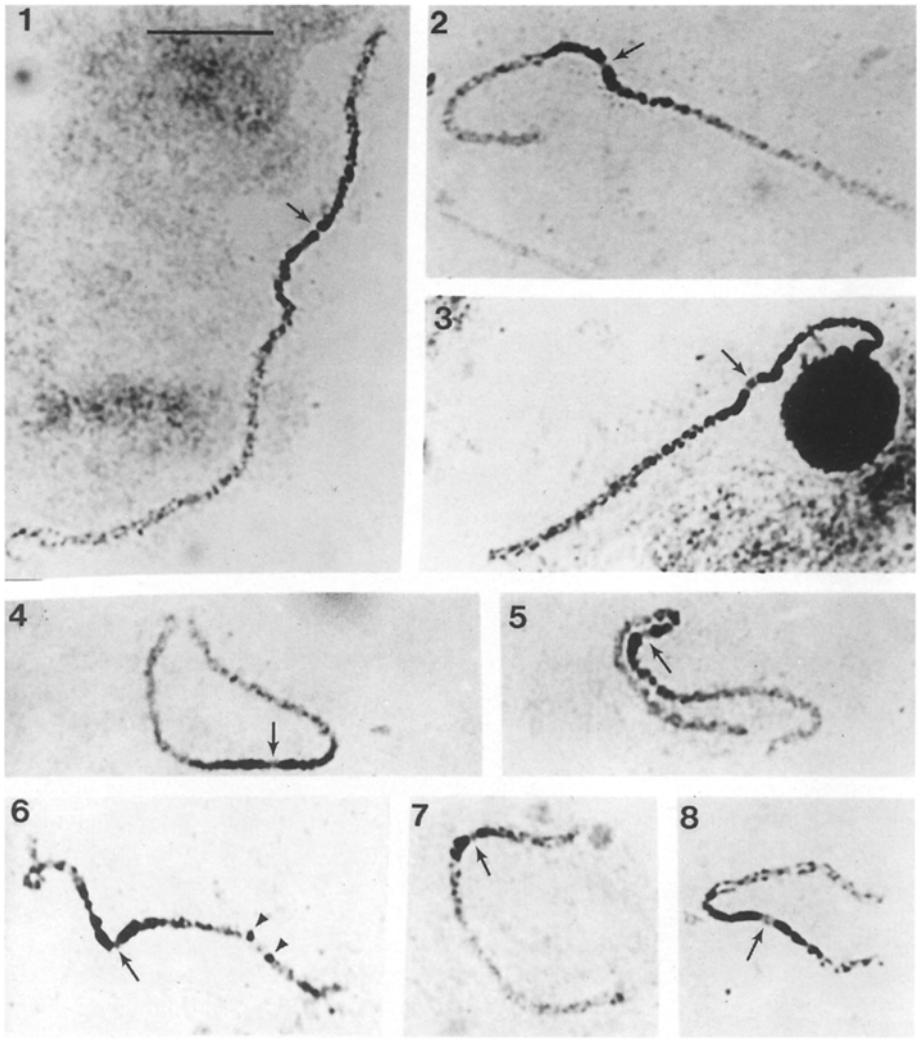

Fig. 1. 1–8. Photomicrographs of the pachytene chromosomes of chickpea. Each figure shows a different chromosome. For example, **Fig. 1.1** is chromosome 1; **Fig. 1.8** is chromosome 8. *Arrow* indicate centromere location; *arrowheads* in chromosome 6 show the small darkly staining heterochromatic block distal ot the centromere. *Bar:* 10 μ

Table 1. Measurements (μ)[a] and other parameters of individual pachytene chromosomes of chickpea

Chromosome number	Long arm	Short arm	Total length	Arm ratio (L/S)	Centromere position[b]	Heterochromatin		
						Long arm	Short arm	Total (%)
1	38.48±5.02	19.68±1.50	58.05±3.94	1.98±0.33	st	8.78	8.08	16.86 (29.1)
2	27.20±1.38	24.75±2.29	51.95±3.31	1.11±0.09	m	6.32	7.16	13.48 (25.9)
3	24.60±1.10	20.00±1.50	44.65±2.61	1.25±0.18	m	5.81	20.00	25.82 (57.8)
4	21.27±2.48	19.65±2.34	40.92±4.66	1.09±0.07	m	6.06	5.29	11.35 (27.9)
5	24.56±3.84	16.16±3.39	40.71±4.71	1.58±0.43	sm	2.20	2.15	4.35 (10.8)
6	19.29±1.99	17.22±1.78	36.45±3.47	1.14±0.10	m	4.67	4.45	9.12 (25.2)
7	21.71±1.51	10.35±1.45	32.07±2.86	2.12±0.21	st	2.22	1.94	4.16 (13.4)
8	19.68±2.60	10.85±1.43	30.53±3.61	1.86±0.22	st	5.70	4.70	10.40 (32.4)

[a] Mean±standard deviation
[b] st, Subterminal; m, median; sm, submedian

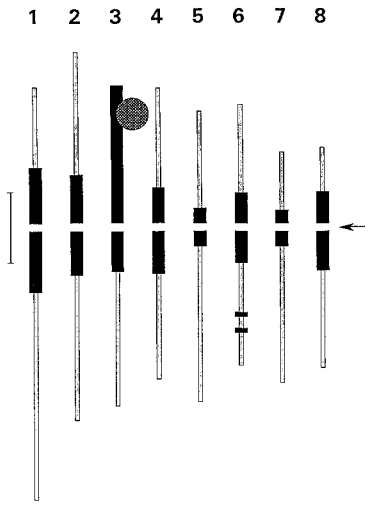

Fig. 2. Proposed pachytene idiogram of chickpea based on Fig. 1 and Table 1. *Arrow* indicates centromere location. *Bar:* 10 μ

some arm ratios were defined according to Sharma and Gupta (1982). Meiotic chromosome configurations were also studied at diakinesis, metaphase I, and anaphase I. Photomicrographs were taken with a Nikon Optiphot-2 microscope using Kodak Technical Pan 2415 film. Pachytene chromosomes from "kabuli" type chickpea cv "Ispanyol" (originating from Turkey), obtained from the United States Department of Agriculture (USDA), were also studied to confirm the pachytene karyotype.

Results

In all of the microsporocytes that were studied at the diakinesis stage only one chromosome pair was seen to be attached to the nucleolus. All of the plants studied were cytologically normal, i.e., they showed normal chromosome pairing, separation, and movement.

At pachytene, it was not possible to trace all of the eight paired chromosomes in a single microsporocyte. Hence, attempts were made to identify cells with one or two isolated chromosomes. Pronounced euchromatin and heterochromatin differentiation and measurements of chromosome parameters such as long and short arm ratio and total length (Table 1) facilitated the identification of individual pachytene chromosomes (Fig. 1) and the construction of an idiogram (Fig. 2). All of the eight chromosomes were numbered from 1 to 8 in descending order of their length. Measurements were made on a total of 82 individual pachytene chromosomes.

The following are the diagnostic features of individual pachytene chromosomes in *C. arietinum*.

Chromosome 1 (Fig. 1.1) is 58.05 μ long; subterminal, and the longest chromosome in the complement (Table 1). The proximal heterochromatic segments of both arms are about equal in length (8–9 μ).

Chromosome 2 (Fig. 1.2) is 51.95 μ long and median. This chromosome is easily distinguishable from chromosome 1 because of its median centromere location (arm ratio = 1.11) relative to an arm ratio of 1.98 for chromosome 1.

Chromosome 3 (Fig. 1.3) is 44.65 μ long and median. This chromosome with a median centromere location is the nucleolus-organizing or satellited chromosome and is easily recognizable as it is attached to the nucleolus. The entire short arm is heterochromatic, and part of this arm contains the nucleolus-organizing region. This chromosome has the longest heterochromatic region (57.8% of the total length).

Chromosome 4 (Fig. 1.4) is 40.92 μ long and median with almost equal arm lengths (arm ratio = 1.09). Darkly stained heterochromatic regions flank the centromere. Approximately 72% of each arm is euchromatic.

Chromosome 5 (Fig. 1.5) is 40.71 μ long and submedian. The heterochromatin in the short arm consists of two adjacent blocks proximal to the centromere, while only one block of heterochromatin is present in the long arm. This chromosome has the shortest heterochromatic region (10.8% of the total length). Arm ratio and heterochromatin distribution are helpful in distinguishing this chromosome from chromosome 2, with which it could be confused at times.

Chromosome 6 (Fig. 1.6) is 36.45 μ long and median. The centromeric regions of both arms are flanked by almost equal amount of densely staining heterochromatin. The long arm contains two darkly staining small heterochromatic blocks at about 11.6 and 14.0 μ from the centromere. This feature is useful in distinguishing chromosome 6 from chromosomes 4 and 2.

Chromosome 7 (Fig. 1.7) is 32.07 μ long and subterminal. The long arm is twice the size of the short arm. The smaller size and heterochromatin distribution are helpful in distinguishing this chromosome from chromosome 1.

Chromosome 8 (Fig. 1.8) is 30.53 μ long and subterminal. It is the shortest chromosome of the complement. Both arms have rather large darkly staining heterochromatic regions proximal to the centromere that are helpful in distinguishing this chromosome from chromosome 5. The euchromatic regions of both arms of this chromosome have small darkly staining chromomeres.

A standard pachytene complement of chickpea with diagnostic features is shown in Fig. 2. The staining patterns in Figs. 1 and 2 were confirmed for the chromosomes of "kabuli" type chickpea cv "Ispanyol". All of the chickpea chromosomes have heterochromatic re-

gions, albeit in different proportions, proximal to and on either side of the centromere. Additionally, the short arm of the nucleolus-organizing chromosome (number 3) is completely heterochromatic. The total length of the chickpea chromosome complement at pachytene is 335.33 μ. The longest chromosome is about twice the size of the shortest chromosome (range = 58.05–30.53 μ, Table 1). About 28.5% of the total chickpea genome is heterochromatic, as judged by staining intensity with propiono-carmine. Chromosome 5 and 3 have the lowest (10.8%) and highest (57.8%) proportion of heterochromatic segments, respectively (Table 1).

Discussion

During the pachytene analysis we did not find any one cell in which all eight pachytene chromosomes could be traced and identified. However, the isolation of one to two chromosomes per cell and our observations on chromosome measurements along with the differentiation and proportion of heterochromatic and euchromatic regions (Fig. 1, Table 1) facilitated the identification and construction of a detailed pachytene idiogram of chickpea for the first time (Fig. 2).

The pachytene chromosomes of chickpea belong to the "differentiated" category with distinct centromeres being flanked by proximal dark-staining heterochromatin and distal light-staining euchromatin. It should be noted that the short arm of the nucleolar-organizing chromosome (number 3) was completely heterochromatic. In this regard, chickpea pachytene chromosomes resemble those of maize (McClintock 1929), tomato (Barton 1950), alfalfa (Gillies 1968), diploid potato (Ramanna and Wagenvoort 1976), pigeonpea (Reddy 1981; Dundas et al. 1983), and soybean (Singh and Hymowitz 1988). Furthermore, the heterochromatin and euchromatin of chickpea pachytene chromosomes are more differentiated than those of barley (Singh and Tsuchiya 1975), sugarbeet (Nakamura and Tsuchiya 1982), and rice (Khush et al. 1984).

On the basis of mitotic metaphase preparations, chickpea has been reported to have none, one, two, and up to three pairs of satellited chromosomes (see Sharma and Gupta 1986; Bahl 1987; Gupta and Sharma 1991). However, recent studies leave little doubt that only one chromosome pair is satellited (Sharma and Gupta 1982; Ahmad 1988; Ohri and Pal 1991). Indeed, in the study presented here only one chromosome pair was found to be associated with the nucleolus, and therefore satellited. While the satellited chromosome was designated chromosome 1 in mitotic cells (Sharma and Gupta 1982; Ahmad 1988; Ohri and Pal 1991), it is designated chromosome 3 at pachytene in the present study. Such differences may be caused by differential chromatin condensation at the two stages of cell division.

Chickpea cytogenetics lags behind that of maize, barley, wheat, rice, and tomato. A few linkage relationships between morphological markers have been reported in chickpea (Muehlbauer and Singh 1987) and a linkage map involving morphological, biochemical, and molecular markers is being developed (Gaur and Slinkard 1990; Simon and Muehlbauer 1991, 1992). The independence of various linkage groups has not yet been tested, and none of the linkage groups have been associated with the respective chromosomes. No cytogenetic stocks, other than tetraploids, are available in chickpea (see Bahl 1987; Gupta and Sharma 1991). Sharma and Gupta (1987) attempted to produce trisomics through triploids in chickpea, following hybridization between tetraploids and diploids, but only one triploid plant could be obtained in spite of repeated pollinations. A set of primary trisomics or translocations involving all of the chromosomes are needed to associate linkage groups with the respective chromosomes. The proposed karyotype and chromosome numbering system, based on pachytene analysis, would be helpful in identifying the extra chromosomes of trisomics, as has been done in rice (Khush et al. 1984) and soybean (Singh and Hymowitz 1991; Ahmad et al. 1992). Moreover, the chickpea pachytene karyotype reported here may be used to compare its chromosomes with those of the related annual and perennial species in the genus *Cicer* in order to get a further insight into the phylogeny of the genus.

Acknowledgements. We thank Drs. A. E. Slinkard and F. J. Muehlbauer for providing chickpea seed samples. The help of Dr. K. P. Kollipara and a useful discussion with Dr. R. J. Singh is also sincerely acknowledged.

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