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The origin of chickpea (*Cicer arietinum* L.): karyotype and nuclear DNA amount

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Karyotypic and feulgen microdensitometric measurements show that the most asymmetric karyotype is correlated with the lowest DNA amount and vice versa in seven annual species of *Cicer*. The 2C nuclear DNA amounts vary from 1.83 pg to 3.57 pg and the values within this range can be categorized into three groups, the means (1.83 pg, 2.59 pg, 3.41 pg) of which are separated by the regular intervals of 0.8 pg. The differences within the groups are insignificant while those between groups are significant, *C. reticulatum*, which is considered to be progenitor species of *C. arietinum*, differs from the latter in having two satellite pairs instead of one and 22.3 per cent less DNA content. The possibility of such concrete changes occurring during domestication, and the fact that *C. arietinum* depicts uniform karyotype and DNA contents in its cultivars underlines the need to reconsider the issue of *C. reticulatum* as the wild ancestor of *C. arietinum*.

Keywords: chickpea, DNA amount, domestication, karyotype.

Introduction

The cultivated chickpea (*Cicer arietinum* L.) is a crop of ancient origin, being the fifth most important leguminous crop after soy-beans (*Glycine max*), groundnuts (*Arachis hypogaea*), dry beans (*Phaseolus vulgaris*), and dry peas (*Pisum sativum*) (Anonymous, 1974). Vavilov (1926) recognized the Mediterranean, Central Asian, Near Eastern, and Indian regions as the probable centres of origin of the chickpea. The plant is known to have eight annual and 34 perennial species (Van der Maesen, 1987). Two main types of chickpea are grown in the world: 'Kabuli' (large ram-shaped, cream coloured) and 'Desi' (small angular and dark coloured) (Van der Maesen, 1972). The 'Kabuli' types are grown in the Mediterranean region and the 'Desi' types mainly in the Indian subcontinent. The problem of the origin of chickpea has attracted the attention of several workers and it is now generally believed that *C. reticulatum* Lad. is its wild progenitor, based on karyotypic (Ahmad *et al.*, 1987), seed storage protein profiles (Ladinzinsky & Adler, 1975; Kabir & Singh, 1988) and interspecific hybridization studies (Ladizinsky & Adler, 1976a,b; Ahmad, 1988). There is no information, however, about the nuclear DNA contents in chickpea and other *Cicer* species. Moreover, certain contradictions were noticed in the earlier karyotypic studies. The present work therefore attempts a detailed study of the karyotype and nuclear DNA contents of six annual species of *Cicer* and five cultivars of chickpea.

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Materials and methods

Materials

Seeds of *C. judaicum* Boiss., *C. cuneatum* Hochst & Rich (SL 157), *C. bijugum* Rech. (No. 200), *C. pinnatifidum* Jaub & Spach. (No. 189), *C. reticulatum* Lad. (JM 2100), *C. echinospermum* Dav. (No. 204), and *C. arietinum* L. (ICC 4918, ICC 4973, ICC 5003) were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The 'Desi' and 'Kabuli' cultivars of chickpea were collected from the market.

Karyotype

Actively growing root tips from 15-day-old seedlings grown in clay pots, were excised and pretreated with a 0.002 M aqueous solution of 8-hydroxy-quinoline for 3 h at 15–17°C. After washing these were fixed in 1:3 acetic–alcohol for 24 h. The root tips were then washed and hydrolysed in 5 N HCl for 30 min at room temperature and stained with feulgen solution for 1 h. As the chromosomes were rather small in size they required intense staining. This was done by squashing

the root tips in 1 per cent iron-acetocarmine. For each material at least two representative cells with well-spread chromosomes were measured. The centromeric nomenclature system of Levan *et al.* (1964), as modified by Schlarbaum & Tsuchiya (1984) was followed. Satellites were included in total length to calculate arm ratios. The degree of asymmetry/symmetry of karyotypes has been estimated following Stebbins (1950, 1971).

Nuclear DNA estimation

For DNA measurements, root tips were fixed for 2 h in 1:3 acetic–alcohol, rinsed in distilled water for 5 min and hydrolysed in 5 N HCl for 30 min at room temperature and stained in feulgen solution (pH 2.2) for 2 h. They were then given three 10 min washes in SO_2 water. Squash preparations were made in glycerol. Three measurements of each of 10 telophase or early prophase from each of three replicate slides were made for all material. Measurements were made on a Vickers M86 scanning microdensitometer set at wavelength 565 nm. *Allium cepa* cv. Nasik Red was used as a standard (2C = 33.5 pg, Van't Hof, 1965).

Results

The somatic chromosome number of each species was found to be $2n = 16$, as previously reported (Mercy *et al.*, 1974; Ladizinsky & Adler, 1976a,b; Ahmed & Godward, 1980; Sharma & Gupta, 1983; Kutarekar & Wanjari, 1983; Mukherjee & Sharma, 1987; Ahmad *et al.*, 1987). Many of the chromosomes of each species had distinctive features which facilitated the determi-

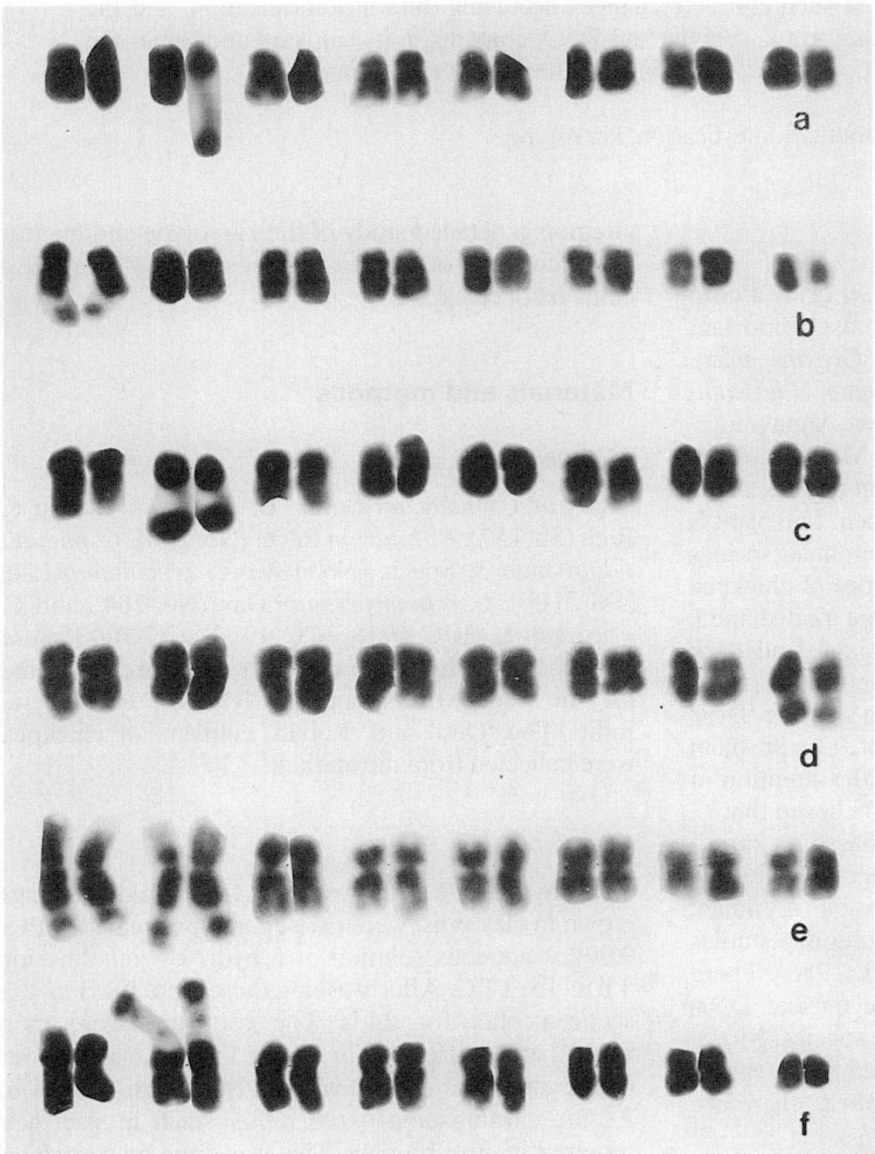

Fig. 1 Karyotypes of (a) *C. judaicum*, (b) *C. cuneatum*, (c) *C. bijugum*, (d) *C. pinnatifidum*, (e) *C. reticulatum*, (f) *C. echinospermum* ×2720.

nation of their pairing in each karyoptype. The most significant finding is the presence of two satellite pairs (1st and 2nd) in *C. reticulatum* (Fig. 1e) while there is only one such pair in the remaining species (Figs 1 and 2; Table 1). The position of the satellite pair in the complement, however, varies. It is the first pair in *C. arietinum* and *C. cuneatum*, second in *C. judaicum*, *C. bijugum* and *C. echinospermum* and eighth in *C. pinnatifidum* (Figs 1 and 2; Table 1). The differences between the karyotypes are also apparent in the relative size differences among chromosomes within the complement and arm ratios (*r*-index; Table 1). The most asymmetrical karyotype is depicted by *C. judaicum* (Fig. 1a; Table 1). Based on a system proposed by Stebbins (1958), the karyotypes can be categorized as 3b (*C. judaicum*), 2b (*C. cuneatum*), 2a (*C. bijugum* and *C. pinnatifidum*) and 1b (*C. reticulatum*, *C. echinospermum*, *C. arietinum*). Minor variations of the *r*-index and relative size are also apparent among the cultivars of *C. arietinum* (Table 1; Fig. 2).

The nuclear DNA contents show a 1.95-fold variation among the species. The lowest amount is depicted by *C. judaicum* (1.83 pg) and the highest by *C. arietinum* ICC 5003 (3.57 pg) (Table 1). The seven species form three DNA groups (Table 1; Fig. 3). Group I is comprised by *C. judaicum* (1.83 pg), Group II by *C. cuneatum* (2.50 pg), *C. bijugum* (2.54 pg), *C. pinnatifidum* (2.56 pg), *C. reticulatum* (2.65 pg) and *C. echinospermum* (2.70 pg) and Group III by five cultivars of *C. arietinum* (3.30–3.57 pg) (Table 1), the group means are separated by regular intervals of 0.8 pg (Fig. 3). Within-group variation in 2C DNA amounts is insignificant while differences between groups are highly significant ($P < 0.01$; Table 2).

Discussion

The present results clearly show that seven annual species of *Cicer* differ from each other in definite karyotypic features (Figs 1 and 2; Table 1). Remar-

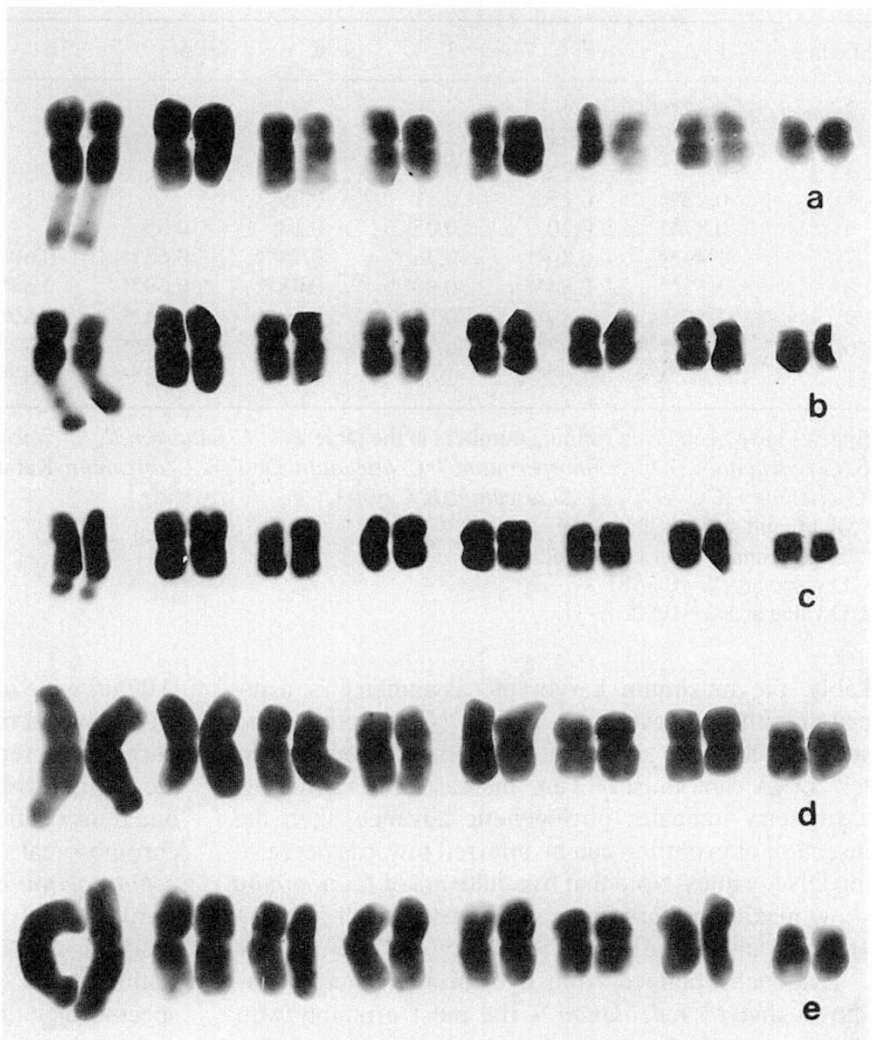

Fig. 2 Karyotypes of the cultivars of *C. arietinum*, (a) 'Desi', (b) 'Kaabuli', (c) ICC 4918, (d) ICC 4973, (e) ICC 5003. × 2720.

Table 1 Karyotype arrangements and 2C nuclear DNA contents in *Cicer*

Species	$2n=$	Number of satellite pairs	Ratio of longest shortest	Maximum *r*-index	Karyotype	Classification (Stebbins, 1958)	2C Nuclear DNA (pg) $\bar{x} \pm$ S.E.	
C. judaicum	16	1(2)*	2.02	3.63(4)*	2m + 1msm, + 3sm + 2st	3b	1.83	0.03
C. cuneatum	16	1(1)	2.31	2.52(1)	2M + 1m + 2msm + 3sm	2b	2.50	0.04
C. bijugum	16	1(2)	1.74	5.53(2)	3M + 1m + 3sm + 1st	2a	2.54	0.05
C. pinnatifidum	16	1(8)	1.74	2.86(8)	1m + 4msm + 3sm	2a	2.56	0.07
C. reticulatum	16	2(1, 2)	2.54	1.97(3)	2M + 1m + 3msm + 2sm	1b	2.65	0.06
C. echinospermum	16	1(2)	3.15	1.78(4)	1M + 3m + 3msm + 1sm	1b	2.70	0.08
C. arietinum 'Desi'	16	1(1)	3.26	1.96(5)	1M + 3m + 2msm + 2sm	1b	3.30	0.09
C. arietinum 'Kabuli'	16	1(1)	2.56	1.87(8)	1M + 4m + 2msm + 1sm	1b	3.34	0.07
C. arietinum ICC 4918	16	1(1)	2.97	1.73(8)	1M + 3m + 3msm + 1sm	1b	3.39	0.09
C. arietinum ICC 4973	16	1(1)	3.05	1.63(3)	1M + 2m + 5msm	1b	3.47	0.08
C. arietinum ICC 5003	16	1(1)	3.05	2.00(8)	1M + 5m + 1msm + 1sm	1b	3.57	0.10

*Number within parentheses denotes the chromosome in order of decreasing size.

Table 2 Comparison and significance of means of 2C DNA amounts of seven *Cicer* species including five cultivars of *C. arietinum*

Species	1	2	3	4	5	6	7	8	9	10
2	0.67**									
3	0.71**	0.04								
4	0.73**	0.06	0.02							
5	0.82**	0.15	0.11	0.09						
6	0.87**	0.20	0.05	0.14	0.05					
7	1.47**	0.80**	0.76**	0.74**	0.65**	0.60**				
8	1.51**	0.84**	0.80**	0.78**	0.69**	0.64**	0.04			
9	1.56**	0.89**	0.85**	0.83**	0.74**	0.69**	0.09	0.05		
10	1.64**	0.97**	0.93**	0.91**	0.82**	0.77**	0.17	0.13	0.08	
11	1.74**	1.07**	1.03**	1.01**	0.92**	0.87**	0.27	0.23	0.18	0.10

Species have been given running numbers in the table as 1. *C. judaicum*, 2. *C. cuneatum*, 3. *C. bijugum*, 4. *C. pinnatifidum*, 5. *C. reticulatum*, 6. *C. echinospermum*, 7. *C. arietinum* 'Desi', 8. *C. arietinum* 'Kabuli', 9. *C. arietinum* ICC 4918, 10. *C. arietinum* ICC 4973, 11. *C. arietinum* ICC 5003.
*Significant at 5 per cent level.
**Significant at 1 per cent level.
CD value at 5% = 0.358.
CD value at 1% = 0.50.

kably, the maximum karyotypic asymmetry is associated with the least DNA amount (*C. judaicum*) and successively more symmetrical karyotypes depict greater DNA amounts. If an increase in karyotypic asymmetry indicates phylogenetic advance, then the direction of evolution can be inferred towards decreasing DNA values. Note that five cultivars of *C. arietinum* show maximum karyotypic symmetry as well as DNA amounts among the species studied.

Evidence obtained from hybridization studies has shown that *C. reticulatum* is the most probably wild progenitor of *C. arietinum*. Ladizinsky & Adler (1976a) were able to produce fertile hybrids with perfectly normal meiosis between *C. reticulatum* and four purple-flowered cultivars of *C. arietinum*. However, the hybrid with one white-flowered cultivar revealed one translocation and a paracentric inversion, implying chromosomal repatterning within *C. arietinum*. *C. echinospermum* on the other hand, yielded completely sterile hybrids with *C. reticulatum* and *C. arietinum* (Ladizinsky & Adler, 1976a). Further work by Ladizinsky & Adler (1976b) assigned seven annual species, including cultivated chickpea, to three crossability groups (Group I: *C. arietinum*, *C. echino-*

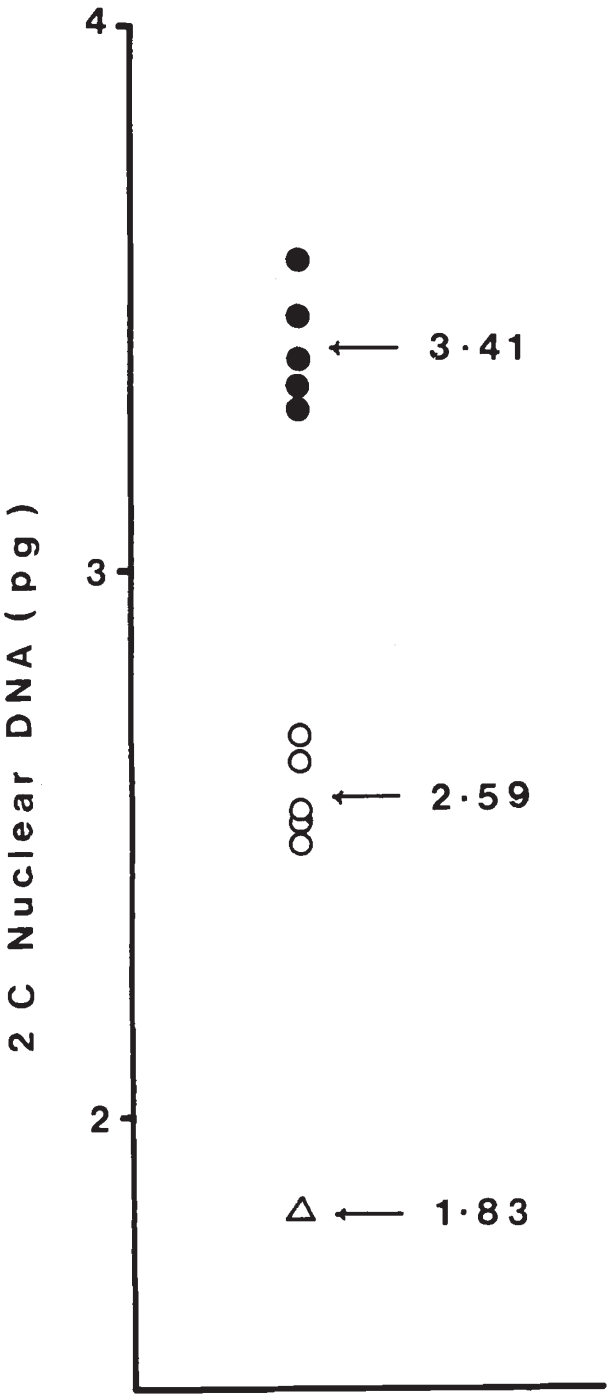

Fig. 3 Distribution of 2C nuclear DNA amounts in seven annual species of *Cicer* including five cultivars of *C. arietinum*. The arrows denote group DNA means. Species in ascending order, (△) *C. judaicum*, (○) *C. cuneatum*, *C. bijugum*, *C. pinnatifidum*, *C. reticulatum*, *C. echinospermum*, (●) *C. arietinum* 'Desi', 'Kabuli', ICC 4918, ICC 4973, ICC 5003.

spermum, *C. reticulatum*; Group II: *C. bijugum*, *C. judaicum*, *C. pinnatifidum*; Group III: *C. cuneatum*). Hybrids with varying fertility were possible within group crosses but between group crosses did not yield any seed. They concluded that *C. reticulatum*, which gives completely fertile F_1 hybrids without breakdown in the F_2, forms the primary gene pool of cultivated chickpea, while *C. echinospermum* forms the secondary gene pool with the rest of the species forming a tertiary gene pool (Harlan & de Wet, 1971). Remarkably the groups based on crossability do not correspond with those based on DNA amounts and karyotypic data (Table 1; Figs 1–3). Therefore in the present case the differences in karyotypes and DNA amounts do not effect species crossability (cf. Ohri & Khoshoo, 1986). The present study explicitly depicts two distinct differences in karyotype and nuclear DNA amounts between *C. reticulatum* and *C. arietinum*.

1 *C. reticulatum* has two satellite pairs in contrast to only one in all the cultivars of *C. arietinum* investigated (Figs 1 and 2).

2 *C. reticulatum* has 22.3 per cent less DNA ($P < 0.01$) than *C. arietinum*.

This means that if *C. reticulatum* is considered as the direct wild progenitor of *C. arietinum*, then we must account for the concrete and distinct changes in karyotype and nuclear DNA contents that might have occurred during this transition. It appears unlikely because the cultivars of *C. arietinum* studied show consistently similar karyotypes (excepting minor variation), and constant DNA amounts with insignificant differences ($P > 0.05$) (Tables 1 and 2; Fig. 2). Most significantly, no such differences have been detected in 'Desi' and 'Kabuli' cultivars of chickpea despite their ancient divergence and disparate cultivation associated with clear-cut phenotypic differences. Moreover, the regular discontinuous manner in which DNA amounts vary among the three groups makes such a precise transition from one DNA group to the other, during domestication from *C. reticulatum* to *C. arietinum*, difficult to comprehend. In other words it is quite remarkable that *C. arietinum* has undergone this concrete DNA change during domestication and attained a stable level in its various cultivars (cf. Narayan, 1988). Such a discontinuous pattern of DNA distribution has been seen in a number of genera (Narayan, 1982, 1988; Ohri & Khoshoo, 1986).

Some earlier contradictory reports on karyotype should be mentioned. The presence of a single satellite pair in *C. arietinum* has been indicated by several workers (Mercy *et al.*, 1974; Ahmed & Godward, 1980; Sharma & Gupta, 1983; Ahmad *et al.*, 1987). The reports of two (Kutarekar & Wanjari, 1983) and

up to three satellite pairs (Mukherjee & Sharma, 1987) lack clear illustrative evidence and could not be confirmed by the present study. In addition, the second satellite pair in *C. reticulatum* has not been observed in earlier studies (Sharma & Gupta, 1983; Ahmad *et al.*, 1987). Similarly, an indication of intraspecific differences in DNA amounts in vars. CSIMF and F10 of *C. arietinum*, as reported by Ahmed & Godward (1980) (without mention of arbitrary units), needs confirmation. The present study unequivocally confirms intraspecific constancy, as has been previously observed in a large number of species (Ohri & Khoshoo, 1986).

The above results provide a tangible evidence for a reconsideration regarding the progenitor species of chickpea. While not discounting completely the possibility of *C. reticulatum*, it is suggested that the search for the wild progenitor must also be made among the perennial relatives of chickpea.

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