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Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation*

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Abstract. Using the HCl-Giemsa banding technique, the total proportion of condensed chromatin was determined by planimetry in 23 plant species and was found to vary from 14–77%. Comparison of condensed chromatin values with DNA content indicated that the latter was involved in determining the interphase nuclear structure. The actual amounts of different DNA components in these species were estimated in terms of picograms. Statistical analysis of condensed chromatin values with quantities of different types of DNA sequences showed the best correlation with highly repeated DNA sequences, suggesting that these sequences could be playing an important role in governing the species-specific chromatin condensation in plants. The amount of DNA packaged per unit length of chromatin was also shown to be a determinant of interphase nuclear structure.

Keywords. Interphase nuclear structure; condensed chromatin; DNA sequences.

1. Introduction

Higher plants, contrary to animals, show species-specific organization of chromatin within the interphase nucleus. They also exemplify several unique features such as wide variations in DNA content, absence of facultative heterochromatin and diverse kinds of DNA sequence organization (Ranjekar 1982). This poses a fundamentally interesting question; can the interphase nuclear structures observed in plants be linked with parameters such as nuclear DNA content, gross DNA architecture and relative abundance of any of the specific DNA frequency classes? Earlier reports in a few plant species (Barlow 1977; Nagl 1979a, b; Nagl and Fusenig 1979; Nagl and Bachmann 1980) have suggested that nuclear DNA content and proportion of different frequency classes of repeated DNA sequences may influence the nuclear organization. This suggestion, however, requires additional screening of a large number of species. We have, therefore, undertaken the work of heterochromatin visualization in the interphase nuclei of 23 plant species using the HCl-Giemsa banding technique (Joshi and Ranjekar 1980, 1982). Molecular analysis of the DNAs in these species has been reported earlier (Ranjekar *et al* 1974, 1976, 1978c; Seshadri and Ranjekar 1979, 1980a; Deshpande and Ranjekar 1980; Bhave *et al* 1984; Lakshmi and Ranjekar 1984; Lakshmi *et al* 1984). The present report gives a consolidated account of a relationship among the three parameters namely interphase nuclear structure, nuclear DNA content and amounts of single copy and reiterated DNA sequences.

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2. Materials and methods

2.1 *Staining with HCl-Giemsa technique*

Seeds of 21 plant species listed in table 1 were germinated on moist filter paper in petri dishes. Root tips (1–2 cm) were fixed in acetic acid:ethanol (1 : 3) for 24 hr and then stored in 70% ethanol till further use. In case of *Allium sativum* and *A. cepa*, root tips were obtained from bulbs and then processed as above.

For air dried preparations, the root tips were squashed in a drop of 45% acetic acid after 10 min maceration in 1 N HCl at room temperature and flattened by mechanical pressure. Precaution was taken to keep the pressure uniform. Coverglasses were detached using liquid nitrogen and the slides were air dried after immersion in 90% ethanol.

HCl-Giemsa treatment was carried out according to the schedule of Joshi and Ranjekar (1980). The treatment time with concentrated HCl varied from 5–30 min. Prior to treatment with concentrated HCl, slides were incubated in distilled water for 10–15 min. After the HCl treatment, the slides were thoroughly washed with cold running water, stained in 4% Giemsa (BDH) diluted with M/15 Sorensen's phosphate buffer, pH 6·8 and mounted in DPX.

To determine the interphase nuclear structure, at least 50 nuclei from different

Table 1. Interphase nuclear structure and condensed chromatin in higher plants.

Plant species	DNA content	Nuclear structure	Number of chromocenters Mean ± SD	condensed chromatin (% nuclear area) Mean ± SD
Raphanus sativus	0·40*	Chromocentric	15 ± 0·33	14·93 ± 1·86
Phaseolus aureus	0.50*	Chromocentric	16 ± 0·50	21·77 ± 2·68
Oryza sativa	0·60*	Chromocentric	11 ± 0·50	13·80 ± 2·11
Setaria italica	0·80	Chromocentric	13 ± 0·80	14·11 ± 2·04
Luffa cylindrica	0·90*	Chromocentric	14 ± 0·50	19·59 ± 5·42
Cucumis sativus	1·00**	Chromocentric	16 ± 0·60	21·64 ± 2·59
Panicum milliare	1.00	Chromocentric	14 ± 0·75	15·58 ± 2·42
Benincasa hispida	1·10*	Chromocentric	20 ± 0·25	15·69 ± 2·63
Trichosanthes anjuina	1·10*	Chromocentric	16 ± 0·27	16·43 ± 2·06
Echinochloa frumentacea	1·30**	Chromocentric	17 ± 0·66	14·44 ± 3·13
Phaseolus vulgaris	1·30*	Chromocentric	15 ± 0·50	19·65 ± 3·33
Eleusine coracana	1·60**	Chromocentric	18 ± 0·33	15·56 ± 2·17
Phaseolus mungo	1·75	Chromocentric	13 ± 0·20	16·11 ± 3·39
Phaseolus aconitifolius	2·00	Chromocentric	16 ± 0·60	15·51 ± 1·91
Luffa acutangula	2·22	Chromocentric	15 ± 0·60	16·11 ± 2·96
Pennisetum americanum	2·45	Reticulate	—	26·82 ± 4·78
Coccinia indica	2·75	Chromocentric	14 ± 0·25	18·85 ± 1·05
Sorghum vulgare	4·60	Reticulate	—	25·08 ± 2·44
Hordeum vulgare	5·50**	Reticulate	—	60·87 ± 8·70
Secale cereale	9·50**	Reticulate	—	66·20 ± 1·96
Allium sativum	15·70*	Reticulate	—	77·28 ± 3·78
Allium cepa	16·75**	Reticulate	—	64·70 ± 4·33
Triticum aestivum	17·30**	Reticulate	—	60·76 ± 7·26

* From Bennett *et al* (1982).

** From Bennett and Smith (1976).

preparations were observed. Area of chromocenters, chromonemata and nuclei were calculated by planimetry. Condensed chromatin amount was calculated by using the following formula. In chromocentric nuclei

$$\% \text{ condensed chromatin} = \frac{\text{Area of chromocenters}}{\text{Area of nucleus}} \times 100$$

In reticulate nuclei

$$\% \text{ condensed chromatin} = \frac{\text{Area of chromonemata}}{\text{Area of nucleus}} \times 100$$

The condensed chromatin values were expressed as % nuclear area and they represented mean of at least 50 nuclei.

2.2 *Determination of* DNA *content*

DNA content was determined only in *Setaria italica*, *Panicum milliare*, *Phaseolus mungo*, *Phaseolus aconitifolius*, *Luffa acutangula*, *Pennisetum americanum*, *Coccinia indica* and *Sorghum vulgare* where the data was not available. DNA quantitation was done by using the standard two wavelength method of Patau (1952) where λ_1 selected was 550 nm. Schiff's reagent preparation (pH adjusted to 3·6) and Feulgen staining were carried out according to Darlington and LaCour (1976). To ensure the absolute amount of DNA per 2C nucleus, late anaphase or telophase figures were selected. *A. cepa* (2C = 33·5 pg) was used as standard. The 1C amounts were obtained from 2C values.

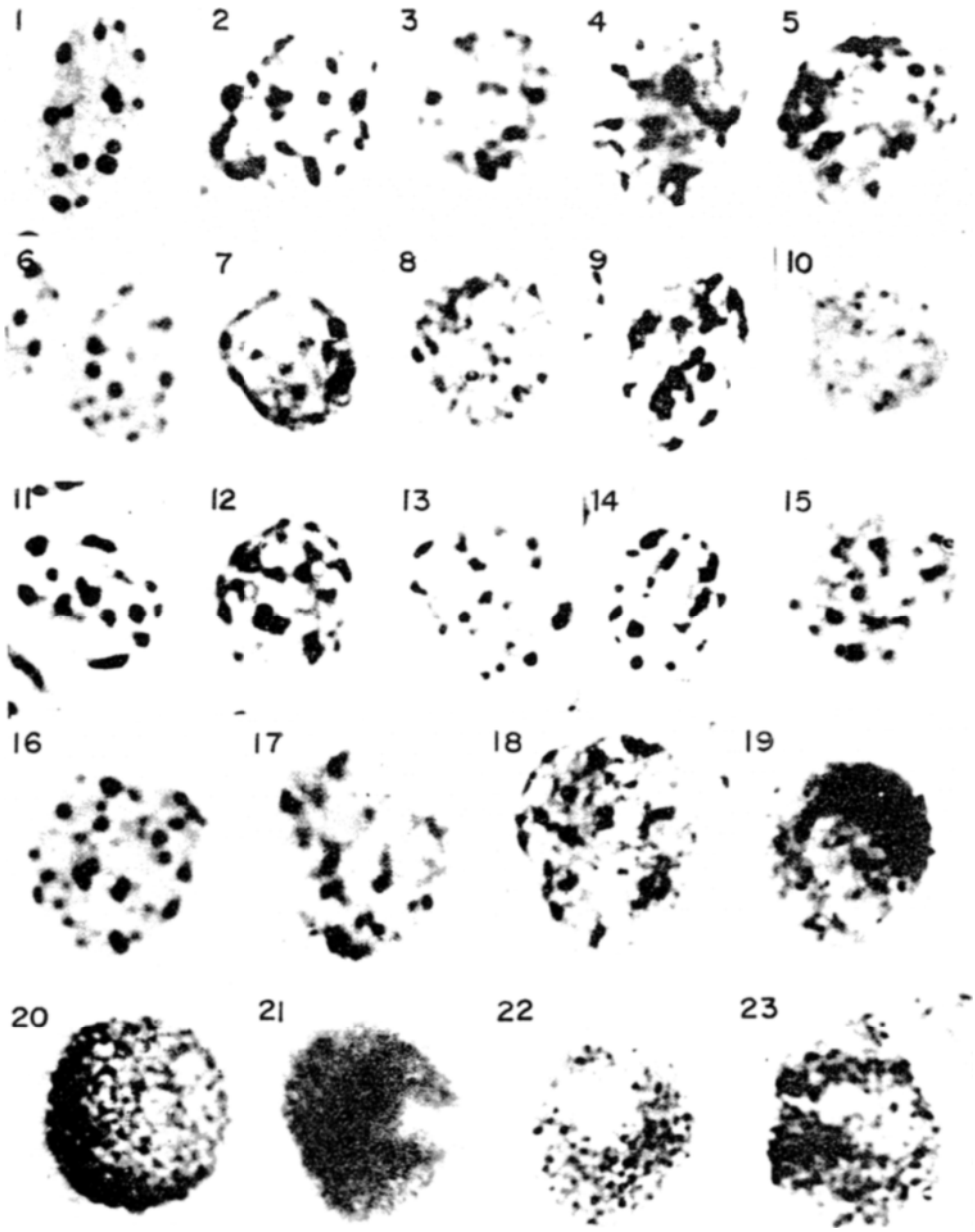
2.3 *Amounts of repetitive* DNA

From our published reassociation kinetics data, the actual amounts of different DNA components were estimated in terms of picograms. The DNA sequences forming duplexes by Cot 0·1 M × sec/l were considered to be highly repetitive. The DNA reannealing between Cot 0·1 M × sec/l and the limit repetitive Cot value was assumed to include intermediately repetitive DNA sequences. The amount of total repetitive DNA represents the sum of the amounts of highly repetitive DNA and intermediately repetitive DNA. The DNA sequences reassociating after the limit repetitive Cot were considered to be mainly unique.

3. Results and discussion

All the species under the present investigation show two types of interphase nuclear organization namely, chromocentric and reticulate. Out of 23 species, 16 species have chromocentric organization and show distinct large and dark chromocenters on a faint euchromatic background (figures 1–16). The remaining 7 species have reticulate nuclei (figures 17–23) and exhibit a dense network of chromomeres and chromonemata.

In chromocentric species, the number of chromocenters is considered to be a species-specific character. Since these chromocenters have been shown to correspond to heterochromatin (Nagl and Fusenig 1979), the condensed chromatin values as determined from chromocenters will, therefore, correspond to heterochromatin amount. These amounts as shown in table 1, vary in a narrow range of 13–21 % of nuclear area. In reticulate nuclei, on the other hand, condensed chromatin corresponds



Figures 1–23. Interphase nuclei, **1–16.** showing chromocentric structure, **17–23.** showing reticulate structure. **1.** *R. sativus* (× 1400), **2.** *P. aureus* (× 10500), **3.** *O. sativa* (× 9800), **4.** *S. italica* (× 6400), **5.** *L. cylindrica* (× 7600), **6.** *C. sativus* (× 1200), **7.** *P. milliare* (× 3000), **8.** *B. hispida* (× 7250), **9.** *T. anjuina* (× 7000), **10.** *E. frumentacea* (× 2250), **11.** *P. vulgaris* (× 7800), **12.** *E. coracana* (× 8250). **13.** *P. mungo* (× 6700). **14.** *P. aconitifolius* (× 7000), **15.** *L. acutangula* (× 7000), **16.** *C. indica* (× 1700), **17.** *P. americanum* (× 8000), **18.** *S. vulgare* (× 2070), **19.** *H. vulgare* (× 1200), **20.** *S. cereale* (× 1200), **21.** *A. sativum* (× 5900), **22.** *A. cepa* (× 5000), **23.** *T. aestivum* (× 6000).

to both heterochromatin and condensed euchromatin (Nagl and Fusenig 1979) and the values, therefore, vary in a wide range of 25–77% (table 1).

The occurrence of different classes of heterochromatin among plants is well known. Furthermore from our earlier work (Joshi and Ranjekar 1982; Patankar and Ranjekar 1984a, b), it is well established that the HCl-Giemsa technique visualizes most of the types of heterochromatin as well as condensed euchromatin. The HCl-Giemsa technique, thus has the advantage over the BSG technique which visualizes only heterochromatic portions as chromocenters in both chromocentric as well as reticulate nuclei. The values obtained using HCl-Giemsa technique, therefore, though not absolute, give a fairly correct picture of condensed chromatin in plants. For example, the values obtained in *P. vulgaris* (19·65 ± 3·33) and *A. cepa* (64·70 ± 4·33) agree well with the reported values of 17% and 52% respectively, calculated by electron microscopic morphometry (Nagl *et al* 1983).

The nuclear DNA content (1C) values show a wide range of 0·4–17·3 pg (table 1). The data also clearly indicate that the chromocentric nuclei are characterized by small genome size (1C < 2 pg, except *Coccinia*) and reticulate nuclei have large genome size (1C > 2 pg). The role of nuclear DNA in determining the nuclear structure has been suggested earlier (Lafontaine 1974; Barlow 1977; Nagl and Fusenig 1979; Nagl 1982). Our data on condensed chromatin also show its strong positive dependence on the nuclear DNA content suggesting thereby a direct role of nuclear DNA content in gross chromatin organization in plant nuclei (table 4, coefficient of correlation $r = 0{\cdot}9053$) (figure 24). This also confirms the nucleotypic effect of DNA content on chromatin structure as suggested earlier by Nagl and Fusenig (1979).

Delay (1948), Lafontaine (1974) and Barlow (1977) have earlier suggested the role of chromosome size and DNA amount per chromatid in determining interphase nuclear

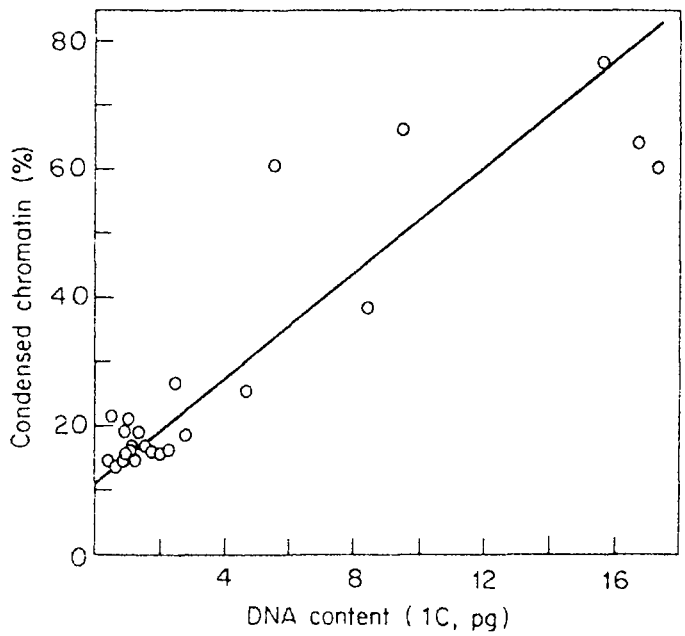


Figure 24. Relation between DNA content (1C, pg) and amount of condensed chromatin (%) in interphase nuclei of 23 plant species.

Table 2. DNA content, chromosome number and chromosome lengths in a few higher plants.

Plant species	DNA content (1C, pg)	Chromosome number (n)	Chromosome length (μ)	Total length of haploid complement (μ)	DNA/unit chromosome length (pg/μ)	Reference*
Chromocentric:						
Raphanus sativus	0·40	09	2·3–1·6	15·66	0·0255	Mukherjee (1979)
Phaseolus aureus	0·50	11	2·8–1·0	18·70	0·0267	Sarbhoy (1980)
Oryza sativa	0·60	12	1·9–1·0	28·40	0·0210	Kurata and Omura (1978)
Setaria italica	0·80	09	2·5–1·6	18·75	0·0426	Chikara and Gupta (1979)
Cucumis sativus	1·00	07	4·0–1·9	20·52	0·0487	Sen and Datta (1978)
Trichosanthes anjuina	1·10	11	3·3–0·9	30·91	0·0355	Singh and Roy (1979)
Phaseolus vulgaris	1·30	11	3·0–1·5	21·00	0·0619	Sarbhoy (1980)
Eleusine coracana	1·60	18	3·9–1·9	43·21	0·0370	Kempanna *et al* (1976)
Phaseolus mungo	1·75	11	2·2–1·0	16·60	0·1054	Sarbhoy (1980)
Reticulate:						
Hordeum vulgare	5·50	07	10·0–8·0	50·02	0·1099	Noda and Kasha (1978)
Secale cereale	9·50	07	8·0–6·0	58·32	0·1628	Vosa (1974)
Allium sativum	15·70	08	11·4–6·6	73·13	0·2146	Choudhary (1978)
Allium cepa	16·75	08	10·0–7·0	72·00	0·2326	Jones and Rees (1968)
Triticum aestivum	17·30	21	6·0–3·0	100·55	0·1720	Pegington and Rees (1970)

Note: Mean amount of DNA (pg/μ) of chromatin length is (i) 0·0449 for chromocentric nuclei, (ii) 0·1784 for reticulate nuclei.

* Reference cited is only for chromosome size and total chromatin length of haploid complement.

structure. The present data confirm the role of chromosome size where chromocentric nuclei are characterized by the small size of the chromosomes with their lengths varying from 1–3 μ (table 2). Reticulate nuclei, on the other hand, show long chromosomes with lengths varying from 3–4 μ or more (table 2). We further suggest that apart from chromosome size, packaging of DNA in chromatin and chromosomes also plays an important role in determining the interphase nuclear structure. There can be limitations on the amount of DNA packaged in the chromosome per unit length and thus the distribution of the total DNA along the chromosomes would reflect the specific nuclear structure. Data on 14 species (table 2) show that as compared to chromocentric nuclei there is nearly 4 times the amount of DNA packaged per unit length of chromosomes in reticulate nuclei. The visualization of extensive network of chromomeres and chromonemata, may, therefore, be attributed to the higher density of DNA along the chromosomes.

From table 3, it can be seen that the higher plants show wide variations of 25–80 % in the amounts of repetitive DNA. From this table, it is also evident that there is an increase in unique as well as repetitive DNA with increasing DNA content. The increase in repetitive DNA is, however, about three times more than that of unique DNA (figure 25). A similar trend has been observed earlier in case of *Allium*, *Anemone*, *Lathyrus*, *Lolium* and members of family Gramineae and Compositae where the rate of increase for repetitive DNA to non-repetitive DNA varies from 1·08 to 5·53 (Hutchinson *et al* 1980). Repetitive DNA, in general, has been suggested to play a major role in chromatin condensation and thereby in interphase nuclear structure in plants (Lafontaine 1974; Nagl 1979b, 1982; Narayan and Rees 1976). Repetitive DNA is, however, highly heterogeneous and consists of different families containing sequences of varying degrees of reiteration. To assess the role of any specific class of repeat sequences in chromatin structure, we compared the amounts of different classes of repetitive DNA with condensed chromatin values. The data in tables 3 and 4 indicate a positive correlation between total repetitive DNA and condensed chromatin (r = 0·8907). The

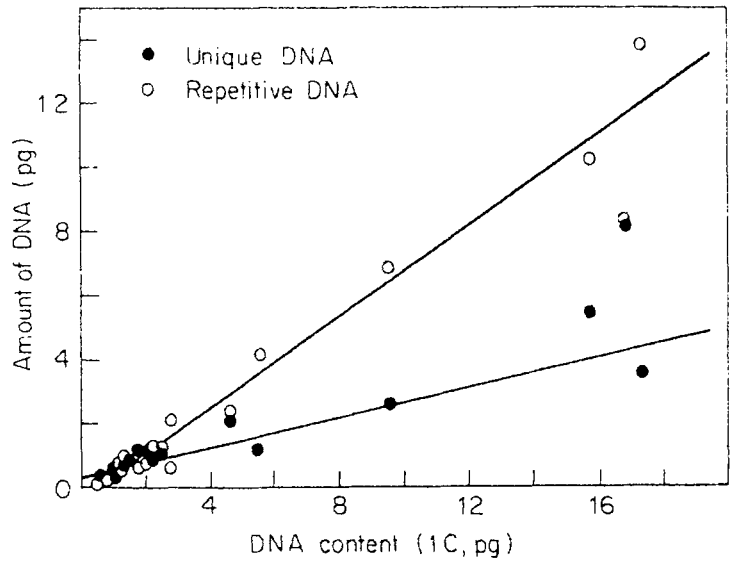


Figure 25. Relation between DNA content (1C, pg) and amount of unique DNA (pg) and total repetitive DNA (pg) in 23 plant species.

Table 3. Relationship between repetitive DNA and condensed chromatin.

Plant species	Highly repetitive DNA		Intermediately repetitive DNA		Total repetitive DNA		Unique DNA		Condensed chromatin
	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	(% nuclear area)
Raphanus sativus	15·0	0·060	30·0	0·120	45·0	0·180	55·0	0·220	14·93
Phaseolus aureus	9·0	0·045	38·0	0·190	47·0	0·235	53·0	0·265	21·77
Oryza sativa	8·5	0·051	43·5	0·261	52·0	0·312	48·0	0·288	13·80
Setaria italica	20·0	0·160	10·0	0·080	30·0	0·240	70·0	0·560	14·11
Luffa cylindrica	11·0	0·099	40·0	0·360	51·0	0·459	49·0	0·441	19·59
Cucumis sativus	20·0	0·200	16·0	0·160	36·0	0·360	64·0	0·640	21·64
Panicum milliare	15·0	0·150	25·0	0·250	40·0	0·400	60·0	0·600	15·58
Benincasa hispida	15·5	0·170	32·5	0·357	48·0	0·527	52·0	0·573	15·69
Trichosanthes anjuina	18·0	0·198	47·0	0·517	65·0	0·715	35·0	0·385	16·43
Echinochloa frumentacea	17·0	0·221	25·0	0·325	42·0	0·546	58·0	0·754	14·44
Phaseolus vulgaris	12·0	0·156	28·0	0·364	40·0	0·520	60·0	0·780	19·65
Eleusine coracana	19·0	0·304	30·0	0·480	49·0	0·784	51·0	0·816	15·56
Phaseolus mungo	7·0	0·122	27·5	0·481	34·5	0·603	65·5	1·147	16·11
Phaseolus aconitifolius	5·0	0·100	36·0	0·720	41·0	0·820	59·0	1·180	15·51
Luffa acutangula	13·0	0·288	46·0	1·020	59·0	1·309	41·0	0·911	16·11
Pennisetum americanum	20·0	0·490	34·0	0·833	54·0	1·323	46·0	1·127	26·82
Coccinia indica	15·0	0·413	10·0	0·275	25·0	0·688	75·0	2·062	18·85
Sorghum vulgare	17·0	0·782	35·0	1·610	52·0	2·392	48·0	2·208	25·08
Hordeum vulgare	19·0	1·036	59·0	3·215	78·0	4·291	22·0	1·219	60·87
Secale cereale	23·0	2·185	50·0	4·798	73·0	6·983	27·0	2·517	66·20
Allium sativum	39·0	6·123	26·0	4·082	65·0	10·205	35·0	5·495	77·28
Allium cepa	20·0	3·350	30·0	5·025	50·0	8·375	50·0	8·375	64·70
Triticum aestivum	18·0	3·115	62·0	10·726	80·0	13·840	20·0	3·460	60·76

Table 4. Statistical analysis--Pearsonian coefficient of correlation and *t* test of significance.

Variable *X*	Variable *Y*	Pearsonian coefficient of correlation*(*r*)	Degrees of freedom** (*d.f.*)	*t* test of significance (*t*)***
1C genome content (pg)	Total unique DNA (pg)	0·8988	21	9·3939
1C genome content (pg)	Total repetitive DNA (pg)	0·9742	21	19·7687
1C genome content (pg)	Condensed chromatin (%)	0·9053	21	9·7660
Condensed chromatin (%)	Highly repetitive DNA (pg)	0·9012	21	9·5287
Condensed chromatin (%)	Intermediately repetitive DNA (pg)	0·8178	21	6·5110
Condensed chromatin (%)	Total repetitive DNA (pg)	0·8907	21	9·4440

* Pearsonian coefficient of correlation, $r = \Sigma xy/(\Sigma x^2 \cdot \Sigma y^2)^{1/2}$ where $x = X - \bar{X}$ and $y = Y - \bar{Y}$.

** Degrees of freedom $d.f.$ = Number of observations $(N) - 2$.

*** t test of significance $t = r\sqrt{d.f./1 - r^2}$ where r = Pearsonian coefficient of correlation.

Standard t values $t_{0.01}$ and $t_{0.05}$ for $d.f.$ = 21 are 2·831 and 2·080 respectively.

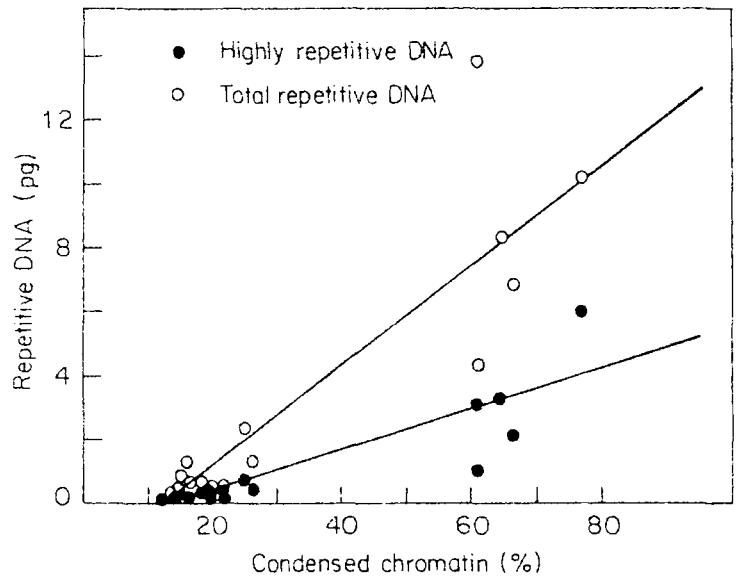


Figure 26. Relation between condensed chromatin and amounts of highly and total repetitive DNA (pg) in 23 plant species.

highest correlation is, however, seen between highly repetitive DNA and condensed chromatin (r = 0·9012) (figure 26), suggesting a major role to the highly repetitive DNA rather than to the total repetitive DNA. The suggested relationship of highly repetitive DNA and/or satellite DNA with centromeric heterochromatin and chromocenters (Timmis *et al* 1975; Nagl and Capesius 1977; Appels and Peacock 1978; Narayan and Durrant 1983) supports our observation that chromatin condensation is dependent on the highly repetitive DNA. The chromatin condensation is, however, a very complex phenomenon and involves several other factors such as ionic changes, protein modifications and specific stages of development.

Table 5. Relationship between satellite DNA presence and interphase nuclear structure.

Plant species	Satellite		Reference
	Natural*	Cryptic**	
Chromocentric:			
Raphanus sativus	+	−	Ranjekar *et al* (1978b)
Phaseolus aureus	+	−	Ingle *et al* (1973)
Luffa cylindrica	+	−	Ingle *et al* (1973)
Cucumis sativus	+	−	Ranjekar *et al* (1978b)
Phaseolus vulgaris	+	−	Ingle *et al* (1973)
Phaseolus mungo	+	−	Seshadri and Ranjekar (1979)
Phaseolus aconitifolius	+	−	Seshadri and Ranjekar (1979)
Reticulate:			
Hordeum vulgare	−	+	Ranjekar *et al* (1978a)
Secale cereale	−	+	Appels *et al* (1978)
Triticum aestivum	−	+	Ranjekar *et al* (1978a)

* Natural satellite is a repetitive DNA fraction which bands in equilibrium CsCl density gradient centrifugation at a buoyant density different than that of main band DNA.

** Cryptic or hidden satellite is revealed only after binding of DNA either with antibiotics or heavy metals followed by equilibrium density gradient centrifugation in CsCl or Cs_2SO_4.

Table 6. Relationship between interspersion pattern and interphase nuclear structure.

Plant species	Interspersion pattern	Reference
Chromocentric:		
Phaseolus aureus	Intermediate	Seshadri and Ranjekar (1980b)
Oryza sativa	Absent	Gupta *et al* (1981)
Setaria italica	long	Lakshmi (1984)
Luffa cylindrica	mixed	Bhave *et al* (1985)
Panicum milliare	mixed	Lakshmi (1984)
Benincasa hispida	mixed	Bhave *et al* (1985)
Echinochloa frumentacea	mixed	Lakshmi (1984)
Phaseolus vulgaris	Intermediate	Seshadri and Ranjekar (1980b)
Eleusine coracana	mixed	Gupta and Ranjekar (1981)
Luffa acutangula	mixed	Bhave *et al* (1985)
Coccinia indica	mixed	Bhave *et al* (1985)
Reticulate:		
Pennisetum americanum	long	Gupta and Ranjekar (1982)
Sorghum vulgare	mixed	Lakshmi *et al* (1984)
Secale cereale	short	Smith and Flavell (1977)
Allium cepa	short	Stack and Comings (1979)
Triticum aestivum	short	Flavell and Smith (1976)

The data presented in table 5 show that species with chromocentric nuclei show the presence of natural satellites whereas those with reticulate nuclei have mainly cryptic satellites. Furthermore, it can be seen from table 6 that species having reticulate nuclei show, in general, predominantly typical short period interspersion pattern of genome organization while species with chromocentric nuclei have either long period or mixed interspersion patterns. Since the highly reiterated DNA (satellite) has a positive role in chromatin condensation, the above findings may have a direct bearing in determining the structural complexity of interphase nuclei in plants.

In conclusion, our data reaffirm the earlier predictions about the role of nuclear DNA content and also propose the involvement of specifically highly repetitive DNA and DNA packaging in chromatin condensation in higher plants. Further work is being undertaken to evaluate the role of highly repetitive/satellite DNAs and DNA sequence organization in chromatin condensation.

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