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Nuclear DNA endoreplication and plastid index in mesophyll of some dicotyledonous species

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Abstract

Cytophotometric studies of nuclear DNA content after Feulgen procedure indicate that in mesophyll of all the seven studied species the highest nuclear DNA endoreplication level occurs in II or III leaf and it varies for particular species. No differences were found in nuclear DNA endoreplication dynamics between the basal and apical parts of the leaf blade. Chloroplast number per cell generally decreases in the successive leaves, and the plastid index is the smallest in the first (oldest) leaves, being similar in both zones. In four species chloroplast number and plastid index show relatively low negative correlation with nuclear DNA contents (expressed as endoreplication index), in two species this correlation is positive, and one species displays very low r value.

Key words: dicotyledonous leaves, nuclear DNA endoreplication, plastid index

INTRODUCTION

Nuclear DNA endoreplication occurs during growth and differentiation of many tissues in angiosperms. Therefore this process is generally reported to be associated with the differentation and morphogenesis. Recognition of DNA endoreplication patterns in particular organs and tissues may be useful in understanding the biological significance of this process.

Many observations show the occurrence of this phenomenon in mesophyll of angiosperms. In monocotyledonous species in bundle sheath cells (Olszewska et al. 1983) as well as in the mesophyll of dicotyledonous one (Olszewska et al. 1986) the attained endoreplication level was

approximately similar to the one found in root parenchyma of the same species.

Various angiosperm species display different maximum nuclear DNA content in the mesophyll cells e.g. 4 C – *Zea mays*, *Vicia faba*, 8 C – *Arachis hypogea*, 16 C – *Triticum durum*, *Beta vulgaris*, *Brassica oleracea*, *Phaseolus vulgaris*, 32 C – *Raphanus sativus* (cited after Olszewska et al. 1986). However, in some species there is no increase in nuclear DNA content over the basic value i.e. *Triticum aestivum* (Dean and Leech 1982) or *Fraxinus americana* (Black and Beckmann 1983), as well as *Muscari comosum*, *Allium porrum*, *Allium cepa*, *Amaryllis belladonna* and *Clivia miniata* (Olszewska et al. 1983).

Many authors studying the relationship between nuclear DNA content in mesophyll and chloroplast number found the positive correlation – increase in ploidy level is associated with an increase in plastid number (Butterfass 1963, 1973, 1980, Kowallik and Herrmann 1974, Rose et al. 1975, Herrmann and Possingham 1980). There are some reports about the lack of relationship between nuclear DNA content and plastid number in a cell. It was found by Cattolico (1978) in the alga *Olisthodiscus luteus*, the cells of which contained constant amount of DNA regardless of chloroplast number, as well as Lamppa et al. (1980) on pea leaves. The studies by Olszewska et al. (1983) also proved that during the leaf development in five monocotyledonous species with different 2 C nuclear DNA content the increase in chloroplast number was not parallel to the increase in DNA level in the nucleus.

The aim of the present studies is to enlarge the dicotyledonous species number in order to enable in future the presentation of more precise view concerning a relationship between DNA content and plastid index.

MATERIAL AND METHODS

The studies were carried out on the leaves of seven dicotyledonous plants with different 2 C nuclear DNA content (Flavell et al. 1974, Olszewska and Osiecka 1983):

1. *Phaseolus vulgaris* L. – 1.0 pg±0.03
2. *Raphanus sativus* L. cv. Chodowianka – 1.25 pg±0.04
3. *Brassica oleracea* L. cv. Amager – 1.55 pg±0.04
4. *Beta vulgaris* L. – 2.7 pg*
5. *Pisum sativum* L. cv. Cud Kalvedonu – 7.6 pg±0.7
6. *Nigella damascena* L. – 21.6 pg±0.5
7. *Vicia faba* L. subsp. *major* cv. Hangdown – 26.7 pg±0.5

Plants were grown in soil in natural photoperiod (April-August). Mature leaves were cut off the plants which entered generative phase. A basal part (1 zone)

* Flavell et al. 1974

and the apical part (5 zone) of the leaf blade of the successive leaves were used for the studies (leaf zone were marked like in the paper by Olszewska et al. 1986), i.e. beginning from the oldest leaves up to the youngest ones – I, II, III, V and VII. In *Vicia faba* the fifth leaf was the youngest one.

Cytophotomery of nuclear DNA after Feulgen reaction was carried out on leaves fixed in ethanol: acetic acid mixture (2:1) for 2 hours. Pieces of fixed leaves were hydrolyzed in 4N HCl for 1 h, at room temperature and stained in Schiff's reagent (prepared from pararosaniline, Gurr) for 40 min. Squashed preparations were analyzed in a Zeiss (Jena) Histophotometer at $\lambda = 550$ nm. 50 nuclei of mesophyll cells from each leaf zone were analyzed. The 2 C and 4 C values were calculated from 20 telophase and 10 prophase nuclei from root meristem of the given species submitted to Feulgen procedure in the same baths together with leaves. Integrated Feulgen densities are presented in histograms in logarithmic scale as arbitrary units (AU).

Basing on the results concerning nuclear DNA contents, expressed in AU, endoreplication index (IE) was calculated according to Olszewska et al. (1988 – in press):

$$IE = \frac{AU}{C}$$

AU – average DNA contents for the studied population expressed in arbitrary units, C – 1/2 of DNA contents of telophase nuclei of 1/4 of nuclear contents of prophase nuclei expressed in arbitrary units.

Chloroplast number, cell and chloroplast areas were calculated from the material fixed in 3% glutaraldehyde and macerated in 0.05M EDTA according to the method described by Possingham and Smith (1972). Plastid index was calculated according to Possingham (1976):

$$IP = \frac{S_{chl} \cdot N_{chl}}{2S_{cell}}$$

S_{chl} – average surface of chloroplasts in μm^2, N_{chl} – average number of chloroplasts per cell, S_{cell} – average surface of cell in μm^2.
IP is a measure of the proportion of the cell surface occupied by chloroplasts.

RESULTS

CHANGES IN NUCLEAR DNA CONTENT IN MESOPHYLL OF LEAVES AT DIFFERENT INITIATION TIMING

In all the studied species the increase in the nuclear DNA content has been found in the mesophyll of the successive leaves from I to II or III depending on the species. In younger leaves (V, VII), however, lower level of nuclear DNA content has been found (Figs. 1-7). The highest nuclear DNA content occurs in II or III leaf and reached 4 C values in *Vicia faba* (Fig. 7), 8 C in *Nigella damascena* and *Pisum sativum* (Figs. 5, 6), 16 C in *Phaseolus vulgaris*, *Brassica*

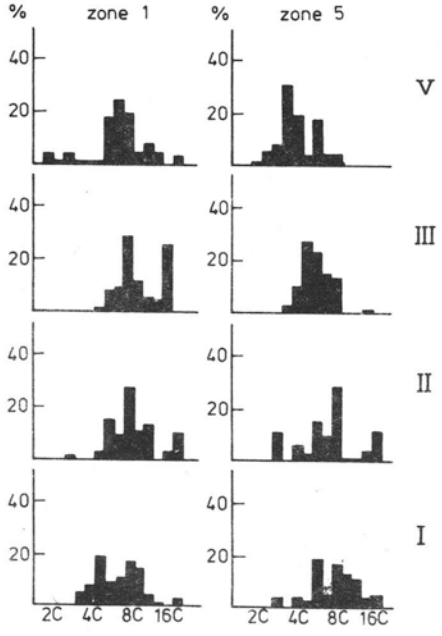


Fig. 1. *Phaseolus vulgaris*. Nuclear DNA contents in mesophyll in basal (zone 1) and apical (zone 5) zones in successive leaves (I-VII). Ordinate – % of nuclei, abscissa – relative DNA content

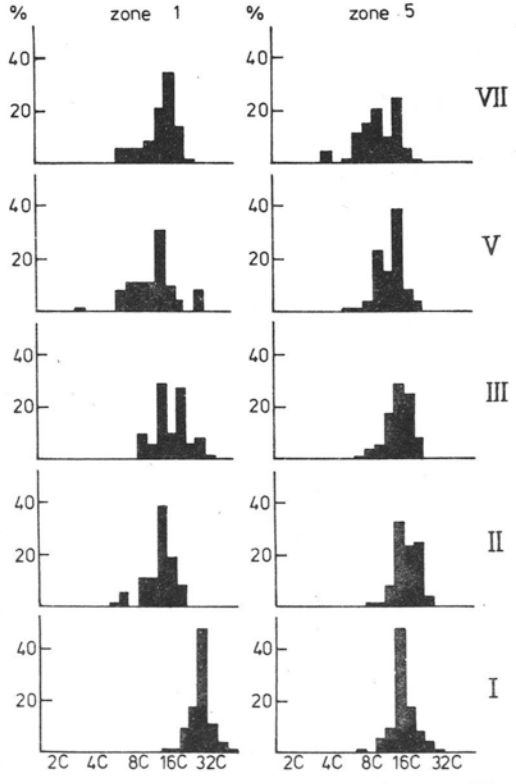


Fig. 2. *Raphanus sativus*. Other explantions see Fig. 1

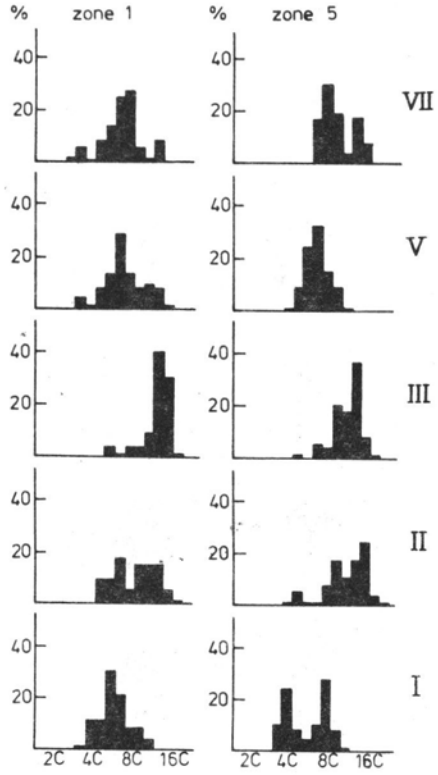


Fig. 3. *Brassica oleracea.* Other explanations see Fig. 1

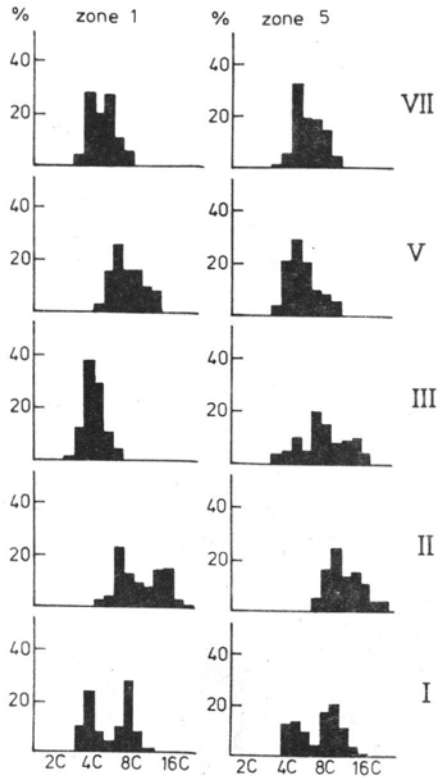


Fig. 4. *Beta vulgaris.* Other explanations see Fig. 1.

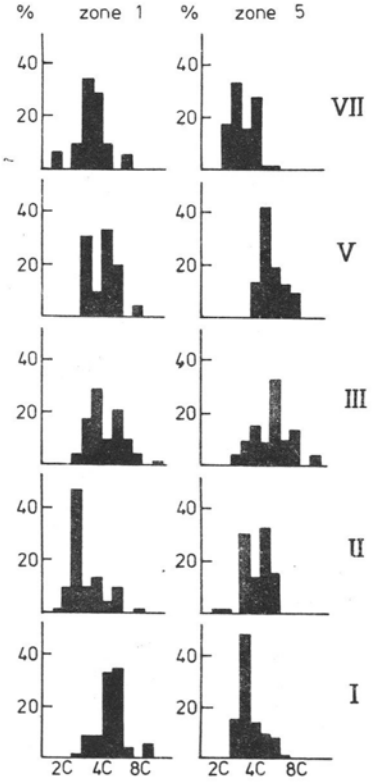


Fig. 5. *Pisum sativum*. Other explanations see Fig. 1

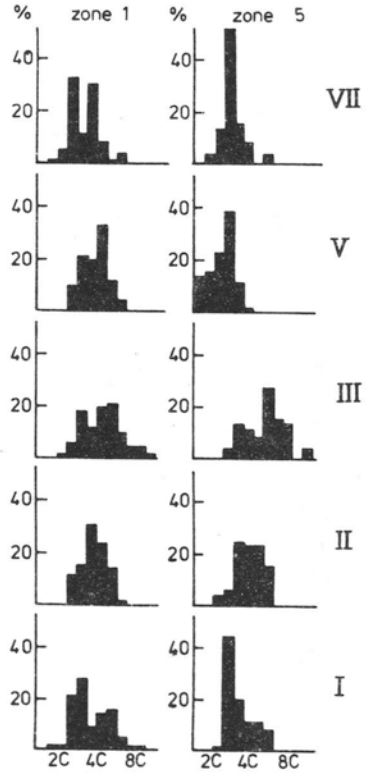


Fig. 6. *Nigella damascena*. Other explanations see Fig. 1

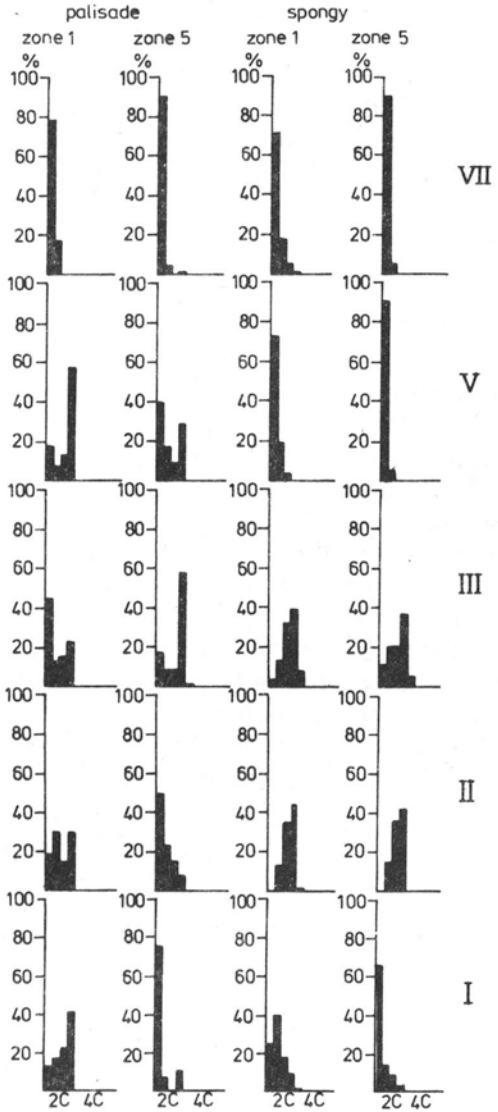


Fig. 7. *Vicia faba.* Other explanations see Fig. 1

oleracea and *Beta vulgaris* (Figs. 1, 3, 4) and 32 C in *Raphanus sativus* (Fig. 2). In *Vicia faba* nuclear DNA content was analyzed separately in palisade and spongy mesophyll of the leaves (Fig. 7). As no significant difference in nuclear DNA content was found in both parenchyma types in the successive leaves, in the remaining species both parenchyma types were not distinguished but considered as mesophyll.

COMPARISON OF NUCLEAR DNA CONTENT IN THE BASAL AND APICAL ZONES OF LEAF BLADES

No essential differences were found in the nuclear DNA content between the basal (1st) and apical (5st) parts of the leaf blades in all the studied species (Figs. 1-7). In both zones of leaves of the same age nuclei attain the same level of DNA contents characteristic of the given species.

CHLOROPLAST NUMBER AND PLASTID INDEX IN MESOPHYLL OF LEAVES WITH DIFFERENT INITIATION TIMING

In general, chloroplast number per cell decreases in the successive leaves of the studied species (Table 1). Mesophyll cells of the oldest (basal) leaves contain the most chloroplasts whereas those of the apical leaves — the fewest.

Average chloroplast surface (μm^2) in the successive leaves of the studied species neither increases nor decreases though there are visible changes between the particular leaves within the given species (Table 1). In the same leaf (zone 1 and 5) of the given species chloroplast surfaces are similar.

The surface of mesophyll cells in the successive leaves of the seven studied species show similar decreasing tendency as that mentioned above concerning chloroplasts. The closer one approaches the stem apex (VII leaves) the smaller dimensiones are reached by the mesophyll cells in both zones of the leaf blade (Table 1). The largest cells were found in the oldest leaves (I) except *Vicia faba* in which the largest cells were present in III leaf.

The parameters discussed above affect plastid index values for particular leaves. Plastid index is the smallest in I, the oldest leaves (Table 1) except *Beta vulgaris*, and it is often similar in both zones of the successive leaves.

CORRELATIONS BETWEEN NUCLEAR DNA CONTENTS, PLASTID NUMBER AND PLASTID INDEX

All the correlations were analyzed by linear regression, in normal coordinates system, carried out by the least square method.

Correlation coefficients and *P* values are presented in Table 2. Except *Brassica oleracea* and *Nigella damascena*, *r* values are low. In four out of studied species, both plastid number and plastid index are negatively correlated with nuclear DNA contents expressed as endoreplication index (*Raphanus sativus*, *Brassica oleracea*, *Beta vulgaris*, *Vicia faba* — Table 2 and Figs. 10-15, 20, 21). In *Phaseolus vulgaris* both plastid number and plastid index are positively correlated with DNA contents (Table 2, Figs. 8 and 9). In *Nigella*, only the number of plastids is positively correlated, while there is no correlation between DNA contents and plastid index (Table 2, Figs. 18 and 19). In *Pisum sativum* the *r* value is extremely low (Table 2, Figs. 16 and 17).

Table 1

Mean of cell surfaces (μm^2, S_{cell}), number of chloroplasts (N. chl.), chloroplast surface (μm^2, S_{chl}) and plastid index (P. i.) in successive leaves (Nr of leaf: I-VII) in basal (1) and apical (5) zones of leaf blade

Species	Nr of leaf	S_{cell}		N. chl.		S_{chl}		P. i.	
		1	5	1	5	1	5	1	5
Phaseolus vulgaris L.	I	1080	1100	35	38	31.8	31.8	0.51	0.54
	II	860	985	43	33	25.4	31.8	0.63	0.53
	III	843	621	32	26	30.1	28.5	0.57	0.60
	V	715	910	27	28	31.9	35.2	0.60	0.54
Raphanus sativus L.	I	1439	1918	45	49	24.8	29.8	0.38	0.38
	II	832	1188	37	26	32.3	35.1	0.71	0.38
	III	1778	1383	37	45	36.7	37.9	0.38	0.61
	V	1412	1453	37	30	38.3	38.9	0.48	0.41
	VII	699	354	28	23	20.6	19.7	0.41	0.63
Brassica oleracea L.	I	8542	5822	91	80	34.1	26.6	0.18	0.18
	II	7363	4342	107	83	33.1	32.6	0.24	0.31
	III	3306	4264	58	73	39.5	36.9	0.34	0.31
	V	1840	1626	88	96	26.8	18.3	0.64	0.54
	VII	1193	2182	65	62	23.2	36.2	0.63	0.51
Beta vulgaris L.	I	2713	3787	91	108	24.8	21.7	0.41	0.31
	II	3161	3153	62	95	22.7	20.2	0.22	0.30
	III	2732	3698	69	77	21.1	24.6	0.26	0.25
	V	2422	3126	76	98	24.4	22.5	0.38	0.35
	VII	1664	1172	61	63	26.3	24.8	0.48	0.66
Pisum sativum L.	I	2430	2605	58	60	35.2	33.0	0.42	0.38
	II	1185	1258	43	52	28.1	24.2	0.51	0.50
	III	2106	1608	66	53	30.0	27.3	0.47	0.45
	V	943	873	40	38	26.4	24.8	0.56	0.54
	VII	615	593	28	25	25.5	26.1	0.58	0.55
Nigella damascena L.	I	1671	1436	157	170	4.0	3.9	0.19	0.23
	II	1372	1524	158	150	8.2	10.2	0.47	0.50
	III	1677	1721	180	201	6.4	5.2	0.34	0.31
	V	1517	1344	156	144	5.5	5.9	0.28	0.31
	VII	1421	1406	140	152	4.8	6.0	0.24	0.32
Vicia faba L.	I pal.	4603	6244	166	158	28.6	28.8	0.52	0.37
	sp.	5642	5900	191	144	20.2	19.8	0.34	0.24
	II pal.	5048	6275	146	143	40.1	35.0	0.58	0.40
	sp.	5842	4843	150	135	29.4	29.3	0.38	0.41
	III pal.	6654	6395	188	155	36.2	38.4	0.51	0.47
	sp.	5916	5795	162	168	30.2	29.4	0.41	0.47
	V pal.	4509	5254	140	151	46.3	40.2	0.72	0.58
	sp.	4849	4455	153	141	36.9	29.6	0.65	0.43
	VII pal.	3786	4762	156	169	31.0	32.0	0.74	0.57
	sp.	3438	4412	157	147	30.1	26.8	0.69	0.47

Table 2

Correlation coefficients (r) and P values calculated for the relationship between plastid number (N. chl.), plastid index (P. i.) and endoreplication index

Species	r		P	
	N. chl.	P.i.	N. chl.	P.i.
Phaseolus vulgaris L.	0.38	0.39	> 0.1	> 0.1
Raphanus sativus L.	−0.47	−0.46	> 0.1	> 0.1
Brassica oleracea L.	−0.64	−0.054	0.05	> 0.1
Beta vulgaris L.	−0.25	−0.47	> 0.1	> 0.1
Pisum sativum L.	−0.15	0.20	> 0.1	> 0.1
Nigella damascena L.	+0.81	0.14	0.005	> 0.1
Vicia faba L.	−0.05	−0.15	> 0.1	> 0.1

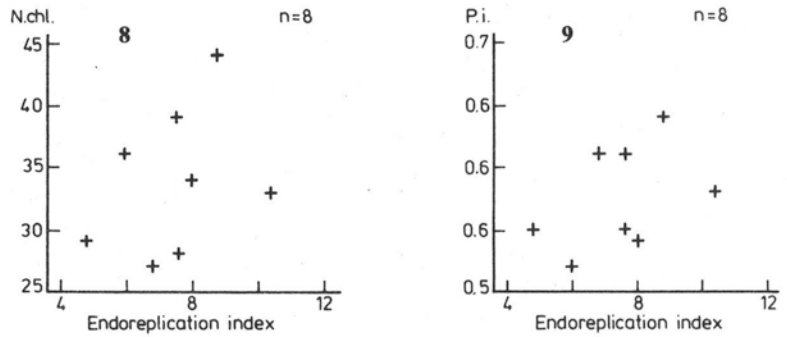


Fig. 8. *Phaseolus vulgaris.* Correlation between chloroplast number (N. chl.) and endoreplication index. Asterisks indicate more than one point of the same value

Fig. 9. *Phaseolus vulgaris.* Correlation between plastid index (P. i.) and endoreplication index. Asterisks indicate more than one point of the same value

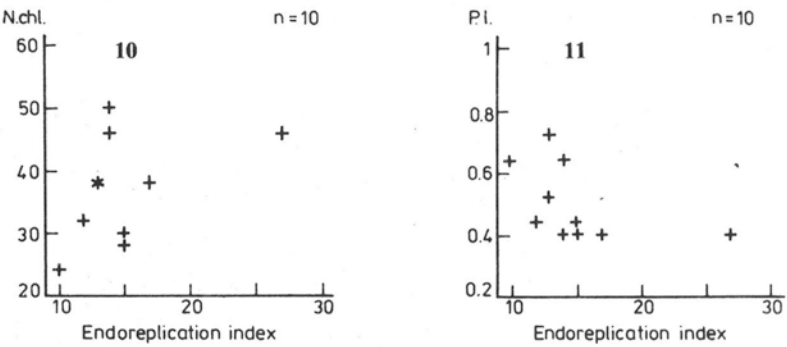


Fig. 10. *Raphanus sativus.* Other explanations see Fig. 8.

Fig. 11. *Raphanus sativus.* Other explanations see Fig. 9.

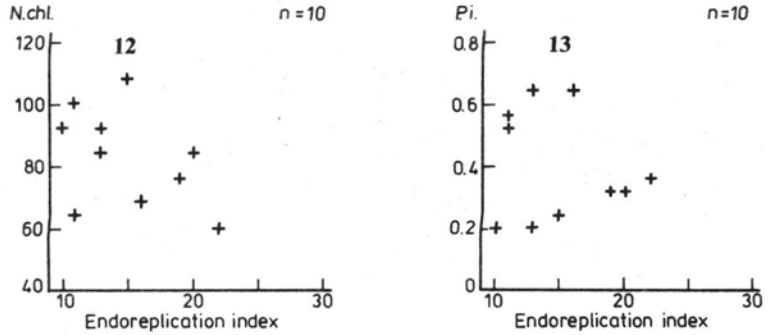


Fig. 12. *Brassica oleracea.* Other explanations see Fig. 8

Fig. 13. *Brassica oleracea.* Other explanations see Fig. 9

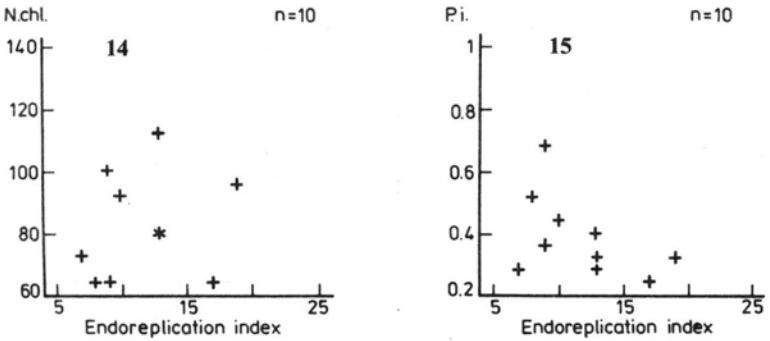


Fig. 14. *Beta vulgaris.* Other explanations see Fig. 8

Fig. 15. *Beta vulgaris.* Other explanations see Fig. 9.

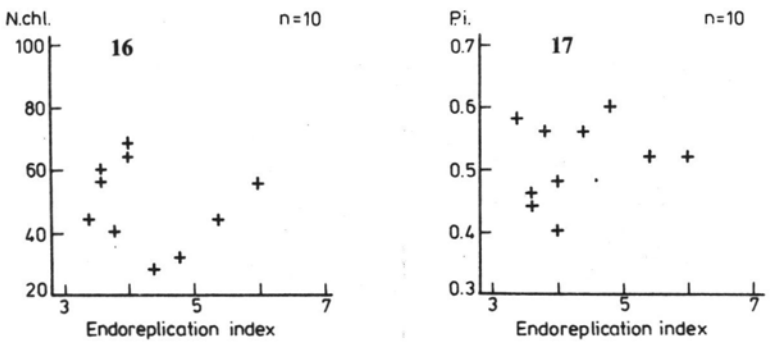
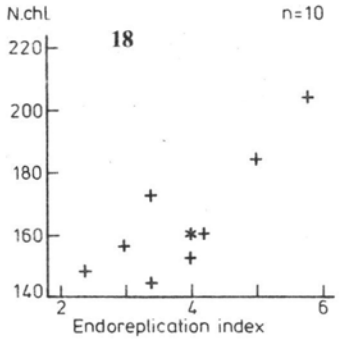


Fig. 16. *Pisum sativum.* Other explanations see Fig. 8.

Fig. 17. *Pisum sativum.* Other explanations see Fig. 9



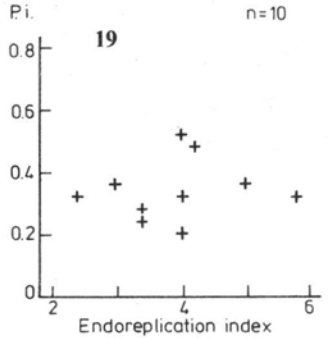
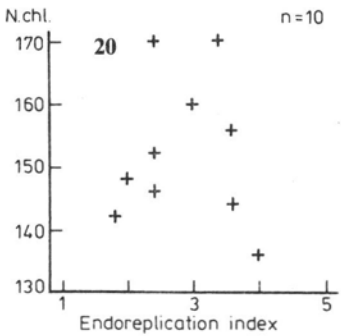


Fig. 18. *Nigella damascena*. Other explanations see Fig. 8

Fig. 19. *Nigella damascena*. Other explanations see Fig. 9



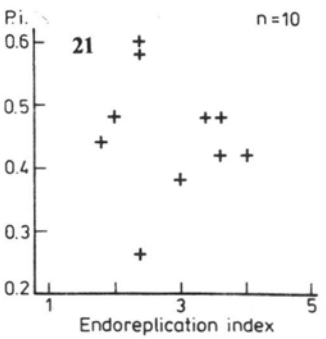


Fig. 20. *Vicia faba*. Other explanations see Fig. 8.

Fig. 21. *Vicia faba*. Other explanations see Fig. 9

DISCUSSION

Observed differences in nuclear DNA endoreplication level in the mesophyll of the successive leaves of the seven studied species may be explained as due to the endomitotic processes following each other during the development of leaves. Diminution of the DNA contents may be caused the decrease in DNA endoreplication dynamics, progressive chromation condensation or its loss during leaf aging. Chromatin condensation during senescence results in the decrease in its sensitivity to HCl hydrolysis in the Feulgen procedure (Olszewska et al. 1986). Several authors stated that diminution of nuclear DNA in mesophyll resulted from a loss of chromosome fragments (Hesemann and Schröder 1982), quicker loss of GC pairs than those of AT (Harris et al. 1984) or increased activity of DNase, pH 6.6 (Kumar and Khan 1984).

Nuclear DNA endoreplication dynamics in leaves is similar to that in root

parenchyma cells in the same species (Olszewska et al. 1983, Olszewska and Osiecka 1983).

Our results failed to establish a significant correlation between nuclear DNA content considered as endoreplication index and plastid index. There may be some causes for that, e.g.: — too few measured points (10 in each case); — the use of average values not referring to particular cells (due to the necessity of employing different method of measuring DNA content and calculating plastid index); — too complex experimental model — the youngest leaves initiated at the end arise from the latest generations of apical meristem, while the oldest ones from the earliest meristem.

Two first causes may be avoided by measuring DNA content and plastid index value in the same cells using in future cytofluorometry. The third cause could be clarified by sampling the material from corresponding leaves at different age.

Results of the present studies would indicate the lack of the positive correlation between nuclear DNA contents considered as endoreplication index and chloroplast number in cells (except *Phaseolus vulgaris* and *Nigella damascena*, comp. Figs. 8 and 18 and Table 1) or plastid index (Figs. 9 and 19). In four out of the species studied there is a negative correlation between the plastid index and the endoreplication index (*Raphanus sativus*, *Brassica oleracea*, *Beta vulgaris*, *Vicia faba*).

Our results show that Butterfass' hypothesis (1963, 1973) about the control of the plastid replication by nuclear DNA dosage does not seem to be universal. Presumably, there are other factors affecting the number of chloroplasts in a cell and their dimensions e.g. genotype, cell type, conditions of growing such as the presence of cytokinins, surose, light intensity, photoperiod, temperature, which was proved by Possingham and Smith (1972) studying *in vitro* the discs from spinach leaves.

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Endoreplikacja jądrowego DNA i indeks plastydowy w mezofilu wybranych gatunków roślin dwuliściennych

Streszczenie

Badania cytofotometryczne zawartości DNA jądrowego po zastosowaniu metody Feulgena wykazują, że w mezofilu u wszystkich badanych gatunków najwyższy poziom endoreplikacji DNA jądrowego występuje w II lub III liściu i jest różny u poszczególnych gatunków. Stwierdzono brak różnic w dynamice endoreplikacji DNA między dolną a szczytową częścią blaszki liścia. Liczba chloroplastów na komórkę na ogół jest mniejsza w kolejnych liściach, a indeks plastydowy jest najmniejszy w pierwszych (najstarszych) liściach i podobny w obu strefach. U czterech gatunków liczba chloroplastów i indeks plastydowy są słabo ujemnie skorelowane z zawartością DNA jądrowego (obliczonego jako indeks endoreplikacji), u dwu gatunków korelacja ta jest pozytywna, u jednego — współczynnik korelacji jest bardzo mały.