Relation between nuclear DNA content and rate of cell growth during interphase in four species of *Angiospermae*

K. MARCINIAK[1], M. J. OLSZEWSKA,[1] R. OSIECKA[1] AND J. BIAŁAS[2]

[1] Department of Plant Cytology and Cytochemistry, Institute of Physiology and Cytology, and [2] Department of Functional Analysis, Institute of Mathematics, University of Łódź, Łódź

Abstract

Among four species of *Angiospermae* with known nuclear DNA content (*Cucurbita pepo* — 2.6 pg, *Helianthus annuus* — 12.0 pg, *Vicia faba* — 38.0 pg, and *Tulipa kaufmanniana* — 93.7 pg) the cell growth in the intermitotic period of the cell cycle has been observed to be the fastest in *Vicia faba*, slower in *Helianthus annuus* and the slowest in *Cucurbita pepo* and *Tulipa kaufmanniana*.

INTRODUCTION

Significant differences of nuclear DNA content in *Eukaryotes* have made it possible to demonstrate correlation between DNA content and cell size in the higher plants (B e n e t t, 1972; P r i c e and coll., 1973) and vertebrates (S z a r s k i, 1976). It is known, however, that apart from increased cell sizes, a higher DNA content also causes a lengthening of the cell cycle in *Angiospermae* (V a n' t H o f, 1965; V a n' t H o f, 1967; V a n' t H o f and S p a r r o w, 1963). The present investigation has been undertaken in order to find out whether there exists a correlation between the rate of cell growth during interphase and nuclear DNA content, i.e. in which species — with low or high content of nuclear DNA —the increase in size of meristematic cells is the fastest. This problem is interesting both from a theoretical point of view and for its practical implications.

As it appears from some investigations of a primitive *Eukaryote*, *Saccharomyces pombe*, the cell size at division and the duration of the cell cycle are homeostatically controlled (F a n t e s, 1977). One may

assume that an analogical mechanism is functioning in the higher plants. It would imply that the volume of a cell initiating mitosis is a constant feature which is characteristic for the given species and for each kind of meristem. Actually, the situation seems to be more complicated because cell sizes and the duration of their division cycles in the root meristem differ significantly depending on the distance from the root tip as well as on the root region. Moreover, there is no certainty that every cell always enters the next division (B a r l o w and M a c d o n a l d, 1973). In this situation we had limited the population of measured cells to certain zones of the meristem and the obtained results were analyzed based on a simplified model represented by the mean volume and the mean duration of the cell cycle. Furthermore, reduplication of the parameters of initial cells in their descendants was taken for granted during the analysis of cell growth.

MATERIAL AND METHODS

On the basis of the value of nuclear DNA content known from the literature (Table 1), the following species had been choosen for investigations: *Cucurbita pepo* L., cv. Bambino, *Helianthus annuus* L., *Vicia faba* L. var. *minor*, cv. Mazur, *Tulipa kaufmanniana* Rgl, cv. Shakespeare. Only the cortex cells from the 1st mm of the meristem from roots of 1.5-2 cm length were taken into consideration.

The duration of the cell cycle was estimated using the method of labelled metaphases. The seedlings of *Cucurbita pepo* and *Helianthus annuus*, and the bulbs of *Tulipa kaufmanniana* were incubated for 1 h in an aqueous solution of ^3H thymidine (20 μ Ci/ml, 21.6 Ci/mM). The seedlings of *Vicia faba* were incubated for 2 h in an aqueous solution of ^3H thymidine (10 μ Ci/ml, 21.6 Ci/mM). After incubation the plants were transferred to a nonradioactive medium. The incubation and post-incubation were performed in darkness at 23°C. The roots were fixed in Carnoy's mixture at 1 or 2 h intervals and stained with Feulgen's method. Squashed preparations were covered with Ilford K2 emulsion and exposed for 21 days (*Cucurbita pepo, Helianthus annuus* and *Tulipa kaufmanniana*) or 16 days (*Vicia faba*).

For the estimation of the volume of cells in telophase (after the formation of the cell plate) and in prophase, measurements of the length of cells and the area of their transverse section were performed on 1 μm longitudinal or transverse sections stained with Azur B. The sections were made with the use of an LKB utrotome III. The roots were fixed in 2% glutaraldehyde buffered with sodium cacodylate to pH 7.2-7.4 for 2 h, postfixed in 2% OsO_4 and embedded in Epon 812. The measurements of the length of cells were made using an ocular micrometer

Table 1

Nuclear DNA content and cellular parameters in root meristem

Species	DNA content per 2C nucleus, authors pg	Teleophase cells			Prophase cells			Duration of cell cycle h
		length μm, ±S.E. $\bar{l}$	area of transv. section μm² ±S.E. $\bar{p}$	approx. volume, μm³ $\bar{v}$	length, μm, ±S.E. $\bar{L}$	area of transv. section, μm² ±S.E. $\bar{P}$	approx. volume, μm³ $\bar{V}$	
Cucurbita pepo	2.6 ref. Lafontaine, 1974	8.8 ±0.2	379.7 ±21.5	3341	17.9 ±0.6	397.5 ±26.9	7115	18
Helianthus annuus	12.0 ref. Essad, 1973	9.2 ±0.2	397.5 ±20.7	3652	19.4 ±0.4	380.5 ±7.9	7382	11.2
Vicia faba	38.0 Van't Hof, 1967	13.4 ±0.2	642.1 ±27.4	8604	25.8 ±0.4	625.0 ±19.9	16125	13.5
Tulipa kaufman-niana	93.7 Van't Hof and Sparrow, 1963	11.0 ±0.2	685.0 ±38.6	7535	22.7 ±0.5	697.4 ±17.2	15831	22.4

under magnification of 1000×. The area of transverse sections of cells was measured using a planimeter on drawings made with the aid of a drawing apparatus under 2700× or 3400× magnification. Each of the measurements was made on at least 6 specimens. Because the length and the width of cells differ in successive meristematic zones in the same specimen, the measurements were limited to the 0.3-0.7 mm segments counting from the junction between the meristem and the root cap.

The shape of cells is almost cylindrical. Therefore, their volume was calculated from equation: $v = \bar{l} \cdot \bar{p}$ and $V = \bar{L} \cdot \bar{P}$. The calculated in this way mean volume of cells in prophase is not exactly a redoubled telophase value (Table 1). It results from the acceptance of $\bar{l}$, $\bar{p}$, and $\bar{L}$, $\bar{P}$ for the calculation of these values. It can be shown, however, on the basis of the experimental data obtained, that, in fact, a doubling of cell volumes occurs during interphase.

Let $\bar{x}$ denote an approximation of an exact number x. An optional number $\Delta\bar{x} > 0$ such that $(x - \bar{x}) \leqslant \Delta\bar{x}$ will be referred to as an estimate of the absolute error of $\bar{x}$. It can be shown that if $\Delta\bar{y}$ is an estimate of the absolute error of the number $\bar{y}$, the

(i) $\Delta(\bar{x} \cdot \bar{y}) = \Delta\bar{x}(\bar{y}) + \Delta\bar{y}(\bar{x}) + \Delta\bar{x}\Delta\bar{y} \cong \Delta\bar{x}(\bar{y}) + \Delta\bar{y}(\bar{x})$

is one of the estimated of the absolute error for the product; according this formula the error of $\bar{v}$ and $\bar{V}$ was calculated.

RESULTS

The differences in the size of metaphase chromosomes in the four studied species are presented in Figs 1-4.

In each of these species a twofold increase of the length of cells occurs in the interphase with no change of the transverse section area (Table 1).

Considering the experimental data (Table 1) we have from (i) that for:
Cucurbita pepo $6399 \leqslant V \leqslant 7831$, $6146 \leqslant 2v \leqslant 7218$,
Helianthus annuus $7084 \leqslant V \leqslant 7680$, $6732 \leqslant 2v \leqslant 7876$,
Vicia faba $15\,373 \leqslant V \leqslant 16\,877$, $16\,206 \leqslant 2v \leqslant 18\,210$,
Tulipa kaufmanniana $15\,083 \leqslant V \leqslant 16\,579$, $13\,932 \leqslant 2v \leqslant 16\,208$
what permits the possibility of the identity V=2v, thus the doubling of volume of cells during interphase.

The volume of cells, and similarly the nuclear DNA content in both studied phases of the cell cycle, can be arranged as follows: Cucurbita pepo < Helianthus annuus < Vicia faba. The volume of cells in Tulipa kaufmanniana is somewhat smaller than in Vicia faba in spite of the fact that the DNA content in this species is higher.

The lapse of time between the first and the second peak of radio-active metaphases has been accepted as the duration of the cell cycle.

A relation between the duration of the cell cycle and nuclear DNA content exists in three species, namely: in *Helianthus annuus*, in *Vicia faba* and in *Tulipa kaufmanniana*. The cell cycle in *Cucurbita pepo* is almost twice as long as that in *Helianthus annuus* though the nuclear DNA content in this species is almost 5 times lower (Table 1).

A very simplified model will be taken into account in the further considerations, namely, the increase in volume of an initial cell and its descendants. One of the purposes of the following considerations it to find out the coefficient of the dynamics of increase in volume of the analyzed population of cells.
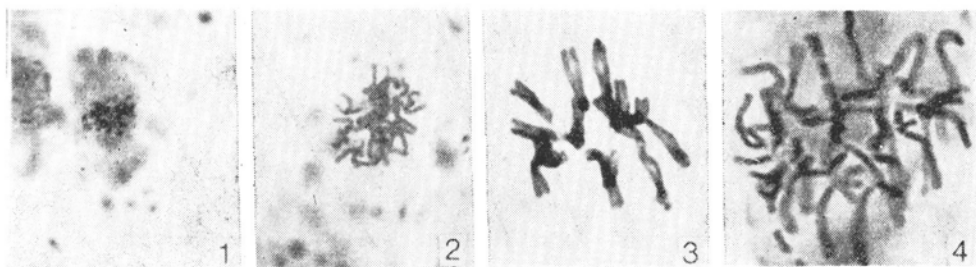

Fig. 1—4. Metaphase plates in root meristem: Fig. 1 — *Cucurbita pepo*; Fig. 2 — *Helianthus annuus*; Fig. 3 — *Vicia faba*; Fig. 4 — *Tulipa kaufmanniana*; 1000 ×

Let $V = V(t)$ denote the function of volume of an observed population, i.e. the function which subordinates the volume of the population at a moment t to the time t with the assumption that at an initial point of time $t = t_o$ the volume of the population was $V(t_o) = V_o$.

At t_o the hypothetical population of cells is composed of one cell just entering interphase (after cytokinesis). This cell has a volume $V_o = V(t_o)$. After a time T, which is equal to the duration of one division cycle, the population will be composed of two cells. Assuming that the volume of daughter cells are equal to each other and identical with that of the initial cell, the total volume of the population will be doubled. After the next period T it will be doubled again and so on. It may be assumed then that after n periods, each of length T, the total volume will be as follows:

$$V(t_o + nT) = 2^n V(t_o) = 2^n V_o.$$

In the model under discussion the parameter n is a variable determining the value of the volume function at the instants t_o, $t_o + T$, $t_o + 2T$... Replacing n in $V(t_o + nT)$ by the continuous variable t as follows:

(1)
$$V(t_o + t) = 2^{\frac{t}{T}} \cdot V(t_o)$$

As it appears from the analysis of the function $V(t_o+t)=2\frac{t}{T}V(t_o)=2\frac{t}{T}V_o$ (Fig. 5), the fastest increase in volume of meristematic cells occurs in *Vicia faba* (Fig. 5, *V.f.*).

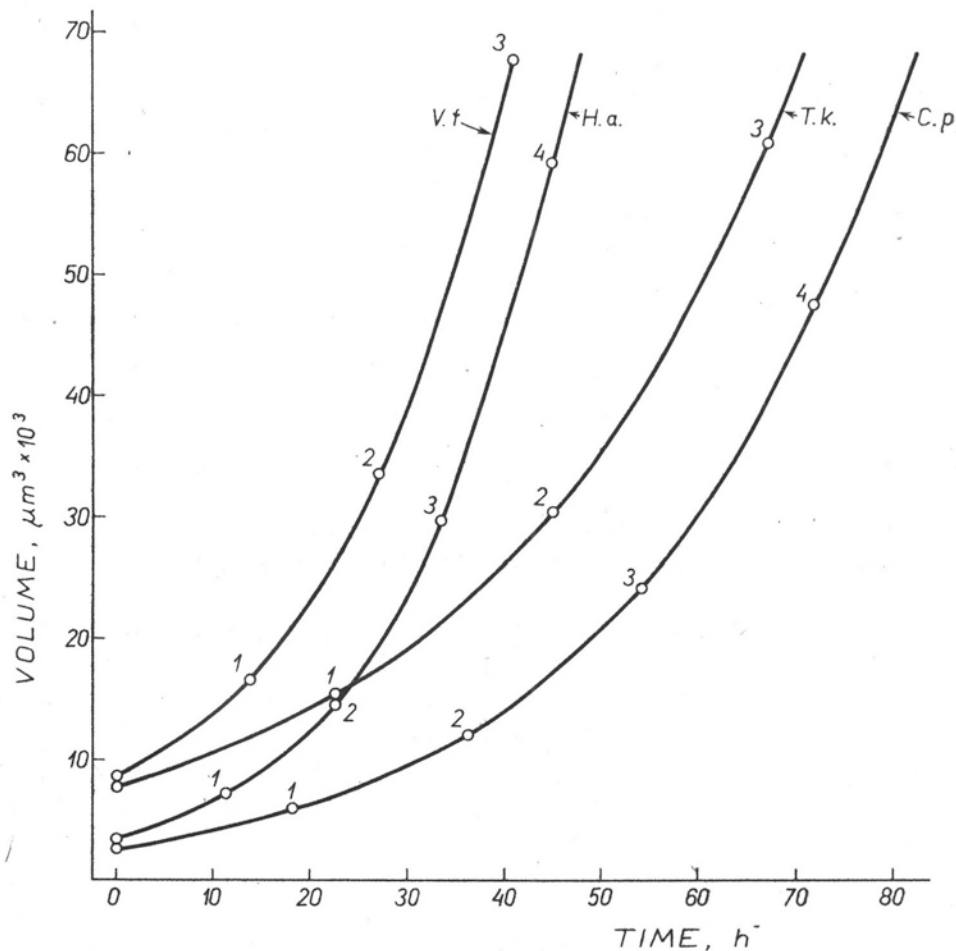Fig. 5. Curve of the function $V(t_o + t) = 2\frac{t}{T} V(t_o) = 2\frac{t}{T} V_o$.

V_o — volume of cells at the start of interphase at the time t_o; t — moment of time to which the volume V is subordinated; *C.p.* — *Cucurbita pepo*, *H.a.* — *Helianthus annuus*, *V.f.* — *Vicia faba*, *T.k.* — *Tulipa kaufmanniana*

The data obtained indicate that in species with very low (*Cucurbita pepo*) and very high (*Tulipa kaufmanniana*) nuclear DNA content, the rate of increase in volume of meristematic cells is slow. In both cases the cell cycle is long and, in *Cucurbita pepo*, the increase in volume of

cells during interphase is very slow as the initial value (volume of telophase cells) is very low also. Among the four species studied, the fastest increment in volume of meristematic cells occurs in *Vicia faba*.

DISCUSSION

In our experiments the duration of the cell cycle at 23°C has been found to be lower or comprised within the time limits reported for the root meristems of the species studied (*Helianthus annuus* — V a n't H o f and S p a r r o w, 1963; V a n't H o f, 1967; B u r h o l t and V a n't H o f, 1971; *Vicia faba* — V a n't H o f and S p a r r o w, 1963; M u r i n, 1966; V a n't H o f, 1967; *Tulipa kaufmanniana* — V a n't H o f and S p a r r o w, 1963). The duration of the cell cycle in *Cucurbita pepo* has not been studied till now; the time found in our experiments is similar to that observed in the stem meristem of another species of *Cucurbitaceae* — *Cucumis melo* cv. Sugar Baby (21.4 h, L i n and L o y, 1976). It is known that in many species the response of the cell cycle duration to the temperature is similar: the cell cycle duration reaches an almost constant value at 20-25°C (ref. O l s z e w s k a, 1977).

The long cell cycle in *Cucurbita pepo*, a species with a low nuclear DNA content, shows that according to suggestions put forward by some authors (B e n e t t, 1972; E s s a d, 1973; N a g l, 1974; N a g l and E h r e n d o r f e r, 1974) the original V a n't H o f's hypothesis (V a n't H o f and S p a r r o w, 1963; V a n't H o f, 1965; V a n't H o f, 1967) concerning the relation between DNA content and duration of the cell cycle should be modified. Many factors are known at present to influence the duration of the cell cycle leading to results different from those expected on the basis of V a n't H o f's hypothesis. Among them are: the rate of DNA replication (V a n't H o f, 1965), the position of cells in the meristem (B a r l o w and M a c d o n a l d, 1973), the content of the condensed chromatin (N a g l, 1974; O l s z e w s k a, 1978) and the life form; the cell cycle in the annual plants is shorter than in the perennial plants with a similar nuclear DNA content (B e n e t t, 1972; N a g l and E h r e n d o r f e r, 1974).

The volume of cells in a meristem is correlated in herbaceous plants with the nuclear DNA content (A l l a n and E n d r i z z i, 1973; P r i c e and coll., 1973). In the species studied in our experiments this correlation appeared in both phases of the cell cycle with the exception of *Tulipa kaufmanniana*. An analogical deviation from this regularity has been discovered in some other species of the herbaceous plants (P r i c e and coll., 1973). The length of cells in the stem parenchyma is greater in the annual than in the perennial plants with a similar nuclear DNA

content (N a g l and E h r e n d o r f e r, 1974). This fact might account for the lower than expected volume of cells in *Tulipa kaufmanniana* — the only perennial species studied in our experiments.

The above results as well as some literature data show that the volume of meristematic cells and their growth rate during interphase may be controlled by genetic factors other than the nuclear DNA content. Such suggestions have been put forward on the basis of some studies on yeasts (N u r s e, 1975) and on the higher plants (B e n e t t, 1972). In our opinion, intensity of transcription and translation might play, among other factors, an important role. The relation between the intensity of transcription and translation and the growth of cells with different nuclear DNA content during interphase will be the object of our further investigations.

REFERENCES

A l l a n E. G., and J. E. E n d r i z z i, 1973. Feulgen cytophotometry of DNA content and a determination of the relationship of DNA content to nuclear, cell and chromosome sizes in the genus *Gossypium*. Genetics 74: 69.

B a r l o w P. and P. D. M. M a c d o n a l d, 1973. An analysis of the mitotic cell cycle in the root meristem of *Zea Mays*. Proc. Roy. Soc. B 183: 385-398.

B e n e t t M. D., 1972. Nuclear DNA content and minimum generation time in herbaceous plants. Proc. Roy. Soc. B 181: 109-135.

B u r h o l d t D. R. and J. V a n't H o f, 1971. Quantitative thermal-induced changes in growth and cell population kinetics of *Helianthus* roots. Amer. J. Bot. 59: 386-393.

E s s a d S., 1973. Variations du cycle mitotique et des teneurs en ADN chez *Vicia sativa* L. Mise en évidence de liaisons entre les durées du cycle mitotique dans différentes groupes botaniques. Caryologia 26: 357-374.

F a n t e s P. A., 1977. Control of cell size and cycle time in *Schizosaccharomyces pombe*. J. Cell Sci. 24: 51-67.

L a f o n t a i n e I. G., 1974. Ultrastructural organization of plant cell nuclei. [In:] The Cell Nucleus, ed. H. Busch, Vol. I, p. 149, Academic Press, New York and London.

L i n P. B. W. and J. B. L o y, 1976. Action of giberellic acid on cell proliferation in the subapical shoot meristem of watermelon seedlings. Amer. J. Bot. 63: 700-704

M u r i n A., 1966. The effect of temperature on the mitotic cycle and its parameter in root tips of *Vicia faba*. Naturwiss. 53: 312-313.

N a g l W., 1974. Role of heterochromatin in the control of cell cycle duration. Nature 249: 53-54.

N a g l W. and F. E h r e n d o r f e r, 1974. DNA content, heterochromatin, mitotic index and growth in perennial and annual *Anthemideae* (*Asteraceae*). Plant Syst. Evol. 123: 35-54.

N u r s e P., 1975. Genetic control of cell size at cell division in yeasts. Nature 256: 547-551.

O l s z e w s k a M. J., 1977. Mechanizmy regulacji cyklu komórkowego u roślin. Post. Biol. Kom. 4: 141-183.

O l s z e w s k a M. J., 1978. Chromatin condensation in late G_2 phase as a factor related to the earlier initiation of mitosis in successive generations of antheridial filaments of *Chara vulgaris*. Microsc. Acta 80: 115-126.

P r i c e H. J., A. H. S p a r r o w and A. F. N a u m a n, 1973. Correlations between nuclear volume, cell volume and DNA content in meristematic cells in herbaceous plants. Experientia 29: 1028-1029.

S z a r s k i H., 1976. Cell size and nuclear DNA content in vertebrates. Intern. Rev. Cytol. 44: 93-111.

V a n't H o f J., 1965. Relationship between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. Exptl. Cell. Res. 39: 48-58.

V a n't H o f J., 1967. Studies on the relationship between cell population and growth kinetics of root meristem. Exptl. Cell Res. 46: 335-347.

V a n't H o f J. and A. H. S p a r r o w, 1963. A relationship between DNA content, nuclear volume and minimum mitotic cycle time. Proc. Natl. Ac. Sci. 49: 897-902.

Authors' address:

Prof. Maria J. Olszewska,
Dr Kazimierz Marciniak, R. Osiecka
Institute of Physiology and Cytology,
University of Łódź
Banacha Str. 12/16; 90-237 Łódź, Poland

Dr Józef Białas,
Institute of Mathematics,
University of Łódź;
Banacha Str. 22, 90-238 Łódź, Poland

Zależność między zawartością DNA jądrowego a szybkością wzrostu komórek w interfazie u czterech gatunków Angiospermae

Streszczenie

Badano zależność między zawartością DNA a szybkością wzrostu komórek w interfazie w korze merystemu korzeniowego u czterech gatunków, u których zawartość DNA była znana z literatury: *Cucurbita pepo* (2,6 pg DNA), *Helianthus annuus* (12,0 pg DNA), *Vicia faba* (38,0 pg DNA) i *Tulipa kaufmanniana* (93,7 pg DNA). Na początku cyklu komórkowego objętość komórek jest najmniejsza u *Cucurbita pepo* (3341 μm³), nieco większa u *Helianthus annuus* (3652 μm³), największa u *Vicia faba* (8602 μm³); objętość komórek u *Tulipa kaufmanniana* jest nieco mniejsza niż u *Vicia faba* (7535 μm³). Podczas interfazy zachodzi podwojenie objętości komórek. Czas trwania cyklu komórkowego, obliczony metodą znakowanych ³H tymidyną metafaz, wynosi 18 godz. u *Cucurbita pepo*, 11,2 godz. u *Helianthus annuus*, 13,5 godz. u *Vicia faba* i 22,4 godz. u *Tulipa kaufmanniana*. Najszybszy wzrost objętości komórek w interfazie ma miejsce u *Vicia faba*, nieco wolniejszy — u *Helianthus annuus*, a najwolniejszy — u *Tulipa kaufamanniana* i *Cucurbita pepo*.