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The Duration of DNA Synthetic (S) Period in *Zea mays*: A Genetic Control*

R.S. Verma
Division of Hematology and Cytogenetics, The Jewish Hospital and Medical Center, Brooklyn, New York and Department of Medicine, State University of New York Downstate Medical Center, Brooklyn, New York (USA)

M.S. Lin
Division of Medical Genetics, Children's Hospital of Los Angeles, Los Angeles, California (USA)

Summary. The nuclear cycle among several diverse genetic stocks of *Zea mays* root meristem cells was compared and it was found that there were no significant differences among the nuclear cycle durations and its component phases. The durations of various periods of their mitotic cycles were studied by autoradiography of cells pulse-labelled with tritiated thymidine (3H-TdR). The total nuclear cycle was 10 to 11.5 hours and mitosis was 0.81 to 1.34 hours at 25°C. The S period is the longest interval (50% of the total time) of the nuclear cycle; of the rest of the cycle, G_2 is longer than G_1 or mitosis among all stocks. The constancy of the nuclear cycle among several stocks was adduced as evidence for strict genetic control of the cycle. Furthermore, it is demonstrated the DNA synthesis period is not dependent upon the amount of DNA present.

Key words: Nuclear cycle – S period – *Zea mays* – Autoradiography

Introduction

The literature provides conflicting views regarding a correlation between DNA synthesis period (S) in both plants and animal tissues: nuclei having more DNA require more time for synthesis than those with less DNA (Prasad and Godward 1965; Van't Hof 1965, 1966; Defendi and Manson 1963; Goldfeder 1965). On the other hand, it has been shown in some organisms that the duration of S period is independent of the nuclear DNA content (Cameron and Stone 1964; Graham 1966; Oehlert et al. 1962; Troy and Wimber 1968). This evidence has been presented from a number of 'unrelated' species. In the present study, we report on the duration of the nuclear cycle with special reference to S period as a function of genotype and polyploidy employing the same inbred line of *Zea mays* at different 'auto'-polyploidy levels. Initially, consideration was given to the view that the nuclear cycle was under specific gentic control. To test this hypothesis, several wide markedly different agronomic and genetic stocks of *Zea mays* were chosen to study the duration of the nuclear cycle.

The investigation reported here was designed to test these two hypotheses indirectly by comparing the nuclear cycle and S period in several inbred lines as well as in autotriploid and autotetraploid inbred lines.

Materials and Methods

We obtained seeds of diploid and autotetraploid stocks of inbred W23 from the Maize Genetics Coop., Urbana, Illinois (courtesy of Dr. R.J. Lambert). Autotriploid seeds were produced after crossing diploid (W23) and autotetraploid (W23) stock. Seneca 60 was provided by Robson Seed Co., Hall, New York, while chromosome 9 tester and KYS were obtained from the Maize Cytogenetics Laboratory, The University of Western Ontario, London (Canada).

Several thousand kernels were germinated at 25°C for 20-25 hours in controlled light. We chose 25°C for the study inasmuch as our previous work indicated that 25°C provided for good resolution of the cycle, i.e., it was neither telescoped nor elongated (Verma 1970). Likewise, experimental error appeared to be minimal at 25°C. The experimental procedures for the pulse labelling and autoradiography have been reported earlier (Verma 1970, 1972; Verma et al. 1977; Verma and Lin 1978). Briefly, following exposure to ^{3}H-TdR (1μCi/ml; specific activity 6.7 Ci/mM) for 30 min., the intact roots were washed thoroughly in distilled water and returned to the germination chamber for further growth. The roots were collected at random from the germination chamber and fixed in freshly made glacial acetic acid: alcohol (1:3) at two hour intervals during the incubation period.

Feulgen stained preparations were made following our standard laboratory procedures (Chen 1969). Slides were coated with Ko-

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dak NTB_2 emulsion, exposed for 14 days at 4°C, and subsequently developed and prepared as permanent autoradiographs. For scoring and data collection, the identifications were covered by a paper label and the slides were recorded by a second party to yield a blind code unknown to the investigator. Three to four slides were scored at each treatment fixation. At least 1000 nuclei were counted from each slide (one root tip per slide). In autotriploid and autotetraploid stocks chromosome counts were also examined. Estimation of the durations of the nuclear cycle and its component phases was made by proportional method (Sparvoli et al. 1966; Wimber 1966). Inasmuch as we are dealing with percentage data, we used a Probit Regression Analysis (PRA) to obtain weighted mean values and standard deviations appropriate to these means (Finney 1962).

Results

We included in our protocol an analysis of the nuclear cycle of several diverse genetic stocks in an attempt to ascertain evidence for genetic control of the cycle. The percent labelled mitotic figure curves from Seneca 60, W23-diploid, a chromosome 9 tester stock and KYS are shown in Figure 1. Each curve contains two peaks, indicating that at least one nuclear cycle has been studied in each stock. No pronounced differences existed among stocks with regard to the appearance of the 'first' and 'second' peak. The various parameters of the nuclear cycle calculated from this figure are presented in Table 1. A total of 98,516 nuclei were scored in this study (Table 2). Standard deviations of the nuclear cycle estimates are presented in Table 3. Harvey's (1970) method was employed for testing the significance of means. There was no significance indicating that the nuclear cycles do not differ among stocks.

To test the contradictory findings regarding amount of DNA and the duration of S period, the nuclear cycle should be studied in autopolyploids. The percent labelled prophase curves from W23-diploid, W23-autotriploid, and W23-autotetraploid stocks were presented in Figure 2. In Figure 2d, the percent labelled prophase curves are superimposed. Each curve appeared together at each level of ploidy tested. The results presented in Table 4 indicate that the duration of the nuclear cycle and in particular, estimates of S period in diploid, autotriploid, and autotetraploid stocks are similar ($p < .05$). Standard deviations of the nuclear cycle and its component phases are presented in Table 5. There is no significant difference in mitotic indices at different ploidy levels (Table 6). In each fixation time, the chromosome counts were also made. The pictorial examples of somatic metaphase chromosomes are presented in Figure 3. A total of 100,000 nuclei were

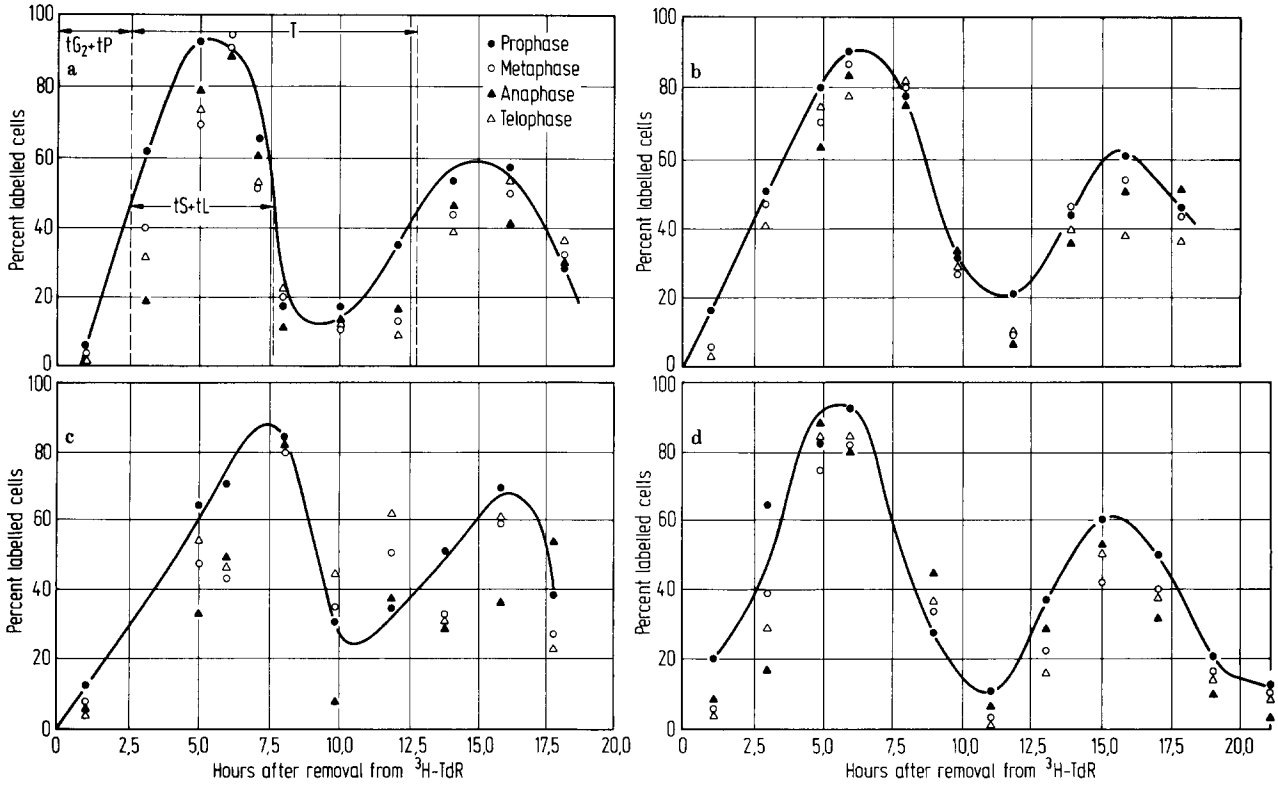

Fig. 1 a-d. The percent labelled prophase curves, following pulse labelling with ^{3}H-TdR for 30 min. at 25°C: **a** Seneca 60; **b** W23-diploid; **c** KYS; **d** Chromosome 9 tester

Table 1. Estimates of the duration (hours) of the nuclear cycle in primary root tips of Seneca 60, 9-tester, W23-2x, and KYS at 25° C

Phase		Stocks			
		Seneca 60	W23-2x	9-tester	KYS
Interphase					
	G_1	1.7	1.8	2.3	0.7
	S	5.0	5.5	4.5	5.5
	G_2	2.1	2.7	2.7	3.2
	Sub-total	8.8	10.0	9.5	9.4
Mitosis					
	Prophase	0.57	0.68	0.57	0.41
	Metaphase	0.23	0.23	0.24	0.18
	Anaphase	0.06	0.06	0.07	0.04
	Telophase	0.24	0.32	0.24	0.18
	Sub-total	1.10	1.29	1.12	0.81
Total		9.9	11.2	10.6	10.2

Table 2. Classification and frequency of nuclei scored from root tips of several stocks following pulse labelling at 25° C

Phase		Stocks			
		Seneca 60	W23-2x[a]	9-tester	KYS
Interphase					
	Labelled	30828	10409	19529	21740
Mitosis					
Prophase					
	Labelled	1639	679	1086	851
	Unlabelled	1818	647	1340	753
Metaphase					
	Labelled	547	210	355	308
	Unlabelled	865	236	681	372
Anaphase					
	Labelled	151	56	87	69
	Unlabelled	218	71	144	100
Telophase					
	Labelled	572	260	381	349
	Unlabelled	896	377	682	364
Total		37534	11791	24285	24906

[a] 2x = diploid

Table 3. Standard deviations (hours) of the nuclear cycle components estimates in several stocks of *Zea mays*

Phase	Stocks			
	Seneca 60	W23-2x	9-tester	KYS
G_1 + Mitosis	0.31	0.20	0.11	0.64
S	0.22	0.15	0.12	0.21
G_2 + ½ Prophase	0.09	0.18	0.04	0.08
Total nuclear cycle	0.40	0.26	0.35	0.68

Table 4. Estimates of the duration (hours) of the nuclear cycle in primary root tips of W23 (2x, 3x, and 4x) at 25° C

Phase		Ploidy		
		Diploid	Autotriploid	Autotetraploid
Interphase				
	G_1	1.8	2.6	2.3
	S	5.5	5.0	5.2
	G_2	2.7	2.8	2.7
	Sub-total	10.0	10.4	10.2
Mitosis				
	Prophase	0.68	0.56	0.68
	Metaphase	0.23	0.28	0.28
	Anaphase	0.06	0.06	0.07
	Telophase	0.32	0.21	0.31
	Sub-total	1.29	1.11	1.34
Total		11.3	11.5	11.5

Table 5. Standard deviations (hours) of the nuclear cycle components in the primary root tips of W23 (2x, 3x, and 4x) at 23° C

Phase	Ploidy		
	Diploid	Autotriploid	Autotetraploid
G_1 + Mitosis	0.20	0.14	0.24
S	0.15	0.14	0.05
G_2 + ½ Prophase	0.18	0.17	0.12
Total nuclear cycle	0.26	0.17	0.27

Table 6. Mean mitotic indices (with standard deviations) in W23 at 25° C

Hours after pulse	Ploidy		
	Diploid	Autotriploid	Autotetraploid
1	6.2 ± 0.53	4.7 ± 0.46	5.4 ± 0.40
3	7.5 ± 0.55	5.2 ± 0.46	8.8 ± 0.56
5	9.6 ± 0.48	5.9 ± 0.38	5.2 ± 0.38
6	8.7 ± 0.59	8.6 ± 0.41	8.5 ± 0.42
8	8.4 ± 0.56	6.4 ± 0.45	8.0 ± 0.47
10	7.4 ± 0.42	7.1 ± 0.57	9.6 ± 0.50
12	8.0 ± 0.39	6.9 ± 0.37	7.3 ± 0.44
14	8.1 ± 0.41	9.5 ± 0.51	8.2 ± 0.43
16	7.4 ± 0.45	7.7 ± 0.46	7.6 ± 0.42
18	7.1 ± 0.46	6.0 ± 0.34	7.7 ± 0.41
Mean	7.89 ± 0.15	6.41 ± 0.13	7.92 ± 0.41

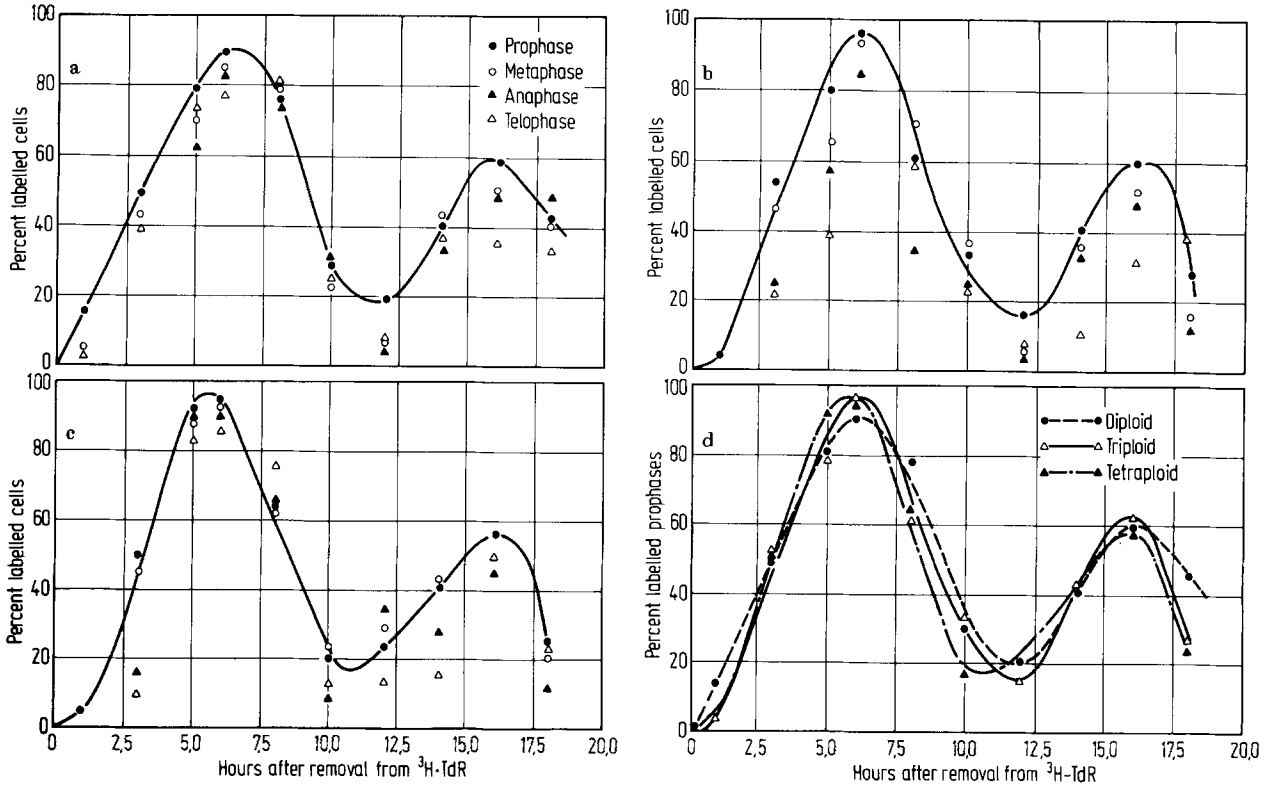

Fig. 2a-d. The percent labelled prophase curves observed in root tip cells of W23-diploid (a), W23-autotriploid (b), and W23-autotetraploid (c) following pulse labelling with ^{3}H-TdR for 30 min. at 25° C (d). All three curves are plotted together

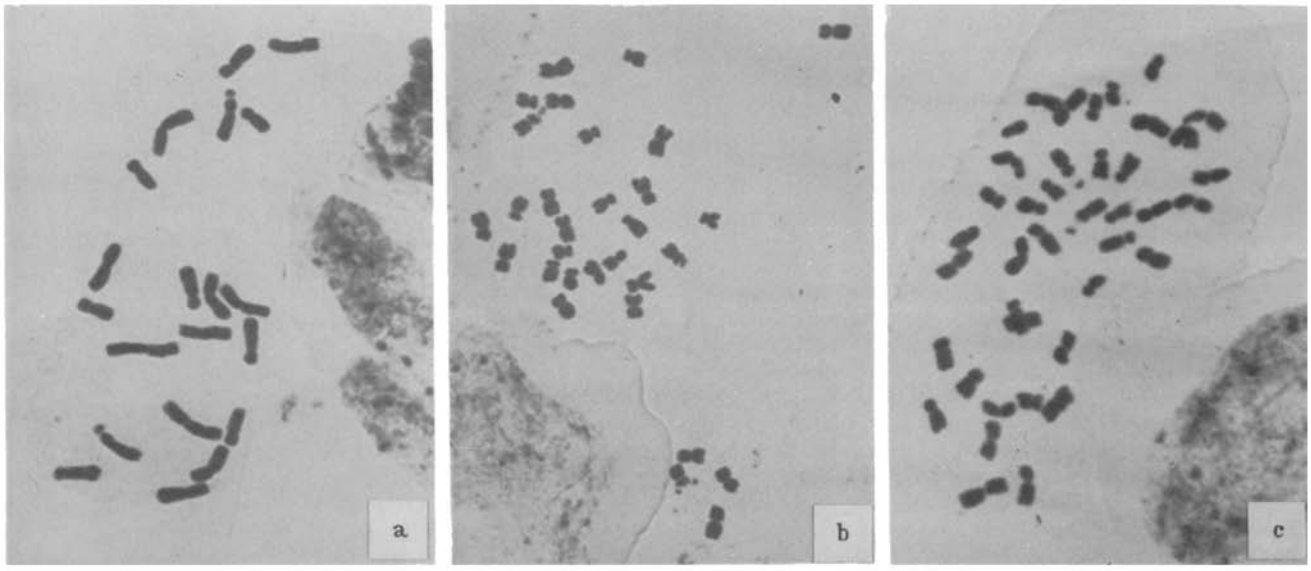

Fig. 3 a-c. Somatic metaphase chromosome of *Zea mays* L. root tips (Inbred W23): **a** early metaphase, diploid; **b** late metaphase, autotriploid; **c** midmetaphase, autotetraploid (× 1400)

Table 7. Classification and frequency of nuclei scored from root tips (*Zea mays* W23) following pulse labelling at 25° C

Phase		Ploidy level		
		2x	3x	4x
Interphase				
	Labelled	10409	9641	10639
	Unlabelled	19209	21747	22150
Mitosis				
Prophase				
	Labelled	679	563	709
	Unlabelled	647	518	736
Metaphase				
	Labelled	210	242	294
	Unlabelled	236	306	287
Anaphase				
	Labelled	56	48	71
	Unlabelled	71	84	75
Telophase				
	Labelled	260	153	274
	Unlabelled	377	236	377
Total		32154	33538	35612

scored (Table 7). At least 3000 nuclei were scored from each collection period. From our data it is clear that there is no increase in the duration of either DNA synthesis period (S) or the entire nuclear cycle among diploid, autotriploid and autotetraploid stocks of *Zea mays* root meristem cells.

Discussion

If the duration of the nuclear cycle was related to the growth characteristics of a stock, judicious choice of a few stocks should permit the identification of different nuclear cycles under identical controlled conditions. A survey of the nuclear cycle of several stocks was undertaken and concommitantly all possible F_1, F_2, BC, and BC_2 were also developed. Upon discovering that no differences in the nuclear cycle of the different stocks at 25°C could be described, the analysis of F_1 and F_2 stocks was deferred. Nevertheless, it remains to be shown whether or not differences can be described at other temperatures and under the influence of other environmental conditions as a prerequisite for heritability studies, although at the moment we must conclude that these differences do not exist and that further information would not be contributed by analysing the F_1 and subsequent generations.

There are conflicting views about the DNA content and the duration of S period. However, it is not appropriate to compare cytogenetically different, but related species with autopolyploids. In the former class, in addition to different amounts of DNA, one may have allele differences which compound the comparison. In the latter class, such genetic differences do not exist.

The asynchrony of chromosomal DNA replication in both plant and animal cells has been known for many years (Taylor 1960; Darlington and Haque 1964; Kusanagi 1966; and several others). Taylor (1963) postulated that a chromosome consisted of many replication units. Cairn (1966) reached a similar conclusion by studying the rate of DNA synthesis in chromosomes of cultured HeLa cells. In the polytene chromosomes of *Drosophila melanogaster*, the replication of DNA at homologous loci is closely synchronized (Plaut and Nash 1964). The observation that a haploid or autopolyploid stock has an S period similar to its diploid counterpart may be explained as follows:

Presumably the number of replication units (replicon) per genome is finite and constant. Thus, increased ploidy levels would not be expected to lengthen the time required for DNA synthesis unless multiple sets of replication units caused genome asynchrony. Gupta (1969) has proposed that the DNA synthesis period of the entire genome of a species is controlled by a regulatory mechanism, and that this mechanism comes under the control of chromosome replicons.

If homologous sites on chromosomes synthesize DNA at the same time, one would expect that with an increase in ploidy, the temporal pattern of DNA replication would remain the same. In theory, corresponding portions of homologous chromosomes should replicate simultaneously, and the pattern of replication throughout the complement would be retained at various euploidy levels. Thus increased ploidy levels would not be expected to lengthen the time required for DNA synthesis.

Apparently the morphological characteristics usually associated with increased ploidy (the gigas characteristics) are not accounted for by the time required for metabolic events. Rather, account can be taken in the autopolyploids by an increase in the quantity of metabolites.

In summary, it is hypothesized that the nuclear cycle is under specific genetic control and it nay be concluded from our observations, that the control is highly specific, as demonstrated by the lack of varietal differences, and further, that the control was not dependent upon the amount of DNA present.

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Dr. R.S. Verma
Div. Hematology and Cytogenetics
The Jewish Hospital and Medical Centre
555 Prospect Place
Brooklyn, N.Y 11238 (USA)