8. Effect of cycloheximide and chloramphenicol on the nuclear cycle in maize root meristems.

The present study reports the effect of cycloheximide (0.001%) and chloramphenicol (0.03%) on the nuclear cycle in maize root-tip meristems.

All treatments were carried out on attached 3 day old singlecross (Seneca 60) primary root-tips. Roots were exposed to ^{3}H-TdR for 30 minutes (1 μc/ml; specific activity 6.3/mM). The roots were then washed for 30 minutes and transferred to either cycloheximide (0.001%) or chloramphenicol (0.03%) for two hours. After incubation the roots were thoroughly washed again and returned to the germination chamber until sacrificed. Experiments were conducted at 25 or 30°C. Root-tips were collected at two hour intervals following treatment and fixed in alcohol-acetic acid, 3:1. Autoradiographs were prepared according to the schedule of Verma (MGCNL 43:186-190, 44:192-195). A minimum of four slides, one root-tip per slide, from each collection period, were coded and scored blindly.

The classification data are presented in Table 1. Employing the proportion method, the nuclear cycle duration and its components were estimated and are presented in Table 2. Treatment with either chloramphenicol (0.03%) or cycloheximide (0.001%) resulted in a delay in the appearance of labelled prophase, metaphase, anaphase, and telophase. In the histograms of labelled prophase from both the cycloheximide and chloramphenicol treatments, the peaks were delayed in comparison with the control. Reference to Table 2, 25°C, shows that tT, tS and tG_2 were affected by the cycloheximide and chloramphenicol treatments. At 30°C, all the stages were affected except tM. Our data confirm the high sensitivity of the tS and tG_2 phases to the treatment. Both chemicals appear to act mainly on the G_2 and S periods to lengthen the duration of the nuclear cycle. We also found that G_1 is affected at 30°C. Several workers have proposed that the primary effect of cycloheximide or chloramphenicol was the inhibition of protein synthesis, and further, that inhibition of DNA synthesis was an indirect effect. These hypotheses, if true, would lead to a delay in G_2 and S periods, such as recorded in our data.

Table 1

Frequency of nuclei scored from root tips following pulse
labelling (^{3}H-TdR, 30 minutes)

	25°C		30°C	
	Cycloh. (0.001%)	Chloram. (0.03%)	Cyclo. (0.001%)	Chloram. (0.03%)
Interphase:				
Labelled	13139	13317	14896	16533
Unlabelled	29200	30785	20985	23891
Mitosis:				
Prophase				
Labelled	639	550	413	627
Unlabelled	717	686	459	616
Metaphase				
Labelled	230	184	160	209
Unlabelled	389	291	264	245
Anaphase				
Labelled	67	56	49	64
Unlabelled	98	95	73	99
Telophase				
Labelled	226	258	135	240
Unlabelled	372	369	194	292
Total	45077	46591	37228	42816

Table 2

Estimated (from the data in Table 1) duration of the nuclear cycle with or without a 2 hr incubation with cycloheximide (0.001%) and chloramphenicol (0.03%) in _Zea_ _mays_ L. (Seneca 60) root tips

	Hours									
	25°C					30°C				
Phase	Control	Cyclo-heximide	Percent increase over control	Chlora-mphenicol	Percent increase over control	Control	Cyclo-heximide	Percent increase over control	Chlora-mphenicol	Percent increase over control
Interphase:										
G_1	1.96	1.77	–	1.89	–	0.59	1.70	188	2.04	245
S	5.00	7.00	40	6.50	30	3.50	5.00	43	6.00	167
G_2	1.84	4.80	161	5.40	194	2.10	4.70	123	3.52	68
sub-total	8.80	13.57	54	13.79	57	6.19	11.40	84	11.56	87
Mitosis:										
Prophase	0.56	0.71	27	0.60	7	0.40	0.30	25	0.49	48
Metaphase	0.23	0.32	39	0.23	–	0.17	0.15	–	0.18	6
Anaphase	0.06	0.09	50	0.07	17	0.04	0.04	–	0.06	50
Telophase	0.24	0.31	29	0.30	25	0.20	0.11	–	0.21	48
sub-total	1.09	1.43	31	1.20	10	0.81	0.60	–	0.94	16
Total	9.90	15.00	52	15.00	52	7.00	12.00	71	12.50	79

Table 3

Standard deviation estimates for the nuclear cycle in root tips of
Zea mays L. (Seneca 60) treated with cycloheximide
and chloramphenicol (hours)

Phase	25°C			30°C		
	Control	Cyclo-heximide	Chloram-phenicol	Control	Cyclo-heximide	Chloram-phenicol
G_1 + M	0.31	0.37	0.15	0.20	0.50	0.52
S	0.22	0.14	0.07	0.33	0.51	0.13
G_2 + P	0.10	0.30	0.23	0.21	0.36	0.23
Total N.C.	0.40	0.49	0.18	0.36	0.71	0.59

M = Prophase + Metaphase + Anaphase + Telophase

P = Prophase

N.C. = Nuclear Cycle

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9. Changes in mitotic index induced by cycloheximide and chloramphenicol.

In addition to the effect noted in the previous note, we were able
from the same experiments to record the data necessary to compute the
mitotic index (MI). For each experiment, cycloheximide (0.001%), chloram-
phenicol (0.03%) and control, the MI was determined. The values are
recorded in Table 1.

The decrease in the mitotic index to 1.5% was apparent between 1
and 4 hours (i.e., 3 to 6 hrs after pulse, accounting for the 2-hour
treatment period) after treatment with cycloheximide or chloramphenicol;
it remained at this level for up to 6 hours, at both temperatures and
with both chemicals. Thus, the mitotic indices are affected immediately
even during the incubation period. If roots were treated with chloram-
phenicol at 30°C, the mitotic indices reached the control level in seven
hours; in the case of cycloheximide (30°C), the control level was reached