10. Duration of mitotic cycle in evolved and primitive varieties of maize.

Techniques for the determination of the duration of the nuclear cycle and some of its component phases, such as the inter- and the mitotic phases, have become available in recent years with the development of the tritium labelling technique of chromosomes. Not only does this technique give an estimate of the length of interphase, it also makes it possible to determine the length of its sub-phases such as G_1 (the pre-DNA synthesis period of interphase), S (the stage during which the chromosomes replicate their DNA) and G_2 (the post-DNA synthetic stage of interphase). In recent years it has been found from observations of a wide variety of materials that the length of the S-phase is directly correlated with the DNA content of the cell. Thus, the S-phase is longer in those materials which have relatively more DNA (Van't Hof, 1965). In this way the length of the S phase provides a measurement of the DNA content of a cell, and if the different varieties or species show varying lengths of the S phase in their cells, it can be concluded that they show a corresponding variation in their DNA content. Two of the primitive varieties SP 2 (Himalayan) and Palomero Toluqueno (Mexican) and two of the improved varieties, KT 41 (Indian) and Mexican June, were analyzed for the length of their nuclear cycle.

Seeds of the four varieties were germinated and incubated at 26 $\pm$ 1°C on filter paper sheets kept moist with distilled water in petri dishes. The germinated kernels with roots at least 1 cm in length were placed at the same temperature for 0.5 hours in a solution of ^{3}H-thymidine (1 μc/ml; specific activity 15.0 c/mM). After the treatment the roots were transferred to a solution of cold thymidine to stop further incorporation of the label, washed thoroughly with distilled water and incubated further on moist filter paper at the same temperature. Roots were fixed subsequently at 2 hour intervals in 1:3 aceto-alcohol solution for 15 minutes and stored in 70% ethanol in a deep freeze until use.

Root-tips were hydrolyzed with 1 N HCl at 60°C for 8 minutes, stained in Feulgen for 2 hours and treated with a 2% pectinase solution for 2 hours. The meristematic portion of the secondary root tips was squashed on gelatinized slides in 0.5% aceto-orcein. Cover glasses were subsequently removed over dry ice and the slides transferred to 95% ethanol kept in a freezer. Autoradiographs were prepared from these slides using stripping film technique on Kodak A.R. 10 emulsions giving an exposure of 25 days in a refrigerator at 4 to 6°C temperature. Nuclei having grains sufficiently distinct from the background grains were taken as labelled. At least 3 roots were examined for each fixation time. The percentage of labelled mitotic cells was recorded at different durations after pulse-labelling of the root-tips and is shown graphically in Figure 1 for each of the four varieties studied. A total of 7237 mitotic cells were scored from the four varieties. The observations presented in Figure 1, together with the observations on mitotic index (Table 1), help to estimate the various parameters of the nuclear cycle (Matagne, 1968). The estimates of the G_1, S, G_2 and the mitotic phase of the different varieties are summarized in Table 2.

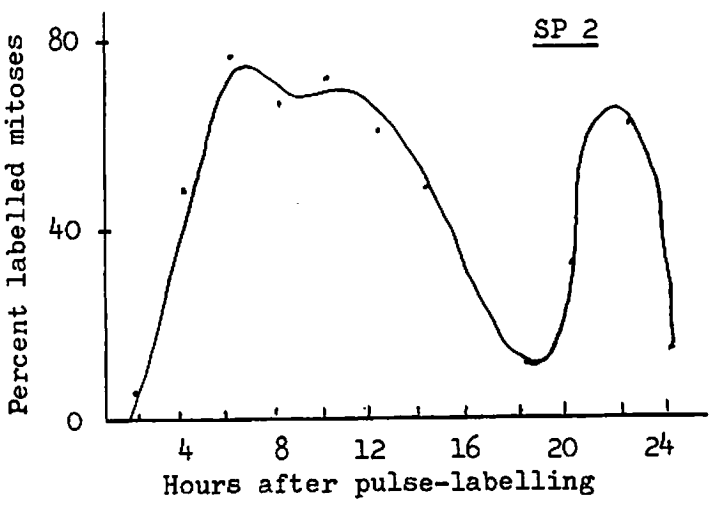
Table 1

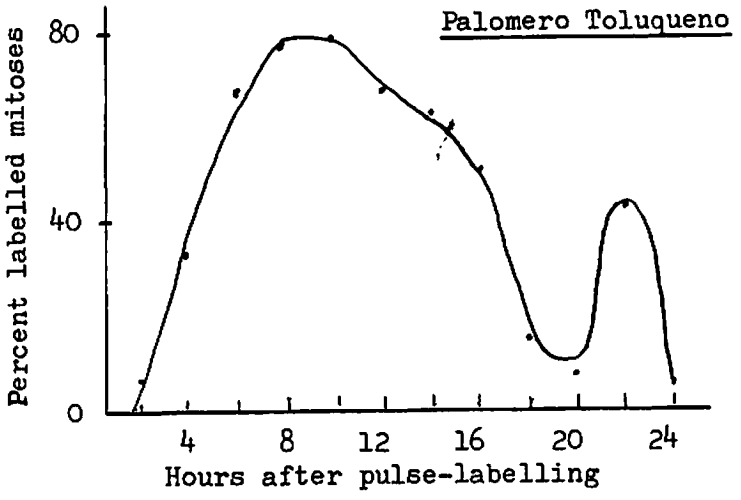
Observations on mitotic index of different varieties obtained from early hours of fixations

Variety	Total cells scored	Cells in mitosis	Mitotic index
SP 2	2112	255	12.07
Palomero Toluqueno	2369	324	13.68
KT 41	2138	297	13.89
Mexican June	2035	250	12.29

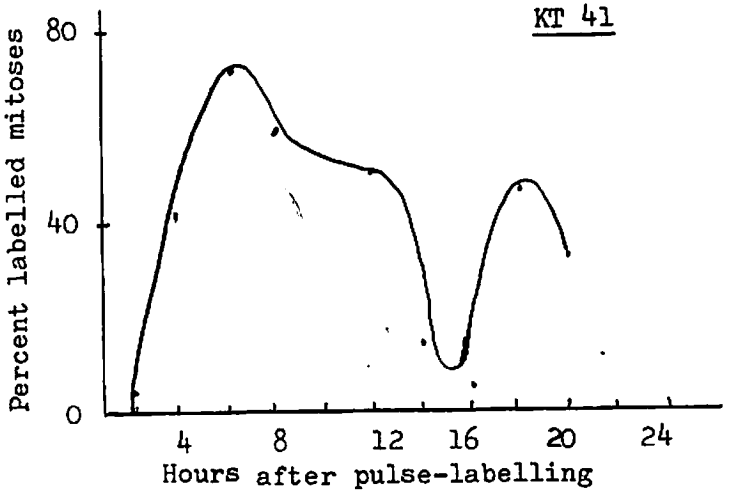
It will be seen that both the primitive varieties show a longer nuclear cycle compared to the improved varieties. It is further observed that the S phase, which represents the period during which DNA is synthesized in the chromosomes, is longer in the two primitive varieties

Primitive varieties





Evolved varieties



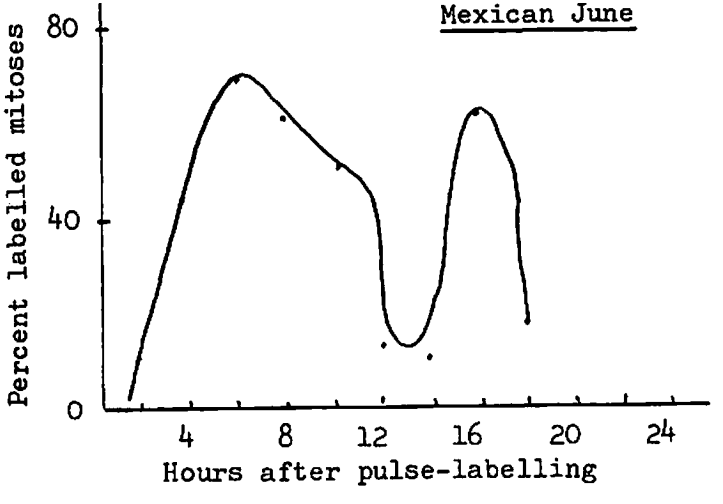


Fig. 1. Graphs showing curves of percent labelled mitosis plotted against duration after pulse labelling in primitive and evolved maize types.

Table 2

Duration of cell cycle in the root-tips of primitive and evolved varieties at 26 ± 1°C.

Phase	Calculated duration (Hours)			
	Primitive varieties		Evolved varieties	
	SP 2	Palomero Toluqueno	KT 41	Mexican June
Interphase				
G_1	0.65	0.01	-0.13	-0.76
S	9.82	10.93	8.35	6.80
G_2	2.87	3.03	2.74	3.09
Sub-total	13.34	13.97	10.96	9.13
Mitosis	2.63	3.17	2.53	1.83
Total	15.97	17.14	13.49	10.96

compared to the improved types. In fact, the longer nuclear cycle of the primitive types is largely accounted for by their longer S phase. These observations, thus, suggest that the two primitive varieties have a greater content of DNA in their cells compared to the two evolved varieties.

It may be added that during the last three years a number of reports have appeared on the duration of the nuclear cycle and its component phases in maize roots grown at different temperatures. It may be observed that the estimates presented on the "Seneca 60" hybrid by Douglas (MNL 42:175) obtained from roots kept at 28°C (which is close to the temperature used in the course of the present studies) show a close correspondence with the values obtained in the present studies with the two evolved varieties, particularly the "Mexican June." Thus, there is evidence that the two primitive varities used in the present studies show a longer nuclear cycle compared to the evolved maize, in general.

This finding is in agreement with the well known observation that the present day commercial varieties of maize show a faster rate

of growth and are more vigorous than the primitive varieties. This fact has been commented upon among others by Stonor and Anderson (1949). The finding is of value in that it helps to establish an important characteristic of the primitive strains. As has been pointed out earlier, it is well known that a longer nuclear cycle corresponds with a greater DNA content. It, therefore, follows that there is more DNA in the chromosomes of the primitive varieties of maize compared to the evolved types. It would appear that in our search for fast growing varieties, suited to agricultural needs, we have selected variation in which there has been some loss of the DNA. It is not very clear at this stage, what the function of the lost DNA was in the primitive varieties. It is possible that some of it did not have any particular genetic information. On the other hand, the possibility cannot be ruled out that some important attribute of the maize plant was lost as a result of the loss of this DNA. Thus, the very prolific nature of the maize plant in which we are now so much interested, and which is commonly found to be present in the primitive varieties, may be a function of this additional DNA.

References

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