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Loss of Nuclear DNA in Leaves of Rye*

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Summary. The nuclear DNA content of rye leaf cells was cytophotometrically determined. At the commencement of differentiation nuclei in rye leaves remain standing at G_1 phase. With further differentiation a remarkable diminution of nuclear DNA content occurs in diploid cells. The largest number of cells showing a loss of nuclear DNA content were found in the top of the leaf. The age of the leaf and the extent of diminution in nuclear DNA content are correlated.

Key words: Leaves of rye – Differentiation – DNA-loss – Cytophotometry

Introduction

In spite of multiple efforts, no one has been successful so far in cereals in obtaining completely fertile plants from isolated protoplasts (Thomas et al. 1979). Most researches derive the protoplasts from the mesophyll cells of young leaves.

In rye we found that such young leaves showed a diminution of nuclear DNA content. We explain the failure of experiments to produce complete plants from isolated protoplasts by the phenomenon of nuclear DNA loss. In the following communication we will show the first results of these DNA measurements.

Materials and Methods

Diploid rye (*Secale cereale* L.), variety 'Somro' was used. The plants were cultivated at 20 °C during October/November in the greenhouse. The DNA content of nuclei from rye leaves was cytophotometrically determined. The measurements were undertaken in three regions of the leaf: base, middle and top. We determined firstly the nuclear DNA content in the three regions of the same leaf and secondly the nuclear DNA content in the same region of leaves of different age. In the first group of investigations nuclei of the youngest leaves of three different young plants were measured. These leaves were, with regard to length, in a similar developmental stage. We used the first leaf (length of 14.0 cm) of the first plant, the third leaf (length of 21.0 cm) of the second plant and the fifth leaf (length of 15.5 cm) of the third plant. The second group of measurements concerned nuclei of the second, third, fourth and fifth leaf of plant older than the three just mentioned. These four leaves, of a different age, where harvested at the same time – when the sixth leaf had obtained a length of 8 cm. We used small pieces of the leaves which were depleted of the epidermis.

For determining the 2C level of the concerned rye variety we measured the telophase nuclei of root tips. Moreover, for controlling comparisons, we determined also the DNA content of *Vicia faba* root tip telophases. The material of the root tips was cultivated as described by Hesemann and Nenninger (1974) and Hesemann (1980). The root tips were not pretreated with colchicine. Fixation, Feulgen staining and production of slides were described by Hesemann (1980).

The DNA measurements were effected by a scanning photometer (UMSP I of Carl Zeiss, Oberkochen). The data obtained from this instrument are the same as that from an earlier publication (Hesemann 1980). Every slide contained on the one side nuclei of the rye leaves and on the other side nuclei of *Vicia faba* root tips. In this manner we could measure the DNA content of both nuclear types of the same staining series. We measured 30–50 nuclei from every region of the rye leaves, 30 telophases in meristematic rye root tip cells and 10–20 telophases in meristematic *Vicia faba* root tip cells. All the DNA values are given in arbitrary units (AU).

Results and Discussion

We found in all DNA measurements of the leaf cells that the nuclear DNA content did not exceed the 2C level ($\bar{x} = 51.56 \pm 2.34$). Evidently the cells of the leaves came to a standstill in the G_1 phase during the transition from the meristematic to the differentiated state.

The results of the nuclear DNA determinations in the three regions of one leaf are presented first. In the first leaf the nuclear DNA content in the base region

* Dedicated to Professor Dr. J. Straub in honour of his 70th birthday

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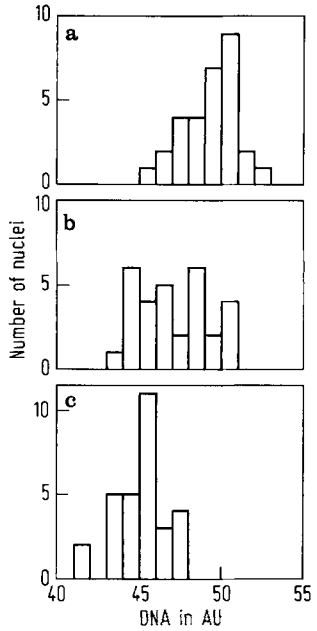
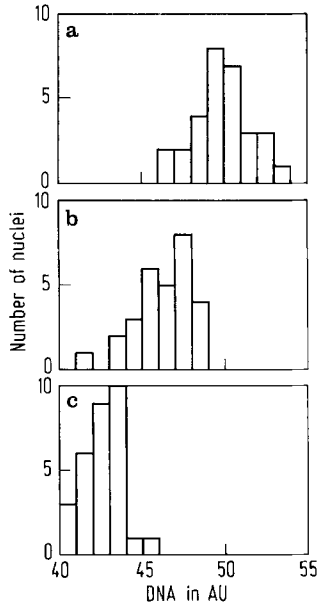


	S.c.	V.f.
$\bar{x}$	49.27	65.69
s	1.56	3.04
n	30	10

	S.c.	V.f.
$\bar{x}$	47.07	67.13
s	2.10	2.04
n	30	10

	S.c.	V.f.
$\bar{x}$	45.01	69.08
s	1.45	2.99
n	30	10

Fig. 1

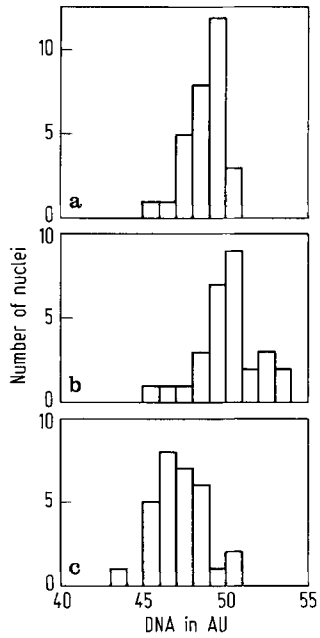


	S.c.	V.f.
$\bar{x}$	49.92	65.69
s	1.71	3.04
n	30	10

	S.c.	V.f.
$\bar{x}$	46.24	69.89
s	1.64	1.08
n	30	20

	S.c.	V.f.
$\bar{x}$	42.60	66.99
s	1.24	3.02
n	30	24

Fig. 2

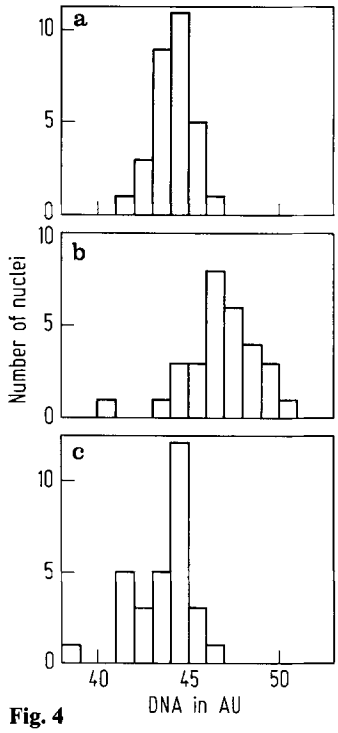


	S.c.	V.f.
$\bar{x}$	48.77	75.97
s	1.20	3.21
n	30	20

	S.c	V.f.
$\bar{x}$	50.13	81.85
s	1.84	1.87
n	30	20

	S.c.	V.f.
$\bar{x}$	47.15	77.70
s	1.58	3.89
n	30	20

Fig. 3



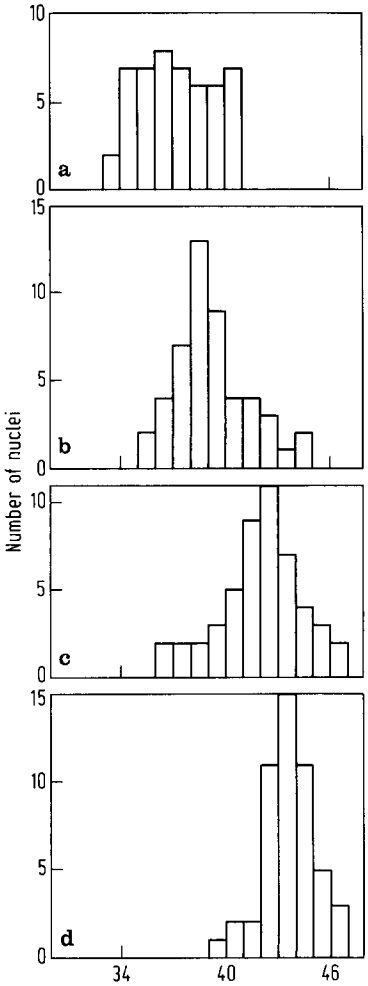
	S.c.	V.f.
$\bar{x}$	44.21	70.70
s	1.04	2.58
n	30	20

	S.c.	V.f.
$\bar{x}$	46.80	79.11
s	2.02	2.39
n	30	20

	S.c.	V.f.
$\bar{x}$	43.69	70.04
s	1.53	2.82
n	30	20

Fig. 4

Figs. 1–4. Frequency distribution and mean values of the nuclear DNA content in different regions of the leaf of *Secale cereale* (S. c.): a = base, b = middle, c = top of the leaf. **1a–c** 1. leaf, 1. staining series, 1. series of slides. **2a–c** 1. leaf, 1. staining series, 2. series of slides. **3a–c** 3. leaf, 2. staining series. **4a–c** 5. leaf, 3. staining series. For comparison the DNA mean values of root tip telophases of *Vicia faba* (V. f.). Abscissa: DNA in arbitrary units (AU), ordinate: number of nuclei



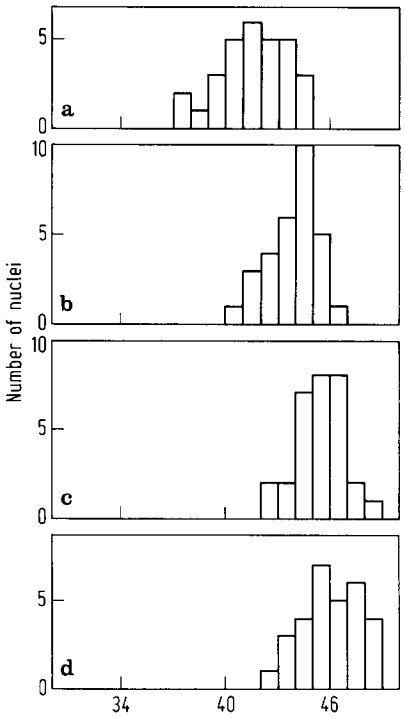
	S.c.	V.f.
$\bar{x}$	37.23	70.79
s	2.09	2.08
n	50	25

	S.c.	V.f.
$\bar{x}$	39.26	69.47
s	2.18	1.89
n	50	25

	S.c.	V.f.
$\bar{x}$	41.94	71.29
s	2.28	1.63
n	50	25

	S.c.	V.f.
$\bar{x}$	43.64	70.80
s	1.49	2.27
n	50	25

Fig. 5



	S.c.	V.f.
$\bar{x}$	41.52	67.90
s	1.97	1.85
n	30	25

	S.c.	V.f.
$\bar{x}$	43.84	68.53
s	1.45	1.97
n	30	25

	S.c.	V.f.
$\bar{x}$	45.53	69.39
s	1.37	1.81
n	30	25

	S.c.	V.f.
$\bar{x}$	46.06	68.78
s	1.62	1.58
n	30	25

Fig. 6

Figs. 5 and 6. Frequency distribution and mean values of the nuclear DNA content in 4 leaves of different age: a = second, b = third, c = forth, d = fifth leaf. **5a–d** base of the leaf, 4. staining series. **6a–d** top of the leaf, 5. staining series. (The rest of the data is the same as in Fig. 1–4)

corresponded with the 2C level (Fig. 1, 2). In the middle region of this leaf we found two different types of nuclear values. The first type corresponded to the 2C level. The DNA value of the second type was placed below the 2C level. The largest number of nuclei with reduced DNA content were contained in the top of the leaf. The measurements were extended to the third and fifth leaf. In these leaves also we found the largest number of cells with reduced DNA content in the top of the leaves (Fig. 3, 4). It is very important for the correct and critical examination of the frequency distribution of the nuclear DNA values in the different leaf regions that the reader bears in mind that the random sample of the measured nuclei has a very limited scale. Therefore, the frequency distribution gives only information about the borders of the highest and lowest analyzed DNA values; the frequency distribution cannot give information about the real frequency of the different classes of DNA values in the leaf regions.

Secondly we present the results of the nuclear measurements in one region of the second, third, fourth and fifth leaf (Fig. 5, 6). These four leaves had a different age and belonged to the same plant. The data of the second series confirmed the results of the first series: the nuclear DNA content decreased in the course of an early beginning differentiation in the leaf tissue of rye plants. In this second series we found a correlation between age of the leaf and extent of diminution in nuclear DNA content.

The presented results demonstrate firstly, that the differentiating cells in rye leaves remain standing at G_1 phase. The second result is more important: the course of the early beginning differentiation of rye leaves is not combined with the appearance of endocycles. In the process of differentiation a remarkable loss of nuclear DNA occurs already at a diploid level. The results of the nuclear DNA determinations in three regions of one leaf show that the maximal loss of

nuclear DNA is 14.66%. The results of the nuclear measurements in one region of four leaves of different ages demonstrate that the maximal loss of nuclear DNA in the base region is 14.69%, in the top region 9.86%. The causes which effect these diminutions of nuclear DNA content in rye leaf cells are still unknown. We favour the following interpretation: the diminished DNA values are caused by loss of chromosome pieces. A great portion could be heterochromatic material, if we especially take into account the results of Roupakias and Kaltsikes (1977) about the loss of telomeric rye heterochromatin in *Triticale*.

A few test measurements of the nuclear DNA content were already made in leaves of barley. The DNA content of the nuclei is also diminished below the 2C level in this crop. We speak for the hypothesis supplied by the preliminary investigations in barley, that these diminutions of the nuclear DNA content occur also in the other crops, which are cultivated in Central Europe. If this assumption is applicable to all crop species, then the diminution phenomene is very important in all cultivation experiments with isolated protoplasts of crop leaves.

Further investigations are being performed in rye and also in other crops as barley or wheat. In these investigations we include in addition to nuclear DNA measurements other cytochemically determinable characters of the leaf cells.

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