BIOLOGIA PLANTARUM (PRAHA)

10 (2) : 112—117, 1968

Endopolyploidy in the Roots of Rye, *Secale cereale* L.

J. DVOŘÁK

Research Institute of Cereal Crops, Kroměříž*

Received April 5, 1967

Abstract. 2,4-D was applied to the roots of diploid and tetraploid corn. After the application the mitotic division in the meristem of root tips was blocked; the mitotic division in differentiated cells of cortex and central cylinder, on the other hand, was provoked. In the cortex of diploid corn (variety České) predominantly tetraploid were found cells with 28 chromosomes and, to a lesser extent, octoploid and diploid ones with 56 and 14 chromosomes respectively. In the cortex of tetraploid corn (variety Bernburger Tetraroggen), most cells were octoploid with 56 chromosomes; the metaphase levels with 112 and 28 chromosomes, e.g. 16-ploid and tetraploid cells, were found less frequently. The relations between the numbers of cells with different polyploidy were similar in both the varieties. The first endoreduplication cycle was found to occur in the region where the cortex cells finish their elongation. In the central cylinder of the roots of diploid corn most cells were found to be diploid, in tetraploid corn most cells were tetraploid.

The differentiation of plant tissue is in many cases accompanied by cellular endopolyploidy. The endopolyploidy is a result of one or several consequent endomitotic cycles. In some tissues of higher plants the endopolyploidy occurs quite regularly, taking place in the cells of tapetum, endosperm (PUNNETT1953; KATO 1957; KAPOOR, TANDOM 1964), cortex cells (HUSKINS, STEINITZ 1948) as well as in endoderm, metaxylem and in other tissues. The occurrence of endopolyploidy in the tissues of higher plants has been reviewed for example by TSCHERMAK- WOESS (1956), LIPAYEVA (1962), ZAKHARYEVA (1962). HUSKINS and STEINITZ found that in the roots of *Rhoeo discolor* the polyploidy gradually increased with the increasing distance of the cells from the root tips. They found that the first endoreduplication cycle was situated 4—5 mm from the root tips. LEVAN and LOFTY (1948) described the polyploid metaphase plates in the differentiated cells of the *Allium cepa* L. roots.

The present work aims at determining the karyological changes during the course of differentiation of the rye roots.

* *Address:* Havlíčkova 2787, Kroměříž, Czechoslovakia.

Material and Methods

The number of chromosomes has been determined during the metaphases of mitotic division of differentiating cells brought about by the application of 2,4-dichlorophenoxyacetic acid (2,4-D). The material used was a diploid rye (variety České) and a tetraploid one (variety Bergenburger Tetraroggen). The seeds were germinated in Petri dishes at room temperature. After four days the roots were immersed for 3, 14, and 24 hours respectively in 2,4-D solution of the following concentrations: 10 p.p.m., 30 p.p.m., 50 p.p.m., 100 p.p.m. The sample treated 3 hours by 2,4-D was immersed in water for 14 hours. During the application of 2,4-D as well as during recovery period in water the plants were kept in a constant temperature chamber at 22° C. Pretreatment was carried out 2·5 hours in 0·002 M solution of 8-oxyquinoline at 18° C (TJIO, LEVAN 1950). Whole seedlings were fixed in alcohol-acetic acid (3 : 1) for 24 hours. The 15—20 mm long pieces of the roots were then cut and stained by the modified Feulgen method. The roots were hydrolyzed for 15 min. in 1N HCl at 62° C, washed in water and stained for 30 sec. by warm Schiff reagent. After the staining they were squashed and differentiated for 5—10 min. in 50% acetic acid. The strips of cortex set free from the squashed roots were separated by a needle on a slide and arranged side by side in order to improve the quality of the preparation.

Results

For the investigation of endopolyploidy in differentiated tissues we can use either indirect methods (determination of the number of heterochromatic regions in the nuclei or the cytophotometrical determination of the amount of DNA in the nuclei) or a direct determination of the number of chromosomes during the metaphase of the postendomitotic mitoses. The method is based on the stimulation of mitotic division in differentiated cells by growth-active substances, e.g. β-indolylacetic acid, α-naphtylacetic acid, 2,4-D and others. During the metaphase of these mitotic cycles we can determine the level of polyploidy. The results may be rendered unreliable by the fact that, apart from the normal mitosis, 2,4-D might bring about the process of endoreduplication. To eliminate this effect, we use four different intervals of the application of 2,4-D. A concentration of 30 p.p.m. was found most suitable and was applied in further experiments. The sample, treated for 3 hours by the 2,4-D and then kept in water for 14 hours, partly recovered its mitotic activity in the meristem of the root tip and exhibited the following characteristics: the cortex contained cells with different levels of polyploidy as shown in Fig. 1. and described further. The number of chromosomes was determined in 114 metaphase plates from the embryonic region (a total of 20 plants from each variety was investigated). None of these plates showed a higher level of polyploidy;

14 chromosomes were found in the 65 metaphase plates of the diploid rye and the number of chromosomes in 49 metaphase plates of the tetraploid rye did not exceed 28.

No differences were found in the frequency of cortex cells with different levels of polyploidy in the samples treated for 3 hours and those treated for 14 and 24 hours by 2,4-D. The first sample showed the following distribution of cortex cells with different endopolyploidy in the diploid rye: diploid cells 11%; tetraploid cells 72%; octoploid cells 17%. The roots of plants treated for 24 hours by 2,4-D showed a similar distribution: diploid cells 12%, tetraploid cells 75%; octoploid cells 13%.

According to the analysis of the metaphase plates of cortex cells of the variety České, one or two endoreduplication cycles occur in the nuclei of differentiating cells. Most cells exhibit a tetraploidy with 28 chromosomes. These cells appear in the zone of termination of the elongation growth of the differentiating cells. At a higher distance from the root tip the octoploid metaphase plates with 56 chromosomes were observed. These cells appeared more rarely than the tetraploid ones. Apart from the cells with pronounced polyploidy, there are also the diploid cells with 14 chromosomes in the cortex. The precise determination of the number of cells with particular numbers of chromosomes is very difficult because of a relatively low mitotic index of the cortex of most roots; 139 metaphase plates were tested for the number of chromosomes. Diploidy was found in 11·5% of the cells; tetraploidy — 72·7%, octoploidy — 15·8%. The cortex cells usually undergo one or two endoreduplication cycles during the course of differentiation, while the cells of the central cylinder retain their diploidy. There the tetraploid cells were observed only rarely.

A similar situation was found in the roots of tetraploid rye variety Bernburger Tetraroggen. In the embryonic region of the root tips of this variety the cells with 28 chromosomes were found. In the cortex cells occurred, in

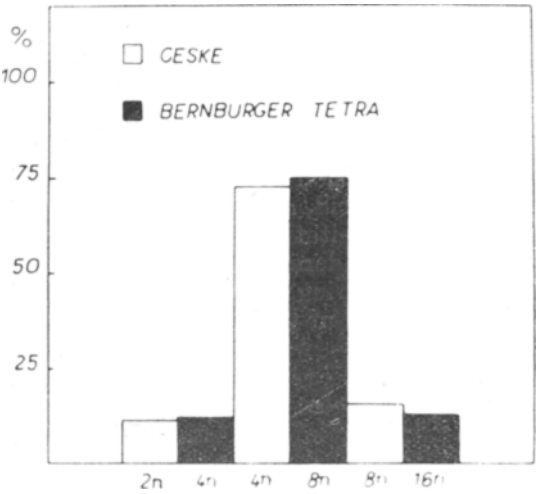

Fig 5. The distribution of endopolyploidy in the cortex of diploid rye (variety České) and tetraploid rye (variety Bernburger Tetraroggen).

addition to the metaphase plates with the original number of 28 chromosomes; most frequently the plates with 56 chromosomes and, to a lesser extent, those with 112 chromosomes. The exact number of chromosomes was determined in a total of 132 cells which gave the following relation between tetraploid, octoploid and 16-ploid cells: 12·1% · 75% : 12.9%. In Fig. 5 the comparison of the percentage of different degrees of cortex polyploidy in diploid and tetraploid rye is given. The relation between diploid, tetraploid and octoploid cells in the diploid variety České and that between tetraploid, octoploid and 16-ploid cells in the tetraploid variety Bernburger Tetraroggen are nearly the same.

In most cells subjected to one endoreduplication cycle the diplochromosomes containing two complete chromosomes attached by a centromere can be observed during the prophase. Bundles of four chromosomes can be found in cells subjected to two endoreduplication cycles. The separation of particular chromosomes takes place obviously in late prophase or early metaphase.

We have confirmed the findings of LEVAN and LOFTY (1949) on the stimulation of mitotic abnormalities by higher concentrations of growth-active substances. In our experiments, the irregularities of the spindle resulting in multipolar mitotic processes and sometimes in the formation of anaphase bridges were observed. These irregularities, however, cannot affect the results because of their very low frequency.

Discussion

As indicated by the above results, the cells of differentiated tissues of rye roots have different levels of endopolyploidy. The endopolyploidy of cortex cells is significantly higher than that of the cells of the central cylinder; in which the endoreduplication takes place only exceptionally. Many cells of the central cylinder have therefore an equal number of chromosomes as the cells of root tips, e.g. 14 in the diploid variety and 28 in tetraploid one.

Further problems concern the effects of growth-active substances on the endomitotic process. The question arises, whether the polyploidy observed in differentiated tissues of rye roots, is natural or evoked by the application of 2,4-D. From the different level of endopolyploidy in the cortex and in the central cylinder, the natural polyploidy can be postulated. The above difference may be also attributed to a different sensitivity of these tissues to the application of 2,4-D, which is not, however, very probable. From the mode of action of 2,4-D (no differences between the endopolyploidy in the samples treated for 3, 14, 24 hours by 2,4-D, the original number of chromosomes in meristem cells of the root tips) we can conclude that 2,4-D acts only as a stimulating agent of the mitotic division but does not affect the endoreduplication. On the basis of his experiments PARTANEN (1965) came to a similar conclusion. In his experiments, the onion roots were subjected for 8 hours to the action of 30 p.p.m. of 2,4-D and then transferred for 3 days to water containing tritiated thymidine. By means of the autoradiographic method the activity of DNA was then determined. The low activity showed that

the DNA synthesis took place before the application of 2,4-D. Similarly, according to D'AMATO et al. (1948), the growth-substances do not affect the endomitotic process.

We found a similar percentage of diploid, tetraploid and octoploid cells in the cortex of diploid rye with that of tetraploid, octoploid and 16-ploid cells in tetraploid rye. On the contrary, PARTANEN (1961) found the changes in the percentage of different polyploidy levels with the increasing total polyploidy in the gametophytes of *Osmunda cinnamomea*. By means of apospory the diploid gametophytes were obtained from a diploid sporophyte; this procedure was repeated several times. By means of a photometric determination of the quantity of DNA in the nuclei was found the increasing cellular endopolyploidy with the increase in the total polyploidy of the gametophyte.

The tissue cultures exhibit also certain relation in the frequency of endopolyploid cells. COOPER et al. (1964) established in two tobacco clones cultivated for 8 years, apart from prevailing diploid cells, the tetraploid and, exceptionally, the octoploid ones. One should realize, however, that the conditions in the culture are not identical with those in the organism. Moreover, the final state is often affected by the composition of the medium (STREET, HENSHAW 1963).

Acknowledgement

I want to express my gratitude to Dr. F. Kuhn for his valuable suggestions and remarks

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J. Dvořák, Výzkumný ústav obilnářský, Kroměříž: **Endopolyploidie v kořenech žita *Secale cereale* L.** — Biol. Plant. **10** : 112—117, 1968.

Na kořeny diploidního a tetraploidního žita byla aplikována 2,4 D. Po ovlivnění kořenů 2,4 D došlo k blokování mitotického dělení v meristému kořenové špičky a naopak vyprovokování mitotického dělení v diferenciovaných buňkách cortexu a centrálního cylindru. V cortexu byly u diploidního žita odrůdy České, zjištěny buňky převážně tetraploidní s 28 chromosomy, v menší míře oktoploidní s 56 chromosomy a diploidní se 14 chromosomy. V cortexu tetraploidního žita — odrůdy Bernburger Tetraroggen, byla většina buněk oktoploidních s 56 chromosomy, méně byly zastoupeny metafázové roviny se 112 a 28 chromosomy, tj. buňky 16ploidní a tetraploidní. Poměr mezi kategoriemi buněk s různou úrovní polyploidie byl u obou odrůd obdobný. Prvý cyklus endoreduplikace byl lokalisován do oblasti, kde buňky cortexu ukončují prodlužovací růst. V centrálním cylindru kořenů diploidního žita byly ve většině případů nalezeny buňky pouze diploidní a u tetraploidního žita buňky tetraploidní.

Я. Дворжак, Научно-исследовательский институт зернового хозяйства, Кромержиж: **Эндополиплоидия в корнях ржи *Secale cereale* L.** — Biol. Plant. **10** : 112—117, 1968.

Корни диплоидной и тетраплоидной ржи обрабатывали 2,4 Д. В результате воздействия прекратилось митотическое деление клеток меристемы верхушки корня и начался митоз в дифференцированных клетках корневой паренхимы и центрального цилиндра. В корневой паренхиме диплоидной ржи сорта Ческа обнаружены клетки в большинстве случаев тетраплоидные — с 28 хромосомами, и диплоидные — с 14 хромосомами. В корневой паренхиме тетраплоидной ржи сорта Бернбургер Тетрарогген большинство клеток было октоплоидными с 56 хромосомами, меньше было метафазных уровней с 112 и 28 хромосомами, т. е. клеток 16-ти-плоидных и тетраплоидных. Отношение между категориями клеток с различным уровнем плоидности было у обоих сортов аналогичным. Первый цикл эндоредупликации осуществлялся в области, где клетки корневой паренхимы заканчивали удлинение. В центральном цилиндре корней диплоидной ржи были в большинстве случаев найдены лишь диплоидные, а у тетраплоидной ржи — тетраплоидные клетки.

Fig. 1. Diploid cell in the cortex of diploid rye.

Fig. 2. Tetraploid cell in the cortex of diploid rye

Fig. 3. Diplochromosomes in the cortex of diploid rye.

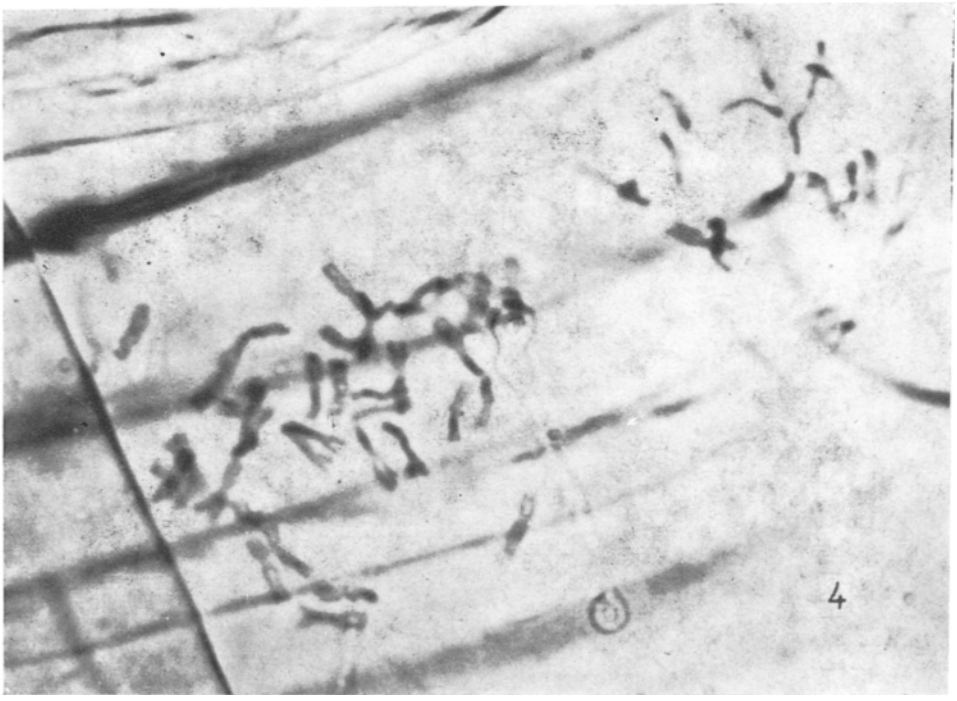

Fig. 4. Octoploid cortex cell in tetraploid rye.