

Effect of Allylthiocyanate on Nuclear Division in *Pisum sativum*

Dedicated to the 100 anniversary of the birth of Professor Němec

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Abstract. In the course of the study of the effect of allylthiocyanate in a 10^{-4} dilution on the mitosis of *Pisum sativum* root tips, some cells with typical meiotic configurations were found. After 92 h of treatment about 2% of the dividing cells were in the state resembling a so called somatic reduction.

Although the reduction division is regularly linked with gamete formation, there are cases of reduction in the number of chromosomes in somatic cells. This is not an exceptional phenomenon. Somatic reduction occurs regularly in mosquito larvae. The existence of somatic reduction was outlined at the beginning of this century, by NĚMEC (1904). PIRROT and BUSCALIONI (1898) were the first to observe this effect in laticifers and in cells, which later differentiated into vascular elements in *Dioscoraceae*. NĚMEC explained some anomalies in the cell division by polyploidy of the nuclei. He observed polycentric mitosis in the cells of galls, where highly polyploid nuclei arise by fusion of diploid ones, and in that of sugar-beet rootlets where the polyploidy was caused by the presence of the parasite *Heterodera radicum*.

A classical example of somatic reduction was *Solanum darwinianum* with 48 chromosomes in two peripheral layers. WINKLER (1916) supposed that it was a genuine graft-hybrid, which originated from *Lycopersicon esculentum* ($2n = 24$) and *Solanum nigrum* ($2n = 72$) and therefore it should have 96 chromosomes or 48 chromosomes, on the assumption of somatic reduction. Although this case is uncertain, there are number of other studies which indicate that the cytogenetically unbalanced nature of hybrid or polyploid plants supports the differentiation of tissues with reduced chromosome numbers. Reduction division does not usually have any relation to normal developmental processes and differentiation.

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Somatic pairing, which includes the close contact and coorientation of homologous chromosomes occurs regularly in *Diptera* (HETTZ and BAUER 1933). The same kind of pairing occurs also in some polyploid plants (BERGER 1941, WITKUS 1945). While somatic crossing over in *Drosophila* was described a long time ago (STERN 1936), references concerning this phenomenon in plants are very rare. The occurrence of chiasmata together with somatic reduction in *Allium cepa* root tips were described by HUSKINS (1948). There is a different situation in homothalic fungi, where not only somatic reduction, but also somatic recombination, caused by mitotic crossing over takes place (PONTECORVO 1954). Somatic reduction results in the equal distribution of chromosomes between two haploid nuclei, without any crossing over and recombination. On the other hand, somatic segregation, caused by crossing over is limited to diploid mycelia and this phenomenon is there quite frequent. Experimentally induced somatic reduction was first described by NĚMEC (1904). He treated the roots of *Vicia faba*, *Pisum sativum* and *Allium cepa* with a 0.75% chloralhydrate solution in distilled water during one hour, the most important observation being polyploidisation. But in addition he observed also an irregular distribution of chromosomes and the distribution of undivided chromosomes, which formed groups of different sizes. Cells with 2—4 nuclei of different size were sometimes formed (Fig. 1).

The question of somatic reduction was investigated again by HUSKINS (1948) who tried to induce somatic reduction by chemical treatment. The

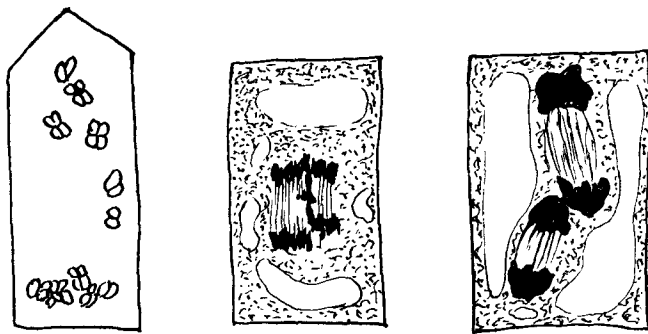


Fig. 1. Illustration reproduced from the paper of NĚMEC 1904: *Pisum sativum* root tip cells treated by 0.75% chloralhydrate solution during 1 h.

somatic reduction induced resembled ordinary stages of mitosis and meiosis. In prophase segregation there are intermediate types between normal prophase and clearly distinguished groups. There are also cases of somatic crossing over in bivalents with terminal and subterminal chiasmata. The phenomena described were induced by solutions of either RNA or its sodium salt in concentrations of 1%—2% over 6 or 12 hour period of treatment.

The same method was also used by KODANI (1948) who used a broader concentration scale 0.05%—8% with different pH (5.4, 6.7). He found that pH differences do not cause any considerable changes in chromosome morphology and cell division. Among the different effects of sodium ribose

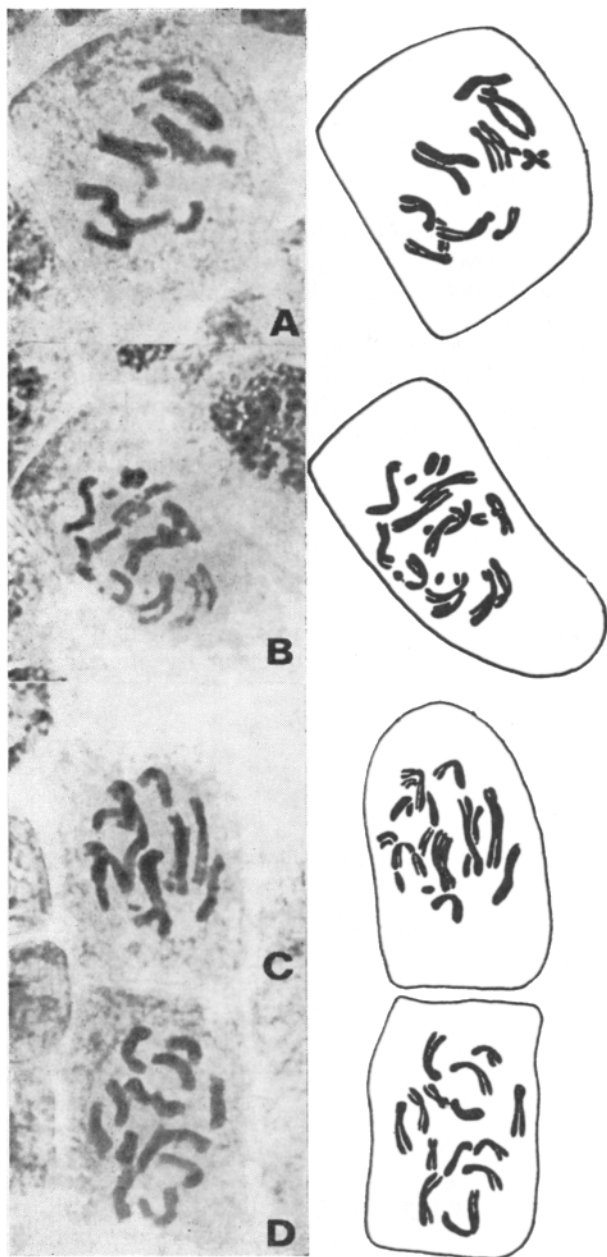


Fig. 2. *A, B, C* — pairing of mitotic chromosomes in *Pisum sativum* root tip cells. *D* — normal metaphase. Description in text.

Fig. 3: see at the end of the issue.

nucleate on chromosomes, besides chromosome pairing and irregular distribution, the inhibition of somatic reduplications and the formation of akinetic fragments was very interesting. KODANI tried to find out, which component of sodium ribose nucleate was effective. He treated the root tips with adenylic acid mononucleotide and observed the production of anaphase bridges, but no fragmentation and abnormal chromosome distribution. He also obtained the same effect with uracil and pyrimidine bases. Some configurations, which resemble meiosis were also found by BERGER and WITKUS (1948) who treated *Allium cepa* root tips with alpha-naphthalene acetic acid in concentrations of 10^{-6} — 10^{-7} . In some cells there were pairing associations of homologous chromosomes, but no chiasmata were formed. The authors explain the formation of pairing associations by reduplication without separation, or by the absence of dissociation.

ARRORA *et al.* (1969) tested the effect of mitomycin C on the root tips of *Vicia faba*. The work was concentrated on the induction of micronuclei. Chromosome aberrations, irregular chromosome distribution and multipolar mitoses were also observed.

Material and Methods

Pisum sativum seeds were allowed to germinate on Petri dishes, on filter paper soaked in water. When the roots were about 5 mm long, they were put into other Petri dishes with filter paper soaked in an allylthiocyanate solution at a concentration of 10^{-4} . The root tips were harvested after 2, 5, 7, 22, 26, 32, 46, 70, 92, 118 and 144 h. The treated root tips were fixed in a solution of alcohol-acetic acid (3 : 1) and stained with acetoorcein.

Observations

Most of the cell divisions in the meristeme of the effected pea root tips showed a typical mitotic character. After 72 h and 92 h treatment there were also some cells with anomalous chromosome distribution. Figs. 2A, B and C show chromosome pairing. In some pairing associations there was relation coiling, in others there were chiasma-like structures. Nonhomologous chromosomes were also sometimes paired (Fig. 2A). The chromosomes show longitudinal splitting into two sister chromatids, centromere is in the narrowed region and chromosomes in the anaphase-telophase show considerable coiling. Some chromosomes show clearly distinguished lighter zones, which correspond to gaps (Fig. 2A, B, C). Chromosome fragments were observed rather frequently; Fig. 2B shows submetacentric chromosomes with a dot satellite, which was not observed in the control karyotype. This dot-like structure is seen in anaphase-telophase in Fig. 3G. Other irregularities were also observed. The association of two pairs of homologous chromosomes and the irregular distribution of chromosomes is illustrated in Fig. 3E. The distribution of undivided chromosomes into two groups with 7 chromosomes in each resembles the first meiotic anaphasis and genuine somatic reduction (Fig. 3F) but such a regular distribution was very rare. The most common cases were partly divided groups like those in Fig. 3G in the stage anaphase-telophase. Fig. 3H shows a tetrapolar anaphase with a chromosome bridge. Clearly-distinguished groups of unequal size in tetrapolar telophase were seen in Fig. 3I and 3J. The latter figure shows another kind of segregation, which takes place during chromosome prophase. In the material studied there were also intermediate types, ranging from normal prophase to clearly-distinguished groups. Fig. 3J shows late prophase with a partial segregation of chromosomes into two groups.

Discussion

The phenomena described in our experiments do not fall strictly into the category of somatic reduction, although in some cases homologous chromosomes are clearly grouped. In mosquito, where somatic pairing is a common

feature, mitotic bivalents are formed through an association of homologous chromosomes in the early mitotic prophase, which shows clear points of contact. In contrast to this, somatic crossing over in mosquito was not detected (MUKHERJEE and REES 1970). On our slices we also observed some points of contact or a clear intimate pairing of chromosomes. The hypothesis of BERGER and WITKUS (1948) that chromosome pairing is caused by reduplication without separation is hardly acceptable here, as not only homologous chromosomes were paired.

Somatic reduction, as described by HUSKINS (1948) is meiotic-like somatic cell division. It includes synapsis and paired chromosomes lie on the metaphase plate. This author observed chiasmata in some somatic bivalents, but genetic effects have not yet been detected. Somatic reduction results in the formation of haploid nuclei. Many of the phenomena described in this paper have a similar character, but the end products are never haploid cells, because in tetrapolar segregation in anaphase there are clusters of different sizes, which certainly contain different number of chromosomes. Chromosome associations are different in different cells. In some of them, there are 5 out of 7 possible chromosome pairs, in others there is a single pair only. Cells, which originated by irregular divisions are capable of normal mitosis as is shown in Fig. 3E, where 17 chromosomes and three acentric fragments are present. However number of cells with aberrant chromosome numbers is small. Cells with haploid or lower chromosome numbers were never found.

The described phenomena merely simulate meiosis but they are not typical cases of reduction division. Nevertheless, there is some reduction of the original volume of chromosome material and, according to NĚMEC (1930) "polycentric division is a potential way of reduction of chromosome number".

This reduction property of allylisothiocyanate was observed in pea only. Chromosomal aberrations on a larger scale than in the described experiments, were found in onion. The chromosome break precedes the appearance of the observable fragment, occurring during DNA replication. Fragmentation can be caused by the partial inhibition of chromosome reduplication, as found by KODANI (1948) after treatment with sodium ribose nucleate and KIHLMAN (1966) after treatment with 5-fluorodeoxyuridine, deoxyadenosine, aminopterin *etc.* All these compounds possess the ability to inhibit deoxyribonucleotide synthesis, as a consequence of the interaction of some sites with molecules of different compounds. According to the gap formation it could be supposed that a partial inhibition of DNA synthesis took place and the mechanism of action may therefore be similar to that in other cases.

Conclusion

A 92 h treatment of *Pisum sativum* root tips with allylisothiocyanate in a 10^{-4} dilution induced, in about 2% of the dividing nuclei, certain structures, which resembled the somatic reduction, described by Huskins. The association of some chromosomes is akin to somatic pairing and their positions in some cases resembled chiasmata. Both homologous and sometimes also nonhomologous chromosomes were paired. Chromosomal aberrations and gaps were frequently observed. Mitosis sometimes resulted in cells with nuclei of different sizes.

References

- ARORA, O. P., SHAH, V. C., RAO, S. R. V.: Studies on micronuclei induced by mitomycin-C in the root cells of *Vicia faba*. — Exp. Cell Res. **56** : 443–448, 1969.
- BERGER, C. A.: Multiple chromosome complexes in animals and polysomaty in plants. — Cold Spring Harbour Symp. Quant. Biol. **9** : 19–21 (sec. SWANSON, C. P., 1957).
- BERGER, C. A., WITKUS, E. R.: Cytological effects of alpha-naphtalene acetic acid. — J. Hered. **39** : 117–120, 1948.
- DARLINGTON, C. D., McLEISH, J.: Action of maleic hydrazide on the cell. — Nature **167** : 407–408, 1951.
- HEITZ, E., BAUER, H.: Beweise für die Chromosomenstruktur der Kernschleifen in den Knäuelkernen von *Bibio hortulanus* L. (Cytologischen Untersuchungen an Dipteran I.). — Z. Zellforsch. **17** : 67–82, 1933.
- HUSKINS, C. L.: Segregation and reduction in somatic tissues. — J. Hered. **39** : 327–335, 1948.
- HUSKINS, C. L., STEINITZ, L. M.: The nucleus in differentiation and development. — J. Hered. **39** : 67–77, 1948.
- KIHLMAN, B. A.: Deoxyribonucleotide synthesis and chromosome breakage. — Chromosomes Today. **1** : 108–117, 1966.
- KODANI, M.: Sodium ribose nucleate and mitosis. — J. Hered. **39** : 327–335, 1948.
- MERZ, T.: Effect of mitomycin C on lateral root-tip chromosomes of *Vicia faba*. — Science **133** : 329–330, 1961.
- MUKHERJEE, A. B., REES, D. M.: Spermatogenesis in the mosquito. — Cytologia **35** : 213–219, 1970.
- NĚMEC, B.: Über die Einwirkung des Chloralhydrats auf die Kern- und Zelltheilung. — Jahrb. wiss. Bot. **39** : 645–730, 1904.
- PIROTTA, BUSCALIONI: Sulla presenza di elementi vascolari multinucleati nelle Dioscoreaceae. — Ann. R. Ist. bot. Roma **7**, 1898 (sec. NĚmec, B., 1904).
- PONTECORVO, G.: Mitotic recombination in the genetic systems of filamentous fungi. — Caryologia **6** (suppl): 192–200, 1954.
- STERN, C.: Somatic crossing over and segregation in *Drosophila melanogaster*. — Genet. **21** : 625–736, 1936.
- SWANSON, C. P.: Cytology and Cytogenetics. — Englewood Cliffs, N.J. Prentice-Hall, Inc. 1957.
- WINKLER, H.: Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen ihrer Keimzellen. — Z. Bot. **2** : 1–38, 1916.
- WITKUS, E. R.: Endomitotic tapetal cell divisions in *Spinacia*. — Amer. J. Bot. **32** : 326–330, 1945.

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ALLYLISOTHIOCYANATE AND NUCLEAR DIVISION

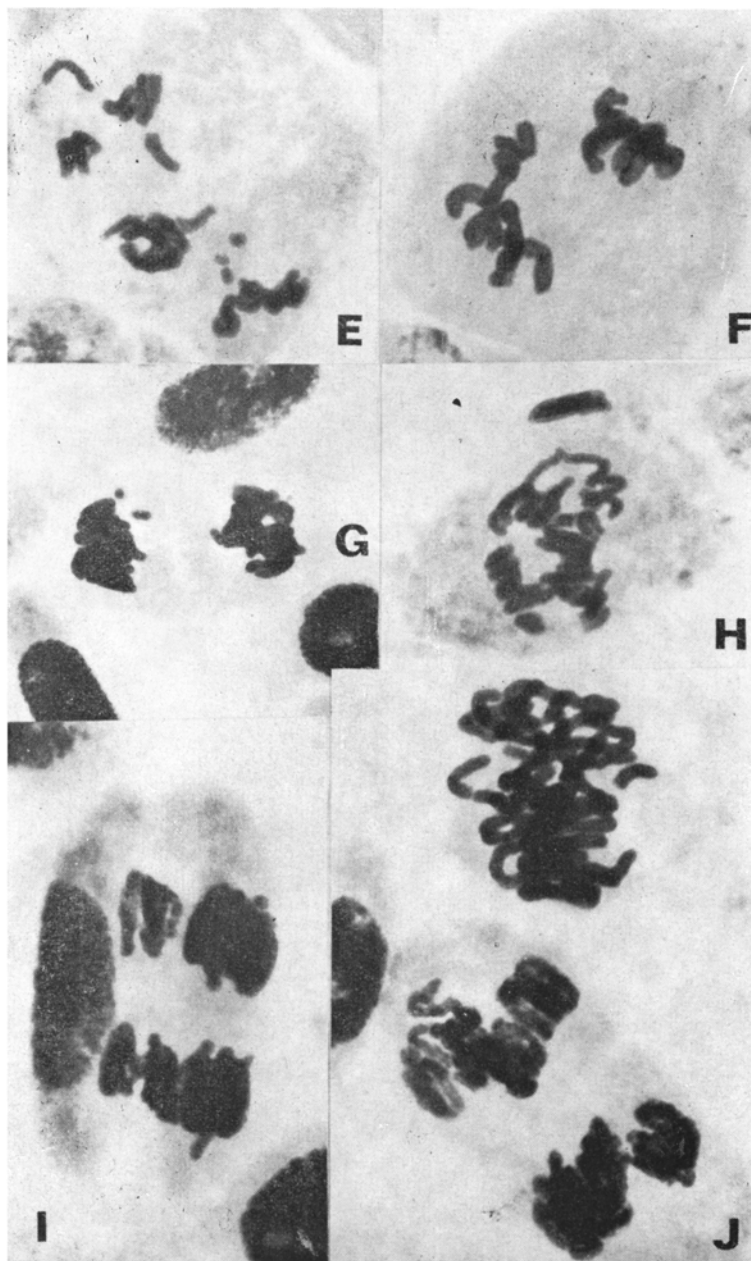


Fig. 3. *Pisum sativum* root tip cells, treated by allyl isothiocyanate. Squashes were stained by acetoorceine. Description in text.