Sensibility towards an Alkylating Agent of Seeds of Different Ploidies

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A number of facts suggest that the cytotoxic action of alkylating agents is due to cross-linking DNA alkylation (Lawley and Brookes, 1965). In a previous work (Simon, 1965) we suggested that these DNA alkylations produce inactivating mutations: the death of an η-ploid cell occurs if at least one vitally important operon (or perhaps cistron) type is inactivated in all the η-chromosomal sets. This hypothesis requires a lowering of the sensibility towards the alkylating agent as the ploidy increases.

There are only few and indirect data (for example Podgajetskaia et al., 1964; Tsukada et al., 1963) concerning this predicted sensibility-ploidy relation. In order to test this lethal mutation hypothesis, we studied the action of a bifunctional alkylating agent, Thio-TEPA, on diploid and tetraploid rye seeds, and on tetraploid and hexaploid wheat seeds. We chose plant seeds as the object of this study, since their ploidy is well-defined and testing of the alkylating agents action is relatively simple.

Materials and Method

Thio-TEPA (N, N', N" - triethylenethiophosphoramide), kindly supplied by Dr. G.Ciustea of the Oncological Institute of Bucharest, is one of the bifunctional alkylating agents used in cancer chemotherapy.

The following seeds, kindly supplied by Dr.C.Grünwald and Dr.C.Miclea, of the Institute of Agronomical Research - Bucharest, were used: diploid rye (noted as DR), Secale cereale, var. Petkus; tetraploid rye (noted as TR); durum tetraploid wheat (TW), Triticum durum, and common hexaploid wheat (HW), Triticum aestivum .

The following test method was used:

Samples of 100 seeds were put in large Petri dishes (20 cm diam.) on filter paper and moistened with aqueous thio-TEPA solutions of various concentrations (for the control samples, distilled water). The thickness of the liquid layer was approximately that of the filter paper; the volume of liquid used in each experiment was the same (10 ml.), irrespective of the thio-TEPA concentration used. The covered dishes were placed at room temperature and after four days the germinated seeds were counted. The length of the plantlet in the fourth day was also measured. The mortality was reported to that of the control: 100 % germination is equated to the number of seeds sprouted in the control samples.

Results and Discussions

The mortalities, relative to the control, for TW and HW, are shown in figure 1, those for DR and TR in figure 2. In figure 3, these mortalities are given in a series of experiments where the solution with which the rye seeds (DR and TR)

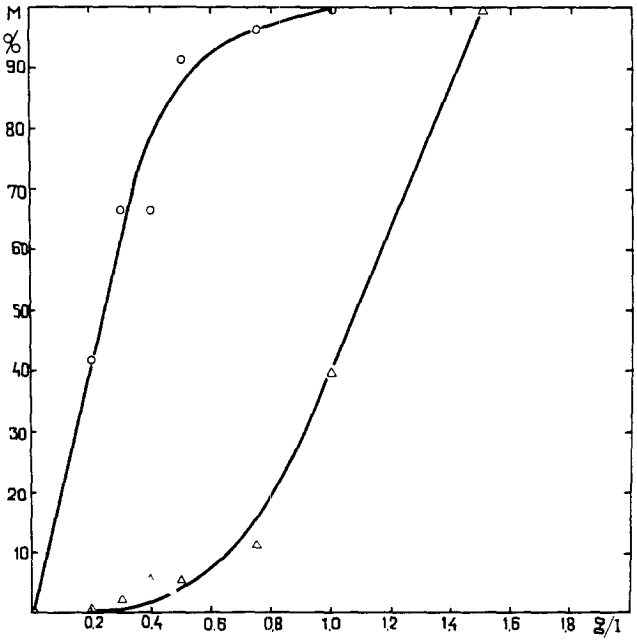

Figure 1. Relative mortality (100Xgerminated seeds in the control minus germinated seeds in treated samples/germinated seeds in the control) vs thio-TEPA concentrations for tetraploid and hexaploid wheat. —o—o— :TW ; —Δ—Δ—:HW

were treated was refreshed by washing twice a day, on the second and third days, with three times the initial volume of the same solution.

In all cases the seeds with higher ploidies (HW and TR) have sensible mortalities only for thio-TEPA concentrations higher than 0.4 g/l, while the sprouting of the seeds of low ploidy (DR and TW) is markedly inhibited for the lowest thio-TEPA concentration used (0.05 g/l.). Germination is inhibited for half of the seeds (sprouted in the control) by concentrations of about 0.25 g/l for TW, 1.1 g/l for HW, 0.5 g/l for

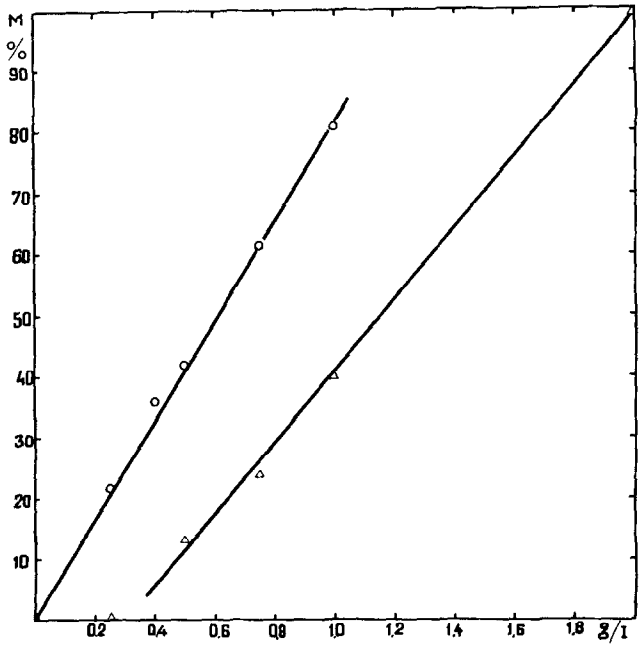

Figure 2. Relative mortality vs thio-TEPA concentrations for diploid and tetraploid rye. —o—o— :DR ; —Δ—Δ— :TR

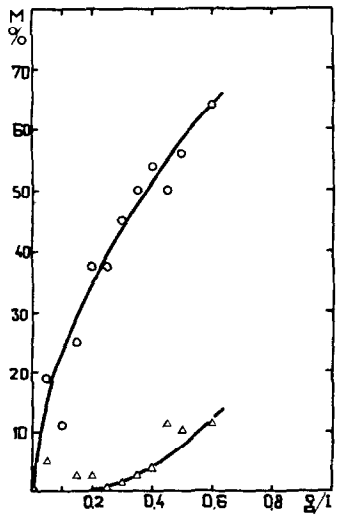

Figure 3. Relative mortality vs thio-TEPA concentrations for diploid and tetraploid rye; first treatment followed by washing (see the procedure in text). —o—o— :DR ; —Δ—Δ— :TR

DR, and 1.1 g/l for TR. Washing (figure 3) seems to raise mortality but only for DR. Besides inhibition of germination, there exists also another effect of the thio-TEPA treatment: from the lowest concentration used (0.05 g/l) the length of the plantlets is reduced to about half of that observed for the control; if the concentration is raised, the length is further reduced but less dramatically.

According to our lethal mutation hypothesis, the sensibility towards alkylating agents must be lowered as the ploidy increases, and from the ratio of concentrations by which LD_{50} is attained for cells which differ only in ploidy, the number of target types (operons of vital importance) could be computed (eq. (6) in the paper of Simon, 1965). The results of the systems which we studied agree with predicted lowering of sensibility as the ploidy increases, but the number of target types cannot be calculated since we used pluricellular organisms, and the mortality is influenced also by repeated washing, by the species used, etc.

References

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