SELECTION IN VITRO FOR ATRAZINE RESISTANCE IN PISUM SATIVUM L.

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Selection in vitro of herbicide-resistant cells and tissues with subsequent regeneration of plants is one of the most effective methods of producing herbicide resistance in crop species (1).

Research was carried out with photoheterotrophic, morphogenic, long term callus cultures obtained from Pisum cultivars Ranny Zeleny, Raport, Viola, and Uzkobobovy as well as from marker lines N. 17 and N. 18. Pieces of callus weighing 20-50 mg were placed in Gamborg medium (2) containing various concentrations of atrazine (10 pieces for each Erlenmeyer flask) to find the dependence on dose. They were incubated at 26 f 2 C under a 16 h photoperiod with a light intensity of 5000 lux. Two to five grams of callus were used in each experiment. Further selection of callus was carried out on selective media with a threshold atrazine concentration (for most genotypes the threshold concentration was 5×10^{-6} M or 1×10^{-5} M). Living parts of callus (about 1.5-8.0% of all callus weight) were detached after 3-4 weeks of cultivation in the herbicide medium and transferred to an atrazine medium with the same concentration. After 4-5 transplantations on selective media, which caused the death of nearly half the selected callus tissue, the surviving callus was transferred to a medium without atrazine to cause organogenesis. The shoots were then grafted in a greenhouse.

In separate experiments, callus cultures were irradiated by X-rays in 2.5 and 5 kr doses prior to transferring to a selective medium. After 3-4 weeks of cultivation the living tissue composed 11-17% of the weight, which means that they grew nearly two-fold compared with non-irradiated variants.

It was found that the extent of suppression of growth and viability of callus tissue depends upon the genotype of the callus, as well as upon the size of explants and the extent of differentiation of the callus. The influence of atrazine was less impressive when big explants or explants with buds were used.

Callus tissues able to grow in a selective medium with threshold concentrations of atrazine were obtained from line N. 17, as well as from cultivars Raport, Ranny Zeleny, and Viola. To find out if the ability of callus tissues to grow in a herbicide medium is connected with their physiological adaptation, the calluses were cultivated for 2 months in a non-selective medium, and later were transferred into media with various concentrations of atrazine. Growth and viability of callus tissues which had not passed through selection were suppressed by a lower concentration of atrazine than callus tissues which had passed through such selection, and callus tissues grown for two months in a non-selective medium were more sensitive to atrazine than the callus tissues grown continually in a medium with the herbicide (Fig. 1). These results do not allow an unequivocal answer regarding a mechanism of atrazine tolerance in callus cultures selected by us. It may be that the ability of callus tissue to grow in a selective medium is connected both with mutational events and adaptation (changes of metabolism of cells). It is not excluded that in the process

{ cultivation in a non-selective medium a reverse selection was started)wards an increase in the proportion of sensitive cells.

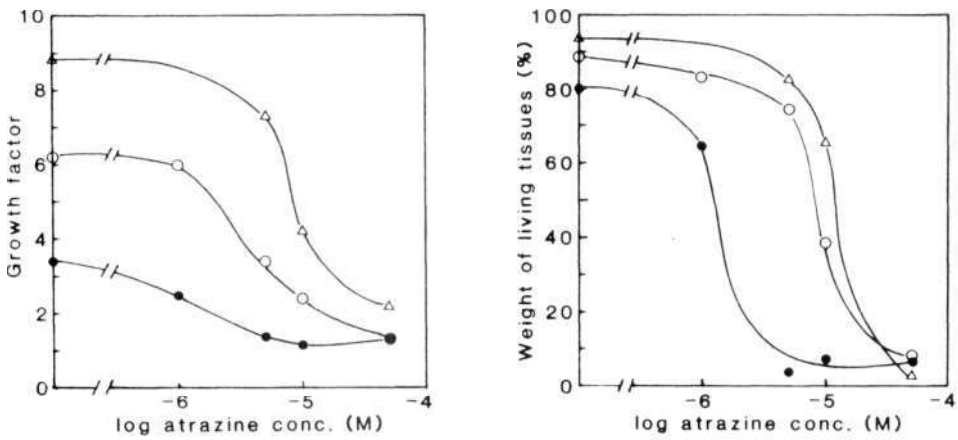

Fig. 1. The influence of atrazine on growth and viability of callus tissue. The "growth factor" is the ratio of final : initial fresh weight after 3 weeks growth.
• - Initial callus
o - Tolerant callus cultivated for 2 months in non-selective medium
Δ - Tolerant callus cultivated constantly in a medium with atrazine

Delayed fluorescence (DF) of chlorophyll was measured in callus tissues passed through selection and cultivation for three months on a medium without atrazine and in regenerant plants (R_G) obtained from them. As was shown earlier (3), suppression of chlorophyll DF components by herbicides can show the efficiency of herbicidal inhibitory action on the oxidizing and reducing sides of photosystem II (PS II). In particular, inhibition of electron transport on the PS II reducing side resulting from binding of atrazine-type herbicides near the Q_B-site of the D-l polypeptide of PS II leads to suppression of millisecond DF. As seen from Fig. 2, 50% inhibition of millisecond DF of atrazine-resistant callus occurs at an atrazine concentration approximately an order of magnitude higher than for the control callus. This fact indicates that the electron transport on the PS II reducing side of atrazine-resistant callus is less sensitive to atrazine. Analogous results have been obtained in the experiments with regenerant plants (R_0).

Table 1. Pollen sterility analysis of regenerants

Regenerants from		Number of regenerants	% of sterile pollen: Limits of variation	% of sterile pollen: Average
Cv. Ranny	control callus	9	0.6-100	33.5
	tolerant callus (clone N 1)	9	4.3-100	43.4
Zeleny	tolerant callus (clone N 2)	18	24.4-100	89.0
Cv. Raport	control callus	9	0-3.0	0.1
	tolerant callus	12	22.7-75.2	45.0
Line N 17	control callus	24	0.4-67.0	14.7
	tolerant callus (clone N 34)	3	10.0-100	49.3
	tolerant callus (clone N 35)	10	10.1-100	32.8

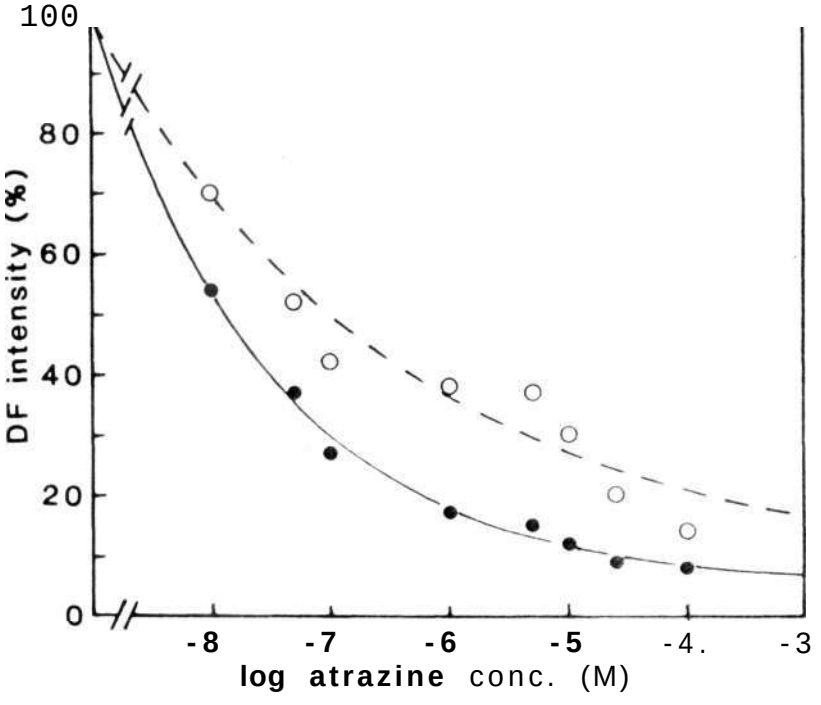

Fig. 2. The intensity of millisecond DF (delayed fluorescence) of atrazine tolerant (o) and control (•) callus tissue as a function of atrazine concentration. Homogenated callus tissues were diluted in the liquid Gamborg medium to a chlorophyll concentration of about 20 mkg/ml and incubated for 2 minutes in the presence of atrazine. The curves are normalized to the DF intensities in the absence of atrazine.

Many regenerant plants growing in a greenhouse were characterized by a changed morphology. The changes in regenerants obtained from tolerant callus tissues of different varieties were of the same type: leaves of the plants were dark green and partially folded, and the morphology of stems and flowers was changed (Fig. 3). The majority of regenerants had a high pollen sterility (Table 1).

A high proportion of sterile pollen was also found in regenerants obtained from control callus tissue, particularly in regenerants from callus of cultivar Ranny Zeleny which had been in culture more than five years from the beginning of the experiments. However, the morphological changes described above were not observed.

The data obtained by us testify to the possibility of obtaining atrazine-resistant lines of Pisum by selection in vitro.

1. Chaleff, R. S. 1986. In: Handbook of Plant Cell Culture, vol. 4. Eds. D. A. Evans, W. R. Sharp, and P. V.Ammirato. MacMillan, New York. pp. 133-147.
2. Ezhova, T. A., A. M. Bagrova, and S. A. Gostimski. 1985. PNL 17:10-11.
3. Vasil'ev, I. R., D. N. Matorin, V. V. Lyadsky, and P. S. Venediktov. 1988. Photosynth. Res. 15:33-39.

Fig. 3. Regenerant (R) plants from tolerant callus (cv. Raport).