RESEARCHES REGARDING THE GERMINATION PROCESS AT SPECIES OF ALIMENTARY PLANTS IN EXPERIMENTAL CONDITIONS

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Abstract. The aim of this study was to identify the genetical, biochemical, morphological and anatomical modifications induced by some biologically active substances with stimulator effect on *Raphanus sativum* L. seedlings, cultivated in laboratory conditions. There were tested three substances (caffeine, kinetine, 2, 4-dichlorophenoxyacetic acid) in concentrations recognized as producers of some functional effects in culture plants, as follows: modifications of cellular division (mitotic index and frequency of the appearance of chromosomal aberrations); the dynamic of the activity of oxidative enzymatic complex (superoxid dismutase, catalase, peroxidase) and the total protein content; morpho-anatomical aspects regarding the germination process (biometric measurements and interpretations of the structure of seedling organs). The applied treatments influence, in generally, the entire process of seed germination and seedling ontogenesis (translated by the cytogenetical, morpho-anatomical and biochemical effects mentioned above), depending on the type and concentration of the utilized substance. The results of this study reveal the complexity of the metabolical transformations of the treated culture plants, whipping up to new explorations of some aspects of practical interest.

INTRODUCTION

Following - up our researches (Zamfirache et al., 2001, 2008; Ştefan et al.,2004) regarding the germination process at species of alimentary plants in experimental conditions, this paper analyzes the cytogentical, biochemical and morpho-anatomical modifications of *Raphanus sativum* L. seedlings cultivated under the influence of three biologically active substances (caffeine, kinetine, 2, 4-dichlorophenoxyacetic acid). The aim of these studies is to contribute to the enlargement of practical possibilities to modulate this process, as starting point in controlled cultivation of respective species.

Caffeine ($C_8H_{10}N_4O_2$) is a purinic derivate, which can be incorporated in DNA macromolecule, inducing serious errors. The purines and the purinic derivates, including caffeine, can act as mutagens due to their capacity to replace the adenine and the guanine of the deoxyribonucleic acid. However, the researches regarding the effect of the caffeine in different biological systems do not end with the same results, some of them being contradictory.

2, 4-dichlorophenoxyacetic acid and kinetine belong to the class of synthetic plant hormones. 2, 4-D is a synthetic auxine, the active ingredient for roots development and vegetative propagation of plant. The auxines influence the cell elongation, the mitotic division and the adventitious roots development.

Kinetine is a synthetic cytokinine, which has a positive influence on numerous aspects of plant development, including cellular division, germination, stem proliferation, chloroplasts differentiation, apical dominance, plant-pathogen interaction, flower and fruit development, foliar senescence. It also activates the genic expression and metabolic activity and inhibits the roots development.

MATERIALS AND METHODS

The investigated biological material is represented by seedlings of *Raphanus sativum* L.., obtained from untreated seeds (control) and treated seeds (experimental variants) with the following biologically active substances in different concentration: caffeine (0,1%, 0,5%); 2, 4 D (1mg/l, 10mg/l); kinetine (1mg/l, 10mg/l).

At the beginning of experiment, the seeds were immersed for 24 h in treatment solution, and then they were placed in Petri dishes (50 seeds / variant) and maintained at room temperature, with periodic adding of distilled water. All the analyses were performed in 4, 11 and 14 -day old seedlings.

For the cytogenetical investigations (mitotic index and frequency of the appearance of chromosomal aberrations), the slides were obtained using the Squash method and were analyzed on a Nikon Eclipse 600 microscope.

Among the biochemical aspects, the protein content and the activity of oxidative enzymatic complex were determined. Superoxid dismutase activity was determined using colorimetric method according to Winterbourn (Winterbourn et al., 1975). This method is based on the ability of superoxide dismutase to inhibit the reduction of nitroblue tetrazolium by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of nitroblue tetrazolium reduction. Catalase activity was assayed using an iodometric method that consist in potassium iodide oxidation by undecomposed hydrogen peroxide, after an incubation interval with catalase, followed by titration of

delivered iodine with sodium thiosulfate, in starch solution presence as titration indicator (Artenie and Tanase, 1981). The catalase activity was calculated knowing that one catalase unit is equivalent to the amount of the enzyme which decomposes 0.034 mg hydrogen peroxide (1 μmol) during one minute. Peroxidase activity was measured using colorimetric method according to Möller and Ottolenghi (Möller and Ottolenghi, 1966). The peroxidase activity was calculated in relation with molecular extinction coefficient. One peroxidase unit represents the enzyme quantity that catalyses 1 μmol hydrogen peroxide decomposition in a minute and in the optimum reaction condition. The proteins from supernatants were quantified with bovine serum albumin as the calibration standard by the method of Bradford (Bradford, 1976).

To investigate the structure of the seedlings, cross-sections through the organs were performed using a manual microtome, coloured with iodine green and ruthenium red and embedded in glicero-gelatine. The obtained permanent slides were analyzed on a Novex (Holland) microscope and photographed at the same microscope with a Sanyo digital camera.

RESULTS AND DISCUSSIONS

I. Cytogenetical aspects in *Raphanus sativum* seedlings

1. The mitotic index (Fig. 1)

Comparing with the control, caffeine (0,1%) and 2, 4 - D (in both tested concentrations) induce a reduction of the mitotic index. Kinetine (in both tested concentrations) determines an increase of the mitosis number, which is more significant for the variant with kinetine (1 mg/l). A less increase of the mitotic index was registered for the variant with caffeine (0,5%).

2. Ana-telophase aberrations (Fig. 2)

The biggest percentage (51,19%) of ana-telophase aberrations reported to the control (16,90%) was noted for the variant with caffeine 0,5%. The maxim concentration of the tested caffeine also induced a big number of aberrations (30,43%). Most types of aberrations were reported at the variant with caffeine (0,5%), in which the most numerous are the bridges and the ana-telophases with expelled chromosomes. A big number of bridges are determined by 2,4 – D, while the expelled chromosomes predominate in the variant with kinetine (10 mg/l).

Caffeine (0,5%) has the most complex picture of chromosomal aberrations and other types of anomalies of cellular division, as follows: ana-telophases with polar deviations, binuclear cells, polyploidy (2n=3x=27 and 2n=4x=36). Caffeine (0,5%) also determines chromatin lysis.

3. Anomalous metaphases (Fig. 3)

The anomalies are represented by metaphases with expelled chromosomes from the equatorial plate and metaphases of C type. The latest ones appeared as a result of inactivation of the spindle fibers and the development of chromosomal configurations similarly with those induced by colchicines.

A big number of anomalous metaphases was registered in the variant with 2,4-D (10 mg/l) and caffeine (0,1%).

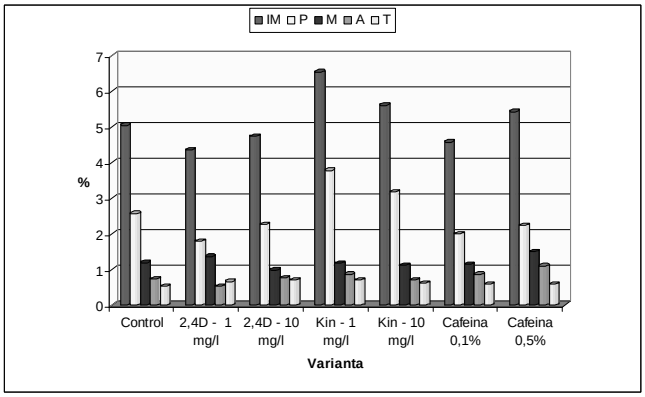

Fig. 1. Influence of 2,4-D, kinetine and caffeine in tested concentrations on mitotic index in *Raphanus sativum* seedling.

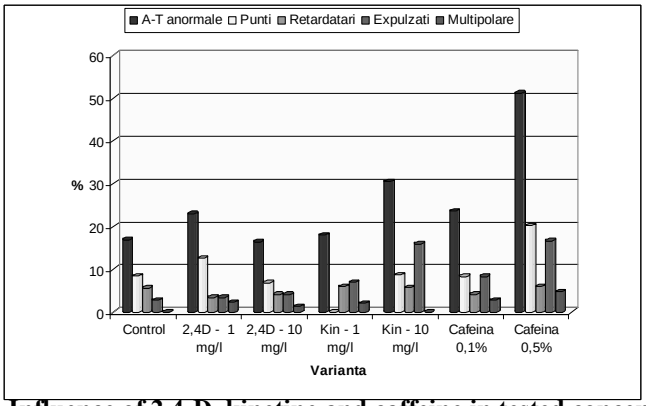

Fig. 2. Influence of 2,4-D, kinetine and caffeine in tested concentrations on the frequency and types of chromosomal aberrations in *Raphanus sativum* seedling.

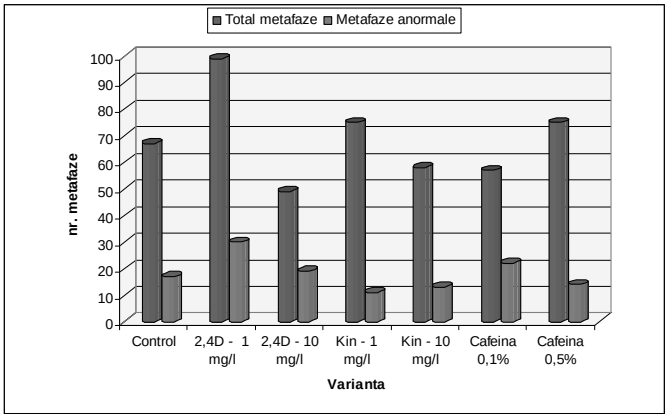

Fig. 3. Influence of 2,4-D, kinetine and caffeine in tested concentrations on the number of anomalous metaphases in *Raphanus sativum* seedling.

II. Biochemical aspects in *Raphanus sativum* seedlings

The activity of the superoxid dismutase is generally superior to the control, with the exception of the 14-day old *Raphanus sativum* seedling. The amplitude of the registered values is dependent from the chemical type of the biologically active substance and the tested concentration (Fig. 4).

Catalase presents maxim activities in the 11 –day old *Raphanus sativum* seedling. Its variation is similar with the superoxid dismutase one (Fig. 5)**.**

The activity of the peroxidase generally exceeds the activities registered in the control. The direction of variation of the peroxidase activities is the same for all the experimental variants; but the amplitudes are different (Fig. 6).

In all experimental variants, the **protein content** diminishes (Fig. 7).

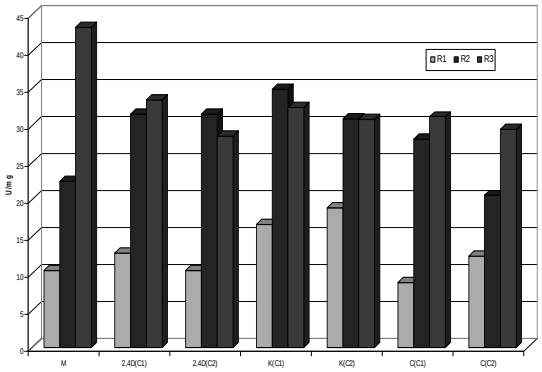

Fig. 4. Dynamics of the superoxid dismutase activity in *Raphanus sativum* seedling during

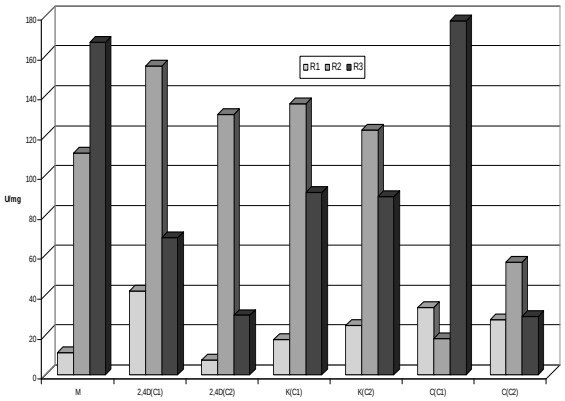

Fig. 5. Dynamics of the catalase activity in *Raphanus sativum* seedling during

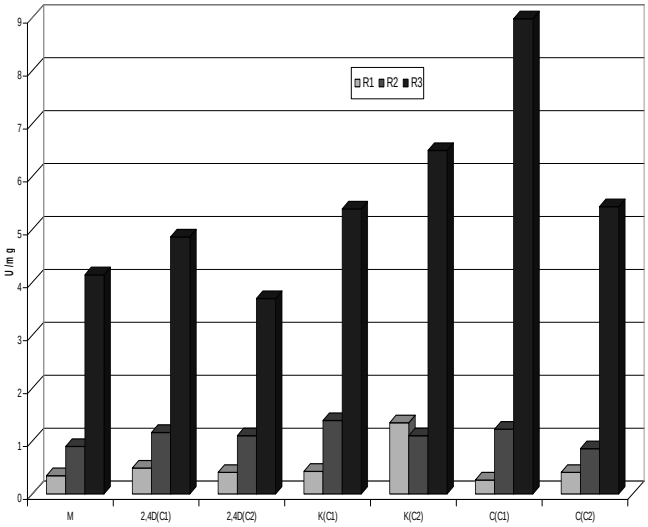

Fig. 6. Dynamics of the peroxidase activity in *Raphanus sativum* seedling during ontogenesis.

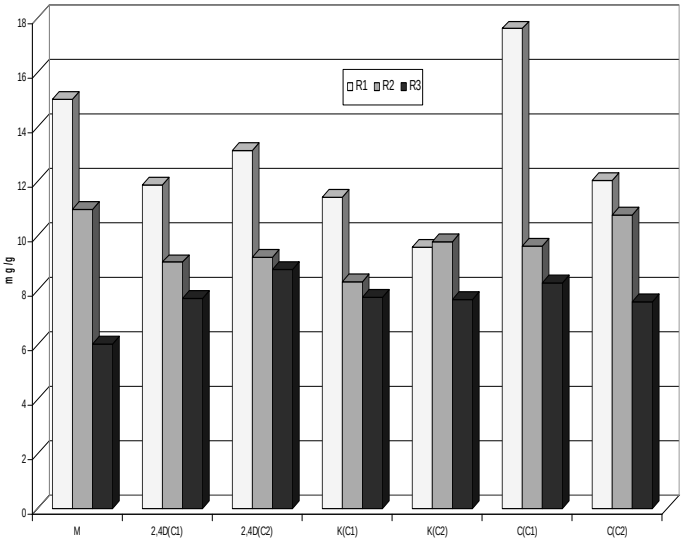

Fig. 7. Dynamics of protein content in *Raphanus sativum* seedling during ontogenesis.

III. Morphological and anatomical aspects in *Raphanus sativum* seedlings

1. Morphological aspects of seed germination

The germination of the seed is epigeous. In the control and all experimental variants, the seedling presents: primary root, hypocotyl and two cotyledons capable to do photosynthesis.

During seedling ontogenesis (in 14 day interval), the tested biologically active substances influence the growth of the primary root and the development of the secondary roots. Thus, caffeine and kinetine (in both tested concentrations) stimulate the growth of the primary root; kinetine (10mg /l) stimulates development of the secondary roots; 2, 4 D (10mg /l) inhibits the growth of the primary root (Fig. 8).

2. Variation of the seedling anatomical characters (Plate I. II)

The structural layout of the seedling is similar in control and all experimental variants. The axial organs (primary root and hypocotyl) present primary structure, the vascular system being in different stages of evolution. The secondary roots have endogenous origin.

Comparing with the control, caffeine (0,1%) and kinetine (1mg/l) stimulate the development of the cortical parenchyma of the axial organs. No other modifications of the seedling structure induced by the tested biologically active substances were observed.

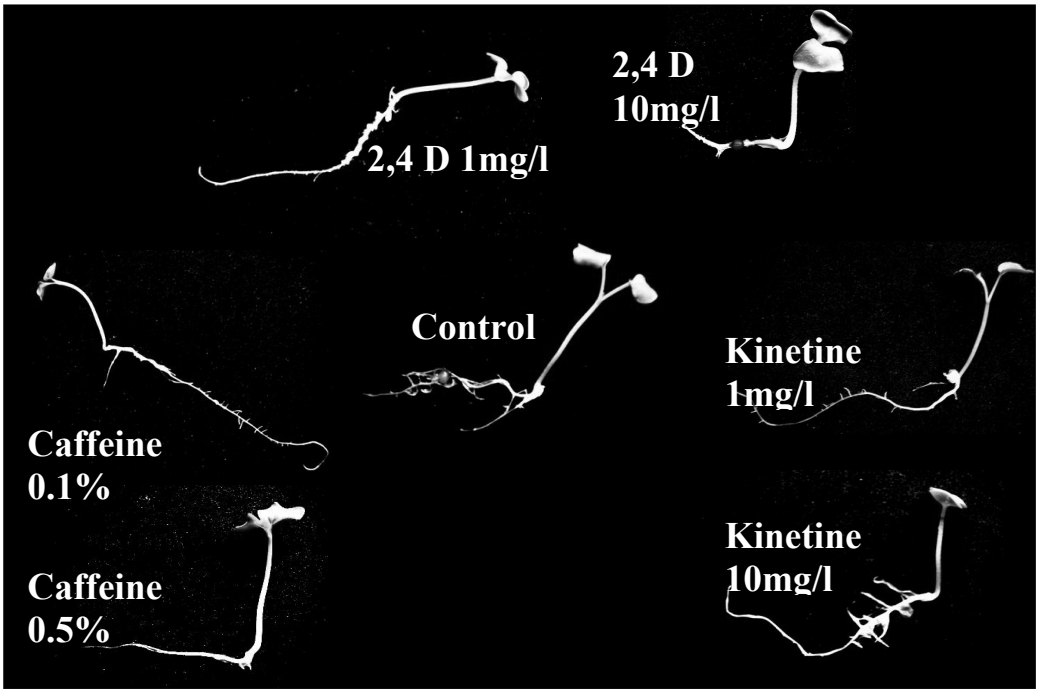

Fig. 8. Morphology of 14- day old *Raphanus sativum* seedling

CONCLUSIONS

The cellular division in the *Raphanus sativum* seedlings is stimulated by kinetine (1mg/l, 10mg/l) and slightly inhibited by 2,4-D (1mg/l, 10mg/l). Caffeine (0,5%) has the most powerful effect of determination of the chromosomal aberrations.

The enzymatic activity in the *Raphanus sativum* seedlings are dependent from the development stage of the seedling, the chemical nature and the concentration of the biologically active substances with which the seeds are treated.

The biologically active substances utilized in the experimental variants influence the morphology and structure of the *Raphanus sativum* seedlings, as follows: (a). stimulate or inhibit the development of the parenchyma, especially of the cortical one; (b) stimulate or inhibit the growth or the primary root and the development of the secondary ones.

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PLATE I . Structure of the primary root in *Raphanus sativum* seedlings (cross-sections)

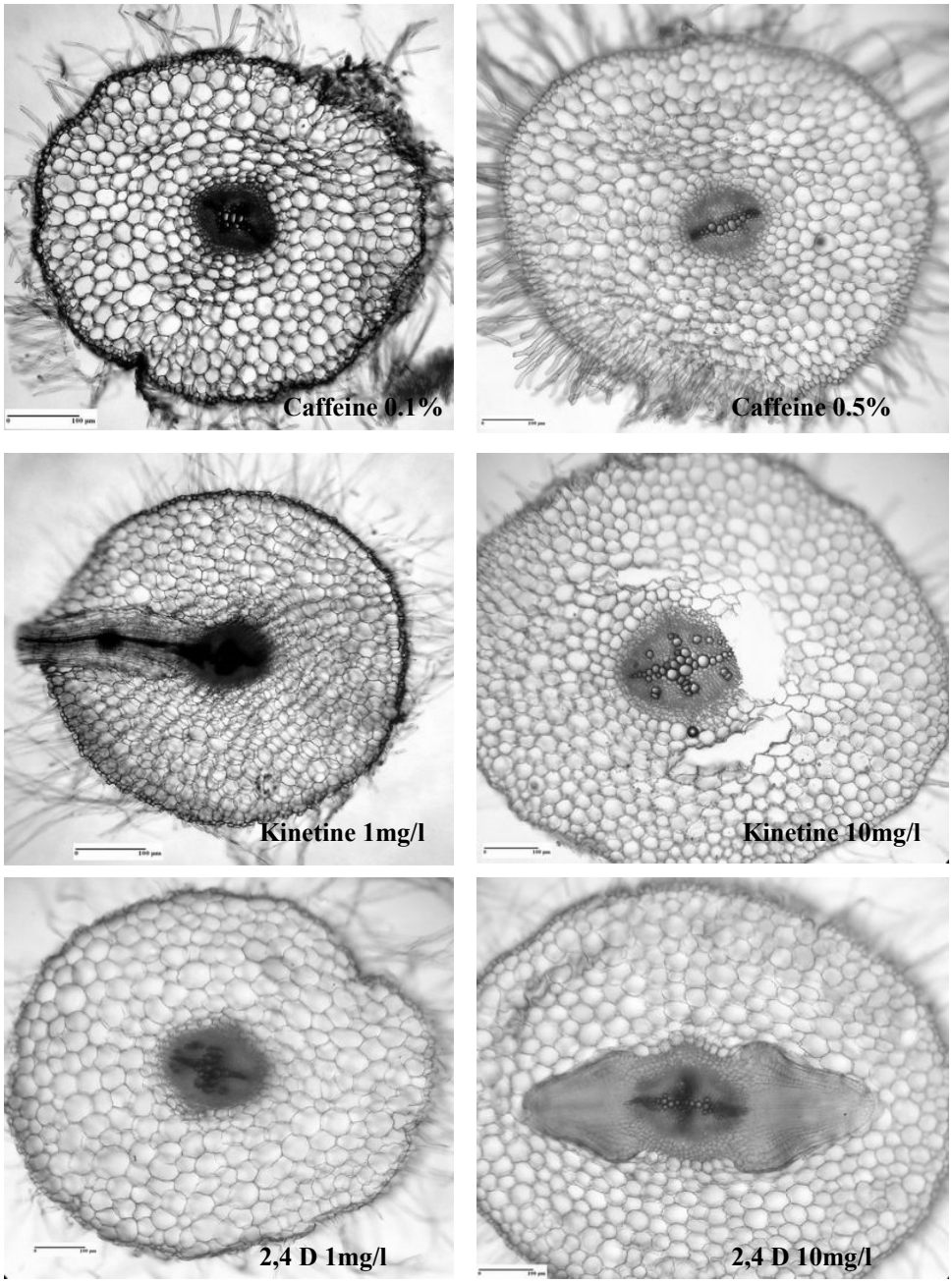

PLATE II. Structure of the hypocotyls in *Raphanus sativum* seedlings (cross-sections)