RESEARCHES REGARDING THE GERMINATION PROCESS IN SPECIES OF ALIMENTARY INTEREST UNDER THE INFLUENCE OF SOME BIOLOGICALY ACTIVE SUBSTANCES

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ABSTRACT. The authors analyze comparatively (control- experimental variants) some morphological, biochemical and physiological features of the germination and development of seedlings in three species of alimentary and fodder interest (*Pisum sativum* L, *Triticum aestivum* L., *Allium cepa* L.) under the influence of some biologically active substances (10^{-5}M phenoxyacetic acid and 50 ppm ascorbic acid). Comparing the experimental data of the species taken into study, the applied treatments induce specifically metabolically modifications of different intensity which have repercussions on the seedlings morphology during the analyzed germination process.

Keywords: germination, seedlings, biologically active substances

INTRODUCTION

Phenoxyacetic acid and its derivatives are recommended to be utilized in very small quantities in agriculture (0.01 – 100 ppm), because of their herbicide effect. These substances are more active against dicotyledonous species than against monocotyledonous species (including cereals). Generally, derivatives of phenoxyacetic acid are utilized, because its physiological activity increase by adding of one halogen atom (chlorine, fluorine) at aromatic part. The position where the halogen atom is attached is essential for the efficiency of compound activity.

In specialized literature, relatively few data exist concerning the effects of phenoxyacetic acid on germination process, the most numerous regarding 2,4-D (2,4-dichlorophenoxyacetic acid), which generally reduces the number of germinated seeds and delays the germination process.

Ascorbic acid – vitamin C – has a special signification in diet. The metabolism and the functions of this compound in plant are incompletely deciphered, although in plant tissues the vitamin C is in enough important quantities - many millimols. The complete knowledge of ascorbate role and biosynthesis in plant can offer the tool for the increasing of its concentration in plant by genetic manipulation.

The effects determined by exogenous applying of ascorbic acid on germination of some plant species depend on studied species, treatment duration, concentration of tested substance, endogenous concentration of ascorbic acid, physiological state of cells etc. These are the reasons for which a general valuable conclusion relative to physiological implications of exogenous applying of ascorbic acid can not be stated.

Ishibashi and Iwaya-Inoue (2006) studied the wheat germination process and proved in a very clear manner the inhibitory effect of ascorbic acid in the case when it was exogenously applied. The tested concentrations in their study were 10, 50, and 100 mM. The authors ascertained that at 50 mM ascorbic acid inhibited the seed germination in a manner similar to that exerted by 100 mM abscisic acid (phytohormone with known inhibitory effect on germination and growth). Also, according of studies of previously cited authors, ascorbic acid inhibited the germination process of seeds, in this case placed to germinate on filter paper moistened with ascorbic acid solutions. This substance is necessary to vital processes in young seedlings after the respective process started.

But the verification of *in vitro* effects of 0.5 mM, 1.0 mM, and 2.0 mM ascorbic acid on alfalfa tissue cultures, placed in saline stress, evidenced the stimulation of seed germination (ARAB and Ehsanpour, 2006), the optimization of certain growth parameters (stem and root length, root number, dry matter), as well as the increase of callus fresh biomass.

In this context, we investigated some morphological, biochemical and physiological parameters which characterize the germination of plants of alimentary and fodder interest, with the aim to contribute to the enlargement of practical possibilities to modulate this process, as starting point in controlled cultivation of respective species.

MATERIALS AND METHODS

The investigated biological material is represented by seeds of (*Pisum sativum* L, *Allium cepa* L.) and caryopses of *Triticum aestivum* L., obtained from private system of production.

The following variants were studied: control (C) – seeds moistened in distilled water; V_1 – seeds moistened in
10^{-5}M phenoxyacetic acid; V_2 – seeds moistened in 50 ppm ascorbic acid.
At the beginning of experiment, the seeds were immersed for 24 h in treatment solution, then they were placed in Petri dishes (50 seeds / variant) and maintained at room temperature, with periodic adding of distilled water.

The analyses were performed on a seven days period from the moment of moistening of seeds in respective solutions (192 h). For each variant, at 48, 96, 144 and 196 h, the germination rate and order of appearance of vegetative organs were established, concomitantly with their characterization, in plant ontogenesis.

From biochemical and physiological aspect, the protein content (Lowry method), the proteinase activity (spectrophotometric method) and catalase activity (iodometric method) were determined, and the intensity of respiratory process was measured (Warburg manometric method).

RESULTS

Morphologic aspectes (Plates I-III)

In *Allium cepa* (Plate I), after 48 hours, the radicle penetrates the micropyle and it is succeeded – between 96 and 144 h – by hypocotyl and cotyledon. The cotyledon tip remains in endosperm, it having a haustorium role, while the hypocotyl – cotyledon axis has a specific growth leading to the formation (144 h) of a 90^0 curvature (so named „knee") which helps for cotyledon extraction from seed and soil. After 192 h the curvature disappears, the „knee" extends and the axis becomes approximately orthotropous. The treatment influences in a little manner the morphological events. So, it determines a reduced inhibition (V_1 – 192 h) or it stimulates (V_2 – 192 h) the length growth of hypocotyl–cotyledon axis – in this case the curvature extension surpasses the control.

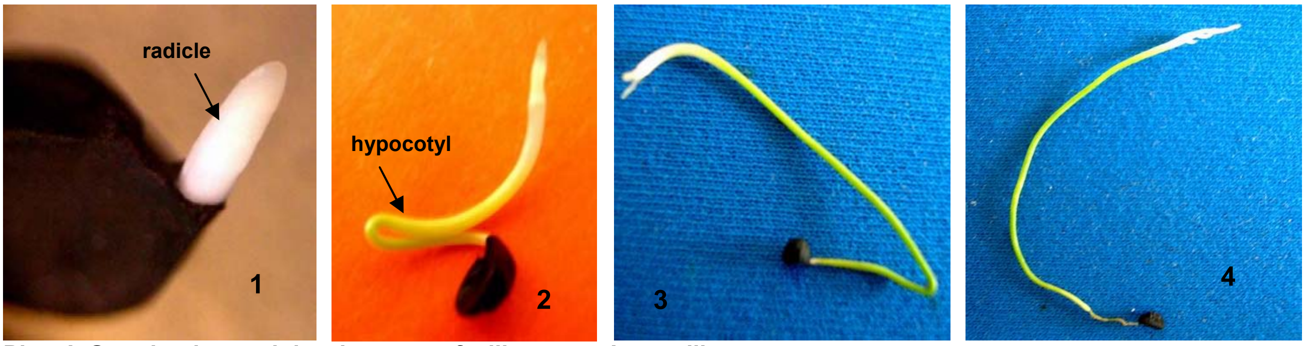

Plate I. Germination and development of *Allium cepa* L. seedlings
Fig. 1. C(48h). **Fig. 2.** C (144h). **Fig. 3.** V_1 (192h). **Fig. 4.** V_2 (192h)

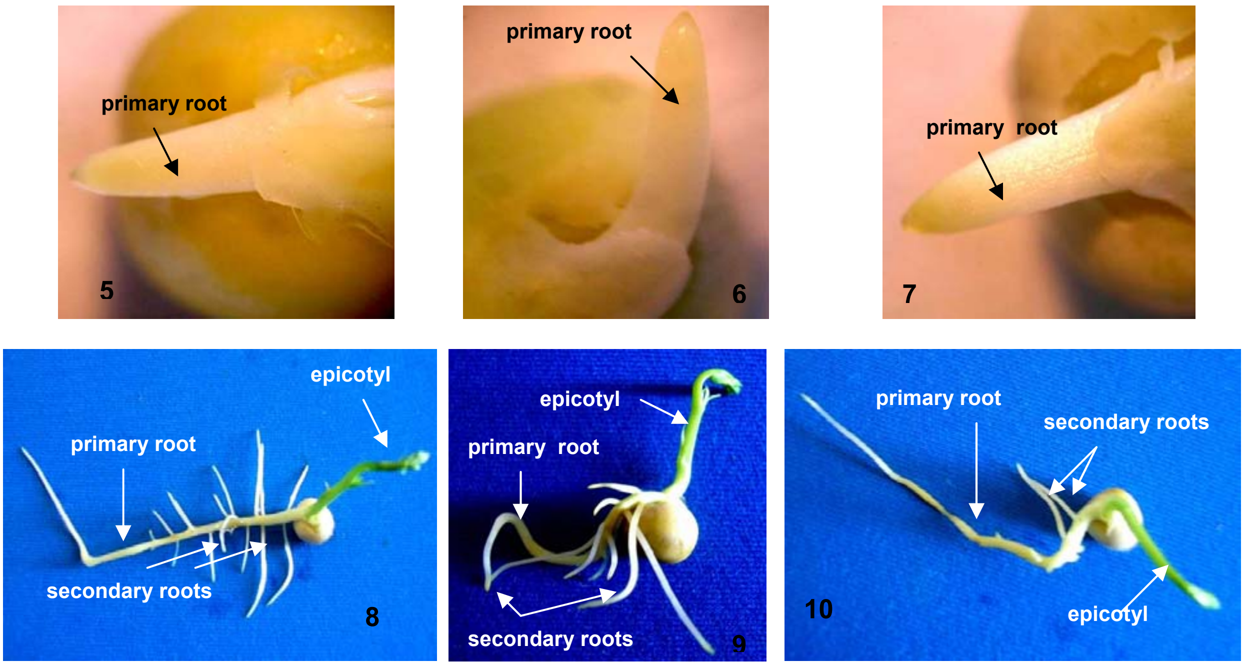

Plate II. Germination and development of *Pisum sativum* L. seedlings
Fig. 5. C (48h). **Fig. 6.** V_1 (48h). **Fig. 7.** V_2 (48h). **Fig. 8.** C (192h). **Fig. 9.** V_1 (192h). **Fig. 10.** V_2 (192h)

In *Pisum sativum* (Plate II), near concomitantly with seed coat and cotyledons imbibition, the primary root appears; all these events are characteristics of germination process. The germination rate is not influenced by treatment, its value being 100% at the two experimental variants and control.

As the growth of primary root takes place, the secondary roots are observed (at 96 h – Control). At the same time, the epicotyl wanders through seed coat, as a consequence of cotyledonary petioles elongation, this phenomenon being easily accelerated in treatment with ascorbic acid (V_2). During this period (48 – 192 h), at the same time with epicotyl growth, primary leaves (trifidate bracts) are evidenced, probably formed before germination.

The length growth of primary root is less inhibited by the treatment, so that at the study interval end (48-192 h), this root represents approximately 50% (V_1), respectively 80% (V_2) from the control primary root length (10 cm – Control). Concomitantly, but especially in interval 144 – 192 h, numerous secondary roots are formed, shorter than in control, especially in the treatment with phenoxyacetic acid (V_1).

In *Triticum aestivum* (Plate III), by imbibition, all caryopsis structures increase till the coleorhiza appearance (48 h), so that the germination process begins. The germination rate is influenced by the treatment in a slight manner, in the sense of its acceleration (Control – 93.4%; V_1 - 95.0%; V_2 – 98.4%).

After appearance of primary root, which wanders through coleorhiza, at 48 h already appears the first pair of seminal roots, visible only in treatment variants. Comparatively with literature data, in our experiment the number of seminal roots is smaller (4 - 5, not 6, as in Hayward, 1967). Afterwards, the development of root apparatus is accelerated in control, so that at 192 h all roots – primary and seminal – have approximately 8 cm in length. Phenoxyacetic acid applied in this dose (V_1) inhibits this process, this effect being visible at the beginning of analysed interval (98 – 144 h).

In both cases, the treatment stimulates the coleoptile appearance, so that this appears earlier (48 h) than in control. Later on, the coleoptile growth slows down, reason for which at 192 h it has only 3 cm in length face to 5 - 6 cm in control.

The first leaf wanders through coleoptile (96 h) only in control and rapidly grows at 7 cm in length.

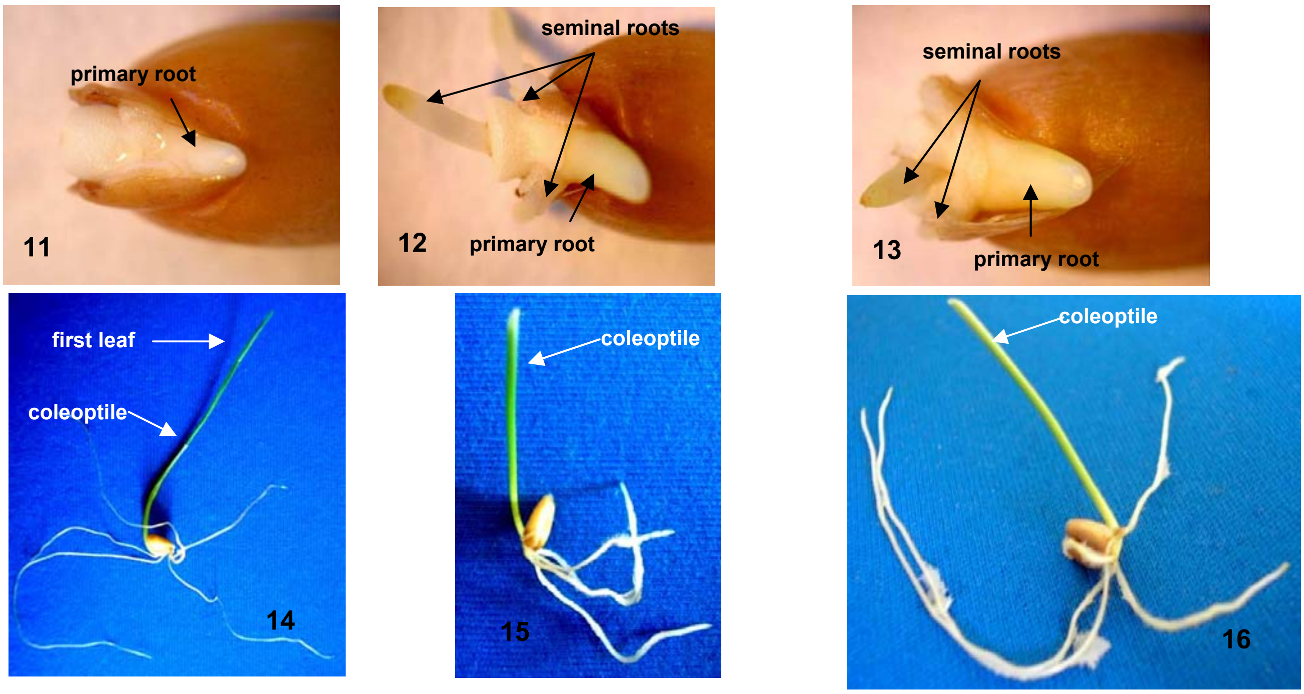

Plate III. Germination and development of *Triticum aestivum* L. seedlings
Fig. 11. C (48h). **Fig. 12.** V_1 (48h). **Fig. 13.** V_2 (48h). **Fig. 14** C (192h). **Fig. 15.** V_1 (192h). **Fig. 16.** V_2 (192h)

Biochemical and physiological aspects (Diagrams 1 - 12)

Allium cepa (Diagrams 1-4). During germination, the protein content display a tendency to increase in the first 144 hours and to decrease at the end (192 hours) of the analysed period, both in control and in experimental variants.

Proteinase activity is reduced in control, being clearly inhibited at the beginning (48 h) and at the end of analysed interval (144 - 192 ore). The higher value of proteinase is registered 48 h after germination in seeds treated with ascorbic acid, but the level of enzyme activity decreases in the following stages, especially at 96 h.

The dynamics of respiratory process is similar for control and in variants treated with ascorbic acid, they having high values at 48 – 96 h. The values suffer a sudden decrease in the next period (144 - 192 h). In phenoxyacetic acid treatment (V_1), the respiration suddenly decreases at 96 h, after that the process increasing at 144 h and 192 h.

Dynamics of catalase activity is also similar in control and experimental variants; the activity of this enzyme registers absolute maximum values at 48 h, diminishes at the middle of the interval (96-144 h) and increases at the end of this.

Pisum sativum (Diagrams 5-6). The protein content has a relatively similar behaviour in control and experimental variants in interval 48 - 96 h, but with superior values in control. In interval 144- 192 h, the protein amount is smaller in treated seeds and it significantly increases in control.

The treatment influences the proteinase activity, in the sense of its amplification in interval 144 - 192 h, especially in V_1 variant – treatment with phenoxyacetic acid. During analysed period (48 – 192 h), the control values are inferior to those of experimental variants.

In the treatment with ascorbic acid (V_2), the respiratory process displays a clear tendency to diminish, in parallel with seedlings growth – at 192 h was registered the absolute minimum absolute value of respiration. In control and in treatment with phenoxyacetic acid (V_1), this process starts (48 h) with relatively high values, followed (96 h) by a decrease and after that an increase of the respiration values. At the final part of analysed interval (144 – 192 h), the respiration diminishes in intensity in treated seeds (V_1) or, contrary, increases in control. (C).

The catalase activity, both in control and in experimental variants, has increase values, especially in control and in variant treated with phenoxyacetic acid (V_1), in this case with maximum absolute values at 192 h. The treatment with ascorbic acid (V_2) induces a tendency to the diminution of catalase activity, sense in which the respiratory process also takes place.

Triticum aestivum (Diagrams 9-12). During germination and seedling growth, the protein level has the same value dynamics, respectively to increase (96 – 144 h) then to decrease (192 h). It seems that in V_2 variant ascorbic acid determines higher values of protein, comparatively with control.

Proteinase activity is small at the germination beginning (48 - 96 h) and it increases in interval 144 - 192 h of seedling growth; the smallest values are registered in the treatment with phenoxyacetic acid (V_1).

The respiration process starts (48 - 96 h) with maximum values, in all variants; then, the values reduce and maintain at this reduced level until the end of analysed interval (96 - 192 h). It seems that the treatment impresses a certain tendency to reduce the respiration process at the middle of the interval (144 ore), especially in variant with ascorbic acid (V_2).

In experimental variants (V_1 and V_2), the catalase activity is intense, comparatively with control, especially at the start of germination (48 h), fact coinciding with an increased respiratory activity.

The dynamics of catalase activity registers a clear descendant curve under the influence of phenoxyacetic acid treatment (V_1). In control and in variant V_2 – treatment with ascorbic acid, at the end of studied interval (192 h), the catalase displays maximum activity.

DISCUSSIONS

Germination is a complex phenomenon of morpho-physiological events, starting with root or hypocotyl appearance and also including (not necessarily) the mobilisation of reserve substances. In this case, in the definition must be included too the imbibition, phenomenon which launches a series of physiological and biochemical processes.

The studied species - *Pisum sativum* L, *Triticum aestivum* L., *Allium cepa* L. - dicotyledonous and monocotyledonous species (the last two) have hypogeal germination.

The morphological manifestations of germination and seedling growth as well as the germination rate are different and have a species character.

The treatment applied by seed imbibition (*Pisum sativum* L, *Allium cepa* L.) and caryopses imbibition (*Triticum aestivum* L.) with phenoxyacetic acid and ascorbic acid proved that these substances influence into a slight degree the morphological events. These substances inhibit or stimulate the plant organ growth.

In applied doses, phenoxyacetic acid inhibited the length growth of primary root and stimulated the growth process for secondary roots. Ascorbic acid stimulates the appearance of *Pisum sativum* epicotyl, accelerates the appearance of *Triticum aestivum* coleoptile, as well as the going up of cotyledon from seed in *Allium cepa.*

Germination is not influenced by treatment in *Pisum sativum,* it is stimulated in *Triticum aestivum* or, by contrary, it is inhibited in a slight manner in *Allium cepa*.

During imbibition and germination, the storage substances will be mobilised in view of structuration of future seedling and then of the plant.

The protein content has a tendency to increase in the first hours from germination (48 – 144 h) and to decrease at the end of the interval (144 - 192 h), both in control and in experimental variant. In *Pisum sativum*, the control has a different behaviour, in the sense of increase of protein level at the end of the analysed period (192 h). At the same time, the two applied substances reduce in a clear manner the protein amount.

The proteinase activity has at the studied species superior values under the influence of the treatment, comparatively with control, an intensification tendency being visible at the end of determinations (192 h); the highest values are registered in *Triticum aestivum.*

The respiratory process (control - treatment) starts with maximum values and it has the tendency to diminish as the plants grow old as in *Triticum aestivum*, or only in ascorbic acid treatment as it happens in *Pisum sativum* and *Allium cepa*.

The dynamics of respiratory process is influenced by phenoxyacetic acid in *Pisum sativum* and *Allium cepa*, in the sense of its diminution and, respectively, of process intensification at the middle of the interval (96 h). In the interval 144 - 192 h, the respiratory process diminishes in intensity.

The catalase activity is influenced by phenoxyacetic acid and ascorbic acid, in the sense of its increasing by comparison with control, in *Triticum* *aestivum* and *Allium cepa* (in this late case the maximum value is registered at 48 h). In *Pisum sativum*, ascorbic acid reduces catalase activity, its values being smaller, comparatively to those of control and variant with phenoxyacetic acid. A clear tendency to decrease the activity of this enzyme is observed as the seedlings grow old, process correlated with the respiration.

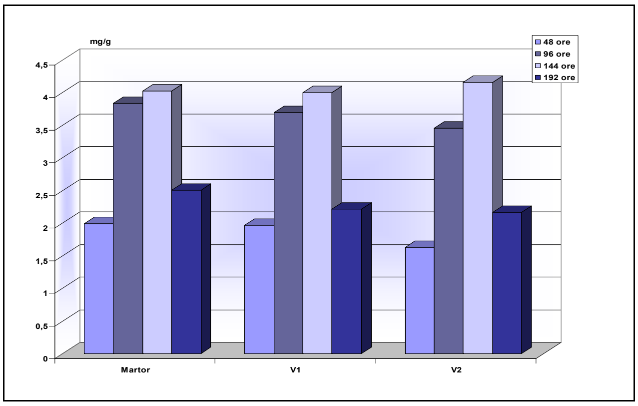

Diagram 1 Dynamics of protein content in *Allium cepa* L. during germination

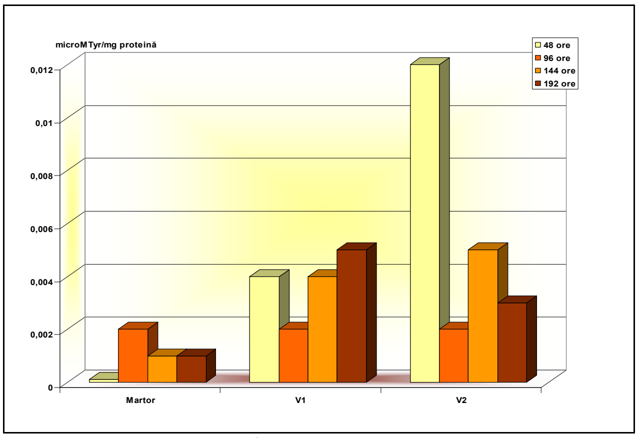

Diagram 2 Dynamics of proteinase activity in *Allium cepa* L. during germination

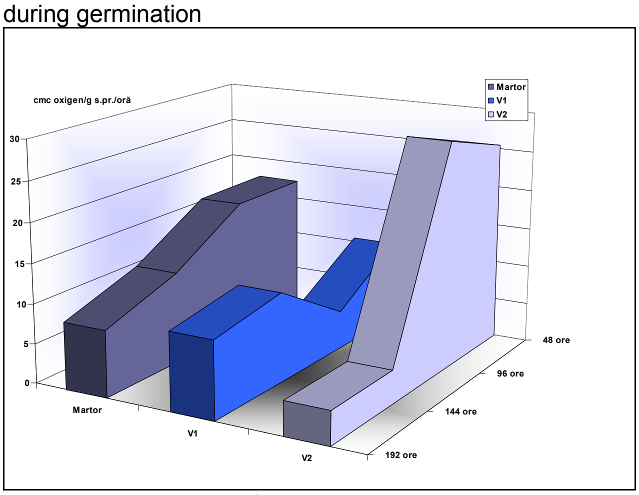

Diagram 3 Dynamics of respiratory process in *Allium cepa* L. during germination

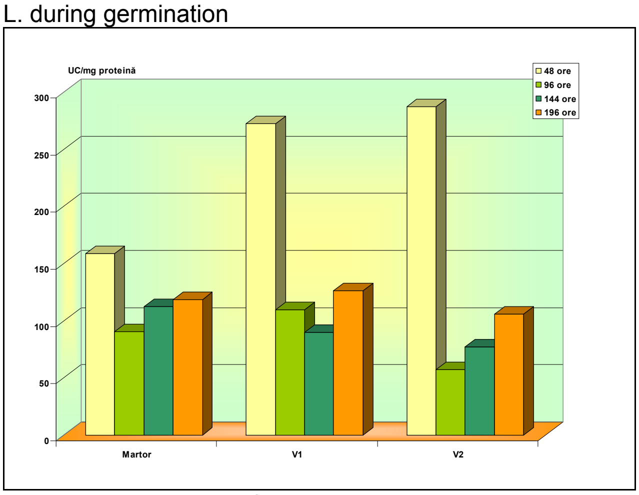

Diagram 4 Dynamics of catalase activity in *Allium cepa* L. during germination

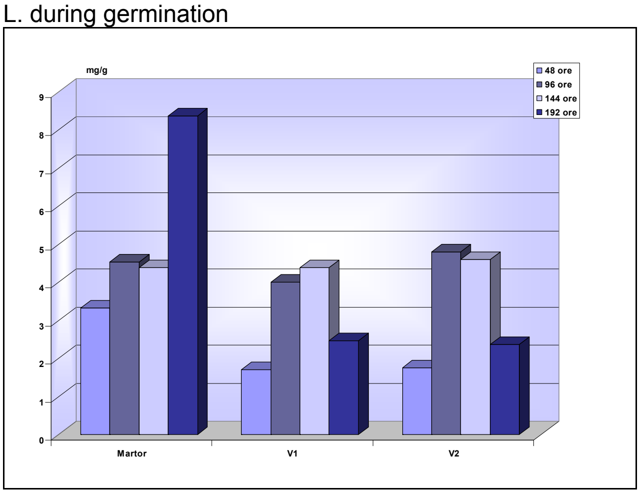

Diagram 5 Dynamics of protein content in *Pisum sativum* L. during gemination

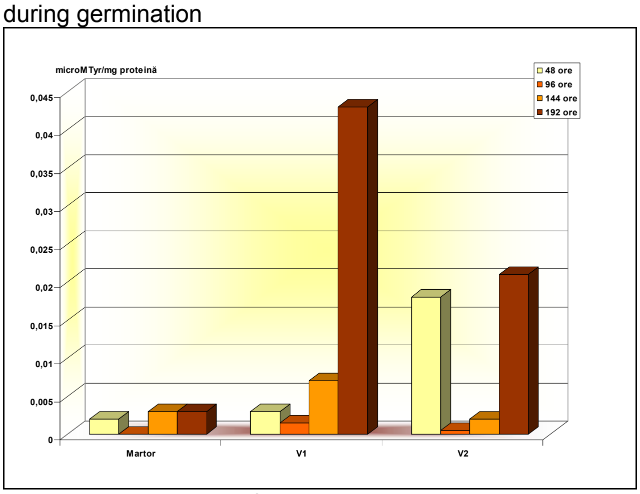

Diagram 6 Dynamics of proteinase activity in *Pisum sativum* L. during gemination

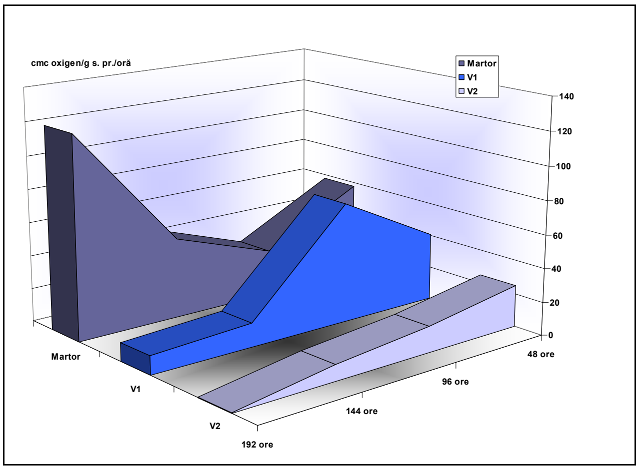

Diagram 7 Dynamics of respiratory process in *Pisum sativum* L. during germination

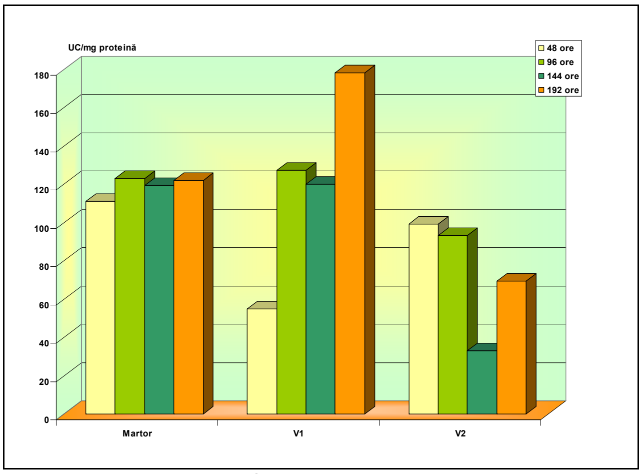

Diagram 8 Dynamics of catalase activity in *Pisum sativum* L. during germination

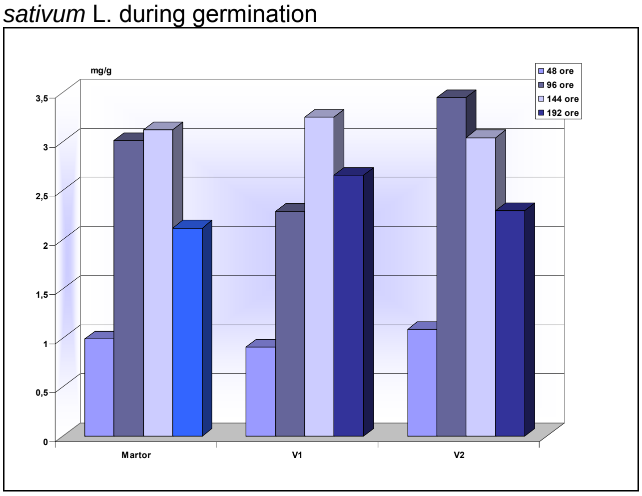

Diagram 9 Dynamics of protein content in *Triticum aestivum* L. during germination

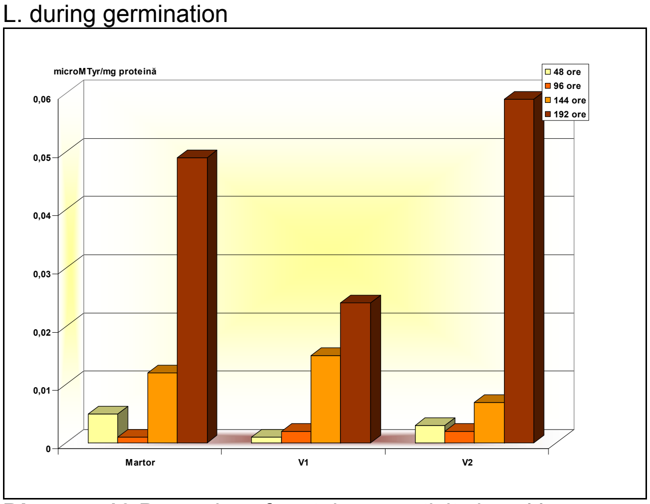

Diagram 10 Dynamics of proteinase activity in *Triticum aestivum* L. during germination

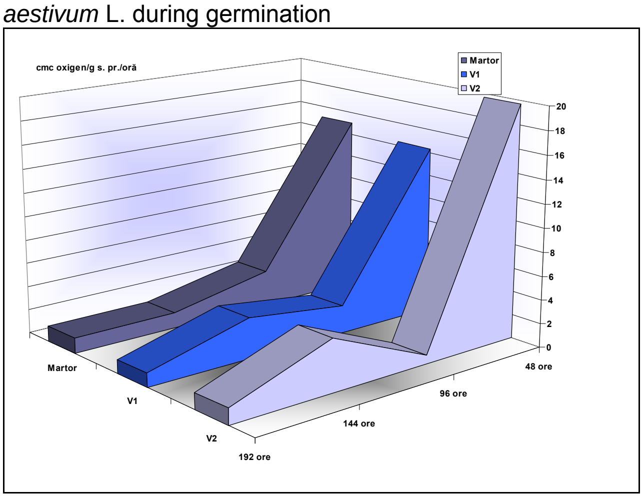

Diagram 11 Dynamics of respiratory process in *Triticum aestivum* L. during germination

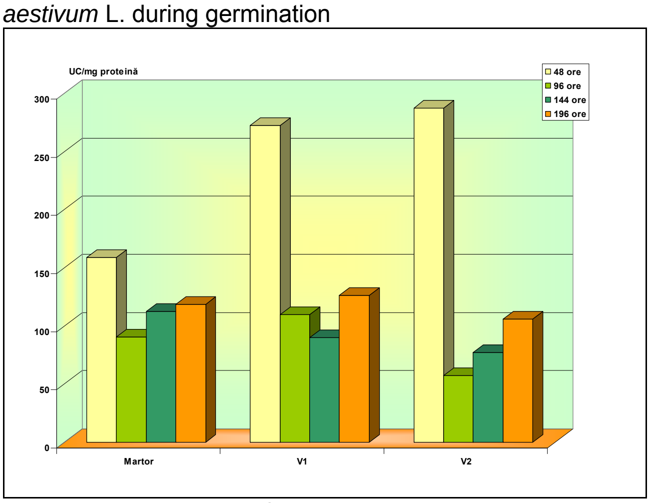

Diagram 12 Dynamics of catalase activity in *Triticum aestivum* L. during germination

CONCLUSIONS

The comparative analysis of the results obtained in experiments organised in the three species leads us to consider that the applied treatments induce specific metabolic modifications, of variable intensities, modifications which have repercussions on morphology of seedlings formed during the investigated germination process. This reality points out the complexity of analysed phenomena and incites to the thoroughgoing study of certain aspects of practical interest.

Generally, the effect of the two tested substances is favourable to germination process. The response of test-species is specific to applied treatments and it is included in the general stimulatory background of metabolic processes characterizing the seed germination.

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