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THE INFLUENCE OF HUMIC ACIDS ON SEED SWELLING PROCESS OF *PISUM SATIVUM* L. cv RAMROD

Abstract. The influence of humic acids on *Pisum sativum* L. seed swelling process was studied. Humic acids (HA) were applied during seed swelling by watering the seeds for one hour in the first, fourth and eight hour of the process. Three different concentrations of HA 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}]; 0.02 [gC dm^{-3}] in buffer phosphate solution at pH=6.5 were used. The control sample was the pea seeds watered in buffer phosphate solution at pH=6.5 without humic acids. All of the pea seeds were placed on Petri dishes onto full water capacity filter paper. Humic acids at concentration 0.02 [gC dm^{-3}] increased the fresh mass of swelling seeds when applied in the first hour of experiment. It was also noted that all concentrations of humic acids applied in the first hour of experiment increased the percentage of pea seeds with visible radicle at the end of experiment.

Humic substances (HS) are a part of inanimate organic matter in the environment. This means in soils, waters, bottom sediments, etc. They are a product of the distribution of transformation and re-synthesis of dead organic matter coming from microorganisms or plant residues and to a smaller degree from animal residues. The final form of humic substances is a product of microbiological processes, as well as mineralization and humification. The influence of humic substances on plants was recognized at the beginning of the twentieth century and then the studies on this problem began [12]. Humic substances may

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influence plants directly and/or indirectly. Indirect effects of humic substances involve changes in physical and chemical properties of soil. Humic substances may be stimulators as well as inhibitors of various processes occurring in plants. Direct effects of humic substances on plants depend on many factors, such as the type and dose of humic substances, method of application or species and the development phase of a plant [7, 10, 4]. The stimulating effect of humic substances on germination, seedling growth or root expansion was observed by [6, 14]. Increased uptake of iron, nitrogen, phosphorus, potassium and other elements essential for normal plant growth was also observed in plants treated with humic substances [17]. Humic acids solutions with different concentrations had a positive effect on swelling and germination of pea seeds [5]. The effect depended on the pH solution which hydrates the swelling seeds.

The aim of this study was to evaluate the effect of humic acids applied in phosphate buffer solution on the process of swelling of the seeds of *Pisum sativum* L. depending on the concentration of humic acids and the time of their application.

MATERIAL AND METHODS

The research material was seeds of *Pisum sativum* L. cv Ramrod. It is one of the most popular pea cultivars grown in Poland characterized by a high seed yield and resistance to lodging until the harvest. In the experiment, the influence of different concentrations of HA and application time on the process of swelling of the seeds was investigated. HA were extracted according to the Schnitzer procedure from low peat. HA application in the seed swelling process was carried out by immersing the seeds in a buffer solution of pH=6.5 for 1 hour in three phases characteristic of the swelling curve. The pea seeds were put into Petri dishes equipped with Plexiglass plates with numbered holes for seeds. It allowed for the observation of the kinetics of swelling of every seed separately. The substrate for the germinating seeds was tissue paper (weight – 65g m^{-2}). Five tissue paper discs were put into each Petri dish and soaked with distilled water to keep 90–100% of maximum moisture of the tissue paper. The Petri dishes were covered with cover slips the seed swelling. The swelling process was carried out in the dark in an incubator with the inside temperature of $(20.0 \pm 0.5)^0$C.

Kinetic examinations began with the measurements of dry seed mass m_0. Then, the masses of particular seeds after 0; 4; 8; 12; 18; 24; 28; 36; and 48 hours of swelling were determined. To apply HA, the seeds at different stages of swelling were immersed for 1 hour in humic acid solutions of pH 6.5 with carbon concentrations correspondingly 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}] and 0.02 [gC dm^{-3}]. Application time of HA was determined experimentally on the basis of swelling curves obtained in preliminary experiments in which the swelling of pea seeds was only carried out in distilled water without application of HA. The exemplary kinetic swelling curve of pea seeds is presented in Fig.1. The choice of application time of HA was dictated by the shape of the swelling curve of pea plants. The curve is a consequence of seed transition through the following phases of swelling: imbibitions; catabolic and anabolic phases that occur in the beginning of germination. Based on the analysis of the swelling curve (Fig. 1) we chose three periods (0 -1)h, (3-4)h and (7-8)h, when HA solutions with carbon concentrations given above were applied to the swelling seeds.

Kinetics of swelling seeds

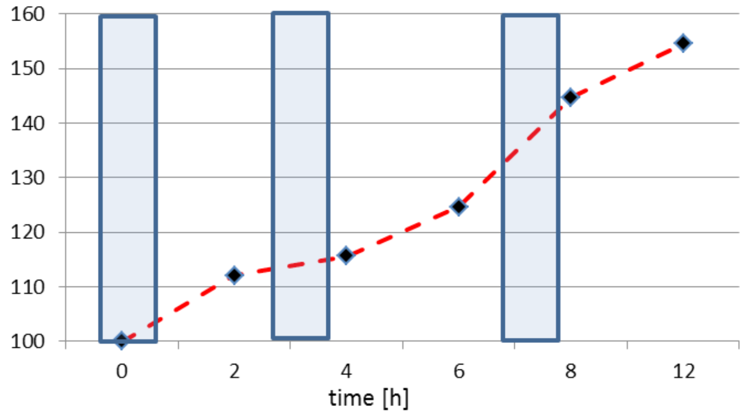

Fig. 1. Kinetics of seed swelling with characteristic phases of the swelling process marked for HA application.

The application was carried out by complete immersion of the seeds in HA solutions in phosphate buffer of pH 6.5 for 1 hour in application times described earlier. The control was the seeds immersed at the same time in phosphate buffer without HA. Then, the seeds were dried on filter paper and put again into Petri dishes on tissue paper soaked with distilled water to allow for continued swelling without contact with HA.

RESULTS AND DISCUSSION

Although the application time of HA on the swelling of seeds was relatively short, their statistically significant effect on swelling and germination processes was observed.

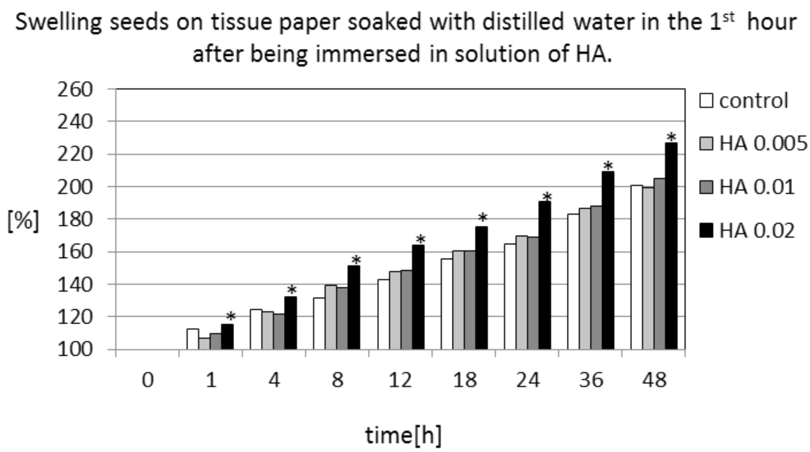

Fig. 2. Swelling of seeds on tissue paper soaked with distilled water in the 1st hour after being immersed in phosphate buffer solution of concentration $(1/15)\cdot 10^{-2}$ [mol dm^{-3}] and pH=6.5. Humic acids were applied in concentrations correspondingly 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}]; 0.02 [gC dm^{-3}]; *signifies an average weight gain of the seeds to which HA were applied and which were statistically different from the control (at a level of significance α=0.05).

The highest concentration of HA 0.02 [gC dm^{-3}] caused a statistically significant relative weight gain of the seeds in relation to the control during the 4th hour of swelling.

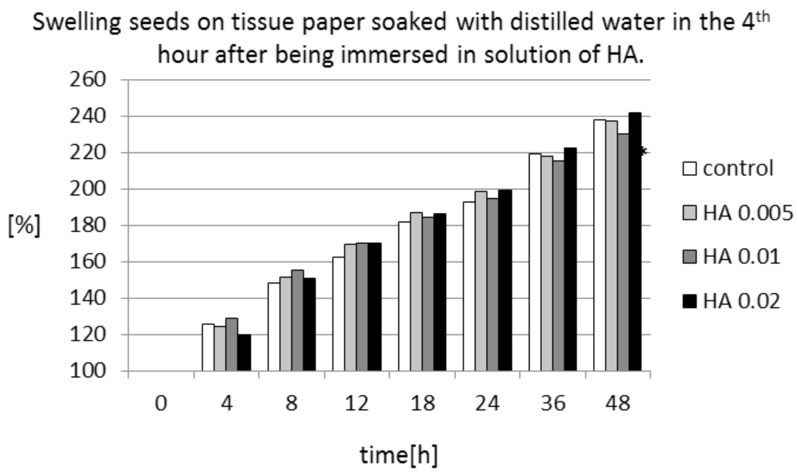

Fig. 3. Swelling of seeds on tissue paper soaked with distilled water in the 4th hour after being immersed in phosphate buffer solution of concentration $(1/15)\cdot 10^{-2}$ [mol dm^{-3}] and pH=6.5. Humic acids were applied in concentrations correspondingly 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}]; 0.02 [gC dm^{-3}]

Immersion of seeds in water solutions of phosphate buffer with HA application carried out in the 4th hour of the experiment did not bring statistically significant changes in the process of seed swelling.

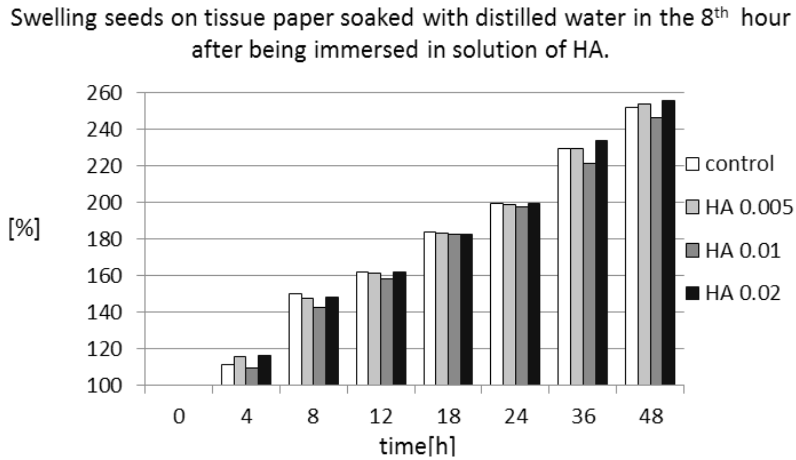

Fig. 4. Swelling of seeds on tissue paper soaked with distilled water in the 8th hour after being immersed in phosphate buffer solution of concentration $(1/15)\cdot 10^{-2}$ [mol dm^{-3}] and pH=6.5. Humic acids were applied in concentrations correspondingly 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}]; 0.02 [gC dm^{-3}];

Statistically significant effects in the kinetics of the seed swelling process in 4th and 8th hour of the experiment were not observed, regardless of whether the HA was or was not present in the phosphate buffer solutions in which the seeds were immersed.

The 48-hour-experiment yielded interesting observations. In the 1st hour of the seed swelling process observed was a significant increase in the number of germinated seeds treated with HA. The number of germinated seeds increased with the increase in the HA concentration. The results are shown in Fig.5.

In the cases in which the seeds were immersed in the 4th and 8th hour of the swelling process, over 90% of the seeds had germs, whatever the HA concentration. Effects of hour-long soaking of the seeds in phosphate buffer solutions with HA application significantly depended on the time of application. At the beginning of the swelling process, in an imbibition phase, anaerobic respiration (glycolysis) is predominant. Hence, observed was a different effect of HA on the swelling of seeds depending on the application time of HA [11]. Accelerating by HA water uptake may be due to their colloidal nature. More efficient imbibition accelerates hydrolysis of macromolecular compounds present in seeds. Simpler sugars, which are the products of hydrolysis of polysaccharides, are used as substrates of glycolysis. The increase in metabolic activity of the seeds in the first phase of their swelling provides better conditions for later water uptake.

Intensive water uptake in the beginning phase of swelling in connection with complete immersion of the seeds in solutions with humic acids application may cause the HA penetration into the seeds.

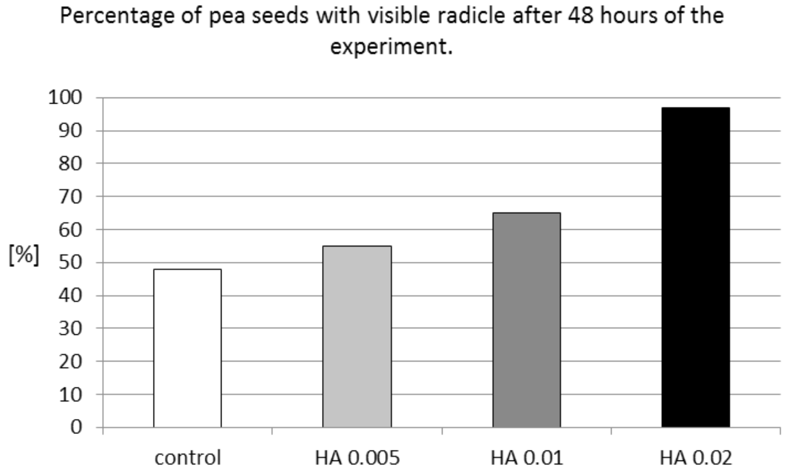

Fig. 5. Percentage of pea seeds with visible radicle after 48 hours of the experiment. Seeds were immersed in the 1st hour of the experiment in phosphate buffer of concentration $(1/15)\cdot 10^{-2}$ [mol dm^{-3}] and pH= 6.5 and in solution with HA application in which carbon concentrations were correspondingly 0.005 [gC dm^{-3}]; 0.01 [gC dm^{-3}] and 0.02 [gC dm^{-3}].

As early as in 1958, Gumińska [9] observed that humic substances influenced the process of swelling of seeds in anaerobic conditions. Humic substances enhance fermentation-anaerobic respiration. The presence of humic substances inside a plant and their influence on the plant metabolism are mentioned by many authors, such as [3, 13, 16]. However, their works discuss experiments on growing plants and not on the swelling of seeds. In later phases of swelling, aerobic respirations become predominant. The seeds enter the catabolic phase characterized by very high, in relation to imbibition, need for oxygen. In this phase, hydrolysis of storage substances and their further transformation are predominant. All the enzymatic processes occurring in the swelling seed are regulated by plant hormones. Gibberellins first activate the synthesis of hydrolytic enzymes. Later, gibberellins, cytokinins and auxins regulate biochemical processes occurring in swelling seeds by stimulation or inhibition of enzyme activity. In the anabolic phase, besides intensive biochemical processes, there is a strong growth of the embryo and, in effect, the germination. The last phase of swelling and the beginning of germination are characterized by high need for water that is collected through osmosis [11]. The seeds immersion in the 4th hour of the experiment, whatever the HA application, did not show any statistically significant differences between the seeds. It could result from limited (as the seeds were immersed only for one hour) availability of oxygen necessary for seeds at that time. Temporary reduction of the amount of available oxygen is not so very harmful when the embryo starts to grow.

The accelerating effect of HA on the process of seed swelling in the first phase of the experiment was due to the change of the protein-lipid structure that causes more intensive absorption of solutions from the environment and improves the transport of water through plant water channels. Changes in permeability of biological membranes under the influence of humic substances were noticed by [1, 2], among others. Cacco *et al*. [1] also recognized the influence of humic substances on changes in permeability of biological membranes that allows for better absorption of elements from solutions. Similar conclusions were reached by Russell *et al.* [15], who studied the HA influence on ion channels of biological membranes of plants. In further phases of germination, the acids might influence the biochemical processes occurring in seeds behaving analogously to plant hormones regarding the acceleration of the seeds' transition from the imbibition to the catabolic phase. It could explain the positive effect of HA on the speed of germination in the first phase of the experiment. HA properties similar to plant hormones were observed by Russell *et al*., who explained them by the presence of polyamines in the HA structure [15], and Young, who explained the properties by the presence of substances capable of interaction with markers of plant hormones [17].

Results obtained in the experiment show that HA has a statistically significant effect on the process of seed swelling when the seeds are soaked in the 1st hour of the experiment. This may be evidence of the HA effect on the permeability of the seed coat and thus on imbibition water uptake by pea seeds. It is likely that humic acids behave analogously to plant hormones - accelerating the transition of the process of swelling seeds into catabolic and anabolic phases. It was also observed that acceleration of the germination correlated with the increase in the HA concentration. HA application in the 4th and the 8th hour did not yield any statistically significant effects on the kinetics of swelling.

CONCLUSIONS

1. HA effect on seed swelling of *Pisum sativum* L. depends on application time and concentration of HA.

2. HA applied at the beginning phase of swelling (in the 1st hour of swelling) accelerate the process of swelling and germination.

3. Percentage of seeds with visible radicle after 48 hours of the experiment increases with the increase in HA concentration applied in the 1st hour of the experiment.

4. HA of concentration 0.02 [mgC dm^{-3}] double the number of seeds with visible radicle in relation to control after 48 hours of the experiment.

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WPŁYW KWASÓW HUMINOWYCH NA PROCES PĘCZNIENIA NASION GROCHU ODMIANY RAMROD (*PISUM SATIVUM* L.)

Badano wpływ kwasów huminowych (KH) rozcieńczonych w roztworach buforu fosforanowego o pH=6,5 na proces pęcznienia nasion grochu *Pisum sativum* L. Kwasy huminowe aplikowano zanurzając pęczniejące nasiona grochu w roztworach o stężeniach: 0,005 [gC dm^{-3}]; 0,01[gC dm^{-3}]; 0,02 [gC dm^{-3}] C_{KH} na jedną godzinę - w pierwszej, czwartej lub w ósmej godzinie trwania doświadczenia. Serie kontrolne stanowiły rośliny kiełkujące z nasion zanurzonych w identycznych roztworach buforu fosforanowego o pH=6,5 bez kwasów huminowych. Wpływ KH zależał zarówno od stężenia, jak i od czasu aplikacji. Najwyraźniej działały kwasy huminowe o stężeniu 0,02 [gC dm^{-3}] C_{KH} podawane w pierwszej godzinie pęcznienia nasion, zwiększając szybkość pęcznienia i kiełkowania nasion.