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ORIGINAL RESEARCH PAPER

# The impact of humic acid fractions on swelling and germination of 'Progres' and 'Nawiko' soybean seeds under salt and water deficit stresses

Andrzej Gawlik[1]*, Dorota Gołębiowska[2], Danuta Kulpa[2], Romualda Bejger[1], Renata Matuszak-Slamani[1], Mariola Sienkiewicz[1], Małgorzata Włodarczyk[3]

[1] Department of Physics and Agrophysics, West Pomeranian University of Technology, Szczecin, Papieża Pawła VI 3, 71-459 Szczecin, Poland
[2] Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Słowackiego 17, 71-434 Szczecin, Poland
[3] Department of General and Ecological Chemistry, West Pomeranian University of Technology, Szczecin, Słowackiego 17, 71-434 Szczecin, Poland

* Corresponding author. Email: andrzej.gawlik@zut.edu.pl

## Abstract

A laboratory research was carried out to examine the impact of humic acids (HA) on swelling and germination of 'Nawiko' and 'Progres' soybean seeds under salt stress (50 mM $dm^{-3}$ NaCl) and water deficit stress (−0.5 MPa) induced by polyethylene glycol (PEG) 6000. HA in the form of a dry preparation was obtained from peat using the IHSS method. Tests on swelling and germination used non-fractionate preparation (NFHA) and two of its molecular fractions obtained using Millipore filters with a 30 kDa cut-off point. This enabled us to obtain two fractions: with a higher molecular weight, above 30 kDa (HMHA), and a lower molecular weight, below 30 kDa (LMHA). The carbon concentration in HA solutions, used in all tests, was 0.005 g $C_{HA}$ $dm^{-3}$. The results showed that HA mitigate the negative impact of salinity and water deficit on swelling and germination of soybean seeds.

## Keywords

humic acids; stress factors; NaCl; PEG 6000; soybean seeds

## Introduction

The soybean is one of the most important crops. Its seeds are a valuable source of nutrients, since they contain 40% of proteins and 20% of fat [1,2]. In terms of area under cultivation, the soybean is ranked fourth in the world [3]. More recent epidemiological, animal, and in vitro studies show that soybean products reduce prostate, breast, and colon cancer risk due to the presence of bioactive substances such as: Bowman–Birk protease inhibitor, lunasin peptide, and isoflavones [4]. The soybean, just as other crops, is exposed to a number of abiotic and biotic stress factors. It is relatively sensitive to shortage of water [5–9] and salt stress, which may disturb the ion concentration in plants. Such factors may cause decay of plants or their slow growth, and consequently reduced yields. Germination is one of the most important growth stages in plants and stress factors prevent or delay the process [10–14]. At the same time, we know that humic substances (HS) stimulate plant growth and improve the yields of agricultural crops. High-molecular-weight HS can also act as root growth promoters via auxin-mediated pathways. HS may have practical application in agriculture as a source of new organic-mineral fertilizers and as inhibitors of plants stress [15–18].

Therefore, the aim of the present study was to assess the protective effect of humic acids (HA) on swelling and germination of soybean seeds under salt (50 mM $dm^{-3}$ NaCl) and water deficit stress (−0.5 MPa) induced by PEG 6000.

## Material and methods

### Biological material

Domestic soybean cultivars 'Progres' and 'Nawiko'. The cultivars were selected after preliminary in vitro tests on six domestic soybean cultivars. These cultivars differed in their response to the stress factors applied.

### Humic acids

Humic acids were extracted from low peat originating from the Babiogórski National Park using a method advised by IHSS [19], namely, a triple extraction of peat samples. The HA preparation, referred to as non-fractionate humic acids – NFHA, was then divided into two molecular fractions using 30 kDa cut-off point Millipore filters and an Amicon device. The molecular weight fraction below 30 kDa was marked LMHA and molecular weight fraction above 30 kDa was marked HMHA. The results of NFHA, LMHA, and HMHA elemental analysis are presented in Tab. 1.

### Germination and swelling of soybean seeds

Soybean seeds were selected for the experiments with particular attention given to the lack of any mechanical damage. Beans visibly bigger and smaller than the average were rejected.

Three experiments were performed using Petri dishes as germination plants. The first and the second dishes were used to test swelling, and the third one to test the germination speed. Each of the experiments included nine different solutions applied during swelling:

- control ($H_2O$ + 1/15 mol $dm^{-3}$ phosphate buffer pH = 7
- $H_2O$ + phosphate buffer + NaCl
- $H_2O$ + phosphate buffer + NaCl + NFHA
- $H_2O$ + phosphate buffer + NaCl + HMHA
- $H_2O$ + phosphate buffer + NaCl + LMHA
- $H_2O$ + phosphate buffer + PEG 6000
- $H_2O$ + phosphate buffer + PEG 6000 + NFHA
- $H_2O$ + phosphate buffer + PEG 6000 + HMHA
- $H_2O$ + phosphate buffer + PEG 6000+ LMHA).

**Tab. 1** Elemental analysis of humic acid preparations used in experiments.

| HA fractions | Elemental compositions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C H N (g 100 $g^{-1}$ HA) | | | | C H N (atomic %) | | | | atomic ratios | | | |
| | C | H | N | O | C | H | N | O | CQ* | C/N | H/C | O/C |
| NFHA | 44.46 | 4.18 | 2.94 | 48.42 | 30.94 | 42.04 | 1.75 | 25.27 | 1.13 | 17.64 | 1.36 | 0.82 |
| LMHA | 27.65 | 3.62 | 1.50 | 67.24 | 19.78 | 43.22 | 0.92 | 36.08 | 1.67 | 21.58 | 2.19 | 1.82 |
| HMHA | 51.53 | 5.09 | 3.28 | 40.12 | 35.58 | 41.71 | 1.94 | 20.77 | 1.04 | 18.35 | 1.17 | 0.58 |

* CQ – internal oxidation coefficient [$CQ$ = 4C / (4C + H + 3N − 2O)] by Kumada [20].

### Concentrations

The concentrations of stress factors were selected in such a manner so that they were not lethal for the plants during germination. NFHA, LMHA, and HMHA were dissolved in a phosphate buffer of pH = 7.0 and applied at a concentration of 0.005 $gC_{HA}$. $dm^{-3}$. In order to create salt stress, NaCl was applied in solutions of 50 mM $dm^{-3}$, whereas to create water deficit stress solutions corresponding to water potential (−0.5 MPa) were applied.

### Experiments

**Experiment 1.** At the beginning of the experiment, soybean seeds at their natural moisture level of 7% were weighted on electronic scales. Soybean seeds in groups of 25 were placed on Petri dishes (10 Petri dishes for each combination of the solutions used in the experiment). Inside each dish on three layers of filter paper (wetted with the relevant solution up to 100% of volume), special plexiglass inserts were placed with numbered "openings" for soybean seeds. This facilitated later statistical assessment of the obtained results. The base filter paper layers were replaced every 4 h. The experiment was conducted at ambient temperature of 20°C. The mass of soybean seeds was determined after 4, 8, 12, 16, 20, 24, 32, 42, and 64 hours of swelling. After the experiment, the number of germinated soybean seeds was counted.

**Experiment 2.** During this experiment, accelerated inhibition was instilled combined with oxygen deficiency [18] by placing beans for 1 h in one of the nine solutions containing stress factors and HA. Just as in Experiment 1, initially each soybean seed was weighted separately. Soybean seeds, in batches of 25, were placed in special plexiglass inserts with numbered "openings" for soybean seeds without filter paper and immersed in one of the solutions. There were 10 Petri dishes for each combination of the solutions used in the experiment. After 1 h, each soybean seed was weighted separately, and three layers of filter paper wetted with distilled water were placed on the bottom of germination plants. The base was first replaced after 3 h, and then every 4 h. The experiment was conducted at ambient temperature of 20°C. The mass of soybean seeds was determined after 1, 4, 8, 12, 16, 20, 24, 32, 40, and 56 hours of swelling. After completion, soybean seeds with visible radicle were counted.

**Experiment 3.** It was carried out to determine the statistical impact of HA and stress factors on the speed of germination. Both soybean cultivars, in the same amount as in the previous experiments, were put on filter paper wetted up to 100% of its volume with the relevant solutions. The base layer was replaced every 6 h. Beans with visible radicle were counted after 48, 60, 72, and 96 h of swelling.

### Statistical analysis

Statistical analysis was performed using Statistica 10.1 and homogenous groups were determined using Tukey's test at the significance level of $\alpha = 0.05$.

## Results

### Results of NFHA, HMHA, and LMHA elemental analysis

The data in the Tab. 1 are average values of three replicates. The maximum uncertainty of the results in the table does not exceed 1%.

The data presented in Tab. 1 indicate that LMHA have a much lower content of C, N, and H compared with HMHA and a significantly higher content of oxygen and internal oxidation in comparison with HMHA. This is due to the fact that the fraction with a molecular weight lower than 30 kDa contains more functional groups, especially carboxylic ones [20].

### Experiment 1

Tab. 2, Tab. 3, Fig. 1, and Fig. 2 present the results of the first experiment.

During the first experiment in which 'Progres' soybean seeds swelled on a base layer saturated with the solutions containing stress factors and HA, the results showed significant susceptibility of those soybean seeds to both stress factors. This was supported by a statistically significant drop of relative fresh mass after 12 and 16 h of swelling and in the final phase of the experiment after 42 and 64 h. Additional NFHA not only reduced the impact of water deficit in the early stage of swelling, but also caused a statistically significant increase in water uptake compared to the control sample. Moreover, it was determined that after 8 h of swelling under the salt stress, similarly to the water deficit stress, NFHA caused an increase in relative fresh mass of soybean seeds. In the case of salt stress, LMHA caused an increase in relative mass already after 4 h of swelling.

When analyzing the number of germinated soybean seeds expressed as percent of the control sample, we can see that 64 h into the experiment the presence of HA, in particular its HMHA, countervails the impact of water deficit stress and salt stress on the number of germinated 'Progres' soybean seeds.

### Experiment 2

Tab. 4, Tab. 5, Fig. 3, and Fig. 4 present the results of the second experiment.

In the case of preliminary immersion of 'Progres' soybean seeds for 1 h in the solutions containing the factors causing water deficit stress, no statistically significant changes of relative increase in fresh mass of germinating soybean seeds was noticed both with and without HA presence. As regards salt stress, a statistically significant decrease in fresh mass was observed after 8 h of swelling. The presence of non-fractionate HA strengthened the process.

The 1-h accelerated imbibition stress significantly influenced the percentage of germinated 'Progres' soybean seeds. The strongest impact was recorded in the control sample. The use of PEG 6000 and NaCl most probably prevented excessively rapid imbibition, and HA, especially its molecular weight fraction higher than 30 kDa, significantly leveled the additional accelerated imbibition.

### Experiment 3

Tab. 6–Tab. 9 present the results regarding the impact of stress factors and humic acids on the speed of germination of 'Progres' and 'Nawiko' soybean seeds.

The observation showed that 80% of 'Progres' soybean seeds in the control series had visible radicle already after 48 h of swelling on filter paper both with water deficit and salt stresses. The water deficit stress caused a significant slowdown of germination in comparison to the control series that could still be seen after 60 h of the experiment.

## Discussion

The analysis of swelling of 'Nawiko' soybean seeds, a cultivar which is more tolerant than 'Progres', showed that swelling soybean seeds responded to water deficit stress with a statistically significant reduction in their fresh mass as compared to the control sample. It was possible to countervail the reduction by adding LMHA after 12 h of swelling (Tab. 2 and Tab. 3). Eight hours into the experiment, the relative fresh mass of 'Nawiko' soybean seeds after 1 h of accelerated imbibition stress, additionally exposed

**Tab. 2** Changes in relative fresh mass of 'Progres' soybean seeds due to swelling expressed as percentage of control sample.

| Series | 4 h | 8 h | 12 h | 16 h | 20 h | 24 h | 32 h | 42 h | 64 h |
|---|---|---|---|---|---|---|---|---|---|
| Control | 126 $^{abc}$ | 165 $^{ab}$ | 206 $^{bc}$ | 230 $^{bc}$ | 245 $^{ab}$ | 247 $^{ab}$ | 250 $^{ab}$ | 257 $^{b}$ | 281 $^{d}$ |
| PEG 6000 | 119 $^{a}$ | 157 $^{a}$ | 178 $^{a}$ | 210 $^{a}$ | 228 $^{a}$ | 232 $^{a}$ | 235 $^{a}$ | 237 $^{a}$ | 243 $^{ab}$ |
| PEG 6000 + HMHA | 124 $^{ab}$ | 169 $^{ab}$ | 200 $^{b}$ | 229 $^{bc}$ | 236 $^{ab}$ | 236 $^{ab}$ | 237 $^{a}$ | 236 $^{a}$ | 241 $^{a}$ |
| PEG 6000 + LMHA | 129 $^{bc}$ | 179 $^{bcd}$ | 206 $^{bc}$ | 233 $^{bc}$ | 236 $^{ab}$ | 235 $^{a}$ | 236 $^{a}$ | 230 $^{a}$ | 245 $^{abc}$ |
| PEG 6000 + NMHA | 139 $^{de}$ | 186 $^{de}$ | 214 $^{bc}$ | 237 $^{bc}$ | 236 $^{ab}$ | 237 $^{ab}$ | 240 $^{ab}$ | 248 $^{ab}$ | 249 $^{abc}$ |
| NaCl | 130 $^{bc}$ | 170 $^{abc}$ | 217 $^{bc}$ | 236 $^{bc}$ | 247 $^{b}$ | 247 $^{ab}$ | 250 $^{ab}$ | 257 $^{b}$ | 276 $^{d}$ |
| NaCl+HMHA | 133 $^{cd}$ | 173 $^{bcd}$ | 210 $^{bc}$ | 226 $^{b}$ | 235 $^{ab}$ | 235 $^{a}$ | 238 $^{a}$ | 243 $^{ab}$ | 263 $^{bcd}$ |
| NaCl+LMHA | 143 $^{e}$ | 196 $^{e}$ | 223 $^{c}$ | 239 $^{bc}$ | 244 $^{ab}$ | 243 $^{ab}$ | 243 $^{ab}$ | 247 $^{ab}$ | 253 $^{abc}$ |
| NaCl+NFHA | 131 $^{bc}$ | 185 $^{cde}$ | 218 $^{c}$ | 245 $^{c}$ | 251 $^{b}$ | 252 $^{b}$ | 256 $^{b}$ | 257 $^{b}$ | 264 $^{cd}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$.

**Tab. 3** Changes in relative fresh mass of 'Nawiko' soybean seeds during swelling expressed as percentage of control sample.

| Series | 4 h | 8 h | 12 h | 16 h | 20 h | 24 h | 32 h | 42 h | 64 h |
|---|---|---|---|---|---|---|---|---|---|
| Control | 133 $^{abc}$ | 167 $^{bcd}$ | 217 $^{cd}$ | 234 $^{b}$ | 247 $^{b}$ | 252 $^{b}$ | 260 $^{b}$ | 259 $^{c}$ | 294 $^{c}$ |
| PEG 6000 | 117 $^{a}$ | 143 $^{a}$ | 174 $^{a}$ | 195 $^{a}$ | 222 $^{a}$ | 229 $^{a}$ | 233 $^{a}$ | 232 $^{ab}$ | 244 $^{a}$ |
| PEG 6000 + HMHA | 120 $^{a}$ | 155 $^{ab}$ | 200 $^{bc}$ | 220 $^{b}$ | 235 $^{ab}$ | 232 $^{ab}$ | 233 $^{a}$ | 233 $^{ab}$ | 240 $^{a}$ |
| PEG 6000 + LMHA | 128 $^{abc}$ | 159 $^{bc}$ | 194 $^{ab}$ | 221 $^{b}$ | 231 $^{ab}$ | 236 $^{ab}$ | 235 $^{a}$ | 234 $^{ab}$ | 240 $^{a}$ |
| PEG 6000 + NMHA | 125 $^{ab}$ | 157 $^{abc}$ | 194 $^{ab}$ | 220 $^{b}$ | 232 $^{ab}$ | 231 $^{ab}$ | 234 $^{a}$ | 227 $^{a}$ | 239 $^{a}$ |
| NaCl | 140 $^{bc}$ | 182 $^{d}$ | 221 $^{cd}$ | 233 $^{b}$ | 240 $^{ab}$ | 243 $^{ab}$ | 247 $^{ab}$ | 249 $^{bc}$ | 269 $^{b}$ |
| NaCl+HMHA | 145 $^{c}$ | 181 $^{d}$ | 223 $^{d}$ | 238 $^{b}$ | 246 $^{b}$ | 248 $^{ab}$ | 250 $^{ab}$ | 254 $^{bc}$ | 275 $^{bc}$ |
| NaCl+LMHA | 128 $^{abc}$ | 164 $^{bcd}$ | 217 $^{cd}$ | 232 $^{b}$ | 244 $^{b}$ | 245 $^{ab}$ | 251 $^{ab}$ | 253 $^{bc}$ | 268 $^{b}$ |
| NaCl+NFHA | 141 $^{bc}$ | 175 $^{cd}$ | 211 $^{bcd}$ | 233 $^{b}$ | 244 $^{b}$ | 249 $^{ab}$ | 248 $^{ab}$ | 250 $^{bc}$ | 269 $^{b}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

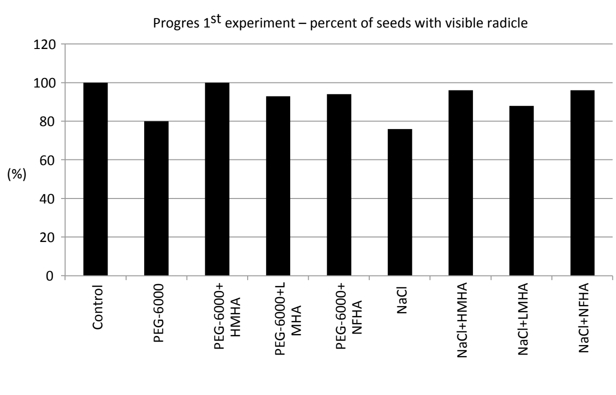


**Fig. 1** Percentage of 'Progres' soybean seeds with visible radicle after 64 h of germination on base layer wetted with solutions containing stress factors and HA.

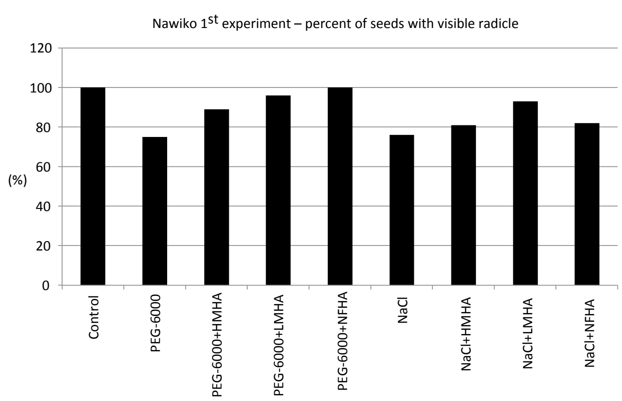


**Fig. 2** Percentage of 'Nawiko' soybean seeds with visible radicle after 64 h of germination on base layer wetted with solutions containing stress factors and HA.

**Tab. 4** Changes in relative fresh mass of 'Progres' soybean seeds during swelling of the 2nd experiment series expressed as percentage of control sample.

| Series | 1 h | 4 h | 8 h | 12 h | 16 h | 20 h | 24 h | 32 h | 40 h | 56 h |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 150 $^{a}$ | 180 $^{b}$ | 205 $^{c}$ | 221 $^{ab}$ | 241 $^{a}$ | 252 $^{a}$ | 251 $^{a}$ | 253 $^{abc}$ | 255 $^{ab}$ | 233 $^{a}$ |
| PEG 6000 | 150 $^{a}$ | 170 $^{ab}$ | 196 $^{bc}$ | 219 $^{ab}$ | 237 $^{a}$ | 244 $^{a}$ | 247 $^{a}$ | 246 $^{abc}$ | 247 $^{ab}$ | 245 $^{ab}$ |
| PEG 6000 + HMHA | 141 $^{a}$ | 164 $^{ab}$ | 189 $^{abc}$ | 220 $^{ab}$ | 228 $^{a}$ | 232 $^{a}$ | 235 $^{a}$ | 236 $^{a}$ | 238 $^{ab}$ | 236 $^{a}$ |
| PEG 6000 + LMHA | 151 $^{a}$ | 174 $^{ab}$ | 201 $^{bc}$ | 222 $^{ab}$ | 242 $^{a}$ | 249 $^{a}$ | 255 $^{a}$ | 263 $^{bc}$ | 261 $^{ab}$ | 262 $^{ab}$ |
| PEG 6000 + NMHA | 150 $^{a}$ | 175 $^{ab}$ | 197 $^{bc}$ | 221 $^{ab}$ | 239 $^{a}$ | 253 $^{a}$ | 257 $^{a}$ | 254 $^{abc}$ | 233 $^{a}$ | 233 $^{a}$ |
| NaCl | 145 $^{a}$ | 169 $^{ab}$ | 185 $^{ab}$ | 208 $^{ab}$ | 230 $^{a}$ | 243 $^{a}$ | 246 $^{a}$ | 242 $^{ab}$ | 251 $^{ab}$ | 234 $^{a}$ |
| NaCl+HMHA | 145 $^{a}$ | 179 $^{b}$ | 200 $^{bc}$ | 211 $^{ab}$ | 237 $^{a}$ | 246 $^{a}$ | 248 $^{a}$ | 249 $^{abc}$ | 258 $^{ab}$ | 252 $^{a}$ |
| NaCl+LMHA | 142 $^{a}$ | 165 $^{ab}$ | 206 $^{c}$ | 228 $^{b}$ | 243 $^{a}$ | 256 $^{a}$ | 257 $^{a}$ | 267 $^{c}$ | 262 $^{b}$ | 271 $^{b}$ |
| NaCl+NFHA | 141 $^{a}$ | 158 $^{a}$ | 174 $^{a}$ | 197 $^{a}$ | 225 $^{a}$ | 238 $^{a}$ | 246 $^{a}$ | 245 $^{abc}$ | 250 $^{ab}$ | 247 $^{ab}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

**Tab. 5** Changes in relative fresh mass of 'Nawiko' soybean seeds during swelling of the 2nd experiment series expressed as percentage of control sample.

| Series | 1 h | 4 h | 8 h | 12 h | 16 h | 20 h | 24 h | 32 h | 40 h | 56 h |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 152 $^{a}$ | 187 $^{b}$ | 215 $^{b}$ | 227 $^{a}$ | 240 $^{a}$ | 246 $^{a}$ | 243 $^{a}$ | 251 $^{a}$ | 251 $^{a}$ | 232 $^{a}$ |
| PEG 6000 | 149 $^{a}$ | 176 $^{ab}$ | 207 $^{a}$ | 227 $^{a}$ | 240 $^{a}$ | 244 $^{a}$ | 242 $^{a}$ | 246 $^{a}$ | 246 $^{a}$ | 244 $^{a}$ |
| PEG 6000 + HMHA | 171 $^{b}$ | 175 $^{ab}$ | 200 $^{ab}$ | 219 $^{a}$ | 233 $^{a}$ | 235 $^{a}$ | 241 $^{a}$ | 247 $^{a}$ | 245 $^{a}$ | 245 $^{a}$ |
| PEG 6000 + LMHA | 148 $^{a}$ | 182 $^{ab}$ | 217 $^{b}$ | 235 $^{a}$ | 239 $^{a}$ | 244 $^{a}$ | 241 $^{a}$ | 249 $^{a}$ | 251 $^{a}$ | 251 $^{a}$ |
| PEG 6000 + NMHA | 141 $^{a}$ | 164 $^{a}$ | 191 $^{ab}$ | 211 $^{a}$ | 229 $^{a}$ | 233 $^{a}$ | 235 $^{a}$ | 237 $^{a}$ | 238 $^{a}$ | 237 $^{a}$ |
| NaCl | 146 $^{a}$ | 174 $^{ab}$ | 200 $^{ab}$ | 224 $^{a}$ | 238 $^{a}$ | 245 $^{a}$ | 246 $^{a}$ | 248 $^{a}$ | 254 $^{a}$ | 239 $^{a}$ |
| NaCl+HMHA | 145 $^{a}$ | 173 $^{ab}$ | 202 $^{ab}$ | 231 $^{a}$ | 242 $^{a}$ | 250 $^{a}$ | 248 $^{a}$ | 252 $^{a}$ | 247 $^{a}$ | 252 $^{a}$ |
| NaCl+LMHA | 145 $^{a}$ | 171 $^{ab}$ | 205 $^{ab}$ | 233 $^{a}$ | 240 $^{a}$ | 244 $^{a}$ | 243 $^{a}$ | 250 $^{a}$ | 250 $^{a}$ | 253 $^{a}$ |
| NaCl+NFHA | 147 $^{a}$ | 178 $^{ab}$ | 199 $^{ab}$ | 219 $^{a}$ | 235 $^{a}$ | 244 $^{a}$ | 244 $^{a}$ | 246 $^{a}$ | 256 $^{a}$ | 251 $^{a}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

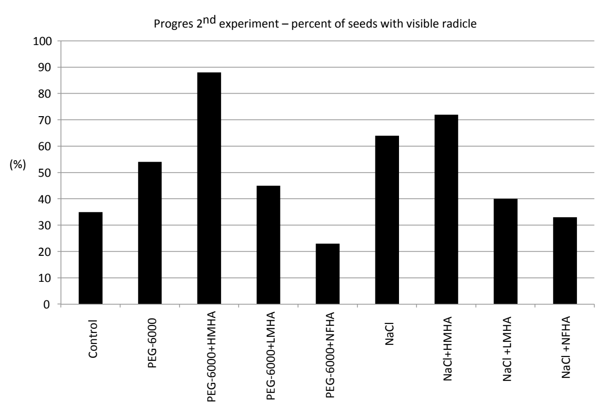


**Fig. 3** Percentage of 'Progres' soybean seeds with visible radicle after 56 h of germination in bath solutions containing stress factors and HA.

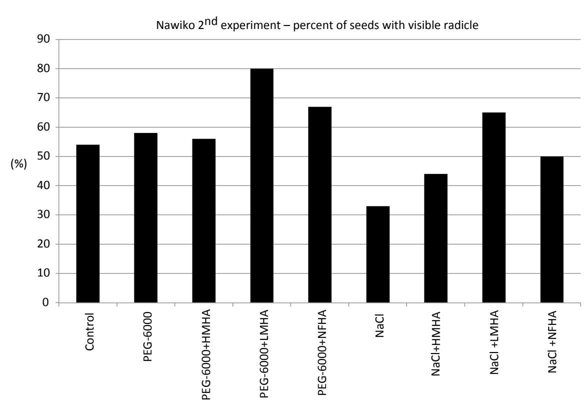


**Fig. 4** Percentage of 'Nawiko' soybean seeds with visible radicle after 56 h of germination in bath solutions containing stress factors and HA.

**Tab. 6** Percentage of germinated 'Progres' soybean seeds under water deficit stress after a certain duration of the experiment.

| Series | 48 h | 60 h | 72 h | 96 h |
|---|---|---|---|---|
| Control | 80.0 $^{b}$ | 91.2 $^{c}$ | 99.6 $^{c}$ | 99.6 $^{a}$ |
| PEG 6000 | 16.4 $^{a}$ | 42.8 $^{a}$ | 80.0 $^{a}$ | 98.4 $^{a}$ |
| PEG 6000 + HMHA | 21.6 $^{a}$ | 59.2 $^{b}$ | 96.8 $^{bc}$ | 100.0 $^{a}$ |
| PEG 6000 + LMHA | 16.0 $^{a}$ | 48.4 $^{ab}$ | 86.4 $^{ab}$ | 98.0 $^{a}$ |
| PEG 6000 + NMHA | 19.6 $^{a}$ | 50.8 $^{ab}$ | 96.4 $^{bc}$ | 100.0 $^{a}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

**Tab. 8** Percentage of germinated 'Nawiko' soybean seeds under water deficit stress after a certain duration of the experiment.

| Series | 48 h | 60 h | 72 h | 96 h |
|---|---|---|---|---|
| Control | 79.6 $^{b}$ | 91.2 $^{c}$ | 99.6 $^{b}$ | 99.6 $^{a}$ |
| PEG 6000 | 15.6 $^{a}$ | 41.6 $^{ab}$ | 82.8 $^{a}$ | 100.0 $^{a}$ |
| PEG 6000 + HMHA | 15.2 $^{a}$ | 52.4 $^{ab}$ | 94.0 $^{ab}$ | 99.6 $^{a}$ |
| PEG 6000 + LMHA | 9.6 $^{a}$ | 40.0 $^{a}$ | 83.6 $^{a}$ | 99.6 $^{a}$ |
| PEG 6000 + NMHA | 20.0 $^{a}$ | 55.6 $^{b}$ | 95.6 $^{ab}$ | 100.0 $^{a}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

**Tab. 7** Percentage of germinated 'Progres' soybean seeds under salt stress after a certain duration of the experiment.

| Series | 48 h | 60 h | 72 h | 96 h |
|---|---|---|---|---|
| Control | 80.0 $^{a}$ | 91.2 $^{b}$ | 99.6 $^{a}$ | 99.6 $^{a}$ |
| NaCl | 64.0 $^{a}$ | 78.4 $^{a}$ | 96.4 $^{a}$ | 96.4 $^{a}$ |
| NaCl+HMHA | 66.0 $^{a}$ | 80.4 $^{a}$ | 99.6 $^{a}$ | 99.6 $^{a}$ |
| NaCl+LMHA | 74.0 $^{a}$ | 90.4 $^{a}$ | 99.2 $^{a}$ | 99.2 $^{a}$ |
| NaCl+NFHA | 68.8 $^{a}$ | 84.8 $^{a}$ | 96.0 $^{a}$ | 96.0 $^{a}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

**Tab. 9** Percentage of germinated 'Nawiko' soybean seeds exposed to salt stress after a certain duration of the experiment.

| Series | 48 h | 60 h | 72 h | 96 h |
|---|---|---|---|---|
| Control | 79.6 $^{a}$ | 91.2 $^{b}$ | 99.6 $^{a}$ | 99.6 $^{a}$ |
| NaCl | 62.8 $^{a}$ | 76.8 $^{a}$ | 98.8 $^{a}$ | 98.8 $^{a}$ |
| NaCl+HMHA | 64.0 $^{a}$ | 78.4 $^{ab}$ | 100.0 $^{a}$ | 100.0 $^{a}$ |
| NaCl+LMHA | 70.4 $^{a}$ | 86.0 $^{ab}$ | 99.6 $^{a}$ | 99.6 $^{a}$ |
| NaCl+NFHA | 68.4 $^{a}$ | 84.8 $^{ab}$ | 99.6 $^{a}$ | 99.6 $^{a}$ |

Letters are used to indicate homogeneous groups determined by Tukey's test for $\alpha = 0.05$ .

to water deficit stress, showed a statistically significant reduction in comparison to the control sample (Tab. 4 and Tab. 5). The presence of HMHA in the solutions containing PEG 6000 caused a statistically significant increase in relative fresh mass of 'Nawiko' soybean seeds already during the first hour compared to the control sample. The presence of NFHA resulted in a similar effect after 4 h of swelling. In the initial phase of swelling, during imbibition anaerobic respiration prevails – glycolysis [21]. Increased water uptake may influence the speed of hydrolysis of high molecular compounds in soybean seeds and cause an increase in their fresh mass. The 1-h accelerated imbibition stress and salinity had the strongest influence on the percentage of germinated 'Nawiko' soybean seeds. The use of LMHA in combination with water deficit stress caused the number of germinated soybean seeds to reach 80%. The lower-molecular-weight fraction (below 30 kDa) had the strongest impact in the case of salt stress (65%). HA boosts anaerobic respiration during immersion of soybean seeds [22], protecting them against the negative impact of their total immersion in solutions, since soybean seeds had contact with HA only during the first hour. Various researchers mention the presence of humic substances inside a plant and HS impact on its metabolism [23–25]. During germination, all enzymatic processes are regulated by plant hormones, i.e., gibberellins, cytokinins, and auxins. Water deficit stress may lead to biochemical changes and the response of plant hormones [26]. HA properties similar to those of plant hormones were observed by Russell who explained the fact by the presence of polyamines in the HA structure [27] as well as by Young who referred to the presence of substances capable of reacting with markers detecting the presence of plant hormones [28]. The analysis of the impact of stress factors and humic acids on the speed of germination of 'Progres' and 'Nawiko' soybean seeds showed that the additional presence of the molecular weight fraction higher than 30 kDa was the most efficient in reducing the germination slowdown of 'Progres' soybean seeds caused by water deficit stress. The LMHA fraction exhibited the strongest impact in the case of salt stress. Already 48 h into the experiment, a positive influence of this fraction was observed (Fig. 1–Fig. 4, Tab. 6–Tab. 9).

As regards 'Nawiko', water deficit stress caused a significant reduction in germination speed. After 60 h into the experiment, the positive influence of non-fractionate HA was the strongest. For this particular type, statistically significant differences between the series with PEG 6000 were observed after 96 h. NaCl added to the solutions caused a relatively smaller interruption of germination than PEG 6000. Soybean seeds exposed to salt stress germinated much better after 48 h, and after 72 h there were no statistically significant differences comparing to the control series for both soybean cultivars. A reduced speed of germination in both soybean cultivars was noted by Michałek in 2002 [29], since the germination of soybean seeds was slower with the increase in PEG 6000 concentration.

## Summary

When compared with the initial assumptions, the results showed that concentrations of the selected stress factors differed between experiments and the plant reactions varied, however plants decay have not been noted. Humic acids modified swelling and germination of soybean seeds exposed to salt and water deficit stresses, and their action depended on the HA fraction used and soybean cultivar. Humic acids mitigated the unfavorable impact of stress factors, both in the case of NaCl and PEG 6000. Particularly interesting are the results of the second experiment. Besides, the results lead to a question why water deficit stress is mitigated by HMHA fraction and salt stress by LMHA. This requires further investigation.

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## Wpływ frakcji kwasów huminowych na proces pęcznienia i kiełkowanie nasion soi odmian 'Progres' i 'Nawiko' w warunkach stresu solnego i stresu spowodowanego deficytem wody


### Streszczenie

Przeprowadzono badania laboratoryjne nad wpływem kwasów huminowych (HA) na proces pęcznienia i kiełkowania nasion soi odmian 'Nawiko' i 'Progres' w warunkach stresu solnego (50 mM $dm^{-3}$ NaCl) i stresu spowodowanego deficytem wody (−0.5 MPa), w wyniku zastosowania glikolu polietylenowego (PEG) 6000. HA uzyskano z torfu metodą IHSS w postaci suchego preparatu. W doświadczeniach z pęcznieniem i kiełkowaniem użyto preparatu nierozfrakcjonowanego (HANF) oraz dwóch jego frakcji cząsteczkowych, uzyskanych za pomocą filtrów Milipore o punkcie odcięcia 30 kDa: frakcję o masach cząsteczkowych większych od 30 kDa – HHA oraz frakcję o masach mniejszych od 30 kDa – LHA. Stężenie węgla w roztworach HA, we wszystkich doświadczeniach, wynosiło 0.005 $gC_{KH}$ $dm^{-3}$. Otrzymane wyniki wskazują, że HA łagodzą negatywny wpływ stresu zasolenia i stresu spowodowanego deficytem wody na proces pęcznienia i kiełkowania nasion soi odmian 'Nawiko' i 'Progres'.


 