Obtaining plant growth biostimulants by hydrolysis of animal raw materials

M Baburina¹, V Nasonova¹ N Gorbunova¹ A Kurzova¹ and A Ivankin²

¹ V M Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia
² Bauman Moscow State Technical University, Department of Chemistry, Moscow, Russia

E-mail: baburina2005@yandex.ru

Abstract. This paper describes the process of obtaining and using complex biostimulants for plant growth, based on enzyme-mediated and acid-mediated hydrolysates of animal raw materials. Animal blood at slaughter was enzymatically hydrolyzed with a substance that contains up to 35% of free amino acids. The effect of biostimulants on development of agricultural crops and forest plants grown from seed after long dormancy was studied. The changes in enzymatic activity of the system were assessed. The prominent positive effect of the biostimulants on the rate of seed germination and formation of green biomass was established.

1. Introduction

The successful development of modern agriculture is largely based on use of chemicals that stimulate the growth and development of plants [1, 2]. Plant growth rate is influenced by various factors, including the influence of microorganisms that can suppress or stimulate the accelerated growth of plant cells [3]. The microbiota of the growth medium and accelerated growth of cell biomass often contributes to intensive development of a plant [4, 5, 6].

Eco-friendly agricultural production now means the issue of fertilizers and other chemicals is a most important factor. This is especially important when using plant growth regulators [7, 8]. Advances in chemical synthesis and the improvement of analytical control methods nowadays allow the use of very low concentrations of growth regulators, which increases the danger of their use from the ecological point of view [9, 10, 11].

Today, research is focused on production of small chemical molecules by hydrolysis of natural biopolymers, the structures of which contain minor
components valuable for biological plant cells [12, 13]. The aim of this work was to establish whether effectors based on animal raw material hydrolysates would have a stimulating effect on development of plant biomass.

2. Materials and Methods

Seeds of beans *Phaseolus vulgaris* (L.) Savi, curled mustard *Brassica juncea* L. and the slowly germinating seeds of Jack pine *Pinus banksiana* were used. Before germination the seeds were kept at 5-10°C for 1 month.

The beans were soaked in water media for 4-7 hours. The swollen seeds were placed on a moistened filter paper in bacteriological Petrie dishes and were germinated for 4 days in a glass box. Seeds of *Brassica juncea* L. were germinated on a filter paper for 3 hours with constant wetting and at the room temperature. The substrate under the seeds was periodically wetted. Sprouted seeds were removed from filter paper and transplanted into soil. After 5 days, the growth was assessed by measuring plant root parameters.

In the work, we utilized acid-mediated hydrolysates (AH) and enzyme-mediated hydrolysates (EH) obtained by us from animal protein [14]. The preparations were diluted with water at a ratio of 1:100.

EH and AH hydrolysates are minor free amino acids with admixed impurities of non-hydrolyzed proteins residues. EH is produced with the help of enzymes, and compared with AH, it usually features lower amounts of amino acids and a significant proportion of peptides and oligopeptides of medium and high molecular weight. AH is produced with the help of mineral acids, which result in a greater proportion of pure amino acids than in EH.

The content of amino acids in AH was as follows, %: Ile – 0.4; Leu – 3.6; Lys – 1.6; Met - 0.5; Cys – 0.6; Phe – 0.8; Tyr – 1.1; Trp – 1.3; Val – 1.4; Ala – 0.7; Arg – 2.8; Asp – 1.4; His – 1.3; Gly – 0.5; Glu – 7.3; Pro – 3.3; Ser – 0.7. Total = 30. In AH, up to 70% of short peptides with a molecular weight of 200-300 kDa were found.

In EH, the following free amino acids were found, %: Ile - 4.6; Leu 5.3; Lys 4.7; Met - 1.7; Cys 0.05; Phe 2.3; Tyr 5.1; Trp - 0.9; Val 5.3; Ala - 7.4; Arg 1.3; Asp - 21.6; His 12.5; Gly 9.9; Glu 2.3; Pro - 3.7; Ser - 1.5, total amount of amino acids – 95%.

The following substances were admixed into the stimulating biological products, g/100 g of concentrate: EH (or AH) – 5; amber acid – 0.3; urea – 3; sodium nitrates – 3; ammonium nitrates – 6; monosubstituted potassium phosphate – 3; magnesium sulfate – 4; ammonium sulfate – 2; manganese nitrate – 0.006; zinc nitrate – 0.6; copper sulfate – 0.5.
The moisture content of the seeds was determined by gravimetric measurement. Amylase was determined by a standard test method.

3. Results and Discussion

Table 1 shows quantitative determination of the standard amylase activity in the germinated seeds in media with added biostimulants.

| Time, hour | Control B. juncea | Control Pinus banksiana | Biostimulant AH B. juncea | Biostimulant AH Pinus banksiana | Biostimulant EH B. juncea | Biostimulant EH Pinus banksiana |
|------------|-------------------|--------------------------|---------------------------|---------------------------------|---------------------------|---------------------------------|
| 0          | 40                | 30                       | 90                        | 75                              | 205                       | 130                             |
| 24         | 70                | 45                       | 110                       | 80                              | 210                       | 150                             |
| 48         | 60                | 50                       | 105                       | 80                              | 215                       | 155                             |
| 72         | 75                | 60                       | 125                       | 87                              | 225                       | 168                             |

The values in Table 1 prove that amylolytic activity in the different systems was expressed in different ways. Maximum amylolytic activity was reached, as a rule, on the third day, and in seeds with biostimulants, this activity exceeded the activity in the control by around 10-15%. The introduction of biostimulants promoted rapid assimilation of nutrients by cells and provided a positive effect on plants’ development and growth. The AH-based biostimulant, with a noticeably high content of free amino acids, quickly activated amylolytic enzymes, which was registered by amylase activity. The use of the biostimulants correlated with the rate of seed germination. It was of scientific interest to assess the efficiency of amylase enzymes on development of the seeds under consideration.

Table 2. Proportion of germinated Phaseolus vulgaris (L.) Savi bean seeds in biostimulant medium, %

| Time, days | Control | Stimulant EH | Stimulant AH |
|------------|---------|--------------|--------------|
| 1          | 30      | 36           | 69           |
| 2          | 32      | 49           | 74           |
| 3          | 61      | 64           | 103          |

Table 2 shows the ratio of germinated seeds to the total number of seeds. From Table 2, it is obvious that a greater percentage of the seeds sprouted in the media with added stimulants, which contained high amounts of amino acids that were released during hydrolysate preparation. AH, based on acid
hydrolysate from animal raw materials, contained more than 90% free amino acids. Their ratio corresponds to those ratios that are specific in natural origin tissues and, therefore, AH is extremely favorable for development of plant cells.

EH had a less significant stimulating effect. EH contains many peptides that are not actively involved in cellular bio-exchange processes. The mass fraction of amino acids in EH amounted only to 30%, and therefore, the stimulating effect was less profound.

**Figure 1.** The development of beans in 4 days at 25 °C, humidity 85%, illumination rate 500 lux, in pure water (left) and in media with the biostimulant EH (right), after subsequent plant growth in soil for 5 days

Figure 1 shows the impact of the stimulant on bean shoot and root formation. The biostimulant EH induced powerful development of the plant root system, which is the key to further successful development of green biomass. The efficiency of AH biostimulant was also tested on hard-to-germinate forest plant seeds, Jack pine. These seeds are known for their poor rate of germination and survival rate. Usually, their germination rate does not exceed 45-60%. Pretreatment of seeds with AH and EH biostimulants resulted in a germination rate of more than 65%.

4. Conclusion

Complex biostimulants were developed for activation of plant cells. These biostimulants are based on enzyme-mediated and acid-mediated hydrolysates obtained from raw materials of animal origin. To increase the efficiency of the biostimulants, inorganic nutritional components were additionally included into the composition of the biostimulants. The impact of AH and EH biostimulants on the growth and development of beans *Phaseolus vulgaris* (L.) Savi, curled mustard *Brassica juncea* L. and Jack pine *Pinus banksiana* was studied. Positive effects of biostimulants on the
rate (percentage) of seed germination and the formation of green biomass were established, and these effects are apparently associated with the amino acids used.

References
[1] Welfle A 2017 Biomass Bioeng. 105 10
[2] Lima M F et al 2017 Biotech. Res. Innov. 1 1
[3] Selanon O et al 2014 Biocatal. Agric. Biotech. 3 4
[4] Laila K M and Elbordiny MM 2009 J. Appl. Sci. Res. 5 9
[5] Lodhi A et al 2013 Soil Env. 32 1
[6] Tunieva E K, Nasonova V V and Spiridonov K I 2019 Vsyo o Myase (Rus) 1 36
[7] Neklyudov A D, Fedotov G N and Ivankin A N 2008 Appl. Biochem. Microbiol. 44 1
[8] Mooij W M and Boersma M Ecol. Model. 93 1–3
[9] Pawlicki-Jullian N et al 2020 J. Courtois Res. Microbiol. 161 2
[10] Chalamaiah M, Yu W and Wu J 2018 Food Chem. 245 4
[11] Aguilar J G and Sato H H 2018 Food Res. Int. 103 1
[12] Taniguchi M et al 2017 Peptides 97 11
[13] Yakhin O, Lubyanov A and Yakhin I 2014 Agrochemistry 7 85
[14] Ivankin A, Vasilyev S, Baburina M et al 2018 Forestry Bulletin 22 5