Effect of Streptomyces Metabolites on Some Physiological Parameters of Maize Seeds

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Abstract. The influence of metabolites of actinomycetes on some physiological parameters of maize seeds of hybrid of Debut variety (roots formation, growth of coleoptiles) has been studied. The strains of actinomycetes genus Streptomyces were isolated from various soil samples of R.Moldova with different content of humus. The obtained data showed that the number of roots increased considerably under the influence of metabolites of Streptomyces sp.47, Streptomyces sp.123, Streptomyces sp.154 and Streptomyces sp.182 (19.4%, 16.6% and 13.8% respectively, compared with the control – water). The increasing by 33.0 and 44.8% of the main root in comparison with the control was determined after the treatment with metabolites of Streptomyces sp.11 and Streptomyces sp.123 strains. The metabolites of Streptomyces sp.154 and Streptomyces sp.182 strains led to an increasing of coleoptiles length on 31.3 and 38.1% respectively. The dry weight of the roots and coleoptiles exceeded the control on 30-80% and 25-75% respectively in experimental variants. Our research showed that the investigated streptomycetes strains are capable to synthesize the substances which stimulate roots forming and development of stems. In summary, the isolated from Moldavian soils strains of streptomycetes can be considered as potential producers of plant growth regulators.

Keyword: Streptomyces, metabolites, plant growth regulators.

INTRODUCTION

One of the perspective directions of the crop productivity management is increasing of plants’ viability and hardness by using natural assistants - soil and rhizosphere microorganisms (Kravchenko et al., 2002; Shirokih, 2007; Tihonovich et al., 2005).

Plant-associated microorganisms play an essential role in the plants’ development; they participate in supply of plants by nutrients, phytohormones, vitamins and other growth factors. Many plant-associated microorganisms are themselves capable of synthesizing compounds inhibiting a pathogenic microflora (toxins, antibiotics, siderofors), and also promote the occurrence in partner plants of the so-called induced resistance, helping with protection against phytopathogens (Cattelan et al., 1999; Shirokih, 2007).

Phytohormones play an important role as regulators of growth and development of plants. According to the conventional classification, there are five groups of phytohormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. Phytohormones contribute to the coordination of diverse physiological processes in plants, including the regulation of quiescence and seed germination, root formation, florescence, branching and tillering, and fruit ripening. They increase plant resistance to environmental factors and induce or suppress the expression of genes and the synthesis of enzymes, pigments, and metabolites metabolites (Boortseva et al., 2002, 2008; Maslobrod et al., 2008, 2010; Tsavkelova et al., 2006).

Actinomycetes make the forth part of all the soil and rhizosphere bacteria. Possessing a powerful enzymatic system and synthesizing a number of biologically active substances,
actinomycetes play an essential role in transformation of organic compounds and in maintenance of potential fertility of the soil (Zenova, 1992). The most common genus among this large group of bacteria is *Streptomyces*. This genus is found worldwide and is considered to play an important role in soil and plant ecology. These organisms have been widely investigated as agents of biological control of several plant diseases (Barreto et al., 2008). Besides the ability to synthesize substances with antimicrobial properties, *Streptomyces* produce growth-promoting metabolites such as auxins, gibberellins, cytokinins, ethylene, siderophores. For example Mansor et al. (1994) and Aldesuque et al. (1998) had found seven strains *Streptomyces alboviridis*, *S. griseoviridis*, *S. olivaceoviridis*, *S. rimosus*, *S. phaeochromogenes*, *S. rochei* and *Streptomyces* sp. No. 20, which possess comparatively high capacities for production of auxins, gibberellins and cytokinin-like substances together with substantial levels of α-amylase and protease.

*Streptomyces lydicus* WYEC108 is a root-colonizing actinomycete originally isolated and studied for its properties as an antifungal biocontrol agent. Recently, was demonstrated that strain WYEC108 is also a plant growth-promoting bacterium in the absence of fungal pathogen challenge. This may be due to the ability of strain WYEC108 to produce hydroxamate-type siderophores and/or other plant growth-promoting metabolites in the rhizosphere (Tokala et al., 2002). The researches made by Brazilian scientists had demonstrated that actinomycetes account for a higher percentage of the total population of culturable bacteria in soil than on cacao roots. *In vitro* cellulytotic, xilanolytic and chitinylotic activity, indolacetic acid production and phosphate solubilization activities were observed in most of the isolates tested (Barreto et al., 2008).

Some investigations in this domain were carried out at the Academy of Sciences of Moldova (the Institute of Microbiology and Biotechnology jointly with the Institute of Genetics and Plant Physiology). It was shown the effect of stimulation of seeds’ germination and of seedlings’ growth (rise of germinating ability of seeds, increasing of roots’ number, growth of stems’ and gain in roots’ length and weight) in the seeds of tomato, cucumber, tobacco, peas, beans, triticale after their treatment with the solutions of metabolites of *Streptomyces* (Boorteseva et al., 2002, 2006, 2008; Maslobrod et al., 2008, 2009).

Maize or corn is a cereal crop that is grown widely throughout the world in a range of agro-ecological environments (http://www.iita.org/maize). In the world wide agriculture, the maize ranks third for its cultivation area (over 147 million hectares) being preceded by wheat and rice (http://euroferma.md/?p=133#ixzz1zkqb5zWq). Drought and infectious diseases are main stress factors that cause a substantial reduction in harvest of this culture. Therefore the increase of resistance to drought and to phytopathogens has a special economic importance.

In the Republic of Moldova research aimed for increasing seeds’ germination capacity plays an essentially important part in these studies, as an effective germination determines a deeper penetration of emerging roots into the soil providing a stable water and nutrition supply for the plant. So the aim of our work was to investigate the possibility of using the *Streptomyces* metabolites isolated from the soils of R. Moldova in order to stimulate the process of germination of corn seeds.
MATERIALS AND METHODS

Maize seeds
A hybrid variety of maize Debuit created in the Institute of Genetics and Plant Physiology (Chalyk, 2003). It is characterized by high productivity and resistance to low and high temperatures.

Soil samples
Soil samples were collected at different locations of the central part of the Republic Moldova; mostly it was chernozem (black soil) of different types and with different content of humus.

Soil sample 1: was collected from the plot with monoculture of maize (based in 1947), humus 2.4 - 2.5 % (no fertilizers, herbicides and pesticides)
Soil sample 2: was collected from Poltava road border, humus 2.6%
Soil sample 3: was collected from ploughed field, water terrace of River Bic, humus 3.5%
Soil sample 4: was collected from vineyard, humus 3.3%
Soil sample 5: was collected from forest reserve, humus 6.8%

Samples were collected at random and brought to the laboratory under aseptic conditions and stored at 4°C for further analysis.

Streptomycetes strains and obtaining of cultural liquid
For our experiment the following strains were selected:
From Soil sample 1: Streptomyces sp. 11 and Streptomyces sp. 22
From Soil sample 2: Streptomyces sp. 47 and Streptomyces sp. 49
From Soil sample 3: Streptomyces sp.123
From Soil sample 4: Streptomyces sp.154
From Soil sample 5: Streptomyces sp.182

The streptomycetes strains were grown in 1 liter flasks with 200 ml of complex medium M-I (basic source of carbon was corn flour) on an agitator within 5 days at 27°C. Biomass has been separated from cultural liquid on a centrifuge (7000 rev/min. during 20 min.). Solution of metabolites was obtained by dilution of cultural liquid with distilled water 1:200. According to our previous data this dilution has the most positive effect on plant growth (Maslobrod et al., 2010).

Testing of biological activity of streptomycetes
The maize seeds were soaked in solution of metabolites during 24 h. As a control group we took a number of corn seeds soaked in distilled water. Then the seeds were put to germinate in Petri dishes with distilled water, in thermostat at 25°C during 4 days. We used 100 seeds for each experimental variant. The calculating of the seeds’ germination was performed on the third day. We measured the length of coleoptiles and roots (main and secondary roots), the number of roots, their total length, wet and dry weight of their roots and coleoptiles (Voznyakovskaya, 1989).

RESULTS AND DISCUSSIONS

The hybrid variety of maize Debuit is characterized by a high level of germination, so in our experiment there were no differences between control and experimental variants by the seeds’ germination parameter. The data on the effect of the metabolites of Streptomyces on the process of root formation are shown in Figure 1. The figure shows that the metabolites had different levels of stimulation of the growth of roots’ number compared with the control. For
example, the metabolites of *Streptomyces sp*. 47, *Streptomyces sp*. 123, *Streptomyces sp*. 154 and *Streptomyces sp*. 182 had a slight increasing effect on the roots’ number (5,5 – 8,3%), whereas under the influence of metabolites of strains from **Soil sample 1** and **2** (*Streptomyces sp*. 11, *Streptomyces sp*. 22 and *Streptomyces sp*. 49) the number of roots increased up to 13,8-19,4%, compared with the control group. Generally it should be noted that the number of roots parameter is decreasing with increasing of humus content in the soil. It may indicate that in soils rich in humus the biological activity of *Streptomyces* is reduced (Shirokih, 2007; Zenova, 1992). This nontrivial fact has been previously observed by us in the experiments with triticale seeds (Maslobrod et al., 2008)

![Fig. 1. The modification of roots’ number under influence of streptomycetes metabolites (% to the control)](image)

The length of main roots was considerably different also (Tab. 1). Thus if the maximal length of primary root in the control group was 20,0 mm, then in the experimental groups (*Streptomyces sp*. 49, *Streptomyces sp*. 123 and *Streptomyces sp*. 182) it was 21,0–24,5 mm. So the average length of the primary roots increased by 24,4-44,8 %.

The metabolites of strains of *Streptomyces* also stimulated the growth of coleoptiles (23,1–38,1 %). The maximal length of coleoptiles was observed at seeds soaked in the solution of cultural liquid with the strain *Streptomyces sp*. 154 (11,8 mm).

Table 1 shows that the length of roots had changed against the control as follows: metabolites of *Streptomyces sp*. 47 didn’t cause any changes, the metabolites of all of the other strains contributed to the increase in the roots’ length compared to the control by 9,7-2,8%. The best results were obtained after treatment the maize seeds with the solution of the metabolites of strain *Streptomyces sp*.123, their roots’ length increased 22,8% compared with the control.

The length of coleoptiles has grown compared with the control unequally, after the influence of the compounds synthesised by the studied strains (Tab. 1). Thus the least stimulating effect was found after the treatment of the seeds with the metabolites of strain *Streptomyces sp*. 123 (by 5,4%). The rest of the studied strains led to the increase of coleoptiles’ length by 9,4-31,3% in the experimental group compared with the control group.
Modification of length of maize seeds’ roots and coleoptiles after their proceeding with metabolites of *Streptomyces* isolated from soils of Moldova

<table>
<thead>
<tr>
<th>Soil sample</th>
<th>Nr. <em>Streptomyces</em> strain</th>
<th>Root length, mm</th>
<th>Max. root length, mm</th>
<th>Length of primary root, mm</th>
<th>Length of coleoptiles, mm</th>
<th>Max. coleoptiles length, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% to control</td>
<td>% to control</td>
<td>% to control</td>
<td>% to control</td>
<td>% to control</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>9.2</td>
<td>100.0</td>
<td>20.0</td>
<td>12.7</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>10.2</td>
<td>110.8</td>
<td>19.5</td>
<td>16.9</td>
<td>133.0</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>10.6</td>
<td>115.2</td>
<td>21.0</td>
<td>15.8</td>
<td>124.4</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>9.2</td>
<td>100.0</td>
<td>18.0</td>
<td>15.3</td>
<td>120.4</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>10.3</td>
<td>111.9</td>
<td>21.5</td>
<td>15.5</td>
<td>122.0</td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td>11.3</td>
<td>122.8</td>
<td>24.5</td>
<td>18.4</td>
<td>144.8</td>
</tr>
<tr>
<td>4</td>
<td>154</td>
<td>10.1</td>
<td>109.7</td>
<td>21.2</td>
<td>15.1</td>
<td>118.8</td>
</tr>
<tr>
<td>5</td>
<td>182</td>
<td>10.8</td>
<td>117.3</td>
<td>24.0</td>
<td>14.5</td>
<td>114.1</td>
</tr>
</tbody>
</table>

The best results were obtained after exposure of corn seeds to the metabolites of *Streptomyces* sp. 182, the length of coleoptiles increased by 38.1%.

The weight of coleoptiles has also changed. This parameter in the experimental variants was higher by 13.8-36.1% compared to the control. Maximal value was noted in the experimental group with *Streptomyces* sp. 182, it was 147.87% (weight of wet coleoptiles) and 175.0% (weight of dry coleoptiles) comparing with the control group (Tab. 2). The weight of wet roots also increased and exceeded the control rates by 27.5–55.0%, reaching the maximum value of 171, 42% after treatment with metabolites of *Streptomyces* sp. 49. The metabolites of this strain also promoted the increase in weight of dry roots by 80.0% compared with the control (Tab.2).

**Influence of metabolites of *Streptomyces* on weight of roots and coleoptiles of maize seeds**

<table>
<thead>
<tr>
<th>Soil sample</th>
<th>Nr. <em>Streptomyces</em> strain</th>
<th>Weight of roots</th>
<th>Weight of coleoptiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wet, g % to control Dry, g % to control</td>
<td>Wet, g % to control Dry, g % to control</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.91 100</td>
<td>0.10 100</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>1.39 152.74</td>
<td>0.15 150.0</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1.38 151.64</td>
<td>0.15 150.0</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>1.29 141.75</td>
<td>0.14 140.0</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>1.56 171.42</td>
<td>0.18 180.0</td>
</tr>
<tr>
<td>3</td>
<td>123</td>
<td>1.41 154.94</td>
<td>0.16 160.0</td>
</tr>
<tr>
<td>4</td>
<td>154</td>
<td>1.34 147.25</td>
<td>0.15 150.0</td>
</tr>
<tr>
<td>5</td>
<td>182</td>
<td>1.16 127.47</td>
<td>0.13 130.0</td>
</tr>
</tbody>
</table>

Our studies have shown that the investigated strains of soil streptomycetes synthesize substances that stimulate not only root formation, but also development of coleoptiles. Moreover we have revealed some strains possessing the ability to stimulate simultaneously roots formation and growth of coleoptiles. These are strains *Streptomyces* sp. 22, *Streptomyces* sp. 49, *Streptomyces* sp. 154, and *Streptomyces* sp. 182. Besides that, these strains cause an increase in length and weight of primary rootlets. Previously we have shown that some other *Streptomyces* strains from the same soil samples have the ability to stimulate
growth of triticale seeds. Therefore, on maize seeds, with the use of the new *Streptomyces* strains, it was shown once again the specific activity of *Streptomyces* strains from different soil samples on plant objects.

**CONCLUSIONS**

Thus, it was reviled that the metabolites of *Streptomyces* isolated from soils of Moldova significantly influence the process of seeds’ germination, which is reflected in growth stimulation of roots and coleoptiles, as well as in increasing of rootlets’ weight. This allows considering the studied strains of *Streptomyces* to be potential producers of growth regulators for plants. Biopreparations created on their basis can be successfully used in crop production.

**REFERENCES**

8. http://euroferma.md/?p=133#ixzz1zkqb5zWq


