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EFFECT OF STREPTOMYCES METABOLITES ON SOME PHYSIOLOGICAL PARAMETERS OF TRITICALE SEEDS

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Abstract

The influence of metabolites of actinomycetes on some physiological parameters of triticale seeds 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.42 (22.11%, compared with the control – water). The increasing by 36.25, 19.9 and 22.9% of the roots length compared to control was determined after the treatment with metabolites of Streptomyces sp.7, Streptomyces sp. 9 and Streptomyces sp.42 strains. The wet weight of the roots and coleoptiles exceeded 2-3 times and on 11.22-37.76% respectively the control values. Our research showed that the investigated Streptomyces 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.

Key words: *triticale, streptomycetes, metabolites, plant growth regulators.*

INTRODUCTION

Triticale is an alternative cereal grain that is a hybrid of wheat and rye. According to many researches, the triticale is a plant species with a high genetic potential for yield and favorable nutritive values so that's why it is considered a promising plant species (Biberdžić et al., 2012). 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).

Microorganisms interact with plants because plants offers a wide diversity of habitats including the phyllosphere (aerial plant part), the rhizosphere (zone of influence of the root system), and the endosphere (internal transport system) (Lynch, 1990, Lindow et al., 2002, Montesinos, 2003). Plant-associated microorganisms play essential roles in agricultural and food safety, and contribute to the environmental equilibrium (Montesinos, 2003). 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 them- selves 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).

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 Mansour *et al*. (1994) and Aldesuquy *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* cellulolytic, xilanolytic and chitinolytic 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., 2009).

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 *Streptomycetes* metabolites isolated from the soils of R. Moldova in order to stimulate the process of germination of triticale seeds.

MATERIALS AND METHODS

Triticale seeds. Were offered by the Institute of Genetics and Plant Physiology. Triticale has an excellent productivity potential and a greater flexibility to adapt to difficult agronomic conditions than wheat (Korver *et al*., 2004).

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 4^0C for further analysis.

Streptomycetes strains and obtaining of cultural liquid. For our experiment the following strains were selected:

From **Soil sample 1**: *Streptomyces* sp. 3, *Streptomyces* sp. 7 and *Streptomyces* sp. 9;

From **Soil sample2**: *Streptomyces* sp. 42, *Streptomyces* sp. 52, *Streptomyces* sp. 73 and *Streptomyces* sp. 120;

From **Soil sample 3**: *Streptomyces* sp.141, *Streptomyces* sp.145;

Soil sample 4:*Streptomyces* sp.155;

Soil sample 5: *Streptomyces* sp. 158, *Streptomyces* sp. 176, *Streptomyces* sp. 178, *Streptomyces* sp. 193.

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 27^0 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 triticale 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^{o}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 triticale seeds are 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 Table 1. The metabolites had different levels of stimulation of the growth of roots' number compared with the control. For example, the metabolites of *Streptomyces* sp. 73, *Streptomyces* sp. 120, *Streptomyces* sp. 141 had a slight increasing effect on the roots' number (3.21-8.74%), whereas under the influence of metabolites of strains *Streptomyces* sp. 7, *Streptomyces* sp. 9 and *Streptomyces* sp. 42 the number of roots increased up to 10.80-22.11%, compared with the control group.

The roots length was considerably different also (Table 1). Thus in the experimental groups the length of the roots exceeded the control length on 19.89 and 36.25% under influence of metabolites from **soil sample 1** (*Streptomyces sp.* 7 and *Streptomyces* sp. 9) and on 4.87, 17.89 and 22.98% under influence of metabolites from **soil sample 2** (*Streptomyces* sp. 42, *Streptomyces* sp. 73 and *Streptomyces* sp. 120).

A slight increasing effect on the roots' length occurs under influence of metabolites of *Streptomyces* sp. 73, *Streptomyces* sp. 141, *Streptomyces* sp. 145 and *Streptomyces* sp. 178 (4.87, 3.11, 8.03 and 5.03% respectively).

Maximal increasing of main root length were determined under influence of metabolites of *Streptomyces sp.* 145 – 8.96%.

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). The metabolites of strains of *Streptomyces* also stimulated the growth of coleoptiles (1.7-33.0%). The length of coleoptiles has grown compared with the control unequally, after the influence of the compounds synthesised by the studied strains (Figure 1). Thus the least stimulating effect was found after the treatment of the seeds with the metabolites of strain *Streptomyces* sp. 145 (by 1.7%). The rest of the studied strains led to the increase of coleoptiles' length by 11.3-33.0% in the experimental group compared with the control group. The maximal length of coleoptiles was observed at seeds soaked in the solution of cultural liquid with the strain *Streptomyces sp.* 52 (33% more, comparing to the control).

The weight of roots and coleoptiles has also changed. In the experimental variants the weight of wet roots was 2-3 times higher than in the control (Table 2). The best results were obtained at seeds soaked in the solution of cultural liquid of the strain *Streptomyces* sp. 9 (327.27% to the control), followed by *Streptomyces* sp. 7 (272.73%), *Streptomyces* sp. 42 (236.36%), *Streptomyces* sp. *52 (286.36%)* and *Streptomyces* sp. 120 (227.27%). The metabolites of *Streptomyces* sp. 73 increased the root wet weight on 54.55%. High values of dry root were determined at triticale seed after treatment with cultural liquid of *Streptomyces* sp. *7, Streptomyces* sp. *9, Streptomyces* sp. *42, Streptomyces* sp. 52 (11.22-29.60% comparing to the control group). A metabolites of the same strains promoted the weight increase of wet and dry coleoptiles. Maximal value was noted in the experimental group with *Streptomyces* sp. 9 (weight of wet coleoptiles increased up to 137.76%) and *Streptomyces* sp. 42 (weight of dry coleoptiles increased up to 187.50%) comparing with the control group.

Table 1. Modification of length of triticale seeds' roots after their proceeding with metabolites of streptomycetes isolated from soils of Moldova

Soil sample	Nr. *Streptomyces* strain	Nr. of roots, % to the control	Roots length, % to control	Length of main root, % to control
	Control, H_2O	100	100	100
1	3	94.60	82.42	76.30
	7	112.60	136.25	91.24
	9	110.80	119.89	102.57
2	42	122.11	122.98	105.37
	52	96.66	83.55	104.09
	73	102.83	104.87	89.95
	120	108.74	117.89	100.58
3	141	103.21	103.11	105.05
	145	100.92	108.03	108.96
4	155	90.37	77.14	78.76
5	158	87.84	92.23	101.03
	176	94.04	87.68	90.24
	178	98.85	105.03	104.94
	193	97.02	90.50	95.41

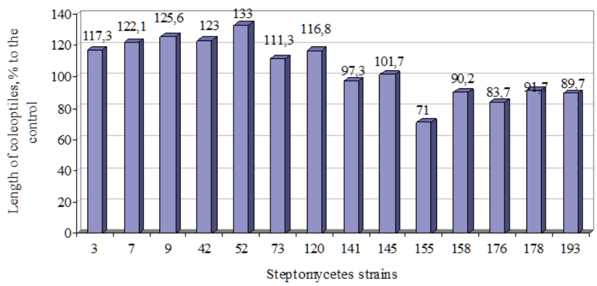

Figure 1. Modification of coleoptiles length after proceeding with metabolites of streptomycetes isolated from soils of Moldova

Table 2. Influence of metabolites of streptomycetes on weight of roots and coleoptiles of triticale seeds

Soil sample .	*Streptomyces* strain	Weight of roots, % to the control		Weight of coleoptiles, % to the control	
		Wet	Dry	Wet	Dry
1	3	54.55	69.15	96.94	62.5
	7	272.73	117.26	111.22	112.50
	9	327.27	134.09	137.76	100.0
2	42	236.36	122.01	129.60	187.50
	52	286.36	123.09	117.35	162.50
	73	154.55	98.17	95.92	70.0
	120	227.27	75.51	100.0	100.0
3	141	79.12	80.0	67.13	111.24
	145	82.72	92.75	104.9	104.9
4	155	76.92	86.43	53.14	81.16
5	158	80.22	83.39	74.83	105.45
	176	74.73	85.57	66.43	68.02
	178	82.72	109.13	85.31	98.26
	193	79.12	80.5	66.43	80.66

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.

7, *Streptomyces* sp. 9, and *Streptomyces* sp. 42. 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 maize seeds (Boortseva et al., 2006, Maslobrod et al., 2009, 2010). Therefore, on triticale 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

Aldesuquy H.S., Mansour F.A. and Abo-Hamed S.A., 1998. Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. F. Microbiol., 43(5), p. 465-470.

Barreto T.R., da Silva A.C.M., Soares A.C. and de Souza J.T., 2008. Population densities and genetic diversity of actinomycetes associated to the rhizosphere of the *Theobroma cacao.* Bras. J. Microbiol., 39, p. 464-470.

Biberdžić M., Maksimović G., Barać S., Jovović Z., 2012. Economic effects of triticale production on acid soils. Economics of Agriculture, 59(4), p. 579-587.

Boortseva S.A., 2002. Biologhicheski aktivnye veshchestva streptomitsetov (biosintez, svoystva, perspektivy primeneniya). Abstr. of a thesis, Kishinau.

Bortseva S.A., Postolaky O.M., Maslobrod S.N., Mihaylov M.A., Rotarenko V.A., 2006. Vliyanie plotnosti moshchnosti millimetrovogo izluchenya na biologicheskuyu aktivnost' *Streptomyces canosus* CNMN-71, otsenivaemuyu poparametram prorastaniya semyan gaploidnoy formy kukuruzy. In: Mat XV Int Simp "Netraditsionnoe rastenievodstvo. Aniologia. Akologiya i zdorov'e", Simferopol, p. 655-656.

Boortseva S.A., Syrbu T.F., Slanina V.A., Namolovan L.N. and Kodreanu C.N., 2008. Antimikrobnaya aktivnost' streptomitsetov pochvy tsentralinoy zony Moldovy. Ştiinţa agricolă, 1, p. 20-24.

Cattelan A.J., Hartel P.G., Fuhrmann J.J., 1999. Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci. Soc. Am. J., 63(6), p. l670-1680.

Korver D. R., Zuidhof M. J., Lawes K. R., 2004. Performance characteristics and economic comparison of broiler chickens fed wheat and triticale based diets. Poultry Science, 83, p. 716–725.

Kravchenko L.V., Azarov T.C., Leonova-Erko E.I., Shaposhnikov A.I., Makarova N.M. and Tihonovich I.A., 2002. Kornevye vydeleniya tomatov i ih vliyanie na rost i antifungal'nuyu aktivnost' shtammov *Pseudomonas*. J. Microbiol. 72(1), 48-53.

Lindow SE, Hecht-Poinar EI, Elliot VJ (eds), 2002. Phyllo-sphere microbiology. American Phytopathological Society, St.

Lynch JM., 1990. The rhizosphere. Wiley, New York.

Mansour F.A., Aldesuquy H.S., Hamedo H.A., 1994. Studies on plant growth regulators and enzymes production by some bacteria. Qatar Univ. Sci. 14(2), p. 281-288.

Maslobrod S.N., Boortseva S.A., Kalkay E.D., Postolaky O.M., Bratuhina A.A., 2009. Как âxometabolity streptomytseta, obrabotannogo millimetrovymi volnami vliyayut na vshojestŢ semyan i bioizomeriyu seyantsev tabaka. Mat. 15 Simp. "Миллиметровые волны в медицине и биологии" Moscow, p. 259-263.

Maslobrod S.N., Korlatyanu L.B. and Ganya A.I., 2010. Vliyanie millimetrovogo izluchenya na jiznesposobnost' rasteniy. 1. Izmerenie metabolizma semyan pri vozdeystvii faktora na suhie semena. Âlektronnaya obrabotka materialov, 5, p. 93-105.

Montensinos E., 2003. Plant-associated microorganisms: a view from the scope of microbiology. Int. Microbiol, 6, p. 221-223.

Tihonovich I.A., Borisov A.Yu., Tsyganov V.E., Ovtsyna A.O., Dolgih E.A., Provorov N.A., 2005. Integratsiya geneticheskih sistem rasteniy i mikroorganizmov pri simbioze. Uspehi sovremennoy mikrobiologii, 125(3), p. 227-238.

Tokala R.K., Strap J.L., Jung C.M., Crawford D.L., Salove M.H., Deobald L., Bailey J.F., Morra M.J., 2002. Novel plant-microbe rhizosphere interaction involving Streptomyces lydicus WYEC108 and the pea plant (*Pisum sativum*). Appl. and Env. Microbiol., p. 2161-2171.

Shirokih A.A., 2007. Izuchenie mikrobnogo potentsiala fitosfery rasteniy dlya ispol'zovaniya v sel'skohozyaystvennoy biotehnologii. Moscow, PhD Diss. Abstr.

Voznyakovskaya Yu. M., 1989. Predposevnaya stimulyatsiya semyan mikroorganizmami-produtsentami vitaminov. Mat Conf "Mikroorganizmy – stimulyatory I ingibitory rosta rasteniy I jivotnyh". Tashkent, 1, p. 67.

Zenova G.M., 1992. Pochvennye aktinomitsety. Moscow: MGU Press, p. 78.