DETECTION OF ARBUSCULAR MYCORRHIZAL FUNGI
IN THE ROOTS OF STRAWBERRY PLANTS FERTILIZED
WITH ORGANIC BIOPRODUCTS

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Summary

In organic farming, mineral fertilizers are replaced by various preparations to stimulate plant growth and development. Introduction of new biopreparations into horticultural production requires an assessment of their effects on the growth and yielding of plants. Among the important indicators of the impact on plants of beneficial microorganisms contained in bioproducts is determination of their effectiveness in stimulating the growth and yielding of plants. Moreover, confirmation of the presence of arbuscular mycorrhizal (AM) fungi in the roots and plant growth promoting rhizobacteria (PGPR) in the rhizosphere is also necessary. In addition to conventional methods, molecular biology techniques are increasingly used to allow detection and identification of AM fungi in plant roots. The aim of this study was identification and initial taxonomic classification of AM fungi in the roots of ‘Elkat’ strawberry plants fertilized with various biopreparations using the technique of nested PCR.

Tests were performed on DNA obtained from the roots of ‘Elkat’ strawberry plants: not fertilized, treated with 10 different biopreparations, or fertilized with NPK. Amplification of the large subunit of ribosomal gene (LSU rDNA) was carried out using universal primers, and then, in the nested PCR reaction, primers specific for the fungi of the genera Glomus, Acaulospora, and Scutellospora were used. Colonization of strawberry roots by arbuscular mycorrhizal fungi was determined on the basis of the presence of DNA fragments of a size corresponding to the types of the fungi tested for.

As a result of the analyses, the most reaction products characterizing AM fungi were found in the roots of plants treated with the preparation Florovit Eko. The least fragments characteristic of AM fungi were detected in the roots of plants fertilized with NPK, which confirms the negative impact of mineral fertilizers on the occurrence of mycorrhizal fungi in the roots of strawberry plants. The roots of plants fertilized with Tytanit differed from the control plants by the presence of one of the clusters of fungi of the genus Glomus and by the absence of a cluster of fungi of the genus Scutellospora. In the roots of plants treated with other biopreparations there were reaction products indicating

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the presence of fungi of the genera *Glomus*, *Scutellospora* and *Acaulospora*, like in the roots of the control plants. The results will be used to assess the suitability of microbiologically enriched biopreparations in horticultural production.

Key words: arbuscular mycorrhizal fungi, strawberry, nested PCR, bioproducts

**INTRODUCTION**

In organic farming, mineral fertilizers are replaced by various preparations to stimulate plant growth and development. Demand for bioproducts in Poland is a result of existent organic production of horticultural crops. In the years 2008-2010 there was an increase in the area of organic vegetable plantations. The area of organic fruit crops plantations, however, has not undergone any significant changes with the exception of raspberry, whose cultivations area doubled in this period (Szymona 2011). Bioproducts are made from natural plant extracts, plant biomass, brown coal, and bacterial and mycorrhizal substrates. Organic waste is also used in the production of biopreparations for use in organic farming, such as ‘Vinassa’, a Polish bioproduct based on the waste from the production of baker’s yeast. In the cultivation of strawberry, bioproducts are used as organic fertilizers (Sas Paszt *et al.* 2011). In addition, bioproducts containing microorganisms that are antagonistic towards plant pathogens and plant extracts with curative action help prevent replantation disease in strawberry cultivation (Zhang *et al.* 2008).

An important indicator of the impact of bioproducts on plants is the extent of colonization of plant roots by arbuscular mycorrhizal (AM) fungi. Mycorrhizal fungi assist plants in the uptake of minerals (P, N, Mg, Zn, Cu, Mn), and have a positive effect on the growth and development of plants by increasing their resistance to biotic stresses (pathogens) and abiotic stresses (cold, salinity, drought) (Newsham *et al.* 1995). AM fungi have a positive effect on root longevity, limit the colonization of roots by pathogenic fungi (of the genus *Phytophthora*), increase lignification of plant cell walls, which protects the roots against infection by pathogens (Harrier & Watson 2004). It has been found that the use of mineral fertilizers and chemical agents to disinfect the soil restricts or inhibits the colonization of strawberry roots by mycorrhizal fungi (Sas Paszt *et al.* 2011, Harrier & Watson 2004, Mark & Cassells 1999). The aim of using bioproducts is to improve the nutritional status of plants in terms of mineral elements by, for example, stimulating the colonization of plant roots by AM fungi and reducing the use of chemical means of production.

AM fungi can be identified on the basis of the morphological features of spores (Błaszkowski 2003). Correct identification by this method can be difficult because the production of spores by AM fungi may depend on environmental conditions (Redecker *et al.* 2003). The increas-
ingly common molecular biology techniques make it possible to detect and identify AM fungi in plant roots without the necessity of obtaining spores of AM fungi from the soil. Molecular analyses are used, for example, to assess variations in the occurrence of AM fungi in crop plants grown organically and conventionally (Galván et al. 2009).

Identification of AM fungi with molecular techniques is based on the amplification of various ribosomal gene fragments: the small subunit (SSU) of rDNA (Lee et al. 2008, Schwarzott & Schüßler 2001), the large subunit (LSU) of rDNA (Kjoller & Rosendahl 2000), the ITS (internal transcribed spacer) regions (Redecker 2000, Renker et al. 2003), and fragments combining SSU, LSU and ITS (Krüger et al. 2009, Ryszka et al. 2010). Analysis of LSU rDNA has allowed the development of specific primers, the application of which makes it possible to obtain a DNA fragment size from 264 to 306 bp, characteristic of the genera *Glomus*, *Scutellospora* and *Acaulospora* (Gollotte et al. 2004). The use of these primers has allowed detection of AM fungi in the roots of the grasses *Agrostis capillris* and *Lolium perenne* (Gollotte et al. 2004). The aim of this study was identification and preliminary taxonomic classification of AM fungi in the roots of ‘Elkat’ strawberry plants fertilized with various biopreparations.

### MATERIALS AND METHODS

#### Plant material

For the tests, roots were taken from ‘Elkat’ strawberry plants that came from the experiment on the effects of biostimulators on the growth of plants grown in rhizoboxes in a greenhouse. The control in the greenhouse experiment was represented by non-fertilized plants (Control 0). In addition, strawberry plants were fertilized with NPK, manure and biostimulators: Micosat, Humus UP, Humus Active, BF Quality, BF Amin, Tytanit, Vinassa, Florovit and Florovit Eko (Sas Paszt et al. 2011). In the molecular studies, the control were the leaves of ‘Elkat’ strawberry plants.

The following treatments were applied (Sas Paszt et al. 2011):

1. Control (no-treatment) - unfertilized podsolic soil, containing: 0.48% C, 0.1% N, 0.2% P and 1.4% K.
2. Standard NPK soil fertilization: 4 g NH₄NO₃∙plant⁻¹, 3 g triple super-phosphate∙plant⁻¹ and 6 g K₂SO₄ ∙plant⁻¹. The soil fertilized with NPK contained: 0.44% C, 2.4% N, 3.8% P and 8.5% K.
3. Dry granulated bovine manure, suitable for organic farming (Doktor O’grodnik), containing: 55% C, 1% N, 0.3% P and 1% K; the product contains also micro-elements and soil micro-organisms. It was applied to the soil, near the root system (1 g∙plant⁻¹), at planting.
4. Micosat (CCS Aosta s.r.l.) – a mixture of beneficial soil fungi and bacteria containing: spores, hyphae and root fragments colonized by five species of AM fungi: *Glomus mosseae* Taxtersensu Gerd. &

The product contains 40% C, 0.15% N, 431 mg·kg⁻¹ P and 9558 mg·kg⁻¹ K. It was applied to the soil, near the root system (10 g·plant⁻¹), at planting. The plants treated with the microbial inoculum received before planting basic soil fertilization (0.5 g·plant⁻¹) with dry manure (containing 1% N, 0.3% P and 1% K).

5. Humus UP (Ekodarpol) – an extract from a vermicompost containing 0.65% C, 0.03% N, 30.8 mg·kg⁻¹ P and 4535 mg·kg⁻¹ K. The product was first applied to the soil at planting as a 2% solution (15 ml·plant⁻¹) and then three times during the growing period (1% solution, 15 ml·plant⁻¹).

6. Humus Active + Aktywit PM (Ekodarpol) – Humus Active is a soil improver with active humus and a population of beneficial microorganisms containing 0.78% C, 0.03% N, 1050 mg·kg⁻¹ P and 4119 mg·kg⁻¹ K. Aktywit PM is a soil improver containing 20.5% C, 0.92% N, 81.2 mg·kg⁻¹ P and 42990 mg·kg⁻¹ K. The products were first applied to the soil at planting as a 2% solution (15 ml·plant⁻¹) and then three times during the growing period (1% solution, 15 ml·plant⁻¹).

7. BioFeed Quality (Agrobio Products B.V.) - an extract from several seaweed species reinforced with humic and fulvic acids containing 0.6% C, 0.07% N, 32.6 mg·kg⁻¹ P. The product was applied to the plants five times during the growing period as a 0.5% solution (25 ml·plant⁻¹). The plants treated with the seaweed extract received before planting basic soil fertilization (0.5 g·plant⁻¹) with dry manure (containing 1% N, 0.3% P and 1% K).

8. BioFeed Amin (Agrobio Products B.V.) - an extract of 100% vegetal amino-acids containing 1.12% C, 0.14% N, 347 mg·kg⁻¹ P. The product was applied to the plants five times during the growing period as a 0.5% solution (25 ml·plant⁻¹). The plants treated with amino-acids received before planting basic soil fertilization (0.5 g·plant⁻¹) with dry manure (containing 1% N, 0.3% P and 1% K).

9. Tytanit (Intermag) – titanium (Ti) 0.8% (5 g Ti in 1 l of working solution), pH 3.40, containing 3163 mg·kg⁻¹ Ti. The product was applied to the plants five times during the growing period as a 0.5% solution (5 ml·plant⁻¹). The plants treated with this bioproduct received before planting basic soil fertilization (0.5 g·plant⁻¹) with dry manure (containing 1% N, 0.3% P and 1% K).

10. Vinassa – molasses residue from yeast production containing 12.0% C, 1.86% N, 949 mg·kg⁻¹ P, 17615 mg·kg⁻¹ K. The product was applied to the plants five times during the growing period as a 0.5% solution (50 ml·plant⁻¹). The plants treated with this
product received before planting, basic soil fertilization (0.5 g·plant\(^{-1}\)) with dry manure (containing 1% N, 0.3% P and 1% K).

11. Florovit Pro Nature contains lignite, urea, potassium sulfate, ammonium phosphate, dolomite, and molasses. The biopreparation was used in an amount of 2 g per rhizobox.

12. Florovit Eko contains lignite, potassium sulfate, phosphorite, dolomite, bentonite and molasses. The biopreparation was used in an amount of 2 g per rhizobox.

DNA extraction

Root samples were collected from the plants growing in rhizoboxes. For each treatment, the samples were taken from 10 plants growing in 5 rhizoboxes, and then a mixed sample containing 5 g of the roots was prepared. After sampling, the roots were rinsed with water and then frozen at -80°C. DNA was isolated from samples constituting 100 g of the roots or leaves using a commercial Plant and Fungi DNA Purification Kit (EURx). DNA concentration was determined spectrophotometrically at a wavelength of 260 nm. For further analyses, DNA dilutions of 10 ng·μl\(^{-1}\) were prepared.

PCR conditions

Amplification of a fragment of the large subunit ribosomal gene (LSU rDNA) was performed in two stages. The first PCR was carried out with the primers LSU 0061 (5' acgatatacaatgcgagga 3') and LSU 0599 (5' tgttcgctttcaagacg 3') (Kjoller & Rosendahl 2000). The reactions were carried out in 20 μl of a mixture containing 1x PCR buffer, 0.2 mM of each dNTP, 0.4 μM of forward and reverse primer, 1.25 U of Taq polymerase (Dream Taq, Fermentas), and 20 ng of DNA.

Nested PCR was carried out with the primers specific for mycorrhizal fungi. The primers used were suitable for amplification of the LSU rDNA of fungi from the class Glomeromycetes. Reactions were performed with the primer FLR4 (5’- tctggaagccctg 3’) in combination with 9 single primers, the use of which makes it possible to obtain DNA fragments of a specific size that correspond to the groups of fungi within the genera Glomus, Acaulospora and Scutellospora (Gollotte et al. 2004). These primers have the following sequences: Glomus mosseae: 5’aaagccttcggatgctg3’, Glomus 2: 5’cttctggaagccctg 3’, Glomus 3: 5’gagegtcgaggttaaaccgc3’, Glomus 4: 5’tcctatttgcaattttac3’, Glomus 5: 5’gccttcgcttgctgtaaa3’; Acaulospora- ceae 1: 5’caacatggagttctgctt3’, Acaulospora 2: 5’tgttcccgccggaga- tctg3’, Acaulospora 3: 5’tctgctgaggtact- tctg3’, Scutellospora 1: 5’gaacctactacctgactgac 3’, Scutellospora 2: 5’agggaaactgaggcact3’. The reaction mixture in the amount of 20 μl contained 1x PCR buffer, 0.2 mM of each dNTP, 0.4 μM of each primer, 0.5 U of Taq polymerase (Dream Taq, Fermentas), and 2 μl of DNA from the first stage of PCR at a dilution of 1:100.

The reactions were carried out in a DNA thermocycler (DNA Engine Dyad, Bio-Rad) in the first stage of PCR in 35 cycles (94°C/1 min., 55°C/1 min., 72°C/1 min.), and the nested PCR in 25 cycles (93°C/1 min.,
55°C/1 min., 72°C/1 min. + 4 s for each cycle). The reaction products were separated in 2% agarose gel, stained in ethidium bromide and visualized under UV light.

After generating electropherograms, the length of the obtained fragments was determined from the 1 kb or 50 bp DNA Ladder (Fermentas). The results formed the basis for establishing in the tested samples of roots the presence (+) or absence (–) of DNA fragments characteristic of the fungi of the genera *Glomus*, *Acaulospora* and *Scutellospora*.

RESULTS AND DISCUSSION

As a result of PCR with the primers LSU 0061 and LSU 0599, a 650 bp product was obtained in all the samples of the roots (Table 1). The least products characteristic of mycorrhizal fungi were observed in the samples of roots of the plants fertilized with NPK. In those samples, there were no reaction products corresponding to the fungi *Glomus mosseae*, *Glomus* cluster 2, and *Scutellospora* cluster 1, which were present in the other samples. However, in the roots of plants fertilized with NPK there were fungi from *Glomus* cluster 4, *Acaulospora* cluster 3, and *Scutellospora* cluster 2. The most reaction products characteristic of mycorrhizal fungi were found in the roots of plants fertilized with the biopreparation Florovit Eko. In those roots there were present fungi from *Glomus* clusters 1, 2, 4 and 5, *Scutellospora* clusters 1 and 2, and *Acaulospora* cluster 3.

Table 1. Results of nested PCR with primers amplifying LSU rDNA of fungi of the genera *Glomus*, *Acaulospora* and *Scutellospora* obtained for DNA from the roots of ‘Elkat’ strawberry plants. (+) presence, (–) absence of reaction products

<table>
<thead>
<tr>
<th>Primers</th>
<th>Length of DNA fragment (bp)</th>
<th>Control 0</th>
<th>Control NPK</th>
<th>Manure</th>
<th>Micosat</th>
<th>Humus UP</th>
<th>Humus Active</th>
<th>BF Quality</th>
<th>BF Amin</th>
<th>Tytanit</th>
<th>Vinassa</th>
<th>Florovit Pro Nature</th>
<th>Florovit Eco</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSU 0061/LSU 0599</td>
<td>650</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FLR4/G. mosseae</td>
<td>290</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>FLR4/Glotamus 2</td>
<td>300</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>FLR4/Glotamus 4</td>
<td>310</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>FLR4/Glotamus 5</td>
<td>290</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FLR4/Acaulosporaceae1</td>
<td>280</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>FLR4/Acaulospora2</td>
<td>280</td>
<td>–</td>
<td>–</td>
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<tr>
<td>FLR4/Acaulospora3</td>
<td>270</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FLR4/Scutellospora1</td>
<td>270</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FLR4/Scutellospora2</td>
<td>260</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>
Among the biopreparations used, only Florovit Eko increased the number of clusters of the mycorrhizal fungi colonizing the roots of strawberry plants. After applying Tytanit, fungi from *Glomus* cluster 5, which were absent in the control plants, were seen to proliferate. At the same time, the roots of plants fertilized with Tytanit did not contain *Scutellospora* cluster 1 fungi identified in the roots of the control plants. The presence of AM fungi in the roots of plants fertilized with the other biopreparations did not differ from their presence in the roots of the control plants.

In the roots of ‘Elkat’ strawberry plants, the most commonly occurring fungi were *Glomus* cluster 4, *Acaulospora* cluster 3, and *Scutellospora* cluster 2. The presence of these fungi was observed in all the tested samples of the roots. *Glomus* cluster 5 fungi were found only in the roots of plants fertilized with Tytanit and Florovit Eko. None of the tested samples was found to contain *Acaulospora* cluster 1 or *Acaulospora* cluster 2 fungi.

The method presented in this study had also been used for the detection of mycorrhizal fungi in the roots of strawberry cultivars ‘Elsanta’ and ‘Honeoye’ (Lisek et al. 2011). As in the cultivar ‘Elkat’, the most frequently occurring fungi were *Glomus* cluster 4 and *Acaulospora* cluster 3. *Glomus* cluster 5 fungi were found only in the cultivar ‘Honeoye’ after applying only some of the biopreparations. Moreover, *Acaulospora* cluster 1 and 2 fungi, like in the cultivar ‘Elkat’, were not seen in either of the two cultivars. Fungi of *Glomus mosseae* and *Glomus* cluster 2, and *Scutellospora* cluster 1 and 2 occurred more frequently in ‘Elkat’ than in the cultivars ‘Elsanta’ and ‘Honeoye’.

For the detection of AM fungi, other primers specific for fungi of the genus *Glomus* have been developed and used. Redecker (2000) developed a set of primers specific for the 5 major subgroups of AM fungi, allowing amplification of gene fragments from the internal transcribed spacers (ITS) and a small subunit (SSU) regions of rDNA. These primers allow detection of AM fungi of the genera *Glomus* and *Acaulospora* in plant roots, excluding amplification of plant DNA or that of other fungi. AM fungi-specific primers have also been developed based on the variation within the large ribosomal subunit (LSU) rDNA, which allow identification of AM fungi in the roots of plants (Turnau et al. 2001). Those primers, like the ones used in this work, can be used for the detection of AM fungi in the field.

In organic as well as conventional farming, bioproducts are increasingly used to promote the growth and development of plants. For example, it has been noted that vermicompost is a bioproduct friendly to the environment, with a positive influence on growth and yielding of plants of bean (Singh et al. 2011). Biopreparations containing antagonistic microorganisms and plant extracts with curative properties are also used to control replantation disease in order to eliminate chemical agents, which destroy beneficial mycorrhizal
fungi (Zhang et al. 2008, Mark & Cassels 1999). Evaluation of the action of these bioproducts should include their effect on the presence of AM fungi in plant roots because these bioproducts stimulate the development of mycorrhizas in the roots of plants. For example, a beneficial effect on the production of spores and root colonization by AM fungi have been observed after application of bioproducts based on mycorrhizal inocula, amino acids, and humic and fulvic acids (Sas Paszt et al. 2011). In addition, field studies have been conducted to assess the effectiveness of some strains of AM fungi in stimulating the growth and yielding of plants with a view of employing these microorganisms in bioproducts. It was found that strains of *Glomus mosseae* applied to alfalfa (*Medicago sativa* L.) plants in the field were present in the roots of the plants two years after inoculation and had a positive effect on root colonization by AM fungi and on the growth and yield of plants (Pellegrino et al. 2012).

The conventional method for the identification of AM fungi consists in collecting spores and examining their morphological characteristics. Identification of AM fungi according to morphological features of the spores may cause difficulties because, depending on conditions such as the time of year, the fungi can produce large amounts of spores or not produce them at all, which means that fungi that are not sporulating may not be detected. In addition, difficulties in the identification of mycorrhizal fungi can be due to the different dynamics of spore production depending on the species of fungus, and dimorphism among certain species of AM fungi (Redecker et al. 2003). For these reasons, the aim is to develop methods for rapid identification of AM fungi in plant roots. The technique used here makes it possible to perform rapid tests for the detection of mycorrhizal fungi in the roots of plants and an initial taxonomic classification of these fungi. The results obtained complement the observations of plants in the field and can be used to assess the effectiveness and usefulness of the biopreparations in the cultivation of strawberry.

CONCLUSIONS

1. The technique of nested PCR with specific primers allowed identification of AM fungi of the genera *Glomus*, *Acaulospora* and *Scutellospora* in the roots of strawberry plants.
2. The most reaction products characteristic of mycorrhizal fungi were found in the roots of plants fertilized with the biopreparation Florovit Eko.
3. The tests confirmed the negative impact of mineral fertilizers on the presence of mycorrhizal fungi in the roots of strawberry plants.
4. The results will be used to further identify AM fungi in the roots of plants in order to determine the species composition of the fungi and their genetic diversity.

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Endogone and Complexipes species deposited in the Department of Plant Pathology, University of Agriculture in Szczecin, Poland. http://www.agro.ar.szczecin.pl/~jblaszkowski/


‘Development of innovative products and technologies for organic fruit production’. The work has been supported by a grant from the EU Regional Development Fund through the Polish Innovation Economy Operational Program.

WYKRYWANIE GRZYBÓW MIKORYZOWYCH W KORZENIACH ROŚLIN TRUSKAWKI NAWOŻONYCH BIOPRODUKTAMI ORGANICZNYMI

Streszczenie

W rolnictwie ekologicznym stosuje się preparaty stymulujące wzrost i rozwój roślin, co zastępuje nawożenie mineralne. Wprowadzanie nowych biopreparatów do produkcji ogrodniczej wymaga oceny ich wpływu na wzrost i plonowanie roślin. Ważnym wskaźnikiem oddziaływania bioproduktów jest określenie obecności w korzeniach roślin arbuskularnych grzybów mikoryzowych (AMF). Do wykrywania grzybów AMF w korzeniach roślin, oprócz metod konwencjonalnych, stosuje się coraz częściej techniki biologii molekularnej, które umożliwiają wykrycie i zidentyfikowanie grzybów AMF w korzeniach roślin. Celem pracy była identyfikacja oraz wstępna klasyfikacja taksonomiczna grzybów AMF w korzeniach roślin truskawki ‘Elkat’, nawożonych biopreparatami przy użyciu techniki zagnieżdżonego PCR.

Testy przeprowadzono na DNA uzyskanym z korzeni roślin truskawki odmiany Elkat, nienawożonych, traktowanych 10 biopreparatami oraz nawożonych NPK. Przeprowadzono amplifikację fragmentu dużej podjednostki genu rybosomalnego (LSU rDNA) z użyciem starterów uniwersalnych, a następnie w reakcji zagnieżdżonego PCR.
zastosowano startery specyficzne dla grzybów z rodzajów *Glomus*, *Acaulospora* i *Scutellospora*. Zasiedlenie korzeni truskawki przez arbuskularne grzyby mikoryzowe określano na podstawie obecności fragmentów DNA o wielkości odpowiedniej dla testowanych rodzajów grzybów.

W wyniku przeprowadzonych analiz najwięcej produktów reakcji charakteryzujących grzyby AMF stwierdzono w korzeniach roślin traktowanych preparatem Florovit Eko. Najmniej fragmentów charakterystycznych dla grzybów AMF wykryto w korzeniach roślin nawożonych NPK, co potwierdza negatywny wpływ nawożenia mineralnego na występowanie grzybów mikoryzowych w korzeniach roślin truskawki. W korzeniach roślin nawożonych Tytanitem obserwowano obecność jednej z grup grzybów z rodzaju *Glomus* oraz brak jednej grupy grzybów z rodzaju *Scutellospora* w porównaniu do korzeni roślin kontrolnych. W korzeniach roślin traktowanych innymi biopreparatami stwierdzono produkty reakcji charakteryzujące grzyby z rodzajów *Glomus*, *Scutellospora* i *Acaulospora*, podobnie jak w korzeniach roślin kontrolnych. Uzyskane wyniki posłużą do oceny przydatności biopreparatów w produkcji ogrodniczej.