DETECTION OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE ROOTS OF STRAWBERRY WITH NESTED PCR TECHNIQUE

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Summary
Organic farming of strawberry fruit crops is being increasingly developed in Poland. There is no possibility of using mineral fertilizers and chemical plant protection products in such a system of cultivation. The Rhizosphere Laboratory of the Research Institute of Horticulture in the Skierniewice has been working on the development and practical implementation of bioproducts enriched with beneficial soil microorganisms that have a positive effect on the growth and development of strawberry plants.

An analysis of the impact of bioproducts on plants requires an assessment of the colonization of the plant roots by beneficial, arbuscular mycorrhizal fungi (AMF). Because of the difficulty in detecting and identifying AM fungi, the mycelium of which cannot be maintained outside the host plant, use is made of molecular biology techniques based on DNA analyses of these fungi.

The aim of this study was to use a nested PCR technique to establish the presence of arbuscular mycorrhizal fungi in the roots of strawberry plants treated with bioproducts.

The tests were performed on the DNA obtained from the roots of strawberry plants of the cultivars Elsanta and Honeoye treated with bioproducts of natural origin. In terms of fertilization, the control combinations were made up of plants that were not fertilized (control 0) and plants fertilized with NPK (control NPK). In the molecular tests, the control was the DNA obtained from the leaves of strawberry plants of the two cultivars. Colonization of strawberry roots by mycorrhizal fungi was determined on the basis of the presence of PCR products of a specific size, which were characteristic of the genera of the tested fungi. Primers amplifying the region of the large subunit ribosomal gene (LSU rDNA), which were specific to the genera *Glomus*, *Acaulospora* and *Scutellospora*, were used.

As a result of the tests, DNA fragments were obtained that confirmed the presence of mycorrhizal fungi in all the samples of roots with the exception of those that came from the control combination of cultivar Honeoye fertilized with NPK. The roots of the plants were found to contain DNA fragments char-
characteristic of three clusters of the genus *Glomus*. The presence of fungi *Acaulospora* was detected in all the samples of cultivar Honeoye roots except the roots of the control plants fertilized with NPK. Reaction products characteristic of the *Acaulospora* fungi in the roots of cultivar Elsanta were observed in the samples taken from the plants treated with bioproducts, with the exception of the control plants and plants fertilized with manure. Moreover, the test samples of both cultivars were found to contain fungi belonging to two clusters of the genus *Scutellospora*.

The tests made it possible to detect the presence of arbuscular mycorrhizal fungi in the roots of strawberry plants. In addition, the analyses have shown the usefulness of most of the primers used for detecting the presence of mycorrhizal fungi of the genera *Glomus, Acaulospora* and *Scutellospora*. The results will be used to assess the effects of bioproducts on the growth and yield of strawberry plants of the cultivars Elsanta and Honeoye, and on the colonization of the roots of these plants by AM fungi.

Key words: arbuscular mycorrhizal fungi (AMF), detection of AMF, nested PCR, strawberry

**INTRODUCTION**

Arbuscular mycorrhizal (AM) fungi are the most important symbionts of higher plants (Allen 1996). The fungi facilitate the transport of inorganic compounds to plants while taking advantage of plant carbohydrates. In addition, AM fungi increase plant resistance to biotic stresses, such as pathogens (Sukhada et al. 2011, Watanarojanaporn et al. 2011), and abiotic stresses, such as cold or salinity (Aroca et al. 2007, Jahromi et al. 2008) and drought (Porcel & Ruiz-Lozano 2004, Aroca et al. 2007, Franzini et al. 2010). Because of the favourable effect on plants, AM fungi are included as a component of bioproducts used in organic farming of strawberries. There is no possibility of using mineral fertilizers and chemical plant protection products on such crops; therefore, studies are being conducted on the development and practical implementation of bioproducts containing beneficial soil microorganisms, including AM fungi (Sas Paszt et al. 2011).

Tests carried out to determine the effects of bioproducts on plants require, among other things, detection of the presence of mycorrhizal fungi in the roots and determination of their affiliation in the systematic classification of fungi. For this purpose, the classical method is used, based on an analysis of the morphological features of spores under a light microscope (Blaszkowski 2003). The production of spores, however, is not always correlated with the extent of colonization of roots by fungi, which is why to detect the presence of AM fungi in the roots, molecular methods based on DNA analysis are now used more and more frequently (Redecker 2000). One advantage of these techniques is the ability to detect and identify AM fungi in the roots of plants without having to acquire spores and conduct a morphological analysis of them.
Molecular techniques have been used to detect and determine the diversity of AM fungi in the roots of wild plants as well as crop plants (Appoloni et al. 2008, Daniell et al. 2001). For the detection of AM fungi in the roots of plants, a technique of nested PCR is used, based on amplification of the large or small subunit ribosomal gene (LSU rDNA, SSU rDNA) (Kjoller & Rosendahl 2000, Krüger et al. 2009). With respect to LSU rDNA, specific primers have been developed for amplification of DNA fragments of fungi of the genera Glomus, Scutellospora and Acaulospora. The use of these primers has made it possible to detect the presence of mycorrhizal fungi colonizing the roots of two species of grass (Gollotte et al. 2004). Moreover, the developed primers allow clustering of fungi within the genera. For the genus Glomus there are 5 clusters, including the species Glomus mosseae, for the genus Acaulospora 3 clusters, and for the genus Scutellospora 2 clusters. The size of the fragments specific to each cluster has been defined in the range from 264 to 306 bp (Gollotte et al. 2004).

The aim of this study was to detect the presence of AM fungi in the roots of strawberry plants treated with various biopreparations and to conduct a preliminary taxonomic classification of those fungi using the nested PCR technique.

MATERIALS AND METHODS

Plant material

The plant material consisted of the roots and leaves of strawberry cultivars Elsanta and Honeoye that were part of an experiment aimed at determining the effect of biostimulators on the growth of the roots of strawberry plants under greenhouse conditions (Sas Paszt et al. 2011). The test samples included roots of unfertilized plants and roots of plants treated with NPK fertilizers, farmyard manure or biostimulators, i.e. Micosat, Humus UP, Humus Active, BF Quality, BF Amin, Tytanit, and Vinassa. Leaves of both strawberry cultivars were also collected to serve as the control in the molecular tests.

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

1) Control (no-treatment)-unfertilized podsollic 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 superphosphate-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. & Trappe, G. intraradices Schenk & Smith, G. caledonium (Nicolson et Gerdemann) Trappe et Gerdemann, G. viscosum Nicolson and G. coronatum (Giovannetti);
Trichoderma viride Pers.; three rhizosphere bacteria species (Bacillus subtilis, Pseudomonas fluorescens, Streptomyces spp.).

1. 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).

DNA extraction
DNA was isolated from 100 mg of roots or leaves of strawberry plants.
using a commercial kit for DNA isolation from plants and fungi: Plant and Fungi DNA Purification Kit (EURx). Roots were taken from plants growing in rhizoboxes. They were taken from 10 plants of each combination growing in 5 rhizoboxes, and then a mixed sample, containing about 5 g of roots, was prepared. For DNA isolation, 100 mg of roots was taken from the mixed sample because that is the required (maximum) amount of plant tissue when isolating DNA with the EURx commercial kit. After collection, the remaining substrate was cleaned off the roots by rinsing them with water. The roots were subsequently frozen at -80°C. DNA concentration was determined spectrophotometrically at a wavelength of 260 nm. For further analyses, DNA dilutions of 10 ng·ml⁻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’ agcatataaagagggagga 3’) and LSU 0599 (5’ tggctcggttcagacg 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 to mycorrhizal fungi. The primers used were FLR3 (5’ tgg aac cgg aac cga tgg aag t 3’) and FLR4 (5’ – tac gtc aac atc ct aac gaa 3’), suitable for amplification of the LSU rDNA of fungi from the class Glomeromycetes. In addition, reactions were performed with the primer FLR4 in combination with 10 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’aaacgcttcggatctcgg3’, *Glomus 2*: 5’catgaggaggaacccctcgg3’, *Glomus 3*: 5’gacgtaggaggttaacg3’, *Glomus 4*: 5’tcctcttgaaattgtatc3’, *Glomus 5*: 5’gcctctgtctggtatc3’, *Acaulosporaceae 1*: 5’caacatgagggtcgcctc3’, *Acaulospora 2*: 5’gttcccccgggagctctc3’, *Acaulospora 3*: 5’tgctctgcttctcgc3; *Scutellospora 1*: 5’gaaccaaccctgaagtc3’, *Scutellospora 2*: 5’aggggaaacttgagctc3’. 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**

**Cultivar Elsanta**

The reactions performed with the primers LSU 0061 and LSU 0599 revealed the presence of a reaction product 700 bp in size on the DNA template from the samples of roots. In addition, as a result of the reactions carried out with the primers FLR3 and FLR4, a product was obtained for all the samples of roots whose size was 380 bp, characteristic of fungi from the class Glomeromycetes.

As a result of the reactions carried out with 9 pairs (out of 10) of the specific primers, DNA products were obtained whose size made it possible to confirm the presence of fungi of the genera *Glomus*, *Acaulospora* and *Scutellospora*, and to distinguish clusters among them. In the tested samples there were products characteristic of two clusters of the genus *Glomus* (Table 1). A product characterizing one of the cluster of this genus (*Glomus* 4) was observed in all the samples of roots. In addition, a DNA fragment characterizing the species *Glomus mosseae* was present in those samples where the plants had been treated with the preparations Humus Active and Tytanit. Reaction products characteristic of the cluster *Acaulospora* 3 were observed in the samples taken from the roots treated with the bioproducts, with the exception of the control plants and plants fertilized with manure. Fungi of the genus *Scutellospora* belonging to two clusters were observed in the roots of the control plants and plants treated with the preparations BF Quality, Humus Active, Tytanit and Vinassa.

**Cultivar Honeoye**

The reactions carried out with the primers LSU 0061 and LSU 0599 revealed the presence of a 700-bp product in all the samples of roots. Nested PCR with the primers FLR3 and FLR4 revealed the presence of a 380-bp product characteristic of fungi from the class Glomeromycetes in all the root samples except the roots fertilized with NPK. The reactions carried out with the primer FLR4 in combination with successive single specific primers revealed the presence of products characteristic of three clusters of the genus *Glomus* (Table 2). The presence of the species *Glomus mosseae* was detected in the samples representing plants for which manure, Humus UP, Humus Active, BF Amin and Vinassa had been used. Products characterizing fungi from *Glomus* cluster 4 were observed in all the samples of roots with the exception of the roots of plants fertilized with NPK. Fungi from *Glomus* cluster 5 were found only in the roots of the control plants. DNA fragments corresponding to *Acaulospora* 3 fungi were present in all the samples of roots except for the control NPK. In addition, the test samples were found to contain fungi belonging to two clusters of the genus *Scutellospora*. In the roots of plants that had been fertilized with manure, Humus UP and BF Quality there were fungi from *Scutellospora* cluster 1, whereas in the roots of the control plants and those treated with Humus UP and BF Amin the presence of fungi from *Scutellospora* cluster 2 was detected.
Table 1. Results of nested PCR with primers amplifying LSU rDNA of fungi of the genera *Gliomus, Acaulospora* and *Scutellospora* obtained for DNA from the roots of ‘Elsanta’ 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>
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<td>+</td>
<td>+</td>
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<td>+</td>
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Table 2. 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 ‘Honeoye’ strawberry plants. (+) presence, (-) absence of reaction products

<table>
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<tr>
<th>Primers</th>
<th>Length of DNA fragment (bp)</th>
<th>Control 0</th>
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As a result of the reactions carried out with the primers FLR4/Glo-
mos 3, a product was obtained in the samples of roots and leaves (negative
control) of both strawberry cultivars. This outcome demonstrated unsuita-
bility of the Glomus 3 primer for detecting mycorrhizal fungi in the roots
of strawberry plants. After applying the other primers, there were no rea-
tion products on the DNA template from the leaves of either strawberry
cultivar, which indicated the usefulness of those primers for analysis and
confirmed that the products obtained on the DNA template from the roots
were the result of amplification of the LSU of fungi from the class Glome-
romycetes.

The analysis of DNA with the primers allowing amplification of the
LSU rDNA, including the primers specific to the genera Glomus, Aca-
ulospora and Scutellospora, made it possible to detect the presence of fun-
gi of these genera in the roots of strawberry plants treated with the
various bioproducts. The specific primers FLR3/FLR4 revealed the
presence of mycorrhizal fungi in all the samples except those of the culti-
var ‘Honeoye’ taken from the roots fertilized with NPK. In those roots,
very low mycorrhizal frequency (3.3%) had been observed (Sas Paszt,
personal communication). Likewise, in ‘Elsanta’ plants treated with NPK
there was also very low mycorrhizal frequency, thus confirming earlier
observations that NPK fertilization adversely affected the colonization of
plant roots by arbuscular mycorrhizal fungi (Sas Paszt et al. 2011). The fact
that the molecular techniques did not detect AM fungi in the roots of ‘Ho-
neoye’ plants fertilized with NPK may have been caused by the limited colo-
nization of those roots by the fungi.

The identification of fungal spe-
cies on the basis of the morphological
characteristics of spores from the
same experiment revealed in the case
of ‘Elsanta’ the presence of several
species of the genus Glomus in all the
treatments and the species Scute-
lospora dipurpureascens in 9 out of 10
treatments (Sas Paszt et al. 2011). The
results of the molecular tests differed
somewhat from the identification of
fungi with the conventional method.
The DNA analysis based on specific
primers revealed in the roots of ‘El-
santa’ plants the presence of fungi that
had not been detected by the acquisi-
tion of spores, such as Acaulospora
and more than one species of Scute-
lospora. Moreover, the DNA tests
revealed the presence of the species
Glomus mosseae in the roots from two
treatments, while the morphological
analysis of spores present in the rhizo-
sphere revealed the presence of this
species in 8 out of 10 treatments (Sas
Paszt et al. 2011).

For the cultivar Honeoye, identifi-
cation of fungal species on the basis
of the morphological features of
spores was also carried out and there
were differences found in the occur-
rence of AM fungi as determined with
the conventional and molecular tech-
niques (Sas Paszt, personal communi-
cation). In the roots fertilized with
NPK, the molecular tests detected no
presence of fungi, whereas the analy-
sis of spores present in the rhizo-
sphere revealed the presence of three
species of AM fungi. Spores of the
species Scutellospora dipurpureascens
were observed in the roots fertilized
with NPK and manure, while the molecular analysis revealed the presence of fungi of the genus *Scutellospora* also in the roots treated with the bioproducts. Similarly, in the roots of the cultivar Elsanta the DNA analysis revealed the presence of fungi of the genus *Acaulospora*, whose presence there had not been detected during the morphological analysis of spores. Moreover, in the roots from several treatments in which *Glomus mosseae* spores had been observed, the molecular analysis did not detect this species in the roots of plants.

The molecular tests revealed differences in the presence of species and genera of mycorrhizal fungi in comparison with the conventional techniques of identifying fungal species. Similar differences have been observed between the identified spores and fungi occurring in the roots of *Hycinthoides non-scripta* and *Arnica montana* collected from natural habitats (Clapp et al. 1995, Merryweather & Fitter 1998, Ryszka et al. 2010). The differences between the mycorrhizal fungi identified with classical methods and those identified with molecular techniques may be due to several causes. Fungi of the genus *Glomus* colonizing some species of plants may not generate spores or produce them only in small amounts, meaning that not always is there a correlation between the number of spores and the extent of root colonization by these fungi (Clapp et al. 1995). In trap cultures, some species may be displaced by other species of fungi, which affects the differences in the results of analyses (Ryszka et al. 2010). Another reason for the differences in the detection and identification of AM fungi with classical and molecular methods may be differences in the biological growth and development of the species of AM fungi. Some species may colonize roots to a very small extent and at the same time produce a large number of spores, whereas in other species we may observe high mycorrhizal frequency in the roots but at the same time a very limited number of spores produced (Sanders 2004). In our experiment, DNA analysis based on specific primers allowed us to detect in the roots of strawberry cultivars Elsanta and Honeoye the presence of fungi of the genus *Acaulospora* and more than one species of *Scutellospora*, i.e. microorganisms that had not been detected by collecting spores from trap cultures and analyzing their morphological features. The reason for that may be that under certain conditions the fungi colonizing the roots of strawberry plants do not produce spores. On the other hand, the fact that the DNA analysis did not detect those species of fungi that had been identified through spore acquisition was most likely the inability of spores to germinate, or if so, only to a small extent. The results thus indicate that a full assessment of the biodiversity of mycorrhizal fungi colonizing the roots of strawberry plants requires both identification of species based on morphological analysis of spores and the use of techniques based on DNA analysis.

In the technique based on nested PCR with primers specific for the detection of mycorrhizal fungi in the roots, the use of a DNA template from leaf tissue makes it possible to verify whether amplification of plant DNA
occurs in the reaction (Gollotte et al. 2004). In the case of the *Glomus* 3 primer we observed reaction products of the same size on the templates obtained from both the roots and leaves, which resulted in the exclusion of that primer from further analysis.

**CONCLUSIONS**

The nested PCR technique with the use of specific primers allowed us to detect AM fungi of the genera *Glomus*, *Acaulospora* and *Scutellospora* in the roots of strawberry plants. The primers used, except the *Glomus* 3 primer, proved useful for carrying out these analyses. The advantage of this technique is the ability to perform tests, in a short time, and obtain information about the colonization of strawberry roots by AM fungi. The obtained results will be used for further analysis of AM fungi in the roots of strawberry plants in order to determine the species composition of the fungi and their genetic diversity.

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WYKRYWANIE OBECNOŚCI ARBUSKULARNYCH GRZYBÓW MIKORYZOWYCH W KORZENIACH TRUSKAWKI TECHNIKĄ ZAGNIEŻDŻONEGO PCR

Streszczenie
Ekologiczna uprawa roślin truskawki jest uprawą coraz popularniejszą w Polsce. Możliwość stosowania nawozów mineralnych i chemicznych środków ochrony w takim systemie uprawy jest wykluczona. W Pracowni Rizosfery Instytutu Ogrodnictwa w Skierniewicach opracowywane są i wdrażane do praktyki sadowniczej biopreparaty wzbogacone o pożyteczne mikroorganizmy glebowe, które wpływają pozytywnie na wzrost i rozwój roślin truskawki. Analiza wpływu biopreparatów na rośliny wymaga oceny zasiedlania korzeni roślin przez pożyteczne, arbuskularne grzyby mikoryzowe (AMF). Z powodu trudności w wykrywaniu i identyfikacji grzybów AMF, których grzybni nie można utrzymywać poza organizmem roślinnym, do analiz wykorzystuje się techniki biologii molekularnej, oparte na analizie DNA grzybów mikoryzowych.
Celem pracy było stwierdzenie obecności arbuskularnych grzybów mikoryzowych w korzeniach roślin truskawki traktowanych biopreparatami, techniką zagnieżdżonego PCR.

Testy przeprowadzono na DNA uzyskanym z korzeni roślin truskawki odmiany Elsanta i Honeoye traktowanych biopreparatami pochodzenia naturalnego. W przeprowadzonym doświadczeniu nawozowym (z którego pobrano korzenie do analiz molekularnych) kontrole stanowiły rośliny nienawozone (Kontrola 0) oraz rośliny nawozone NPK (Kontrola NPK). W testach molekularnych kontrolą był DNA uzyskany z liści roślin obu odmian truskawki. Zasiedlenie korzeni truskawki przez grzyby mikoryzowe określano na podstawie obecności produktów PCR o określonej wielkości, charakterystycznych dla testowanych rodzajów grzybów. Zastosowano startery amplifikujące region dużej podjednostki genu rybosomalnego (LSU rDNA), specyficzne dla rodzajów Glomus, Acaulospora i Scutellospora.

W wyniku przeprowadzonych testów uzyskano produkty PCR charakterystyczne dla grzybów mikoryzowych z rodzaju Glomus we wszystkich próbach korzeni obu odmian oprócz korzeni odmiany Honeoye z kombinacji NPK. Obecność grzybów Acaulospora stwierdzono w korzeniach odmiany Honeoye z wyjątkiem roślin nawożonych NPK. Produkty reakcji charakterystyczne dla grzybów Acaulospora w korzeniach odmiany Elsanta obserwowano w próbach pobranych z roślin traktowanych biopreparatami z wyjątkiem korzeni roślin kontrolnych i nawożonych obornikiem. Ponadto, w korzeniach testowanych roślin stwierdzono obecność grzybów z rodzaju Scutellospora.

Przeprowadzone testy umożliwiły wykrycie obecności arbuskularnych grzybów mikoryzowych w korzeniach roślin truskawki. Ponadto, wykonane analizy wykazały przydatność większości zastosowanych starterów do wykrywania obecności grzybów mikoryzowych z rodzajów Glomus, Acaulospora i Scutellospora.

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