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ORIGINAL RESEARCH PAPER

Response of tomato seedlings inoculated with mycorrhizal fungi on the photosynthetic activity, growth, and health status of plants after infection with the fungus *Colletotrichum coccodes*

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Abstract
The aim of this study was to estimate the effect of mycorrhizal fungi (MF) on the photosynthetic activity, growth, and health status of tomato seedlings ('Pelikan F_1') infected with the pathogenic fungus *Colletotrichum coccodes*. A commercial mycorrhizal inoculum (Mycoflor, Poland) containing spores and dormant mycelium of MF was used in the experiment. It was carried out in a growth chamber where 1-week-old seedlings were inoculated with 3 mL of mycorrhizal inoculum applied into the soil. Three-week old mycorrhized and nonmycorrhized tomato seedlings were infected with the pathogenic fungus. Seedlings planted in sterile horticultural soil without the mycorrhizal inoculum constituted an absolute control. The growth, disease index, and photosynthetic activity of the plants were measured after 4 weeks. There was no significant effect of the mycorrhiza on the photosynthetic parameters analyzed. However, it was noted that the mycorrhized and pathogen-infected seedlings had higher maximum quantum yield of PSII (F_v/F_m), higher effective quantum yield (Y) and more favorable photochemical (qP) and nonphotochemical fluorescence quenching (qN) coefficients than did the pathogen-infected seedlings. The experiment showed that the mycorrhizal commercial inoculum had varied effects on the health status of tomato seedlings depending on the pathogenic fungus isolate. Mycorrhiza did not have a significant effect on the length of roots and stems, whereas the roots inoculated with MF were better developed than those infected with the pathogenic fungus. It can be assumed also that not only the pathogen but also the mycorrhiza is a stress factor towards the seedlings and affects the growth performance parameters mentioned above.

Keywords
tomato seedlings; brown root rot; mycorrhizal fungi; disease index; photosynthetic activity of seedlings

Introduction

Tomatoes have great economic importance worldwide. The tomato is a major proportion of the vegetable production for the fresh and processed food markets. In Poland, from 40% to 60% of the annual tomato fruit production are intended for processing. For many years, an increase in the yield in this crop plant has been noted. Despite the application of new efficient technologies in tomato cultivation, there are new phytosanitary problems. One of these is the anthracnose disease of tomato roots caused by

Colletotrichum coccodes (Wallr.) S. Hughes. This fungus causes root rot of tomato at the seedling stage. The cortical layer in the roots of plants infected with the fungus is loosened and filled with numerous sclerotia and is therefore easily separated from the rest of the root. Three types of brown root rot damage are observed in infected tomato roots: rot of lateral roots, corky roots, and rot of the stem base [1,2]. The fungus is in contact with the underground part of the plant and occurs in a complex with other soil pathogens [3].

One of the elements of integrated plant protection is the use of biological methods, including mycorrhiza. Mycorrhizal fungi (MF) are widespread in the soil environment and play an important role therein. One of their functions is to protect plants against soil pathogens. These beneficial soil microorganisms directly and indirectly exert on phytopathogenic fungi [4]. The aim of the present study was to assess the effect of MF on the photosynthetic activity, growth, and health status of tomato seedlings infected with *Colletotrichum coccodes*.

Material and methods

Plant material

The experiment was carried out in the growth room at the University of Life Sciences in Lublin, Department of Plant Protection in 2016. Tomato seedlings of 'Pelikan F_1' cultivar were used in the experiment. Seeds were obtained from the Horticultural Seed and Nursery Company (PNOS) in Ożarów Mazowiecki, Poland.

MF treatment

A commercial mycorrhizal inoculum (Mycoflor, Końskowola, Poland) containing spores and dormant mycelium of MF such as *Rhizophagus aggregatus* (syn. *Glomus aggregatum*), *R. intraradices* (syn. *G. intraradices*), *Claroideoglomus etunicatum* (syn. *G. etunicatum*), *Endogone mosseae* (syn. *G. mosseae*), *Funneliformis caledonium* (syn. *G. caledonium*), and *Gigaspora margarita*, was mixed with peat.

Colletotrichum coccodes inoculum

Randomly selected *C. coccodes* isolates were used in the experiment as a group representing diversity within the species. The *C. coccodes* isolates (EIR32, PCL21, PCL22, PER19, PER31, EES22, PCR14) which originated from the Department collection, were obtained from tomato plants grown in the field in 2015. The fungal colonies used for plant infections originated from 10-day spore cultures grown on Potato Dextrose Agar (Difco PDA) in a thermostatic chamber at a temperature of 22°C. The surface of the tomato seeds was disinfected with 0.1% sodium hypochlorite for 1 minute and then rinsed three times in distilled water [5]. Next, the seeds were germinated on plates filled with sterilized horticultural soil (peat moss) and quartz sand at a ratio of 2:1. When the first leaves appeared (after 1 week), the seedlings were planted into multiplates. Fifty mL of sterilized horticultural soil were mixed with 3 mL of the mycorrhizal inoculum and added under each seedling, in accordance with the producer's instructions. The control seedlings were transplanted into sterile horticultural soil without the mycorrhizal inoculum. The seedlings were placed in a growth chamber at 22–23°C and 85% air humidity with a 14-h photoperiod and watered with sterile water as needed. Three-week old mycorrhized and nonmycorrhized seedlings were transferred with overgrowth soil to pots (0.5 L, diameter 10 cm) filled with sterilized horticultural soil where *C. coccodes* grown on PDA slices were placed and then covered with soil according to the method used by Jamiołkowska et al. [6]. In the control combination, slices of PDA without the pathogenic fungus were used. The following experimental combinations were used in the study: absolute control, i.e., seedlings growing without the mycorrhiza and without the pathogenic fungus (C); relative control, i.e., seedlings inoculated with the MF (M);

seedlings infected with the pathogenic fungus (Cc); seedlings inoculated with the MF and infected with the pathogenic fungus (Cc+M).

The experiment was carried out in a randomized block arrangement. Fifteen plants (three plants in five replicates) were tested for each fungal isolate within each experimental combination. The tomato seedlings were grown in the growth chamber at 23–25°C, 85% air humidity with a 14-h photoperiod and photon flux density of ca. 350 µmol m^{-2} s^{-1}. All plants grew in the pots for 4 weeks. After this time, the size of plants and the number of healthy and diseased seedlings were recorded, and the photosynthetic activity of leaves was measured.

Estimation of the photosynthetic activity of leaves

Fluorescence parameters such as the maximum quantum yield of PSII (F_v/F_m), the effective quantum yield (Y), and the photochemical (qP) and nonphotochemical fluorescence quenching coefficient (qN) were measured with the pulse amplitude modulated (PAM) fluorometer (PAM-2000 Waltz GmbH, Germany). The fluorescence was measured after dark adaptation (leaves were shaded for about 20 min with manufactured clips; actinic light for 0.7 s). The study included five randomly selected leaves from each experimental combination, selecting one leaf from the same position on the plant. Fluorescence parameters were calculated according to the following equations: the maximum quantum yield of PSII: $F_v/F_m = (F_m - F_0)/F_m$, where F_v equals the fluorescence increase induced by the saturation pulse; the effective quantum yield: $Y = (F_m' - F_s)/F_m'$; coefficient of photochemical fluorescence quenching: $qP = [(F_m' - F_s)/(F_m' - F_0')]$; coefficient of nonphotochemical quenching: $qN = [(F_m - F_m')/(F_m' - F_0')]$.

Estimation of seedling growth

The evaluation of the growth of tomato seedlings was carried out by measuring the length of roots and the aboveground parts of plants (cm).

Estimation of the disease index (DI)

The plants were evaluated for the degree of infection after 4 weeks growth in the pots, using a 4-grade scale: 0 – no symptoms; 1 – small necrosis spots on lateral roots, no symptoms on leaves or stem; 2 – necrosis of tap root and stem base, chlorotic leaves; 3 – completely rotten roots and stem base, death of plants. Then, the number of seedlings in each class was counted. The disease index was estimated for each replication using the Townsend and Heuberger formula [7]: *Disease index* = $(\sum a/\sum b) \times 100$, where a = sum of products of the numerical scale index (infection degree) and the corresponding number of plants and b = total number of tested plants multiplied by the highest numerical scale index. The mean disease index was then computed for each combination. The percentage of plants with disease symptoms was counted. Koch's postulate was met by reisolation of the fungi from the diseased plants.

Statistical analysis

The results from the obtained data were analyzed statistically using the SAS software. The significance of treatment differences were evaluated on the basis of multiple Tukey's test at $p \leq 0.05$.

Results

Photosynthetic activity of tomato seedlings

The photosynthetic activity of the seedlings varied and depended on the pathogenic fungus isolate and the presence or absence of mycorrhiza (Tab. 1). The mean value of F_v/F_m for seedlings mycorrhized and infected with the pathogenic fungus (Cc+M) was 0.562 and it was higher than the mean value of F_v/F_m for seedlings infected with the pathogenic fungus (Cc) – 0.557, but not higher than values noted for the control (C – 0.594, M – 0.576) (Fig. 1). The differences in the potential photochemical efficiency between the combinations studied were not statistically significant (Fig. 1).

There was a positive effect of the MF on the effective quantum yield (Y) in the tomato seedlings. The highest mean Y value (0.528) was noted for the relative control (M). It was statistically different from the mean values of Y in the other experimental combinations. The mean Y value for seedlings mycorrhized and infected with the pathogenic fungus (Cc+M) was higher (0.490) than the mean Y for plants growing only with the pathogenic fungus (Cc – 0.484) and for control plants (C – 0.479). However, these differences were not statistically significant (Fig. 1). The mycorrhiza also exerted an effect on the process of photochemical fluorescence quenching (qP). The highest mean value of qP was noted for control seedlings (C – 0.585; M – 0.580). Statistically lower values of qP indicating a fast rate of photochemical fluorescence quenching were found for the pathogen-infected seedlings (Cc – 0.548). This process, qP, in the mycorrhized seedlings infected with the pathogenic fungus (Cc+M) was slower than in the case of the pathogen-infected seedlings. However, the differences were not statistically significant. A reverse result was noted for the qN coefficient. The lowest rate of nonphotochemical fluorescence quenching was observed for the relative control seedlings (M – 0.134),

Tab. 1 Parameters of chlorophyll fluorescence in the leaves of tomato seedlings.

Experimental combination	Isolates	F_v/F_m	Y	qP	qN
Cc	EIR32	0.551 [a]	0.479 [a]	0.565 [a]	0.137 [a]
	PCL21	0.553 [a]	0.458 [a]	0.517 [a]	0.130 [a]
	PCL22	0.583 [a]	0.522 [a]	0.598 [b]	0.135 [a]
	PER19	0.576 [a]	0.509 [a]	0.572 [a]	0.145 [a]
	PER31	0.584 [a]	0.519 [a]	0.567 [a]	0.139 [a]
	EES22	0.527 [a]	0.467 [a]	0.525 [a]	0.156 [a]
	PER14	0.527 [a]	0.432 [a]	0.492 [a]	0.164 [a]
Cc+M	EIR32	0.533 [a]	0.463 [a]	0.528 [a]	0.141 [a]
	PCL21	0.557 [a]	0.483 [a]	0.562 [a]	0.137 [a]
	PCL22	0.579 [a]	0.478 [a]	0.544 [a]	0.138 [a]
	PER19	0.577 [a]	0.481 [a]	0.545 [a]	0.132 [a]
	PER31	0.577 [a]	0.535 [b]	0.593 [b]	0.134 [a]
	EES22	0.555 [a]	0.497 [a]	0.533 [a]	0.140 [a]
	PER14	0.555 [a]	0.490 [a]	0.545 [a]	0.140 [a]
C		0.594 [a]	0.479 [a]	0.585 [b]	0.146 [a]
M		0.576 [a]	0.528 [b]	0.580 [a]	0.134 [a]
LSD		0.0833	0.0922	0.089	0.0377

Number of measurements in each experimental combination: $n = 5$; F_v/F_m – maximum quantum yield of PSII; Y – effective quantum yield; qP – photochemical fluorescence quenching coefficient; qN – nonphotochemical fluorescence quenching coefficient; Cc – seedlings inoculated with the pathogenic fungus; Cc+M – seedlings with the mycorrhiza and pathogenic fungus; C – absolute control; M – relative control. Values marked with the same letters for chlorophyll fluorescence parameters do not significantly differ at a significance level $p \leq 0.05$ (Tukey's test).

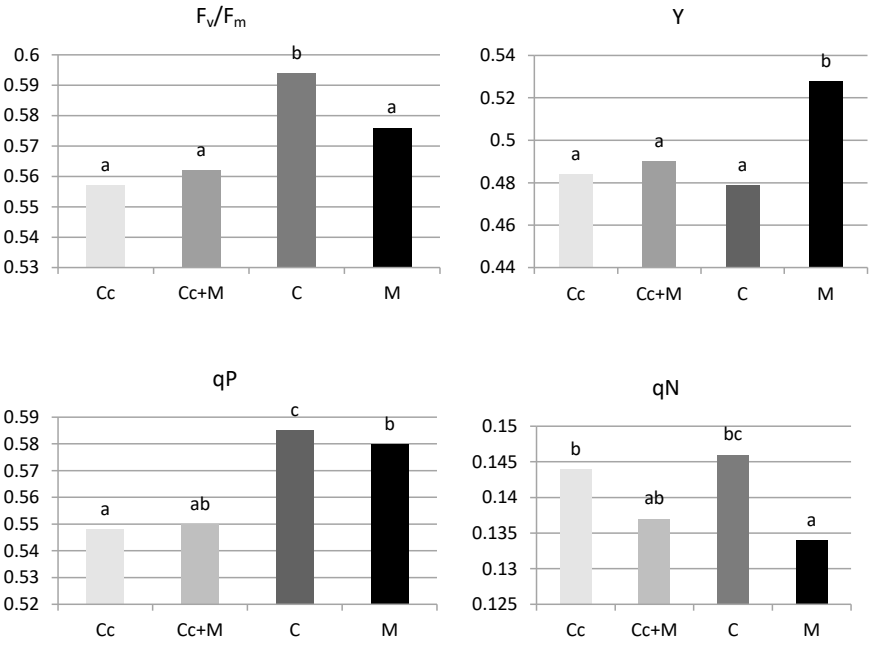

Fig. 1 Photosynthetic activity of tomato seedlings in the experimental combinations. Values marked with the same letters for chlorophyll fluorescence parameters do not differ significantly at $p \leq 0.05$ (Tukey's test); For description of abbreviations, see Tab. 1.

whereas the highest rate of the process was noted for the absolute control seedlings (C – 0.146) and those infected with pathogen (Cc – mean 0.144) (Fig. 1).

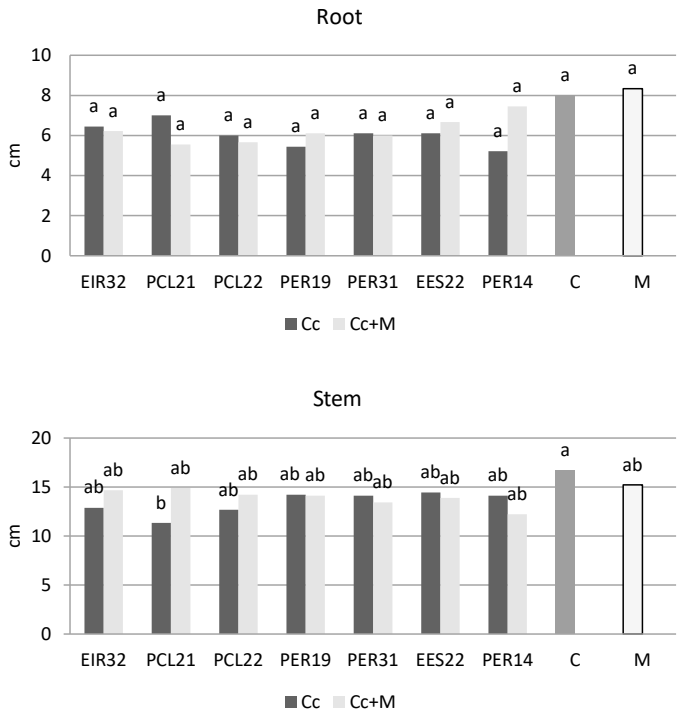

Fig. 2 Size of tomato roots and stems of seedlings inoculated with the pathogenic fungus (Cc), seedlings with the mycorrhiza and pathogenic fungus (Cc+M), control combinations (M, C); values marked with the same letters for the size of plants do not differ significantly at $p \leq 0.05$ (Tukey's test).

Size of roots and stems

Macroscopic assessment of the plants showed strongly reduced roots with visible symptoms of necrosis in the pathogen-infected seedlings (Cc) (length in the range from 5.22 to 7.0 cm) (Fig. 2, Fig. 3). In turn, the mycorrhized seedlings infected with the pathogenic fungus (Cc+M) had a longer and better-developed root system (length from 5.55 to 7.44 cm) (Fig. 2, Fig. 3). The best quality of roots was noted in the control plants (C) (mean 8.0 cm). The relative control seedlings (M) had the longest but poorly developed roots (mean 8.33 cm). However, there were no statistically significant differences between the lengths of the roots within the experimental combinations.

There was no significant effect of the mycorrhiza on the increase in the length of the tomato seedling stems. The stems of the pathogen-infected seedlings (Cc) and the mycorrhized and pathogen-infected seedlings (Cc+M) had varied length, depending on the fungal isolate used. A beneficial effect of the mycorrhiza on the stem length was noted only in the case of seedlings infected with isolates EIR32, PCL21, and PCL22 (Fig. 2). The stems of seedlings from the absolute control (C) were longer and developed better than the stems of mycorrhized seedlings (M), as well as the stems of mycorrhized seedlings infected with the pathogenic fungus (Cc+M). However, these differences were not statistically significant (Fig. 2, Fig. 3).

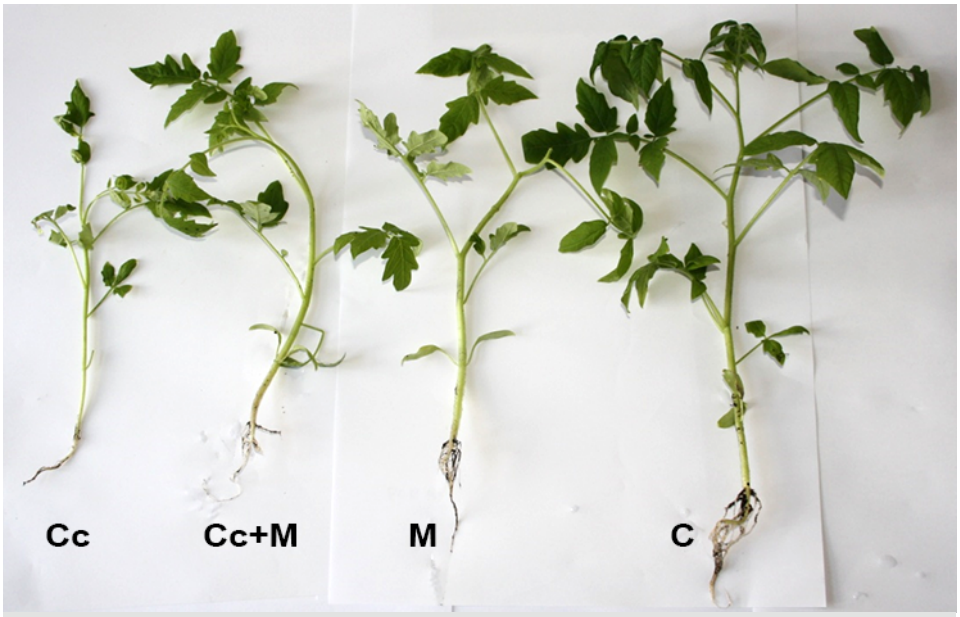

Fig. 3 Tomato seedlings grown with the pathogenic fungus (isolate PER14), mycorrhiza, and in the control groups. For description of abbreviations, see Tab. 1.

Disease index (DI)

The study showed varied degrees of pathogenicity of *C. coccodes* isolates against seedlings as well as varied protective effects of the mycorrhiza against seedlings were observed. There were no significant differences between the combinations studied within the given parameter, with the exception of the isolate PER31, where the addition of MF to the soil reduced the DI of seedlings infected with pathogenic fungus (Tab. 2). Isolates PER31, PER19, and PCL21 exhibited the highest pathogenicity (DI 83.3–75.0%), whereas PCL22 was the least pathogenic (52.8%). The mean DI in the mycorrhized and pathogen-infected seedlings (Cc+M) was 68.1%. This value was lower than the DI of plants growing only in the presence of the pathogen (Cc) – 69.8% (Tab. 2, Fig. 4).

The best protective effect of the mycorrhiza was noted in the case of seedlings infected with pathogenic strains PCL22 and PER31, where the mycorrhization contributed to

Tab. 2 Disease index (%) of tomato seedlings in the experimental combinations.

	Disease index (%)			
Isolates	**Cc**	**Cc+M**	**C**	**M**
EIR32	66.7 ^{ab}	66.7 ^{ab}		
PCL21	75.0 ^{ab}	88.9 ^{b}		
PCL22	52.8 ^{ab}	44.4 ^{ab}		
PER19	77.8 ^{ab}	83.3 ^{b}		
PER31	83.3 ^{b}	61.1 ^{ab}		
EES22	69.4 ^{ab}	71.4 ^{ab}		
PER14	63.9 ^{ab}	61.1 ^{ab}		
Mean	69.8	68.1	35.4 ^{a}	33.3 ^{a}
LSD	44.98			

Number of measurements in each experimental combination: $n = 15$; values marked with the same letters for disease index do not significantly differ at $p \leq 0.05$ (Tukey's test). For description of abbreviations, see Tab. 1.

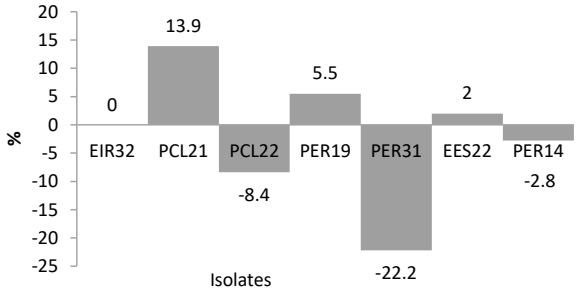

Fig. 4 Difference in the plant disease index (%) in the pathogen-infected seedlings and the mycorrhized and pathogen-infected seedlings.

a decrease of DI by 8.4% and 22.2%, respectively, in comparison with DI of pathogen-infected seedlings (Fig. 4). There was no favorable protective effect of the mycorrhiza in the case of seedlings infected with isolates PCL21, PER19, and EES22.

Discussion

Mycorrhizal fungi are an important component of the rhizosphere. They penetrate the substrate and improve the quality of soil aggregates. They also improve the water and nutrient flow and cycling in the soil, thereby contributing to better plant growth. Additionally, these fungi enhance the resistance of the root system to soil-borne pathogens [8–10].

The present investigations demonstrated a varied effect of the mycorrhiza on the photosynthetic activity of tomato pathogen-infected seedlings. The mycorrhized and pathogen-infected seedlings exhibited higher maximum quantum yield of the PSII (F_v/F_m), higher effective quantum yield (Y), and more favorable photochemical (qP) and nonphotochemical fluorescence quenching (qN) coefficients than the pathogen-infected seedlings. Varied pathogenicity of *C. coccodes* against tomato plants has been reported by many authors [11]. Some isolates caused severe infections in plants, whilst others exhibited less pathogenicity. Similar results were obtained in the present study, where the *C. coccodes* isolates were characterized by varied pathogenicity towards the tomato seedlings. The results showed that the mycorrhiza affects the disease severity caused by *C. coccodes* depending on the fungal isolate. Głuszek and coauthors [12] reported changes in the population of pathogens and reduction of the disease severity of host plant in the presence of arbuscular mycorrhiza fungi (AMF), as well as higher plant resistance to the stress caused by the pathogen.

The mycorrhiza had no significant effect on the growth of tomato seedlings. Different results have been presented by other researchers. Cieślińska and coauthors [13] conducted an experiment on mycorrhized energy plants cultivated in an area with heavy metal contamination and reported a beneficial effect of the mycorrhiza on the development of these plants in adverse growth conditions. Upon the application of the mycorrhizal inoculum, the plants developed a highly specialized root system. Thus, the root-external hyphae penetrating the substrate enabled the fungi to absorb substantial amounts of mineral compounds. Shartiati and coauthors [14] carried out an experiment with the use of mycorrhizal inoculum in safflower cultivation and observed a beneficial effect of the inoculum on the plant, which was manifested in increased chlorophyll content in leaves, longer roots and shoots, and increased root dry matter. The best safflower growth rate was recorded when the plants were inoculated with a mixture of MF, and the plants developed without exposure to water stress. An experiment on the addition of mycorrhiza in pine cultivation demonstrated better plant growth and a greater number of shoots. Mycorrhiza contributes to improvement of plant fitness and health status, regardless of changes in the environmental conditions [15].

The present study has shown that mycorrhiza can also be a stress factor for seedlings. Similar results were reported by Gołcz and Bosiacki [16], who demonstrated that

mycorrhiza used in an experiment with thyme had a negative effect on fresh and dry thyme yields. The investigations showed that nonmycorrhized plants were characterized by higher thyme yields than those in inoculated plants. However, the experiment demonstrated that the inoculum contributed to increased uptake of nutrient elements (nitrogen, calcium, zinc, sulfur). The development of mycorrhiza significantly contributed to an increase in the content of phosphorus, which was unavailable to the plants [9,16].

The present study showed that mycorrhizal inoculation has varied effects on the disease severity of tomato seedlings, which depended on the pathogen fungus strain. Similar results were presented by Jamiołkowska and Michałek [17]. Many researchers, however, show that the presence of MF has a beneficial effect on the health status of plants studied when infected with *C. coccodes*. The reduction in the disease severity caused by *Phytophthora capsici* in pepper was reported by Ozgonen and Erkilic [18]. Other mechanisms may be involved in plant defence during AM symbiosis, for example, differential regulation of chitinases and β-1,3-glucanase isoforms as a result of hormone changes and/or synthesis of specific elicitor/suppressor molecules [19].

Mycorrhiza is especially important for horticultural crops. Symbiotic MF introduced into plantations contributes to substantially better growth and health status of cultivated plants. Mycorrhization of plants growing in multiplates is important. This mode of seedling production results in thick and robust plants. Mycorrhized seedlings do not have much stress when replanting to containers, in comparison with seeded nonmycorrhized plants whose root system may be damaged and the stress is most often reflected in growth inhibition [12]. The present investigations indicate a need to carry out further detailed research in this field.

Conclusions

- The mycorrhized and pathogen-infected seedlings exhibited higher maximum quantum yield of the PSII (F_v/F_m), higher effective quantum yield (Y), and more favorable photochemical (qP) and nonphotochemical fluorescence quenching (qN) coefficients than the pathogen-infected seedlings.
- The mycorrhiza had no significant effect on the growth of tomato seedlings
- The mycorrhizal commercial inoculum had a varied effect on the disease index of tomato seedlings infected with the pathogen, depending on the *C. coccodes* isolate.

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Reakcja siewek pomidorów szczepionych grzybami mykoryzowymi na aktywność fotosyntetyczną, wzrost i zdrowotność roślin po ich zakażeniu *Colletotrichum coccodes*

Streszczenie

Celem pracy była ocena wpływu grzybów mykoryzowych na aktywność fotosyntetyczną, wzrost i zdrowotność siewek pomidora ('Pelikan F_1') infekowanych grzybem chorobotwórczym *Colletotrichum coccodes*. Do eksperymentu użyto szczepionki mykoryzowej zawierającej spory i uśpioną grzybnię grzybów endomykoryzowych (Mycoflor/Polska). Eksperyment przeprowadzono w fitotronie. Tygodniowe siewki podczas przesadzania do multiplatów inokulowano szczepionką mykoryzową (3 ml szczepionki/roślinę). Następnie trzytygodniowe mykoryzowane i niemykoryzowane siewki pomidorów były zakażane grzybem chorobotwórczym. Kontrolę bezwzględną stanowiły siewki wysadzane do sterylnego podłoża bez dodatku szczepionki mykoryzowej i grzyba chorobotwórczego, zaś kontrolę względną siewki inokulowane szczepionką mykoryzową. Po 4 tygodniach wspólnego wzrostu mierzono długość łodyg i korzeni, indeks chorobowy i aktywność fotosyntetyczną siewek. Wykazano brak istotnego wpływu mykoryzy na badane parametry fotosyntezy. Zanotowano jednak, że siewki zaszczepiane mykoryzą i zakażane patogenem miały wyższą potencjalną wydajność fotochemiczną PSII (F_v/F_m), wyższy całkowity

zysk kwantowy (Y) oraz korzystniejsze parametry fotochemicznego (qP) i niefotochemicznego współczynnika wygaszania fluorescencji (qN), niż siewki rosnące w obecności patogenu. Eksperyment wykazał, że inokulum mykoryzowe miało zróżnicowany wpływ na zdrowotność siewek w zależności od badanego izolatu grzyba. Mykoryza nie miała znaczącego wpływu na długość korzeni i łodyg, chociaż korzenie siewek zaszczepionych grzybami mykoryzowymi były lepiej rozwinięte, niż siewki zakażane patogenem. Należy również przypuszczać, że nie tylko grzyb chorobotwórczy, ale i mykoryza, działają na siewki jak czynnik stresu, wpływając na wielkość badanych parametrów.