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Interaction between *Rhodotorula mucilaginosa* and *Alternaria alternata* on *Phaseolus vulgaris-Glomus mosseae* association

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Abstract: A glass house experiment was chosen to study the influence of *Alternaria alternata* and *Rhodotorula mucilaginosa* on the activities and functions of mycorrhizal fungus *Glomus mosseae* during growth of *Phaseolus vulgaris*. This investigation revealed that mycorrhizal plants exhibited improvements in growth compared with non-mycorrhizal ones. In addition, the paired inoculation of *G. mosseae* with *R. mucilaginosa* improved plant growth compared with either single inoculation with *G. mosseae* or paired with *A. alternata*. Phosphatases activity, dry weight, chlorophyll, carbohydrates and protein contents of mycorrhizal plants were significantly increased in the presence of *R. mucilaginosa*. It was also noticed that *R. mucilaginosa* exhibited higher mycorrhizal colonization including; higher percentage in mycorrhizal infection (F%), more colonization intensity (M%) and higher arbuscular formation (A%) compared with single inoculation of AM fungus as well as in case of *A. alternata* all over the experimental periods. Mycorrhizal plants dependency was increased all over the experimental periods in the presence of *A .alternata* while, it was decreased in the presence of *R. mucilaginosa* during the current work. It could be concluded that the benefits of symbiosis of AM fungus and *Phaseolus* plants was increased in the presence of *R. mucilaginosa*, while it was decreased in the presence of *A. alternata.*

Key words: Arbuscular mycorrhiza, *Glomus mosseae*, *A. alternata. R. mucilaginosa,* soil fungi, symbiosis.

Introduction

Arbuscular mycorrhizal (AM) fungi are known to influence and to be influenced by the activities of microorganisms in the soil (Andrade *et al*. 1997, 2004). Mycorrhiza formation can affect the microbial population in the rhizosphere directly or indirectly through changes in root exudation patterns, or through fungal exudates (Barea *et al*. 2002 a, b). Conversely, numerous soil microorganisms interact with mycorrhizal fungi by producing substances that stimulate plant growth or inhibit root pathogens (Berta *et al*. 2005). Soil microorganisms mainly influence AM fungi when these fungi are in the extrametrical phase. Volatile and soluble exudates produced by soil microorganisms are involved in these effects (Fortin *et al*. 2002, Boby *et al.* 2008).

Most studies have dealt with interactions between selected bacteria or saprophytic fungi in relation to AM colonization enhancement (Fracchia *et al*. 2000, Giri and Mukerji 2004). Soil micro-organisms affect the development and function of AM symbiosis (Martinez *et al*. 2004, Rabie *et al.* 2005). Saprophytic fungi are important and common components of rhizosphere soil. Saprophytic and AM fungi are important because they represent a substantial proportion of microbial biomass in soil. Some experimental results have confirmed the existence of synergistic effects of saprophytic fungi on spore germination of AM fungi and plant root colonization (McAllister *et al*. 1996, Sampedro *et al.* 2004, Ablasse *et al*. 2012). Yeasts are a common component of the rhizosphere in all geographic zones (Slavikova and Vadkertiova 2003); however, there is little knowledge of their role in nutrient cycling and their interaction with other soil microorganisms (Slavikova *et al*. 2002). Only a few studies have investigated AM fungi interactions with soil yeasts (Fracchia *et al.* 2003, Sampedro *et al*. 2004, Boby *et al.* 2008).

The aim of this study was to examine the influence of two soil fungi: *Alternaria alternata* and *Rhodotorula mucilaginosa* inoculation on levels of root colonization of *Phaseolus vulgaris* by AM fungus *Glomus mosseae* as well as its effects on plant growth.

Material and Methods

Microorganisms

AM inocula

Two-month-old (10 g per pot) of mycorrhizal inocula of *Glomus mosseae* (local strain obtained by G.M. Abd-El Fattah, Botany Deptartment, Faculty of Science, Mansura University, Egypt) were used. The mycorrhizal inocula consisted of AM colonized root fragments from stock culture with guania grass, rhizosphere soil having extrametrical mycelium and spores (10 spores/g of soil) were taken (Gerdemann and Nicolson 1963).

Alternaria alternata and *R. mucilaginosa* were isolated from different types of soils in Sharkia Governorate by dilution plate method (Johnson *et al.* 1959) and chosen due to their high frequency in the tested soils. *R. mucilaginosa* strain was identified by Prof. El-Shahat Ramadan, Prof. of Microbiology, Ain Shams University, Egypt) according to Barnett *et al.* (2000).

Seed

Seeds of a local variety of *Phaseolus vulgaris* were obtained from Agronomy Deptment, Agriculture Research Centre, Giza, Egypt. These were surface sterilized with 0.01% $HgCl_2$ (Boby *et al*. 2008) and washed 3-4 times with distilled water and grown in black plastic pots containing 1000 g of soil.

Growth conditions

The experiment included six inoculation treatments with five replicates for each treatment. The experiment was carried out with the following treatments: non-inoculated (control), inoculated with *G. mosseae* and *A. alternata* as a single and paired inocula or inoculated with *G. mosseae* and *R. mucilaginosa* as a single and paired inocula. *Phaseolus* seeds were sown in pots containing soil (garden sandy loamy soil) and were thinned to five plants per pot after one week of germination. The pots were arranged in growth chamber at 25/20°C day/night, 11 h day, 60-70% relative humidity and watered to 75% of water-holding capacity (WHC) two times per week. Observations were recorded weekly until 60 days after inoculation. The plants were harvested after 60 days of sowing date.

Mycorrhizal measurement

Mycorrhizal root colonization was determined by the grid line intersect method (Giovannetti and Mosse 1980) after staining the roots with trypan blue (Philips and Hayman 1970). Determination of mycorrhizal dependency (MD) of plants was calculated according to Gerdemann (1975). Determination of R/S ratio according to root shoot lengths was also determined.

Plant analysis

Fresh weight and dry weight were determined during the experimental periods. Chlorophyll content of leaves was estimated by method of Arnon (1949). Carbohydrates content was determined by method of Said *et al.* (1964). Protein content was determined by method of Lowery *et al*. (1951). Activities of acid and alkaline phosphatases were estimated according to Weimberg (1975).

Statistical analysis

The data of the experiments were analyzed by using One- way ANOVA and L.S.D. (Least Significant Difference) according to Kautsoyiannis (1981).

Results

The levels of root colonization by AM fungus were expressed in three ways: Firstly as frequency of mycorrhizal infection in root segments (F%) which reflects the proportion of roots colonized with AM fungus; secondly as intensity of mycorrhizal infection in root tissues (M%) and thirdly as the rate of arbuscular formation (activity of mycorrhizal infection) in root segments (A%) which reflects the potentiality of exchange with symbiosis. As shown in the results of Table (1), dual inoculation of *Rhodotorula mucilaginosa* with AM fungus recorded the highest percentage of F%, M% and A% along the experimental periods of inoculation compared with dual inoculation of AM fungus with *Alternaria alternata*.

R. mucilaginosa revealed significant increase in frequency of mycorrhizal infection (F%) that reached up to 94% after 60 days of inoculation. The results also revealed that the presence of *A.alternata* co-inoculated with AM fungi decreased mycorhhizal infection than that of AM fungus alone at all the experimental periods. In addition, the maximum intensity of mycorrhizal infection (M%) was estimated in case of paired inoculation of AM fungus and *R. mucilaginosa* after 60 days of inoculation. The M% was decreased by about 58% in case of paired inoculation of *A. alternata* and AM fungus at the end of experiment. On the other hand, the rate of mycorrhizal activities (A%) of AM fungus was increased in the presence of *R. mucilaginosa* by about 200%, while it was decreased by about 25% in the presence of *A. alternata* at the end of the experiment.

The results in Table (2) showed that there are significant differences in mycorrhizal dependency (MD) between the different treatments along the periods of experiment. Dual inoculation of *A. alternata* with AM fungus showed the highest percentage of MD compared with single inoculation by AM fungus either singly or paired with *R. mucilaginosa* all over the experimental periods. Moreover, the MD of growing plants increased with the time of experiment and the highest percentage of MD

was observed in *A. alternata* accounting for 420% at the end of experiment. On the other hand, the MD accounted for 180% and 170% at the end of experiment for mycorrrhizal plants in absence and presence of *R. mucilaginosa* respectively. These results indicate an increase of dependency of *Phaseolus* plant on AM fungus in the presence of *A. alternata*.

The data of Table (2) also show that inoculation of plants with AM fungus caused an increasing in root shoot ratios than non-mycorrhizal one. Generally, these rates slightly decreased at 60 days of inoculations compared with the rates at 20 and 40 days. As well, the plants inoculated with AM fungus either singly or paired with *R. mucilagino*sa revealed the highest R/S ratio compared with R/S ratio of single AM fungus as well as that paired with *A. alternata*. On the other hand, R/S ratio of mycorrhizal plants in the presence of *A. alternata* fell down compared with single AM fungus**.**

The data in Table (2) also indicate that the dry weight of all mycorrhizal plants increased significantly compared with non-mycorrhizal ones at all the experimental periods. In addition, dual mycorrhizal inoculation of *R. mucilaginosa* induced significant increase of dry weight over that single inoculated with AM fungus or dual mycorrhizal plants inoculated with *A. alternata*. Also, dry weight of mycorrhizal plants in the presence of *A. alternata* decreased significantly compared with single inoculation with AM fungus at 60 days of inoculation.

The results of Table (3) show that acid phosphatase activity in mycorrhizal plants co-inoculated with either *R. mucilaginosa* or *A. alternata* was higher compared with that in non-mycorrhizal plants. The maximum acid phosphatase activity was observed after 60 days of inoculation in AM plants co-inoculated with *R. mucilaginosa.* On the other hand, AM plants inoculated with *A. alternata* showed decline in acid phosphatase activity compared with that in AM plants.

Table 1: Effect of different inoculants on the level of mycorrhizal colonization: frequency of mycorrhizal infection (F%), intensity of mycorrhizal infection in the root tissues (M%) and rate of mycorrhizal activity of root segments (A%).

Periods (days) Treatments		F%				M%				A%			
		10	20	40	60	10	20	40	60	10	20	40	60
P	NM	-	-	-	-	-	-	-	-	-	-	-	-
	M	83.3	85	89	87	32.7	34.3	36.6	29.85	2.1	10.8	18.4	10.9
Y	NM	-	-	-	-	-	-	-	-	-	-	-	-
	M	87.5	89	91	94	38.2	39	42.1	45.4	4.6	12.6	19.1	21.8
A	NM	-	-	-	-	-	-	-	-	-	-	-	-
	M	76.7	78	79.5	72	10.2	11.3	18.4	17.3	1.9	4.6	7.5	2.7

NM = non-mycorrhizal plant, M = mycorrhizal plant, P = *Phaseolus* plant, Y = yeast (*R. mucilaginosa*), A = *A. alternata*.

Table 2: Mycorrhizal dependency of AM colonization in *Phaseolus vulgaris,* root-shoot ratio and dry weight (g) of *Phaseolus* plant during periods of investigation.

Periods (days) Treatments		MD%				R/S ratio				Mean of dry weight (g)			
		10	20	40	60	10	20	40	60	10	20	40	60
P	NM	-	-	-	-	0.14	0.16	0.2	0.16	0.08e	0.13c	0.2de	0.34c
	M	150	154	175	180	0.18	0.24	0.25	0.14	0.12d	0.2b	0.35bc	0.52a
Y	NM	-	-	-	-	0.18	0.19	0.23	0.15	0.15c	0.15c	0.22d	0.3c
	M	140	158	166	170	0.19	0.24	0.25	0.16	0.21a	0.27a	0.42a	0.51a
A	NM	-	-	-	-	0.13	0.14	0.14	0.14	0.10e	0.15b	0.2de	0.1d
	M	170	180	200	420	0.18	0.19	0.17	0.13	0.17b	0.27a	0.40ab	0.42b
LSD		-	-	-	-	-	-	-	-	0.019	0.054	0.032	0.057

NM = non mycorrhizal plant, M = mycorrhizal plant, P = *Phaseolus* plant, Y= yeast (*R. mucilaginosa*), A = *A. alternata*

LSD at significant level (0.01); same symbols (a. a) means non-significant difference, different symbols (a, b) means significant difference.

Alkaline phosphatase activity increased significantly in mycorrhizal plants in the presence of *R. mucilaginosa* or *A. alternata* more than that of AM plants all over the periods (Table 3). Moreover, the best value of alkaline phosphatase activity was observed in AM plants co-inoculated with *R. mucilaginosa* compared with either AM plants or AM co-inoculated with *A. alternata*. On the other hand, alkaline phosphatase activity decreased significantly in mycorrhizal plants inoculated with *A. alternata* compared with that of AM plants.

As shown in Table (4), the total chlorophyll content was significantly promoted in mycorrhizal plants than non-mycorrhizal ones at all experimental periods. Also the chlorophyll content of mycorrhizal plants in the presence of *R. mucilaginosa* exceeded significantly that with mycorrhizal plants in the presence of *A. alternata.*

The total carbohydrates content was significantly stimulated in mycorrhizal plants over that in non-mycorrhizal ones (Table 4). The total carbohydrates content of mycorrhizal plants in the presence of *R. mucilaginosa* with AM fungus exhibited non-significant difference compared with single inoculation with AM fungus, except at 10 days of inoculation it was significantly decreased. On the other hand, mycorrhizal plants with *A. alternata* recorded significant decrease in total carbohydrates content compared with single AM fungus. However, the inoculation of plants with *R. mucilaginosa* induced significant increase in carbohydrates content compared with the plants inoculated with *A. alternata*.

The protein content increased significantly in all mycorrhizal plants compared with non-mycorrhizal ones. The highest concentration of protein was estimated in mycorrhizal plants co-inoculated with either *R. mucilaginosa* or *A. alternata* all over the experimental periods.

Table 3: Effect of different inoculants on acid and alkaline phosphatase enzymes of *Phaseolus vulgaris* during periods of investigation.

Periods (days)		Mean of acid phosphatase (mg pi/mg protein/min)/0.1 g fresh weight				Mean of alkaline phosphatase (mg pi/mg protein/min)/0.1 g fresh weight			
Treatments		10	20	40	60	10	20	40	60
P	NM	9.40f	12.80e	20.80g	12.30e	9.40bc	12.60d	14.20d	9.00f
	M	14.40c	22.60b	48.10a	35.40c	12.70a	24.50b	28.50a	31.50b
Y	NM	12.90d	17.80c	20.10g	12.30e	12.10a	17.00c	12.10e	10.20e
	M	21.77b	28.10a	43.10b	48.00a	12.90a	27.70a	27.70a	43.30a
A	NM	12.80d	12.90e	24.40e	22.00d	10.20b	8.93e	21.30b	26.10d
	M	12.90d	14.20d	31.70d	22.33d	10.30b	12.90d	21.80b	29.50c
L.S.D		0.14	0.15	0.16	0.14	0.17	0.14	0.16	0.14

Legends as those in Table (2), pi means inorganic phosphorus.

Table 4: Effect of different inoculants on total chlorophylls, total carbohydrates and protein contents of *Phaseolus vulgaris* during the periods of investigation.

Periods (days)		Total chlorophyll (mg chl./1gm of fresh weight)				Mean of total carbohydrates (mg/0.1 g dry weight plant)				Protein content (mg/0.1 g dry weight plant)			
Treatments		10	20	40	60	10	20	40	60	10	20	40	60
P	NM	0.68c	1.03d	1.30d	1.77d	0.57d	0.74e	0.97d	1.07d	0.44d	0.53c	0.60cd	0.62c
	M	2.30ab	2.43ab	2.50ab	2.60a	0.69a	0.84b	1.22a	1.48a	0.53c	0.59ab	0.65bc	0.73b
Y	NM	0. 69c	1.30c	1.60c	1.87c	0.52ee	0.65g	0.90e	1.01e	0.44d	0.58b	0.62c	0.62c
	M	2.40a	2.50a	2.63a	2.67a	0.60c	0.84b	1.17ab	1.48a	0.56b	0.62a	0.65b	0.77a
A	NM	0.63c	0.97cd	1.00e	0.37e	0.45g	0.58h	0.74f	0.49h	0.39e	0.44d	0.41e	0.25e
	M	2.33ab	2.53a	2.57a	2.63a	0.51e	0.78c	1.10c	1.21c	0.58a	0.63a	0.71a	0.78a
LSD		0.11	0.14	0.13	0.076	0.016	0.017	0.054	0.016	0.019	0.033	0.054	0.021

Legends as those in Table (2).

Discussion

Several experimental results indicated interactions between AM and saprophytic fungi in the soil rhizosphere and in plant root colonization (Gryndler 2000). The changes detected in the present study suggest a direct effect of AM colonization, as well as an indirect effect through changes in mycorrhizal inoculation with tested inoculants. Whereas, the best percentage of root colonization was found in paired inoculation of AM fungus with *R. mucilaginosa*.

In the current results, dual inoculation of AM fungus with *R. mucilaginosa* lead to an increase in root colonization compared with single inoculation by AM fungus alone. This is in accordance with that recorded by Andrad *et al*. (2004). Fracchia *et al.* (2003) and Sampedro *et al*. (2004) noticed an increase in mycorrhizal colonization of plant roots inoculated with *R. mucilaginosa*. These results suggest that the number of yeasts present in the rhizosphere when AM colonization of roots is initiated seems to determine the extent of the beneficial effect of these yeasts on the AM symbiosis. In this context, soluble exudates had different natures and effects on the AM symbiosis, and both can be important with respect to the role of yeasts in AM colonization of plants (Fortin *et al.* 2002).

In the current study, dual inoculation of AM fungi with *A. alternata* activated the root colonization but with lower percentages than single AM fungus. Similar results have been proposed by McAllister *et al.* (1996) about activation of mycorrhizal root colonization by *A. alternata* after establishment of mycorrhizal fungi in plant roots. On the other hand, they reported that inoculation of plants with *A. alternata* decreased mycorrhizal root colonization when inoculated with plants before inoculation of AM fungi.

The mycorrhizal dependency in the presence of *A. alternata* increased all over the experimental periods. In this connection, mycorrhizal inoculation protects the plant against the detrimental effect of the pathogenic organisms in soil due to the root development and higher nutrient acquisition in response to AM fungi colonization (Ghazi and ALkaraki 2006). In addition, the results showed that under stress condition of pathogenic microorganisms, plants need mycorrhiza not only for acclimatization but also for continued nutrient uptake during progressive growth stages and these results have been in agreement with those obtained by Giri *et al.* (2003). On the other hand, in the present study the percentage of MD decreased in case of inoculation with *R. mucilaginosa*. Where, the inoculated plants dependency on mycorrhizal fungi was highly decreased due to its positive influence on plants indirectly by encouraging the growth and enhancement of root colonization of legumes by native AM fungi as reported by Fracchia *et al.* (2003) and Silvio *et al.* (2010). Also, Gemma *et al.* (2002) found that mycorrhizal dependency of plant related to the type of fungal species colonization of the root and the levels of nutrient supply. In this respect, it could be concluded from the current results that the benefits of symbiosis of AM fungus and *Phaseolus* plants were increased in the presence of *R. mucilaginosa***,** while it decreased in the presence of *A. alternata*.

The present study also revealed that mycorrhizal plants showed enhanced acid and alkaline phosphatase activities in all treatments which might lead to higher uptake of phosphate from soil. This enhanced uptake of nutrients led to increase in growth of plants inoculated with AM fungus similar to that reported by Uetake *et al*. (2002) and Saito *et al.* (2004). In this connection, Ezawa *et al.* (2001) reported that absorption of P by external hyphae from soil is the first step, followed by translocation along hyphae and the final exchange for sugar in arbuscules.

It is now well established that AM fungal inoculation had significant effect on plant growth variables and improve growth and nutrition of several plants that are important in agriculture and horticulture (Araim *et al.* 2009). The present results showed an enhancement in the fresh and dry weights of the mycorrhizal plants more than in the non-mycorrhizal ones. These results are in agreement with those of Cho *et al.* (2006) and Cavagnaro (2008).

Numerous studies revealed that interactions between soil microorganisms and AM fungi are important for plant growth (Azcon-Aguilar *et al*. 2002, Rabie *et al.* 2005, Boby *et al.* 2008). In this context dual inoculation of AM fungus with *R. mucilaginosa* considered as the best treatment compared with improvements in plant biomass and dry weight in the present study. Similar results were also reported by Sampedro *et al.* (2004). Also, Bhowmik and Singh (2004) recorded that yeast inoculation showed positive effects on dry weight and plant biomass.

The results indicated that AM symbiosis could enhance the chlorophyll content of plant leaves that agreed with the results of other studies (Sannazzaro *et al.* 2006, Sheng *et al.* 2008). Feng *et al.* (2002) and Colla *et al*. (2008)

reported that the increase in chlorophyll content was due to enhanced mineral nutrition, thus helping in higher photosynthetic rate consequently. Our results also revealed that *R. mucilaginosa* co-inoculated with AM fungus gained more chlorophyll content than mycorrhizal plants inoculated with *A. alternata*. These results are in accordance with those obtained by other works (Feng *et al*. 2002, Ablasse *et al*. 2012).

All heterotrophic microorganisms are ultimately dependent on the carbon that originates from the fixation of carbon by photosynthetic plants. An AM fungus is believed to be obligate biotrophs in respect to C, but the extraradical AM mycelium forages in soil for other nutrients and possesses some degradative capabilities (Hodge *et al*. 2001).

Extrapolation of our results revealed that there was an increase in protein content in mycorrhizal plant compared with non-mycorrhizal all over the experimental periods. Evidence from the previous studies (Johanson *et al*. 2004, Rabie *et al*. 2005, Fritz *et al*. 2006, Liu *et al*. 2007) indicated that the presence of AM fungi was known to enhance mineral nutrients, nodulation and nitrogen fixation and then protein content by legumes and consequently promotion of root and mycorrhizal development. In the present study, co-inoculation of *R. mucilaginosa* with AM fungus exhibited higher protein content compared with that of co-inoculated with *A. alternata*. These results are in harmony with the work of Fracchio *et al*. (2003) and Boby *et al*. (2008).

In conclusion the benefits of symbiosis of AM fungus and *Phaseolus* plants were increased in the presence of *R. mucilaginosa***,** while they declined in the presence of *A. alternata*. Good ecological adaptation of mycorrhizal fungus with yeast and to soil in the rhizosphere of *Phaseolus* plants and continue along the age of plant compared with single AM fungus.

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