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Biological Relationship of *Potato virus Y* and Arbuscular Mycorrhizal Fungus *Glomus intraradices* in Potato

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ABSTRACT

The effects of systemic infection by *Potato virus Y* (PVY) and arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* on host reaction were quantified in potato symtomatologically, morphologically, serologically, and physiologically. Reduction in plant height and root development was significantly greater with the AMF+PVY combination than with single PVY infection. Inoculation of AMF increased the symptoms of PVY infection. The *G. intraradices* x PVY pathosystem significantly increased disease severity and reproduction of the virus in potato. The chlorophyll contents increased in AMF plants by 18.8% more than the control. The results suggest that the inoculation of AMF reduced vegetative development in the presence of PVY infection in potato but it increased viral activity considerably.

Keywords: arbuscular mycorrhizal fungus (AMF), *Glomus intraradices* x *Potato virus Y* (PVY) interaction
Abbreviations: AM, *Glomus intraradices* colonized; **AMF**, arbuscular mycorrhizal fungus; **AMV**, *G. intraradices* + PVY; **C**, control; **G.i.**, *G. intraradices*; **PVY**, *Potato virus Y*; **Vr**, PVY only

INTRODUCTION

Arbuscular mycorrhiza (AM) fungi are obligate symbiotic microorganisms that form associations with plant roots in a host-nonspecific manner (Shaul *et al*. 1999). Nowadays, about 80% of all terrestrial plants, including most agricultural, horticultural, and hardwood crop species are able to establish this mutualistic association (Smith and Read 1997; Pozo and Azcón-Aguilar 2007) and their occurrence has been reported in many ecosystems (Brundrett 1991). Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs because they rely on their host plant to proliferate and survive (Pozo and Azcón-Aguilar 2007). The fungi have been shown to promote plant growth, mainly by enhancing nutrient acquisition (especially phosphorus), producing plant growth hormones, improving rhizospheric and soil conditions, altering host physiological and biochemical properties and defending roots against soilborne diseases and nematodes (Shaul *et al*. 1999; Demir and Akkopru 2007). In contrast, formation of the symbiosis mostly leads to higher susceptibility to shoot pathogens including powdery mildew and rust fungi (Linderman 1994; Dugassa *et al.* 1996) viruses (Dehne 1982), and aphids (Gange and West 1994). This mycorrhizal side effect of promoting shoot pathogens is worth investigating in more detail since individual plants may be able to compensate negative influences but species or cultivars may show high variability (Gernns *et al.* 2001; Pozo and Azcón-Aguilar 2007).

Potato virus Y (PVY) causes one of the most important viral diseases of potato (*Solanum tuberosum* L.). The virus is the type member of the Potyvirus group, one of the largest plant virus groups (Riechmann *et al.* 1992). The PVY genome is a positive-sense single-stranded RNA of 10 Kb size, encapsidated into long flexuous rods of about 2000 capsid proteins (Dougherty and Carrington 1988). PVY infects different Solanaceae such as potato, tomato, pepper and tobacco (Marchoux *et al.* 2000; Bougatef *et al.* 2005).

There are numerous reports of interactions (Damsteegt and Bonde 1992; Dehne 1982) between two etiological agents infecting a common host. Such interactions produce antagonistic or protective, mutually exclusive, additive, or synergistic affects in the host plant. No reports could be found describing co-infections of potato by PVY although they occur in the same habitats. The studies reported here were conducted to evaluate the effects of PVY infection on mycorrhizal and non-mycorrhizal potato plants and to obtain quantifiable data on the effects of double infections on pathogen and host. We have analyzed the effects of infection with PVY on mycorrhizal and non-mycorrhizal potato plants. The aim was to verify as symptomlogically, morphologically, physiologically, and serologically whether AM colonization can modify plant response to infection by virus.

MATERIALS AND METHODS

Plant material, AM fungus, and pathogen inoculation

The experiments were carried out with the plant-AMF-pathogen system *Solanum tuberosum* L. cv. 'Marfona', *Glomus intraradices* (*G.i.*) (Isolate no. OM/95, collection of the Department) and PVY. Potato plants were grown in plastic pots (18 cm × 18 cm) containing a sterilized mixture of soil and sand (1:1, v/v). Ten experimental replicates were prepared for each treatment (each potato plant was in a separate pot) according to a completely randomized design. PVY-infected seed tubers of potato were surface-sterilized with 0.05% sodium hypochloride. For the breaking of dormancy of seed tubers and to promote sprout development from eyes of tubers were plunged into water containing 0.1% gibberellic acid for 10 min. Then tubers were kept either in the dark or light for a 15-days period (Demir and Levent 2002). In the AMF-inoculated samples, 5 g (25 spores g^{-1}) of inoculum was placed in the growth medium before seed tubers were sown (Demir and Onogur 1999).

All pots were placed in a growth chamber under standard conditions (15 h light, 25°C, 60% relative humidity) for 10 weeks. Plants were watered twice a week with deoinized water and 200 ml of the nutrient solution [containing 720 mg $MgSO_4$ $7{\cdot}H_2O$, 12.2 mg KH_2PO_4, 295 mg $Ca(NO_3)_2.4H_2O$, 240 mg KNO_3, 0.75 mg $MnCl_2{\cdot}4H_20$, 0.75 mg KI, 0.75 mg $ZnSO_2{\cdot}H_2O$, 1.5 mg H_3BO_3,

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0.001 mg $CuSO_4 \cdot 5H_20$, 4.3 mg FeNaEDTA and 0.00017 mg $Na_2MoO_4 \cdot 2H_2O$; modified from Vosátka and Gryndler (1999) was applied three times into each pot during the experiment.

PVY isolate was obtained from Van province, Turkey, identified from a preliminary survey and maintained in *S. tuberosum* 'Marfona'. The isolate was used as a positive source for molecular diagnosis of PVY. Leaf tissues of systemically infected shoots of *S. tuberosum* 'Marfona' were used in ELISA and RT-PCR assays.

Determination of chlorophyll

At the end of the experiment randomly collected young potato leaves were homogenized with mortar and pestle in mixture of $CaCO_3$, 80% acetone and quartz sand and extracted two times in 5 ml acetone, each followed by centrifugation (5000 rpm, 4°C, 10 min). The supernatants were adjusted to 12 ml with 80% acetone. Chlorophyll content was calculated from the absorption at 645 and 663 nm (Smith and Benitez 1955).

Evaluation of disease severity caused by PVY and root colonization by *G. intraradices*

The symptom development of virus on each potato plant grown in the growth chamber for 10 weeks was recorded on a scale of 0 to 4 , where 0 = no visible lesions on leaf, 1 = up to 25% leaf area affected, 2 = 50%, 3 = 75%; and 4 = more than 75% leaf area affected or dead leaves (Demir and Levent 2002). The disease index was calculated based on the following formula:

$$K = \frac{\Sigma(a \times b)}{(c \times d)} \times 100$$

where K is disease index, a is rating, b is number of plants rated, c is total number of plants and d is highest rating.

Potato roots were dyed with lactophenol blue solution in order to determine the existence of *G. intraradices* by a modified method of Phillips and Hayman (1970) and the colonization rate was determined by the Grid-Line Intersect Method (Giovanetti and Mosse 1980).

Determination of morphological parameters in potato plants

Shoot length, fresh and dry weight of shoots (70°C, 48 h) and tuber size of potato plants were determined at the end of the study. The data were analyzed by ANOVA using SAS statistical program (SAS 1998). Before the analyses were carried out data of disease severity were transformed using log-transformations. Differences between treatments were determined by Duncan's multiple range test at 5% significant level.

Determination of PVY concentration

Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) method was used to quantify PVY in the pathosystems and applied according to Clark and Adams (1977) and instructions of the antiserum manufacturer (Bioreba AG, Switzerland) using plant extracts at a dilution of 1:10, 1:50, 1:100, 1:250, and 1:500 for detecting the virus. Leaf samples were ground (1 g leaf/5 ml buffer) in extraction buffer containing 0.05% Tween-20 added to wells of microplate after coating with PVY specific polyclonal antisera diluted in coating buffer (pH: 9.6) and incubated at 4°C overnight. Plates were washed three times with PBS-Tween-20 buffer and coated with alkaline phosphatase conjugated antibody diluted in conjugate buffer and incubated for 2 h at 37°C. After washing three times, *p*-nitrophenyl phosphate in substrate buffer (0.5 mg/ml; pH: 9.8) was added to wells and incubated at room temperature for 30-120 min. Absorbance values were read at 405 nm using a microplate reader (Awareness Technology, USA). Samples were considered positive when the absorbance values at 405 nm exceeded the mean of the negative controls by least a factor of two.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Verification of PVY in pathosystems was tested by RT-PCR. A modified silica-capture based RNA preparation procedure was adopted from a previously reported RNA extraction method (Foissac *et al.* 2001). Reverse transcription was made as described by Glais *et al.* (2002). To detect PVY, oligonucleotide primers (sense 5′-ACGTCCAAAATGAGAATGCC-3′ and antisense 5′-TGGTGT TCGTGATGTGACCT-3′), published in Singh and Singh (1998), were used to amplify the region between 8717-9196 nucleotides by RT-PCR. The amplified fragment was 480 bp in length. Samples were amplified in a ThermoHybaid PX2 thermo cycler. Aliquots of 10µl PCR products were separated on 1.5% agarose gel in TAE buffer (40 mM Tris pH 7.8, 20 mM acetic acid, 2 mM EDTA) and the DNA was visualized by ethidium bromide staining (Sambrook *et al.* 1989).

RESULTS

Morphologically and physiologically parameters

Shoot length was strongly and significantly reduced in AMV (*G.i.* + PVY) plants in comparison with C (control), AM (*G.i.*), Vr (PVY) demonstrating a 36.4, 38.2 and 39.3% decrease, respectively (**Table 1**). The only PVY inoculation had a significant effect on the shoot fresh and dry weights (36.71 and 5.06 g plant^{-1}, respectively) compared than the other treatments. Plants infected with only PVY had more leaf area than AMV plants (**Fig. 2**). Significant host reactions were recorded between the root systems of AMV and Vr plants. Plants infected with only PVY exhibited a vigorous root system compared to AMV plants. Tuber weight was significantly affected by the *G.i.* inoculation. It increased in AM plants by 10% compared with those of the C plants while it decreased in Vr plants and AMV plants by 15 and 82% compared with those of the AM plants, respectively. Almost no tuber development was observed on the root system of AMV plants (**Fig. 3**). The contents of chlorophyll increased in AM plants by 18.8 and 4.4% compared with Vr plants and AMV plants, respectively.

Colonization of *G.i.* and disease severity of PVY

The degree of mycorrhizal colonization was 54 and 58% in AM and AMV plants, respectively (**Table 2**). None of these differences were statistically significant. The percentage efficacy of the AMF against PVY is seen in **Table 2**. Disease severity was significantly increased by mycorrhiza in AMV plants. The percentage increase in disease severity was 50% compared with the pathogen alone.

Effect of AMF on PVY concentration

The presence of PVY in AMV and Vr plants was verified by RT-PCR (Nie and Sing 2001) before testing by ELISA for

Table 1 Length, shoot fresh and dry weights, tuber sizes and chlorophyll contents of potato plants affected by *G.i* and PVY.

Treatments	Shoot length (cm)	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Tuber weight (g tuber^{-1})	Chlorophyll content (mg g^{-1})
C	28.1 a*	27.90 bc	3.96 c	8.1 ab	0.0090 a
AM	29.0 a	30.71 b	4.25 b	9.0 a	0.0090 a
Vr	29.5 a	36.71 a	5.06 a	7.6 b	0.0073 b
AMV	17.9 d	19.10 d	3.16 cd	1.6 c	0.0086 ab

* Means values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 5% significance level (*C* control, *AM Glomus intraradices* colonized, *Vr* PVY only, *AMV G. intraradices* + PVY)

Table 2 Colonization of AMF and disease severity of potato plants affected by *G.i.* and PVY.

Treatments	Colonization of AMF (%)	Disease severity (%)
C	--	--
AM	54 a	--
Vr	--	30 a
AMV	58 a	45 ab

* Means values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 5% significance level (*C* control, *AM Glomus intraradices* colonized, *Vr* PVY only, *AMV G. intraradices* + PVY)

Table 3 Mean A_{405} values of AMV and Vr plants at different plant extract dilutions.

Dilutions	A_{405} value with AMV pathosystem	A_{405} value with Vr plants	Positive control	Negative control
1/10	1.035	0.490	1.784	0.044
1/50	1.233	0.577		
1/100	0.860	0.373		
1/250	0.545	0.200		
1/500	0.295	0.132		

virus concentration. All tested plants reacted positive for PVY infection resulting in a 480 bp DNA fragment in agarose gel (**Fig. 1**). The concentration of PVY in AMV and Vr plants was investigated using extracted preparations at a 1:10 dilution to 1:500 in DAS- ELISA. PVY was readily detected in AMV plants at the highest dilution (1:500) which gave an A_{405} value (0.295), well above the negative control (0.044) (**Table 3**).

Because there was little variation in ELISA values (A_{405}) between treatments, the values for the repetitions were pooled for analysis. The absorbance values obtained with AMV plants demonstrated high levels of PVY concentration in co-inoculated plants. By contrast, we obtained low absorbance values in the Vr plants tested in the same way (**Table 3**).

DISCUSSION

The influence exerted by AM fungus on plant disease development was studied in potato plants inoculated with PVY. Mycorrhizal potato plants were more susceptible to the PVY (**Table 2**). Virus multiplication and even the sensitivity to viral infection were enhanced. Jabaji-Hare and Stobbs (1984) found that electron microscopic examination of tomato roots infected by tobacco mosaic virus demonstrated increased virus titers when roots also were infected by *Glomus* sp.

Contrary to AM fungus, the PVY reduced photosynthesis of potato plants (**Table 1**). Gernns *et al.* (2001) described the same effects with another biotrophic leaf pathogen, *Erysiphe graminis* f.sp. *hordei*. The mechanisms underlying this AM effect may be multiple. Although phytohormones are likely to be important (Gernns *et al.* 2001), it was not possible to perform analyses in this direction during our experiments.

Growth was measured as height from the soil to the tip of the extended upper leaves. PVY infection in combination with AMF resulted in a significant reduction in plant height compared to PVY infection alone. Linderman (1994) stated that the soil-borne diseases caused by fungal and nematode pathogens most often are reduced by AMF, while those caused by viral and other shoot pathogens are generally increased in mycorrhizal plants.

The higher reproduction of PVY in mycorrhizal potato plants could be attributed (to a certain degree) to the improved nutrient status of these plants. However, some other experiments (Schoenbeck 1978) with split root systems showed that the increase in viral disease is not only due to better nutrition. Dehne (1982) indicated that whether the infection by different viruses was systemic or local, mycorrhizal host plants exhibited increased disease intensity. Foliage diseases caused by both obligate and non-obligate leaf pathogens can be increased on AM compared to nonmycorrhizal plants, likely due to enhanced development of the pathogens rather than to increased incidence or frequency of infections.

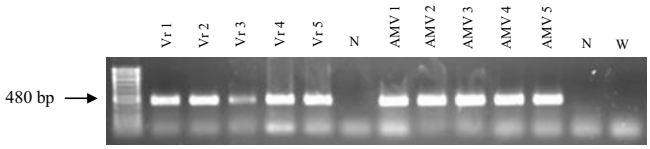

Fig. 1 Verification of PVY infection in AMV and Vr plants by RT-PCR. N: Negative control, W: water control.

Fig. 2 Reduction in plant height and leaf area in AMV plants respect to Vr plants.

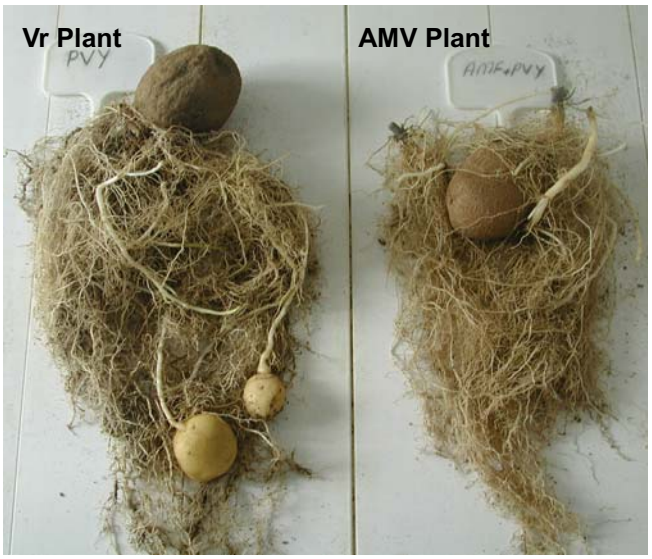

Fig. 3 The effect of AMF+PVY combination and PVY infection on root system of potato.

The cultivar tested was known to be susceptible to PVY when singly inoculated. Inoculation of AMF to PVY infected potatoes resulted in an increase in susceptibility of the cultivar. PVY production was prominent in mycorrhizal plants respect to only PVY infected plants. Linderman (1994) stated that VAM effects on viruses occur throughout the plant due to changes in the host physiology.

The increased exchange of substances from the fungus into the host cell and *vice versa* can be characterized by higher phosphate metabolism and high concentrations of nucleic acids and proteins (Schoenbeck 1980). Virus multiplication may benefit from this high physiological potential in the plant tissue. The increased synthesis of nucleic acid and proteins may promote virus multiplication and thus be responsible for better spread over the whole plant (Dehne 1982).

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REFERENCES

Bougatef K, Marrakchi RT, El Khil HK, Jarboui MA Elgaaied ABA (2005) Detection of *Potato virus Y* in pepper by ELISA-RT-nested PCR. *Phytopathologia Mediterranea* **44**, 75-79

Brundrett M (1991) Mycorrhizas in natural ecosystems. *Advanced in Ecological Research* **21**, 171-313

Clark MF, Adams AN (1997) Characteristics of microplate method of enzyme–linked immunosorbent assay for detection of plant viruses. *Journal of General Virology* **34**, 475-483

Damsteegt VD, Bonde MR (1992) Interactions between *Maize straeak virus* and Downy mildew fungi in susceptible maize cultivars. *Plant Disease* **77**, 390-392

Dehne HW (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* **72**, 1115-1119

Demir S, Levent R (2002) Reactions of different potato cultivars against to early blight disease. *The Journal of Turkish Phytopathology* **31**, 97-103

Demir S, Akkopru A (2007) Using of arbuscular mycorrhizal fungi (AMF) for biocontrol of soil-borne fungal plant pathogens. In: Chincholkar SB, Mukerji KG (Eds) *Biological Control of Plant Diseases*, Haworth Press, NY, USA, pp 17-37

Demir S, Onogur E (1999) *Glomus intraradices* Schenck&Smith: A hopeful vesicular-arbuscular mycorrhizal (VAM) fungus determined in soils of Turkiye. *The Journal of Turkish Phytopathology* **28**, 33-34

Dugassa GD, Von Alten H, Schönbeck F (1996) Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L. infected by fungal pathogens. *Plant and Soil* **185**, 173-182

Dougherty WG, Carrington JC (1988) Expression and function of potyviral gene products. *Annual Review of Phytopathology* **26**, 123-143

Foissac X, Savalle-Dumas L, Gentit P, Dulucq MJ, Candresse T (2001) Polyvalent detection of fruit tree Tricho, Capillo and Faveaviruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR). *Acta Horticulturae* **357**, 52-59

Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolota* L. *New Phytologist* **128**, 79-87

Gernns H, Von Alten H, Poehling HM (2001) Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen-is a compensation possible? *Mycorrhiza* **11**, 237-243

Giovanetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489-500

Glais L, Tribodet M, Kerlan C (2002) Genomic variability in *Potato potyvirus Y* (PVY): evidence that $PVY^{N}W$ and PVY^{NTN} variants are single to multiple recombinants between PVY^{O} and PVY^{N} isolates. *Archives of Virology* **147**, 363-378

Jabaji-Hare SH, Stobbs W (1984) Electron microscopic examination of tomato roots coinfected with *Glomus* sp. and *Tobacco Mosaic Virus*. *Phytopathology* **74**, 253-378

Linderman RG (1994) Role of VAM fungi in biocontrol. In: Pfleger FL, Linderman RG (Ed) *Mycorrhizae and Plant Health*, APS Press, St Paul, Minnesota, pp 1-26

Marchoux G, Selassie KG, Gognalons P, Arteaga ML (2000) Le virus Y de la pomme de terre s'adapte à d'autres solanacées. *Phytoma* **533**, 45-47

Nie X, Singh RP (2001) A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers. *Journal of Virological Methods* **91**, 37-49

Phillips JM, Hayman DS (1970) Improved procedure for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection *Transactions of the British Mycological Society* **55**, 158-161

Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* **10**, 393-398

Riechmann JL, Lain S, Garcia JA (1992) Highlights and prospects of potyvirus molecular biology. *Journal of General Virology* **73**, 1-16

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (2^{nd} Edn), Cold Spring Harbor Laboratory Press, New York

SAS (1998) SAS/STAT Software: Hangen and Enhanced. Sas, Ins. Inc. Cri. NCI

Schoenbeck F, Spengler G (1978) Detection of TMV in mycorrhizal cells of tomato by immunofluorescence. *Phytopathologische Zeitschrift* **94**, 84-86

Schoenbeck F (1980) Endomycorrhiza: Ecology, function, and phytopathological aspects. *Forum Microbiology* **3**, 90-96

Singh RP, Singh M (1998) Specific detection of *Potato virus A* in dormant tubers by reverse transcription polymerase chain reaction. *Plant Disease* **82**, 230-234

Smith JHC, Benitez A (1955) Chlorophylls analysis in plant materials. In: Paech K, Tracey MV (Eds) *Modern Methods of Plant Analysis*, 59 pp

Smith SE, Read DJ (1997) Vesicular-arbuscular mycorrhizas. In: Smith S, Read DJ (Eds) *Mycorrhizal Symbiosis*, Academic Press, London, pp 9-161

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease severity and PR protein expression in tobacco leaves. *Molecular Plant-Microbe Interactions* **12**, 1000-1007

Vosátka V, Gryndler M (1999) Treatment with culture fractions from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. *Applied Soil Ecology* **11**, 245-251