

Glycosidation of apigenin results in a loss of its activity on different growth parameters of arbuscular mycorrhizal fungi from the genus *Glomus* and *Gigaspora*

J.M. Scervino^a, M.A. Ponce^b, R. Erra-Bassells^b, M.J. Bompadre^a, H. Vierheilig^c,
J.A. Ocampo^{d,*}, A. Godeas^a

^aDepartamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, 4P Ciudad Universitaria, (1428) Buenos Aires, Argentina

^bCIHIDECAR-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, 3P Ciudad Universitaria, (1428) Buenos Aires, Argentina

^cInstitut für Pflanzenschutz, Department für Angewandte Pflanzenwissenschaften und Pflanzenbiotechnologie, Universität für Bodenkultur Wien, Peter-Jordan-Strasse 82, 1190 Wien, Austria

^dDepartamento de Microbiología, Estación Experimental de Zaidín, CSIC, (18008) Granada, Spain

Received 30 January 2006; received in revised form 6 April 2006; accepted 2 May 2006

Available online 5 June 2006

Abstract

The effect of different concentrations (0.5, 2 and 8 μM) of apigenin and its glycosidated form 5,7,4'-hydroxy flavone glycoside on arbuscular mycorrhizal (AM) fungal spore germination, hyphal growth, hyphal branching, the formation of entry points and root colonization of *Gigaspora rosea*, *Gi. margarita*, *Glomus mosseae* and *G. intraradices* was tested. The lowest apigenin concentration (0.5 μM) nearly doubled hyphal branching, the formation of entry points and root colonization of all four tested fungi, whereas higher concentrations (2 and 8 μM) nearly doubled the hyphal growth of *Gi. margarita*, *G. mosseae* and *G. intraradices*. In none of the treatments with the apigenin-glycoside any effect on AM fungi could be observed. Our data show that apigenin exhibits an AM fungal genus and even species activity and we provide strong evidence that glycosidation results in a loss of its activity towards AM fungi. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Arbuscular mycorrhiza; Glomeromycota; Flavonoid; Apigenin; Signal molecule; Symbiosis

Flavonoids in roots and root exudates have been suggested to play a role as signalling compounds in the arbuscular mycorrhizal (AM) symbiosis (Phillips and Tsai, 1992; Vierheilig and Piche, 2002; Scervino et al., 2005a–c). Contradictory data are available on the activity of the flavonoid apigenin on AM fungi (Gianinazzi-Pearson et al., 1989; Bécard et al., 1992; Baptista and Siqueira, 1994). Recently, Vierheilig and Piché (2002) suggested that contradictory results on the effect of flavonoids might be due to an AM fungal genus- and even species-specific effect of flavonoids. Scarce information is available about what determines the activity of flavonoids on AM fungi.

Comparing the effect of different flavonoids on AM fungi, Bécard et al. (1992) and Chabot et al. (1992) attributed the activity of flavonoids, such as quercetin and kaempferol, to the hydroxyl group on position 3 of the C aromatic ring (see Fig. 1) as a glycosidation on this position resulted in a complete loss of activity. In the present study we tested whether apigenin exhibits an AMF genus- or species-specific effect on different AM fungal growth parameters and whether an alteration of the apigenin structure (glycosidation) alters its activity.

We used the flavonoids apigenin from SIGMA and apigenin-glycoside (5, 7, 4'-hydroxy flavone) from *Brassica alba* root (Ponce et al., 2004). Flavonoids were tested on *Gigaspora rosea* (BEG 9), *Gi. margarita* (J7; Fracchia, 2002), *Glomus mosseae* (BEG 12) and *G. intraradices*

*Corresponding author. Tel.: +34 58121011; fax: +34 58 129600.

E-mail address: jocampo@eez.csic.es (J.A. Ocampo).

Compound	R ³	R ⁷	R ^{3'}	R ^{4'}	
Quercetin	OH	OH	OH	OH	Act.
Kaempferol	OH	OH	OH	none	Act.
Apigenin	none	OH	OH	none	Act.
Quercitrin	glycoside	OH	OH	OH	Inact.
Rutin	glycoside	OH	OH	OH	Inact.
Apigenin-glycoside	none	glycoside	OH	none	Inact.

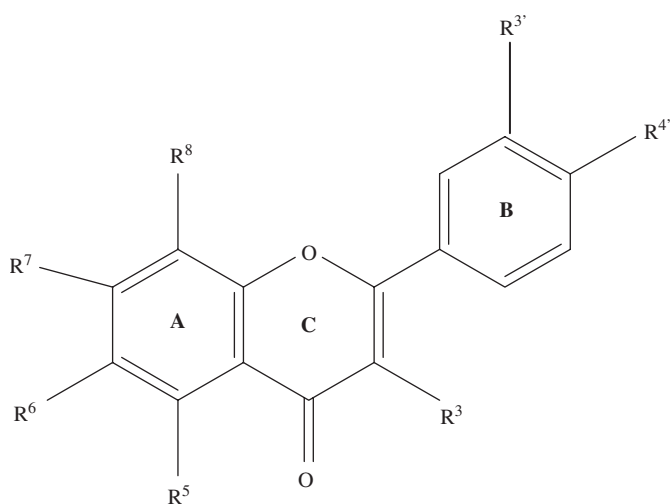
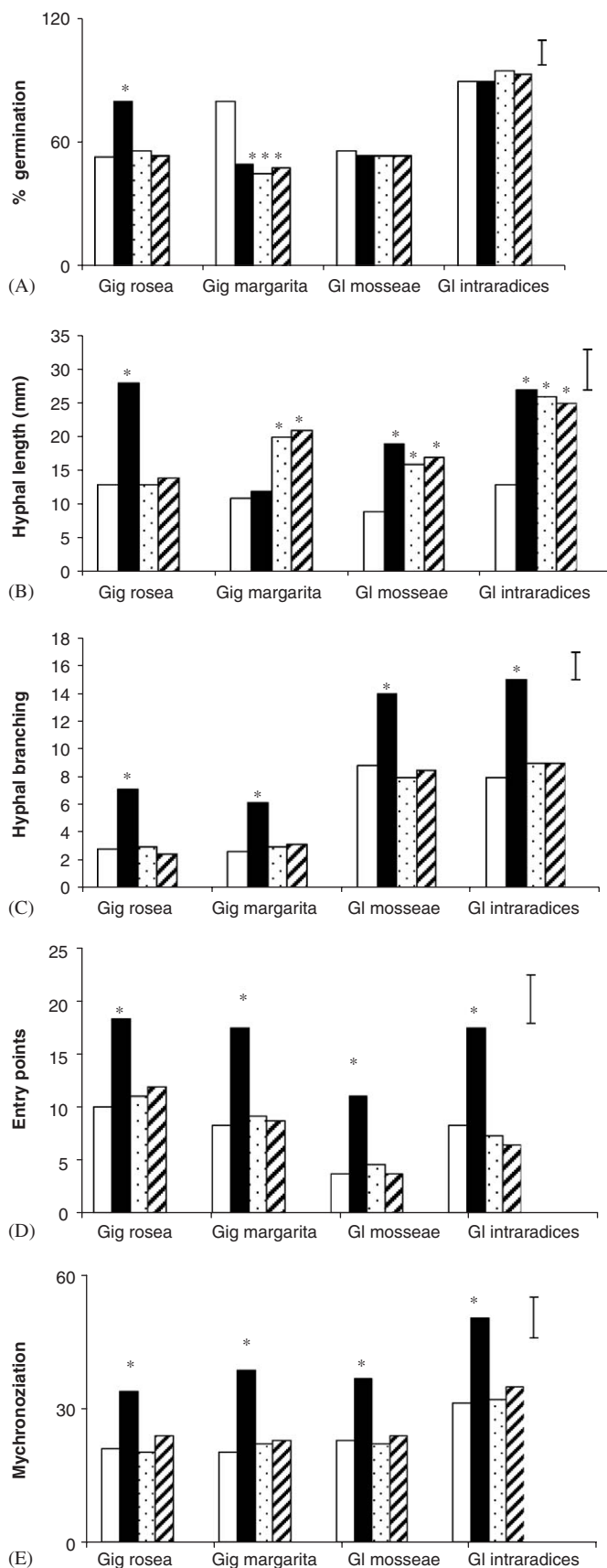


Fig. 1. Structure of differing sidegroups of active (Act.) and inactive (Inact.) flavonoids tested on arbuscular mycorrhizal fungi.

(DAOM 197198) spores in Petri dishes. Spores were isolated (Gerdemann, 1955) from a sorghum (*Sorghum vulgare*) pot culture. Flavonoids were dissolved in 0.05% ethanol at a final concentration of 0.5, 2 and 8 μ M. These concentrations were selected for being the concentrations with reported effects on different steps of the AM fungal development (Morandi, 1996; Vierheilig et al., 1998). Ten replications and 10 controls of each treatment were prepared. The effect of the different flavonoid concentrations on spore germination, hyphal growth and hyphal branching was tested (for details see Scervino et al., 2005b).

The effect of the flavonoids on the formation of AM entry points and on root colonization of tomato (*Lycopersicon esculentum*) was tested in 5 cm diameter Petri

Fig. 2. Effect of apigenin on the percentage of germination (A), hyphal length (mm) (B), number of hyphal branches (C), number of entry points (D) and percentage of root length colonization of tomato (E) by *Gigaspora rosea*, *Gi. margarita*, *Glomus mosseae* and *G. intraradices*. Vertical bar is the least significant difference ($P = 0.05$). (*) = Significantly different from control. □ = Control without flavonoids, ■ = 0.5 μ M of flavonoids, ▨ = 2 μ M of flavonoids and ▩ = 8 μ M of flavonoids.



dishes using a monospore culture technique (Fracchia et al., 2001). Twenty replicates per treatment and controls were used. Plants were harvested and the root system was cleared and stained (Phillips and Hayman, 1970). Ten replicates per treatment and control plants were harvested when hyphal of the AM fungi contacted the root (2 weeks after seedling transplanting), and the number of entry points per 30 cm of root was assessed (Ocampo et al., 1980). The rest of the plants were harvested 6 weeks after transplanting and the percentage of root colonization was measured (Giovannetti and Mosse, 1980).

Results were statistically analysed by a multi-factorial analysis of variance using dose and AM species as the main effects. Significance was determined according to the Fisher's least significant difference (LSD) test.

The water control and the 0.05% ethanol treatments had the same effect on all fungal parameters (data not shown). Fig. 2 shows that at 0.5 μM apigenin increased spore germination, hyphal length and the number of hyphal branches of *Gi. rosea*, whereas all concentrations decreased the spore germination of *Gi. margarita*. Hyphal length of *Gi. margarita* was increased at 2 and 8 μM whereas 0.5 μM of apigenin increased the number of hyphal branches. Apigenin did not affect the spore germination of the *Glomus* species. All doses of apigenin increased the hyphal length, but only 0.5 μM increased the number of hyphal branches of both *Glomus* species.

The dry weights of tomato grown in Petri dishes were similar in all treatments (Data not shown). At 0.5 μM apigenin increased the number of entry points and the root colonization of all four AM fungi (Fig. 2), however, higher concentrations exhibited no effect. Apigenin glycoside never exhibited an effect on any of the parameters tested.

The effect of apigenin on spore germination and hyphal length of the tested AM fungi was complex and depended on the concentration of the flavonoids and on the genus and even the species of the AM endophyte. With *G. rosea* the lowest apigenin level showed a stimulation of spore germination, whereas all apigenin concentrations inhibited the spore germination of *G. margarita* and showed no effect on the spore germination of the two *Glomus* species. All concentrations stimulated the hyphal length of the two *Glomus* species but the stimulation of the two *Gigaspora* species depended on the concentration and differed between each other. Hyphal branching of AM fungi has been suggested as one of the events in host root recognition that precedes successful root colonization (Giovannetti et al., 1996; Buee et al., 2000; Nagahashi and Douds, 2000), and our data strongly support this hypothesis. With all four AM endophytes the lowest concentration of apigenin increased hyphal branching and at the same concentration a close relationship between hyphal branching, the number of entry points and the degree of root colonization was found. In none of the treatments with the apigenin-glycoside any effect on AM fungi could be observed. Interestingly the active apigenin has been reported from root exudates of plants from the fabaceae which is a family

of AM host plants (Scheidemann and Wetzel, 1997; Steele et al., 1999; Suominen et al., 2003), whereas the inactive apigenin-glycoside has been recently detected in roots of the AM non-host plant *Brassica alba* (Ponce et al., 2004).

Bécard et al. (1992) and Chabot et al. (1992) attributed the activity of the compounds quercetin and kaempferol on AM fungal growth parameters not only to the presence of at least one hydroxyl group on the B aromatic ring, but also to the hydroxyl group on position 3 of the C aromatic ring, as quercitrin and rutin with a glycosidation on position 3 exhibited no hyphal growth stimulating effect. Our data clearly question this hypothesis because apigenin with no hydroxyl group on position 3 exhibited a clear stimulatory effect on different AM fungal growth parameters of all the four AM fungi tested. Moreover, apigenin-glycoside which is glycosidated at position 7 of the A aromatic ring (Fig. 1) and not at position 3 of the C aromatic as in quercitrin and rutin, did lose its activity on the AM fungi. These data indicate that the glycosidation independently of their position is responsible for the lack of activity.

To summarize, we could show that apigenin exhibits an AM fungal genus and species activity and we provide evidence that glycosidation of flavonoids can result in a loss of activity towards AM fungi.

Financial support for this study was provided by the Comisión Interministerial de Ciencia y Tecnología, Spain, by the Acciones integradas program Spain–Austria and by CONICET, Argentina.

References

- Baptista, M.J., Siqueira, J.O., 1994. Efeito de flavonóides na germinação de esporos e no crescimento assimbiótico do fungo micorrizico arbuscular *Gigaspora gigantea*. Revista Brasileira de Fisiologia Vegetal 6, 127–134.
- Bécard, G., Douds, D.D., Pfeffer, P.E., 1992. Extensive in vitro hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO₂ and flavonols. Applied and Environmental Microbiology 58, 821–825.
- Buee, M., Rossignol, M., Jauneau, A., Ranjeva, R., Becard, G., 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Molecular Plant Microbe Interactions 13, 693–698.
- Chabot, S., Bel-Rhild, T., Chenevert, R., Piche, Y., 1992. Hyphae growth promotion in vitro of the VA mycorrhizal fungus, *Gigaspora margarita* Becker & Hall, by the activity of structurally specific flavonoid compounds under CO₂-enriched conditions. New Phytologist 122, 461–467.
- Fracchia, S., 2002. Hongos saprotrofos del suelo como microorganismos auxiliares de la micorrización. PhD Thesis, Universidad de Buenos Aires.
- Fracchia, S., Menendez, A., Godeas, A., Ocampo, J.A., 2001. A method to obtain monospore cultures of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 33, 1283–1285.
- Gerdemann, J.W., 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47, 619–632.
- Gianinazzi-Pearson, V., Branzanti, B., Gianinazzi, S., 1989. In vitro enhancement of spore germination and early hyphal growth of a

- vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. *Symbiosis* 7, 243–255.
- Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* 84, 489–500.
- Giovannetti, M., Sbrana, C., Citernesi, A.S., Avio, L., 1996. Analysis of factors involved in fungal recognition responses to host-derived signals by arbuscular mycorrhizal fungi. *New Phytologist* 133, 65–71.
- Morandi, D., 1996. Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions, and their potential role in biological control. *Plant and Soil* 185, 241–251.
- Nagahashi, G., Douds, D.D., 2000. Partial separation of root exudate components and their effects upon the growth of germinated spores of AM fungi. *Mycological Research* 104, 1453–1464.
- Ocampo, J.A., Martin, J., Hayman, D.S., 1980. Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. 1. Host and non-host plants grown together. *New Phytologist* 84, 27–35.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158–161.
- Phillips, D.A., Tsai, S.M., 1992. Flavonoids as plant signals to the rhizosphere microbes. *Mycorrhiza* 1, 55–58.
- Ponce, M.A., Scervino, J.M., Erra-Bassels, R., Ocampo, J.A., Godeas, A., 2004. Flavonoids from shoots, roots and roots exudates of *Brassica alba*. *Phytochemistry* 65, 3131–3134.
- Scervino, J.M., Ponce, M.A., Erra-Bassels, R., Vierheilig, H., Ocampo, J.A., Godeas, A., 2005a. Flavonoids exclusively present in mycorrhizal roots of white clover exhibit different effects on arbuscular mycorrhizal fungi than flavonoids exclusively present in non-mycorrhizal roots of white clover. *Journal of Plant Interaction* 15, 22–30.
- Scervino, J.M., Ponce, M.A., Erra-Bassels, R., Vierheilig, H., Ocampo, J.A., Godeas, A., 2005b. Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*. *Mycological Research* 109, 789–794.
- Scervino, J.M., Ponce, M.A., Erra-Bassels, R., Vierheilig, H., Ocampo, J.A., Godeas, A., 2005c. Arbuscular mycorrhizal colonization of tomato by *Gigaspora* and *Glomus* species in presence of roots flavonoids. *Journal of Plant Physiology* 162, 625–633.
- Scheidemann, P., Wetzels, A., 1997. Identification and characterization of flavonoids in the root exudate of *Robinia pseudoacacia*. *Trees* 11, 316–321.
- Steele, L., Werner, D., Cooper, J.E., 1999. Flavonoids in seed and root exudates of *Lotus pedunculatus* and their biotransformation by *Mesorhizobium loti*. *Physiologia-Plantarum* 107, 251–258.
- Suominen, L., Luukkainen, R., Roos, C., Lindstrom, K., 2003. Activation of the nodA promoter by the nodD genes of *Rhizobium galegae* induced by synthetic flavonoids or *Galega orientalis* root exudate. *FEMS Microbiology Letters* 219, 225–232.
- Vierheilig, H., Piche, Y., 2002. Signalling in arbuscular mycorrhiza: facts and hypotheses. In: Buslig, B., Manthey, J. (Eds.), *Flavonoids in Cell Function*. Kluwer Academic/Plenum, New York, pp. 23–39.
- Vierheilig, H., Bago, B., Albrecht, C., Poulin, M.J., Piche, Y., 1998. Flavonoids and arbuscular-mycorrhizal fungi. In: Manthey, J.A., Buslig, B.S. (Eds.), *Flavonoids in the Living System*. Plenum Press, USA, New York, pp. 9–33.