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BRIEF COMMUNICATION

Effect of flavonoids on *in-vitro* proliferation of hyphae of *Glomus fistulosum*

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Abstract

Biochanin A, luteolin, naringenin and quercetin significantly decreased the percentage of root segments bearing the intraradical proliferating hyphae of *Glomus fistulosum* at the concentrations up to 10 μmol dm^{-3}. The growth of hyphae was inhibited by biochanin A and luteolin whereas no significant effects of quercetin and naringenin were observed.

Additional key words: biochanin A, luteolin, maize, naringenin, quercetin, *Zea mays*.

Plants have a capacity to synthesize flavonoids which play a role in plant defense mechanisms and probably in initiation of arbuscular mycorrhizal (AM) symbiosis (Bel-Rhlid *et al.* 1993). Some flavonoids support the germination and germ-tube growth of spores of AM fungi (Bécard *et al.* 1992, Gianinazzi-Pearson *et al.* 1989). Their possible role in the regulation of infection events in AM was discussed (Morandi *et al.* 1992, Siqueira *et al.* 1991, Tsai and Phillips, 1991) but the exact mechanisms of their effects on AM fungi are poorly understood. In particular, further information on the effects of flavonoids on growth of mycelium of AM fungi is needed. In the presented study we have therefore investigated the effect of four selected flavonoids on proliferation of intraradical mycelium of *Glomus fistulosum* Skou and Jakobsen (isolate BEG 23) in surface disinfected maize root segments.

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Abbreviations: AM - arbuscular mycorrhiza(l); GH - growth of proliferating hyphae, P% - percentage of root segments bearing proliferating hyphae.

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Mycorrhizal root segments were obtained from 4-week-old plants inoculated with *G. fistulosum*. The root systems were washed and selected yellow-colored roots (mycorrhizae) were shaken 5 times with sterile distilled water for 2 min and kept for 4 h in a volume of 100 cm^3 of a solution containing 1 drop of Tween 80, streptomycin, polymyxin B, penicillin G and neomycin, all at a concentration of 500 mg dm^{-3}, and rolitetracycline at a concentration of 250 mg dm^{-3}. The roots were then disinfected in a 1:50 solution of sodium hypochlorite (5 % available chlorine) with traces of Tween 80 for 3 min, washed with 500 cm^3 of sterile glass-distilled water, cut into segments 1 - 2 mm long and washed 6× with water deionized by reverse osmosis and ion exchanger (Mix-bed column, *GORO Ltd.*, Czech Republic). The root segments were incubated in 0.03 cm^3 drops of a medium containing buffers, minerals, vitamins and glucose at concentrations described previously (Gryndler *et al.* 1997). Flavonoids (biochanin A, luteolin, naringenin and quercetin) were suspended in alkalinized deionized water (2 drops of 1 M KOH 100 cm^{-3}), sonicated for 15 min and diluted in the above medium (pH 6.3) to obtain the concentration of 0, 1, 3 and 10 μmol dm^{-3}. Drops of the filter-sterilized incubation medium were placed on the inside bottom of the cover of a polystyrene Petri dish. The dishes, each containing 16 hanging drops of the medium, were incubated in the dark in a humid chamber for 5 d at 25 °C.

After the incubation, the root segments placed in the drops of the incubation medium were observed under a microscope (magnification 63×) with an eyepiece equipped with a grid net focal plate. The length of AM hyphae per root segment (GH) was estimated on non-contaminated segments using grid-line intersect method and mean percentage of non-contaminated root segments bearing proliferating hyphae (P%) was calculated. Only the non-contaminated root segments were taken into account because of possible interference of growth of AM hyphae with contaminating saprophytic microorganisms. One intersection corresponded to a hyphal length of 0.059 mm. One Petri dish was taken as an experimental unit. The data were analyzed by a second-order polynomial regression analysis and Duncan's multiple range test. Results with $P \leq 0.05$ were taken as significant.

Increased concentrations of all flavonoids significantly inhibited P% (Table 1). The strongest negative effect was observed when the root segments were exposed to naringenin. The negative effect of naringenin on hyphae of *Gigaspora margarita* was already observed by Poulin *et al.* (1993), however, the neutral effects of this compound on the growth of hyphae of *G. margarita* and *Glomus intraradices* were also reported (see Morandi 1996 for review).

Quercetin inhibited P% (Table 1), but it did not cause a significant decrease of GH at concentration 10 μM (Table 2) what is in contradiction with the results of Poulin *et al.* (1997), Bécard *et al.* (1992) and Tsai and Phillips (1991) who found stimulation of hyphal growth of *G. margarita* and *G. intraradices* by this compound. Our results indicate that such a stimulation may not be generally observed for all AM fungi or for all fungal structures.

The results of our experiment indicate that luteolin decreases the growth of hyphae of *G. fistulosum*. This compound was shown to cause positive or no effects on the

germ tube growth (Tsai and Phillips 1991), depending on the species of the fungus used. No significant effect of this compound was observed on the development of mycorrhizal infection (Siqueira *et al.* 1991).

Table 1. Effect of flavonoids on percentage of root segments bearing proliferating hyphae (P%) of *G. fistulosum*. Means in columns followed by the same letter do not differ significantly by Duncan's multiple range test at $P \leq 0.05$ ($n = 7$).

Concentration of flavonoids [μM]	P% biochanin A	quercetin	luteolin	naringenin
0	39.3 a	39.3 a	26.8 ab	15.4 a
1	25.1 ab	25.4 ab	32.4 a	6.5 b
3	20.1 b	19.5 b	20.2 bc	5.1 b
10	15.5 b	15.2 b	12.6 c	0.0 [1])
Polynomial regression: $y = a + bx + cx^2$				
a	36.77	37.0	29.9	13.5
b	-7.92*	-8.2*	-2.8**	-4.1***
c	0.58	0.6	0.1	0.3*
F	4.370	4.157	6.246	14.634
r^2	0.259	0.250	0.333	0.539
P	0.024	0.028	0.006	0.000

*, **, *** - coefficients of regression formula significant at $P \leq 0.05$, 0.01 or 0.001, respectively.
[1]) Data not included into statistic treatment.

Table 2. Effect of flavonoids on length of hyphae (GH) of *G. fistulosum* at the end of the experiment. Means in columns followed by the same letter do not differ significantly by Duncan's multiple range test at $P \leq 0.05$ ($n = 7$).

Concentration of flavonoids [μM]	GH [mm per root segment] biochanin A	quercetin	luteolin	naringenin
0	2.47 a	2.47 a	1.95 a	1.10 a
1	1.60 ab	1.05 a	1.95 a	0.73 a
3	1.21 b	1.05 a	1.55 ab	0.63 a
10	1.09 b	1.5 a	0.67 b	n.d.
Polynomial regression: $y = a + bx + cx^2$				
a	2.36	2.17	1.00	1.03
b	-0.54*	-0.61	-0.14**	-0.20
c	0.04	0.05	0.00	0.01
F	4.269	3.318	4.565	1.154
r^2	0.289	0.240	0.268	0.142
P	0.028	0.056	0.020	0.344

*, ** - coefficients of regression formula significant at $P \leq 0.05$ or $P \leq 0.01$, respectively. n.d. - not determined.

Biochanin A stimulated the development of mycorrhizal infection (Siqueira *et al.* 1991) and the hyphal growth of *G. intraradices* (Nair *et al.* 1991, Poulin *et al.* 1997). Our experimental results (Tables 1, 2) do not support a hypothesis of biochanin A being an activating signal for *G. fistulosum* intraradical hyphae because no positive effect on P% or GH was observed. If such an effect really exists, it is either restricted to other fungal structures or it should act together with other unknown factors.

Our results support the findings of others (Bécard *et al.* 1995, Morandi 1996) that the tested flavonoids are not the signal molecules responsible for the growth of hyphae of AM fungi in host root tissue. The effect of flavonoids on AM fungi may be thus restricted on initial phases of the AM infection (Tsai and Phillips 1991, Morandi *et al.* 1992).

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