

Characterization of Glomalin related Compounds in Australian Clover Grown in Sterilized Sand, Alkaline Soil Mixture

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A study of organic compounds produced by arbuscular mycorrhizal fungi (AMF) on the roots of clover

1. Introduction

U.S. scientists reported in 1996 that the product of AMF activity on roots of plants is the glycoprotein glomalin (1-5).

Glomalin is released by hyphae emerging from root spores into the soil, thus improving fertility of soils.

Glomalin is found in the alkaline insoluble fraction of humic substances (HS) of soils and is extracted into sodium citrate solution by an autoclave procedure.

Glomalin was characterized by electrophoresis techniques and reported to have an unknown structure of molecular weight of 90 000 Dalton.

This study reports a range of organic compounds produced by roots of clover plants grown in sterilized soil/sand mixture and sand compartments in a controlled glasshouse experiment (6)

2. Methods

Clover growth system was carried out as per the method developed by Rasmussen *et al.* (6). This system consists of a root compartment (RC) and a hyphae compartment (HC) as shown in Figure 1.

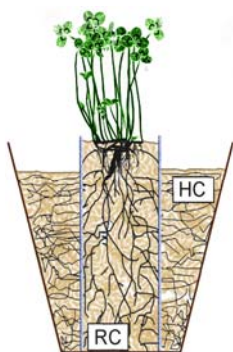


Figure 1: Clover Growth System with cleared roots after 9 weeks

The root compartment, is a nylon mesh bag of 75 mm diameter and 25 µm pore size, filled with 1000 g of sterilized soil/quartz sand mixture (1: 9) and 10 g of clover inoculum from South Australia. Clover seeds were placed in the RC.

HC is the hyphae compartment. A low P fertilizer was placed on the surface of the HC to initiate hyphae growth. The system was kept moist with double distilled (DD) water at 21 °C for 9 weeks.

After 9 weeks the sand of HC, sand/soil mixture of RC, mesh bag and clover roots were air dried and extracted with 50mM Na-citrate solution and autoclaved at 121 °C for 4 hours. The solution was treated with icy cold trichloroacetic acid to precipitate the proteins.

After centrifugation, the remaining pellets were dialyzed in dilute Na-borate solution, followed by dialysis in DD water, further centrifugation and freeze dried as per the total protein extraction method of Wright and Upadhyaya (3).

Glomalin Sample Origin	%C	%H	%N	%O	%S
Root Compartment	9.26	2.37	0.85	20.91	<0.2%
Hyphae Compartment	10.38	2.33	0.98	19.2	<0.2%
Clover Roots	20.64	3.34	1.71	25.92	<0.2%

Table 1 Microanalysis of freeze dried glomalin samples

Microanalysis of extracts were conducted by the Chemistry Dept. University of Otago, New Zealand (Table 1).

Solid State-¹³C NMR spectra of freeze dried extracts of clover roots and the sand/soil mixture of RC were acquired on a Varian Unity INOVA 400 spectrometer (shown in Figure 2)

Pyrolysis-GC- MS were obtained with a Varian CP-3800 GC-Varian Saturn 2200 GC/MS/MS system fitted with a Varian capillary column (VF-5MS 30Mx0.25 mm ID-DF=0.25) and pyrolysis probe CDS pyroprobe, which was heated for 5 sec at 600°C. The pyrolysis products were separated on the column using a helium carrier gas and an oven temperature of 40°C, raised at a rate of 7°C min⁻¹ to 320°C and kept at that temperature for 20 minutes. GC traces of the pyrolysis products of HC and mesh bag lining are shown in Figure 3.

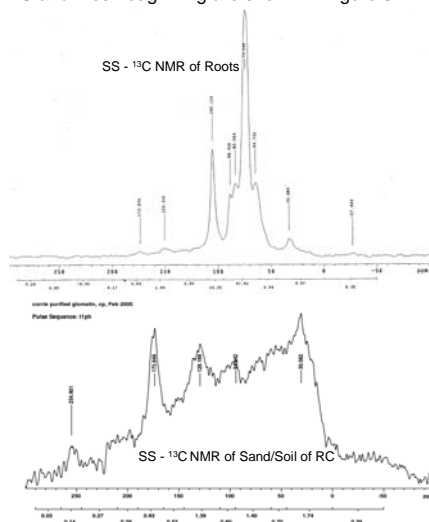


Figure 2: C¹³ NMR Spectra

3. Results and Discussion

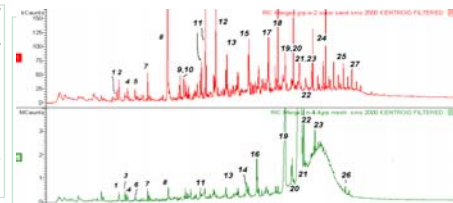
Microanalysis results show higher nitrogen content in roots than in RC and HC extracts, but are in all cases too low for abundant presence of proteins.

High carbon and oxygen content in the extracts of RC and HC roots indicate the presence of carbohydrates and polysaccharides, confirmed by the chemical shifts (50-120 ppm) in the SS-¹³C NMR spectrum.

The small peak at a chemical shift of 174 ppm in the root sample may be associated with -C=O of an amide.

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No	Compound	Source	Mol.Weight
2	3-eicosene, (E)-	alkene	280
3	4-dodecene, (E)-	alkane	168
5	3-furaldehyde	polysaccharides	96
9	phenol, 4-methyl-	lignin	108
10	phenol, 3-ethyl-5-methyl-	lignin	136
12	2,6-diethyl pyridine	protein	135
15	3-eicosene, (E)-	alkene	280
16	9-hexadecenoic acid, 9-octadecenyl ester(Z,Z')	carbohydrates	504
17	octadecane, 1-(ethenyl)-	lipids	296
18	octadecane nitrile	Protein/am.sugar	265
19	9, hexadecenoic acid, eicosyl ester, (Z)	lipids	534
20	oleic anhydride	lipids	546
22	Phytol	polysaccharides	296(E)-
23	Betulin	plant hormone	442
26	stigmast-5-en-3-ol, oleate	plant horm./lipid	678

Figure 3: ID of pyrolysis products of HC extract (red) and mesh bag (green)

3. Results and Discussion

Presence of a significant amount of amide and hence protein in the roots can only be supported if the nitrogen content was higher.

Minor pyrolysis products containing nitrogen are present in all extracts as represented by peaks 12 and 18 in the GC trace shown here.

Most pyrolytic products are from polysaccharide, lipid, lignin and plant hormone origin.

Occurrence of betulin and the stigmastens plant hormones in the extracts was confirmed by running commercially available reference samples.

4. Conclusion

There is little evidence that juvenile mycorrhizal fungi produce Glomalin. A complex mixture of organic compounds was produced by the roots, spores and hyphae of the fungi in 9 weeks.

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