

WHITE CLOVER SAPONINS AND THEIR VARIATION WITH SHADING OR NITROGEN APPLICATION LEVEL.

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ABSTRACT

Saponins are widely present in leguminous plants. Several kinds of saponins which were different from soybean saponins were identified in white clover (*Trifolium repens* L.). These white clover saponins were varied in quality and quantity by the change of cultural conditions such as shading or nitrogen application. In particular, the concentration of saponins in white clover showed tendency to increase under high light intensity and low nitrogen conditions.

INTRODUCTION

Saponins are widely contained in leguminous plants and are regarded as pathogenic substances of bloat in ruminants. Many studies have been carried out on soybean and alfalfa saponins (Quazi, 1975, and Price and Fenwick, 1987), but very little information is available on saponins in white clover (*Trifolium repens* L.) (Sakamoto et al., 1992). In a mixed stand of white clover and grasses, white clover particularly suffered physiological effects by light conditions, by competition with grass and on nitrogen nutrition by fertilization. Thus, the composition of saponins in white clover and their variations in quality and quantity by treatments with shading and fertilizing nitrogen were studied.

MATERIAL AND METHOD

Five plants of white clover clone (cv. Fuia) were planted in a 1/5000a wagner pot filled with 3.5kg of soil on April, 1994. Basal fertilizers, Dolomitic limestone: 14, N: 0.9, P2O5: 0.88 and K2O: 0.16 g/pot had been added to the soil prior to planting. Plant tops were removed three times before the beginning of treatments. A top dressing of N: 0, P2O5: 0.18, and K2O: 0.20 g/pot were given at every cutting. Treatments for light intensities were, 100 (no shading), 60 and 40% of RLI (Relative light intensity), by covering with shading screen and for nitrogen levels were, 0N (nodressing), 1N: 0.13 and 3N: 0.39g of nitrogen/pot by top dressing, were carried out in August. Three pots were used for each treatment.

Nitrogen fixation activity, nodule number, saponin concentration and plant growth were measured on the potted plants.

Nitrogen fixation was measured by acetylene reduction method with Gas chromatography (Sugawara and Duke, 1992). Analysis of saponin was carried out by the method of TLC (Kieselgel 60F-254 plate, Merck, solvent: ethyl acetate/acetic acid/water=7:2:2) and HPLC (YMC-ODS-AM303 column, solvent: acetonitrile/2-propanol/water/acetic acid=34:6:60:0.1) to 70% aqueous ethanol extract of a lyophilized plant. Spots on the TLC plate were detected by spraying methanol/water/sulfuric acid (30:5:2) and heating at 120°C for 10 min. Soybean saponin (Wako Pure Chem. Ind. Ltd.) was used as a standard.

RESULT AND DISCUSSION

Dry matter production of white clover decreased with the decrease of RLI, but did not show a significant difference among plants treated with nitrogen levels. The top/root ratios of dry weight increased with a decrease of RLI at each nitrogen level. The concentration of nitrogen was slightly higher in top and root of 3N than in those of 0N and 1N, but it was higher in leaf and lower in root of 100% RLI than in that of other RLI. Nitrogen fixation activity of white clover decreased considerably with shading and high level of nitrogen application.

Nodule number of root decreased with shading treatment, but did not show significant change among nitrogen application levels (Table 1). Above results showed that the white clover in mixed grassland was subject to suffer physiological damage for nitrogen fixation and photosynthesis by shading.

By TLC development of crude extract of white clover (Fig. 1), 9 spots were detected on the plate. Band 1, 2, 3, 4, band 6, 7, 8 and band 5 were suggested as saponins, substances regarded as saponins and an unknown, respectively, according to Rf values and color of spot. The differences between white clover and soybean were found in the TLC patterns. Rf values of band 3 and 4 were different from those of soybean saponins. By comparison among nitrogen treatments, band 3 and 4 were higher in 0N than in 1N and 3N. In shading treatments, band 1, 4 and 6 were higher in 100% than in 60% and 40% of RLI. Namely saponins accumulate in white clover under an active condition of photosynthesis and nitrogen fixation.

In this study, 4 kinds of saponins were identified in white clover, but 2 kinds of saponins and 5 kinds of saponins in white clover had been reported by Shibata and Myoga (1977) and by Sakamoto et al. (1992), respectively. On identification of clover saponins, further studies are needed under the analytical condition with the prevention of hydrolysis of glycoside.

The results of HPLC analysis also showed 7 peaks regarded as saponins at 34, 41, 46, 52, 64, 70 and 85 min. of retention time. These also varied in amount according to treatments of shading and nitrogen level. In particular, peaks appearing on and after 41 min. were high in 100% of RLI.

The results showed that white clover contains saponins which are different from soybean saponins and the biosynthesis of those saponins are appreciably affected by cultural conditions such as light intensity and nitrogen nutrition.

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Figure 1

TLC patterns of 70% ethanol extracts of white clover.

SS: soybean saponins. 100%, 60% and 40% show relative light intensities, and 0N, 1N and 3N show nitrogen treatments.

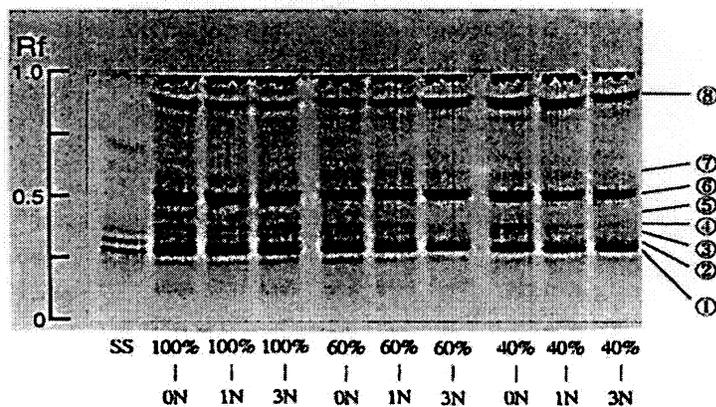


Table 1				
Dry matter production and relative activity of nitrogen fixation of white clover.				
Treatment	Dry matter(g/pot)		N2 fixation (%)	
	Top	Root		
100	0N	5.2±1.1	2.9±0.7	100
	1N	5.3±1.3	3.0±0.7	117
	3N	4.6±0.3	2.9±0.2	53
60	0N	2.6±0.7	1.4±0.3	24
	1N	3.1±0.9	1.7±0.7	25
	3N	2.9±1.0	1.5±0.6	24
40	0N	1.7±0.6	0.8±0.2	34
	1N	1.7±1.1	0.8±0.6	14
	3N	1.9±0.5	1.0±0.2	12