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Saponins of the genus Medicago

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Abstract

The 24 lucerne species were investigated for their saponin content by means of the thin layer chromatography method as well as by haemolytic and fungistatic tests. Wide interspecific differences were found in the content, the chemical composition and biological activity of saponins.

INTRODUCTION

In plant breeding more and more crossings are practised with distant forms. Introduction of genes from wild species into the culivated forms is often desirable as regards resistance to diseases, better wintering or more luxuriant growth of primitive forms.

Interspecific crossing is facilitated by the use of new ways of crossing as well as by breeding of isolated embryos in vitro and other techniques.

The widening of gene resources of a cultivated species by the genetic variability of the wild forms, besides undoubtful advantages, carries also many dangers. The selective powers which acted on species growing without the protection of man often against his will, such as weeds are different from those acting in populations of cultivated plants. Thus, many wild species and weeds contain substances which assure their survival (K e e l e r 1975).

Species of the genus *Medicago* contain variable amounts of saponins including highly toxic glycosides of medicagenic acid. Consequently, we considered it useful to analyse chemically the potential sources of genes which may be available for plant breeders.

MATERIAL AND METHODES

Seeds of 24 lucerne species, from a collection, were sown into pots on 5th March 1975. 20 seedlings from each species were planted on plots

at the end of April. Plants for analyses were cut at the beginning of the flowering stage, dried at 60°C and ground.

Biological test for saponins. A lucerne meal sample of 0.5 g was treated with 100 ml of medium consisting of potato-dextrose-agar (OXOID ltd. England), left to stand until the next day and afterwards sterilized at 121°C for 20 min. The samples were tested with the *Trichoderma viride* fungus according to the method described by Zimmer et al. (1967) (see Table 1).

Isolation of saponins. Milled dry plants (3 g) were extracted with 60 ml of 80% methanol on a boiling water bath during 3 hours. Alcohol was distilled off from the filtrate and the remaining water solution was extracted with methylene chloride, and afterwards 3 times with 15 ml of *n*-butanol saturated with water. Butanol was distilled off under reduced pressure and the remaining dry matter was dissolved in 1 ml of 90% methanol.

Chromatographic analysis of saponins. 4 µl of saponin solution was applied to plates covered with silica gel (Pre-coated TLC Plates, Silicagel 60 Merck). The chromatograms were developed in an ethyl acetate — acetic acid — water (7:2:2) system, dried for one hour at 100°C and sprayed with Liebermann-Burchard's reagent or 10% blood suspension (Kołodziejski and Stecka, 1965) (Fig. 1).

Hydrolysis of saponins and chromatographic analysis of aglycones. The saponin solution (0.5 ml) was evaporated to dryness and after addition of 5 ml of 1 N sulphuric acid in a solution of dioxane-water (1:3) was boiled for 5 hours (Shany et al. 1970). The hydrolyzate was twice diluted with water and extracted with chloroform. After concentration to a small volume aglycones were analysed on Silica gel plates (Fig. 2), with chloroform — petroleum ether — acetic acid (2:7:1) as solvent (Shany, et al. 1970).

RESULTS AND DISCUSSION

The 24 analysed lucerne species may be divided into 4 groups in dependence on the fungistatic activity of water extracts of the lucerne meal towards the *Trichoderma viride* fungus.

Group I. Slight inhibition of fungus growth (< 10%): *Medicago coerulea* (8%), *M. disciformis* (5%), *M. laciniata* (3%) and *M. sativa* (4%), *M. minima* (6%).

Group II. Medium inhibition of fungus growth (10-30%); *M. aculeata* (19%) *M. blancheana* (30%), *M. ciliaris* (14%), *M. coronata* (15%).

Group III. High inhibition of fungus growth (30-70%); *M. glutinosa* (57%), *M. hemicycla* (70%), *M. rugosa* (58%), *M. scutellata* (67%), *M. tornata* (49%), and *M. truncatula* (59%).

Group IV. Very high activity (> 70% of fungus growth inhibition): *M. arabica* (82%), *M. carstiensis* (87%), *M. falcata* (78%), *M. hybrida* (83%), *M. lupulina* (75%), *M. orbicularis* (100%), *M. polymorpha* (90%), *M. rigidula* (86%) and *M. turbinata* (90%).

Table 1

Nr Inhibition of *Trichoderma viride* growth and estimated content of medicagenic acid in *Medicago* species

No.	*Medicago* species	*Trichoderma viride* inhibition in %	Estimated content of medicagenic acid
1	*M. aculeata* Willd. v. *aculeata*	19	medium
2	*M. arabica* L. Huds.	82	high
3	*M. blancheana* Boiss.	30	low
4	*M. carstiensis* Wulf.	87	high
5	*M. ciliaris* L.	14	medium
6	*M. coerulea* Less.	8	low
7	*M. coronata* L.	15	low
8	*M. disciformis*	5	low
9	*M. falcata* L.	78	v. high
10	*M. glutinosa* M. B. ssp. *glutinosa*	57	v. high
11	*M. hemicycla* Grossh.	70	v. high
12	*M. hybrida*	83	high
13	*M. laciniata* L. var. *laciniata*	3	low
14	*M. lupulina* L. var. *lupulina*	75	high
15	*M. minima*	6	low
16	*M. orbicularis* L.	100	high
17	*M. polimorpha* L. var. *polimorpha*	90	traces
18	*M. rigidula* L. var. *rigidula*	86	high
19	*M. rugosa*	58	medium
20	*M. sativa*	4	low
21	*M. scutellata* L.	67	high
22	*M. tornata* L. var. *spinosa*	40	high
23	*M. truncatula*	59	medium
24	*M. turbinata* L. var. *turbinata*	90	high

All the species of lucerne with low fungistatic activity (group I) are not very rich in saponins, which are characterized by a relatively low haemolytic activity and have small or only trace amounts of medicagenic acid. Hydrolysates of saponins from *M. coerulea* and *M. minima* have a distinctly higher content of aglycone, the R_f coefficient of which equals the value of soyasapogenol A. However, glycosides of soyasapogenols, according to the data from literature (Gestetner et al. 1970) and our investigations (Jurzysta 1973a, 1973b), have a lower biological activity as compared with that of the glycosides of medicagenic acid.

Plants with a moderate fungistatic activity (10-30% of inhibition) and a high one (30-70%), are characterized by a corresponding high content of medicagenic acid in the saponin fraction and their haemolytic activity is also high.

Among the 24 analysed lucerne species as many as 9 show a very high fungistatic activity (70-100% inhibition). Extracts from the plants *M. carstienis, M. falcata, M. hybrida, M. lupulina* and *M. turbinata* have a high haemolytic activity, and their saponins contain high or very high quantities of medicagenic acid.

M. arabica, M. orbicularis and *M. rigidula* species have a low haemolytic activity, notwithstanding their high fungistatic activity and a high content of medicagenic acid. Chromatographic analysis of saponins in the above mentioned species is evidence, that the haemolysing components exhibit high R_f coefficients as compared with those of other lucerne species, at the same time the intensity of spots on the chromatograms corresponding to the blood suspension is slight, scarcely visible. The high R_f coefficients of saponin glycosides indicate their low hydrophylic properties which probabaly prevent the penetration of relatively hydrophobic particles to the suspension of blood cells and retard the haemolytic reaction.

M. polymorpha differs distinctly from the species discussed above. The saponin extracts from this species are characterized by a very high fungistatic activity (90%) although they contain only traces of medicagenic acid and hardly cause any haemolysis. Chromatographic analysis of saponin hydrolysates shows the presence of three aglycones at least, not found in other species of the genus *Medicago*. Probably, it is those compounds that have a high fungistatic activity.

Up to date the biological activity of lucerne saponins was believed to be due to the presence of medicagenic acid, while soyasapogenols also present in lucerne were considered as biologically not very active. However, Horber et al. (1974) report that lucerne (*Medicago sativa*) contains, besides soyasapogenols and medicagenic acid, several other aglycones, one of which exhibits fungistatic activity.

In the analysed species of the genus *Medicago* wide differences may be observed in the qualitative composition of saponins. On the other hand, some species have a very similar chemical composition. It seems, therefore that saponins may be a good diagnostic factor in chemotaxonomic investigations of lucerne.

As shown by our ealier investigations (Jurzysta et al. 1973) the hybrid *M. sativa* × *M. falcata* was almost as toxic for animals as *M. falcata.* However, by a proper selection the frequency of forms with a high content of saponins may be decreased. It is probable that in other interspecific crosses, the biochemical features would be inherited in a similar

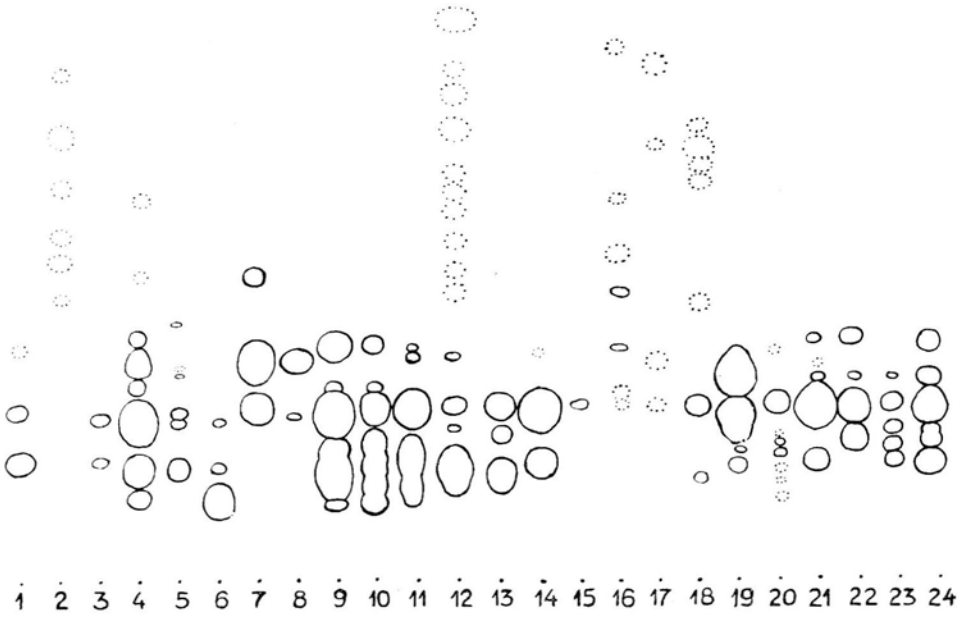

Fig. 1. TLC separation of the genus *Medicago* saponins (see Table 1)

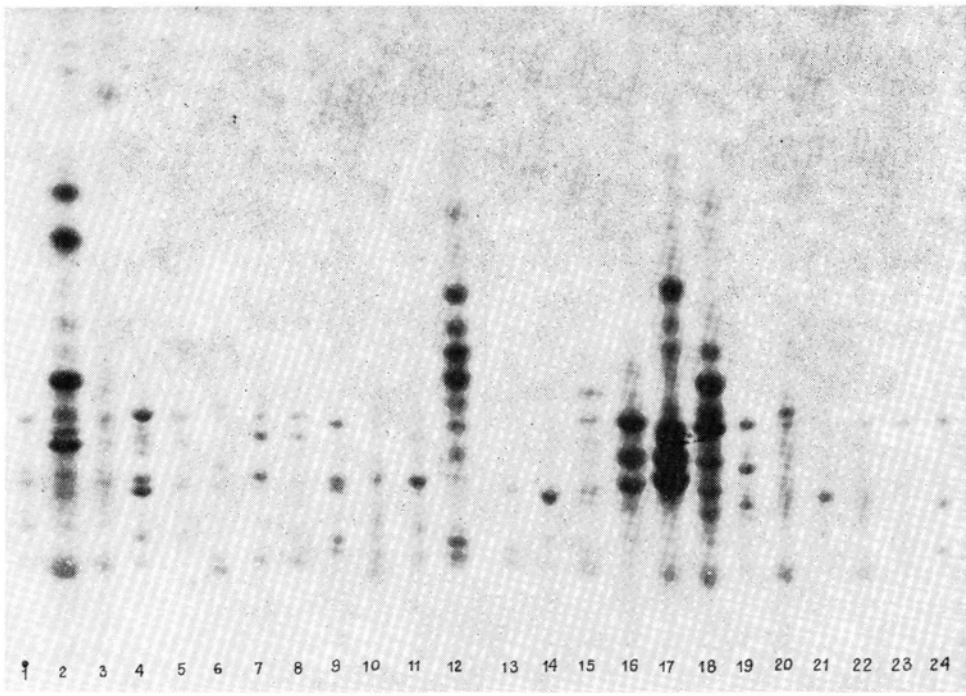

Fig. 2. Thin-layer chromatogram of the genus *Medicago* sapogenins (see Table 1)

way. Attention should be devoted to the unidentified sapogenins of the *M. polymorpha* species. In the genus *Medicago* we may expect the presence of substances with still other toxic and teratologic effects than those described above.

CONCLUSIONS

1. The analysis of 24 species from the genus *Medicago* revealed a significant differentiation in the saponin content of the investigated species as well as a great differentiation in the biological activity of saponins (biological test on the *Trichoderma viride* fungus and the test of blood haemolysis).

2. While enlarging the gene sources of the cultivated lucerne species by genes of wild forms one must take into consideration the possibility of introduction of undesirable, harmful substances of saponin type.

3. The biochemical analysis of the interspecific hybrid progeny will be essential.

4. The chromatographic picture of saponins may be a diagnostic factor for the identification of species for chemotaxonomic purposes.

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Saponiny rodzaju Medicago

Streszczenie

Posługując się metodą chromatografii cienkowarstwowej oraz stosując test hemolityczny i fungistatyczny, przebadano występowanie saponin u 24 gatunków lucerny. Stwierdzono duże zróżnicowanie międzygatunkowe zarówno w zawartości, jak też w składzie chemicznym i aktywności biologicznej saponin.