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Cyanogenesis in Lotus and Trifolium species

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Abstract

The occurrence of cyanogenic plants was determined in 48 *Trifolium* species, 12 *Lotus* species, in wild population as well as in varieties of *T. repens* L. and *Lotus corniculatus* L. species. In the genus *Trifolium* only *T. nigrescens* Viv. proved to be high-cyanogenic, all the remaining species are acyanogenic or low-cyanogenic. In the *T. repens* species varieties and wild populations include an insignificant per cent of cyanogenic plants.
The genus *Lotus* comprises both high-cyanogenic and acyanogenic species. In the *L. corniculatus* species varieties include much more high-cyanogenic plants than do wild populations. It seems that in *L. corniculatus* the breeding went in a wrong direction, because of lack of plant selection regarding the presence of toxic compounds.

INTRODUCTION

Populations of *Trifolium repens* L. and *Lotus corniculatus* L. frequently contain cyanogenic and acyanogenic plants. HCN is released from cyanogenic plants by the action of a β-glucosidase on two cyanogenic glucosides, linanarin and lotaustralin.

The polymorphism of cyanogenesis in *Lotus* and *Trifolium* was the object of many studies. These studies were mostly concerned with to species of *Lotus corniculatus* L. (Dawson 1941; Jones 1962, 1968, 1972, 1973) and *Trifolium repens* L. (Corkill 1940, Daday 1954, 1955; Foulds and Grime 1972), but not many works were found in the available the literature dealing with the genus *Lotus* and *Trifolium* (Grant 1967; Phillips 1968; Gibson 1972) and frequency of cyanogenic plants in Polish varieties.

This investigation was undertaken to determine the frequency of cyanogenic plants in species, varieties and wild populations of *Lotus* and *Trifolium* grown under Polish environmental and climatic conditions.

MATERIAL AND METHODS

The occurrence of cyanogenic plants was determined:

a) In 48 *Trifolium* species and 12 *Lotus* species. The seeds originated from the collection in Gatersleben, G.D.R.

b) In 5 varieties of common bird's foot trefoil *(Lotus corniculatus* L.); 'Bursztyn', 'Puławska', 'Skrzeszowicka', 'Viking', 'Empire' as well as in 3 varieties of white clover *(Trifolium repens* L.) 'Radzikowska', 'Ala', 'Podkowa' and in a variety of Swedish clover *(Trifolium hybridum* L.) 'Zorza'. The seeds of bird's foot trefoil were obtained from the Department of Fodder Plants IUNG in Puławy and the clover seeds from IHAR (Institute of Plant Breeding and Acclimatization) at Radzików and from SHR (Plant Breeding Stations) at Antoniny, Śmiłów and Kowróz.

The plants were grown in pots under greenhouse conditions. In May the seedlings were planted on the plots. The plants were tested in the first and second year of vegetation before flowering. The results in both years were alike.

c) In plants collected from wild populations.

The test for cyanogenesis followed closely the techniques reported by Dawson 1941.

Four drops of toluene or chloroform were dropped into a vial (1.5×5 cm), several top leaves (0.1 g) of the analysed plant were put into it and a strip of filter paper (3×0.9 cm) saturated with sodium picrate solution was placed over the sample. The vial was then tightly closed and incubated at room temperature. A 24-hour period was allowed for HCN to be released. HCN from the leaves reddened the paper strip in proportion to the amount released.

The colour of the papers was visually compared with a standard, prepared from a KCN solution. The plants were classified according to HCN content into four groups: acyanogenic, low-, medium- and high--cyanogenic.

The HCN content in 1 g of fresh material ranged in the group of low-cyanogenic plants from 0.01 to 0.1 mg, medium-cyanogenic from 0.10 to 0.30 mg, and high-cyanogenic from 0.30 to 0.70 mg.

RESULTS AND DISCUSSION

In the genus *Trifolium* no distinct interspecies differentiation in the frequency of cyanogenic plants was found. Only in *Trifolium nigrescens* Viv. all the plants were high-cyanogenic all the remaining analysed species contained both acyanogenic and low-cyanogenic or only acyanogenic plants (Table 1).

The situation in the genus *Lotus* is different, the interspecies dif-

Table 1
Frequency of cyanogenic plants in *Trifolium* species

Species	Number of plants			
	acyano-genic	low-cyano-genic	medium-cyano-genic	high-cyano-genic
Trifolium alexandrinum Jusl.	15	—	—	—
T. alpestre L.	10	4	—	—
T. angustifolium L.	12	3	—	—
T. apertum Bobr.	14	1	—	—
T. arvense L.	11	4	—	—
T. campestre Schreb.	8	7	—	—
T. carmeli Boiss.	7	8	—	—
T. cernuum Brot.	10	4	—	—
T. cherleri Jusl.	14	1	—	—
T. clypeatum L.	6	9	—	—
T. curvisepalum Tackh.	9	6	—	—
T. desvanxii Boiss et Bl.	12	3	—	—
T. dichroanthum Boiss.	13	2	—	—
T. dubium Sibth.	12	3	—	—
T. echinatum M. Bieb. ssp. *supinum* (Sar.) Aschers. et Graeb.	12	3	—	—
T. fragiferum L. Australia	13	2	—	—
T. glomeratum L.	10	5	—	—
T. heldreichianum Hausskn.	11	4	—	—
T. hybridum L.	15	—	—	—
T. incarnatum L. Bernburger *Incarnat.*	14	1	—	—
T. laevigatum Desf.	10	4	—	—
T. lappaceum L.	11	4	—	—
T. leucanthum M. Bieb.	12	3	—	—
T. ligusticum Balb.	9	6	—	—
T. martimum Huds.	14	1	—	—
T. medium Grufb. var. *medium*	10	3	—	—
T. medium Grufb. var. *sarosiense* (Hatsl.) Nyar	8	7	—	—
T. michelianum Sari s.l.	6	9	—	—
T. miegeanum Maire	14	1	—	—
T. montanum L.	13	1	—	1
Trifolium nigrescens Viv.	—	—	—	15
T. ochroleucon Huds.	13	2	—	—
T. pannonicum Jacg.	14	1	—	—
T. phlecides Pourr.	4	9	2	—
T. pratense L. Lembkes Rotklee	14	1	—	—
T. repens L.	1	7	7	—
T. resupinatum L. var. *majus* Boiss.	11	4	—	—
T. resupinatum L. var. *minus* Boiss.	13	2	—	—
T. resupinatum L. var. *resupinatum*	12	3	—	—
T. rubens L.	15	—	—	—
T. scabrum L.	12	3	—	—

Table 1 continued

T. sossonei Savi	13	—	—	—
T. spumosum L.	12	3	—	—
T. stellatum L.	12	2	—	—
T. striatum L.	12	3	—	—
T. tomentosum L.	15	—	—	—
T. trichocephalum M. Bieb.	10	3	—	—
T. xerocephalum Fentl.	2	13	—	—

ferentation is very distinct here. Beside high-cyanogenic species acyanogenic ones occur (Table 2). Other authors report similar results. The *Lotus* species which were considered by Grant and Sidhu in 1967 and by Phillips in 1968 as cyanogenic proved to be cyanogenic in our country as well, with the exception of *L. tenuis* it is possible that we met with an acyanogenic population of this species.

Investigating the genus *Lotus* we took into consideration three species of *Tetragonolobus*, because in older works this genus was classified as *Lotus*.

Table 2
Frequency of cyanogenic plants in *Lotus* species

No.	Species	Number of plants			
		acyanogenic	low-cyanogenic	medium cyanogenic	high-cyanogenic
1	*Lotus corniculatus* L. Gützower Hornklee	—	—	—	15
2	*L. edulis* L.	—	—	—	15
3	*L. hispidus* Desf. em. Lam. et S. C.	10	5	—	—
4	*L. krylovii* Schischk. et Serg.	—	11	4	—
5	*L. ornithopodioides* L.	—	—	1	14
6	*L. pedunculatus* Cav.	13	2	—	—
7	*L. tenuis* Waldst. et Kit.	15	—	—	—
8	*L. uliginosus* Schkuhr	15	—	—	—
9	*L. weilleri* Maire	—	—	—	15
10	*Tetragonolobus requienii* (Mauri ex Sang) Sang	—	—	—	14
11	*T. purpureus* Moench	13	—	—	—
12	*T. siliguosus* Roth.	12	3	—	—

When analysing the interindividual variability in respect to cyanoglucoside content, within the species *Lotus corniculatus* L. and *Trifolium repens* L., from which the varieties grown in our country are derived, we found wide intervarietal and interindividual differentiation only within the *L. corniculatus* L. species (Fig. 1).

In all the investigated varieties of *T. repens* L. almost exclusively acyanogenic plants occur and the per cent of cyanogenic individuals is negligible. Therefore it may be accepted, that our varieties of white clover and the Swedish one are acyanogenic (Fig. 1).

In bird's foot trefoil, all the varieties are cyanogenic although to a different degrees. In the *L. corniculatus* L. species only about 2% of

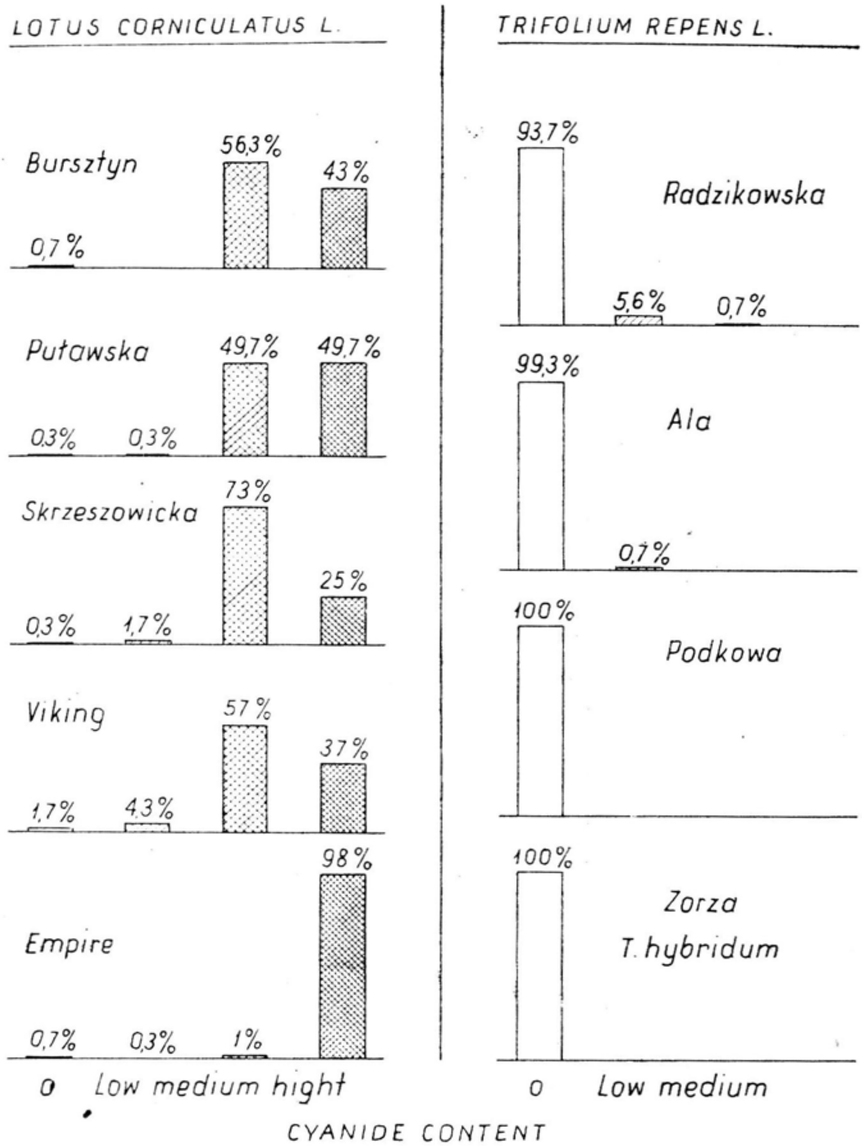

Fig. 1. Frequency of plants with different cyanoglucoside content in *L. corniculatus* and *T. repens* varieties

acyanogenic or low-cyanogenic individuals were found, while medium- and high-cyanogenic ones amounted to 98% (Fig. 2).

The ratio of low to high-cyanogenic individuals was not the same in all the varieties. In the 'Empire' variety for instance there were 98% of high-cyanogenic individuals and in the 'Skrzeszowicka' variety only 25% (Fig. 1). This is the cause of the great intervarietal differences in the content of cyanogenic glucosides.

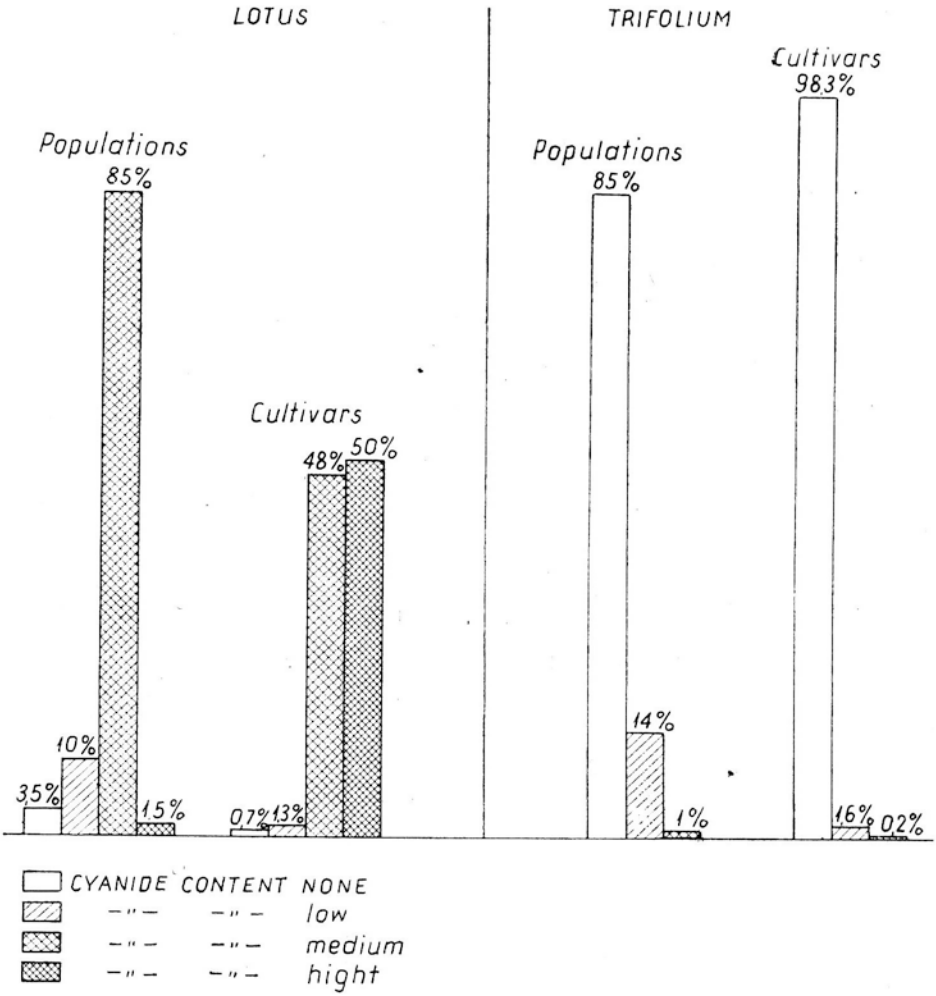

Fig. 2. Frequency of plants with different cyanoglucoside content in the cultivars and wild populations of *Lotus corniculatus* and *Trifolium repens*

Previous work has indicated (B l a i m 1975) that the Polish variety 'Skrzeszowicka' as well as the American variety of European origin 'Viking', contain similar amounts of HCN ranging between 25-32 mg%

calculated on the dry mass basis. The second Polish variety — 'Bursztyn', contains 35-45 mg%, that is somewhat more than the previously mentioned ones, while 'Empire', a variety derived from America, differs by an exceptionally high HCN content, amounting to 180-240 mg%.

In the *T. repens* L. species the wild populations as well as varieties are almost acyanogenic (Fig. 2), whereas, the wild populations and varieties of *L. corniculatus* L. contain a high per cent of cyanogenic plants.

However, in the cultivated there are considerably more high-cyanogenic plants than in the wild populations. On the average in these varieties the number of acyanogenic and low-cyanogenic plants amounts to about 2%, and that of high-cyanogenic ones — to 50%, while in the wild populations we have found about 13% of acyanogenic and low-cyanogenic plants and 1.5% of high-cyanogenic plants. This allows the conclusion that the breeding of bird's foot trefoil varieties has been conducted in a wrong direction because of the lack of plant selection with regard to the presence of toxic compounds.

The cyanogenesis of bird's foot trefoil forms under natural conditions is explained by Jones (1962) by the fact that the cyanogenic forms are not damaged by snails. We have obtained similar results in our nutrition experiments; the animals even after a period of hunger, did not eat the whole quantity of the given high-cyanogenic bird's foot trefoil. Thus it seems that the accumulation of glucosides is protective limiting the demaging of plants by herbivorous animals.

The situation is different as regards white clover. Populations from natural habitats in Poland contain a negligible per cent of cyanogenic plants and our varieties are practically acyanogenic. This phenomenon is frequently explained by unfavourable ecological conditions for cyanogenic forms.

It was observed as early as 1950 that in southern Europe the cyanogenic plants may constitute even 100% of white clover population, in north-eastern Europe this phenotype hardly ever occurs. Daday (1954), investigating the polymorphism of the *Trifolium repens* L. species established a direct correlation between the number of cyanogenic plants and the isoterm of January. Each decrease of the mean January temperature by 1°F corresponds to decrease of the number of cyanogenic plants in the population by 4.23%. It can be concluded that the mean winter temperature played an important role in the evolutional adaptation of *Trifolium repens.*

Thus, there are various opinions on the subject of cyanogenesis in wild populations. On the other hand, in bred populations the genetic factor is most significant, and it is independent of the geographical situation. Therefore, there always exists the danger of breeding a toxic variety when during the selection of plants their quality is not taken into consideration.

CONCLUSIONS

1. Among the analysed species of the genus *Trifolium* only *Trifolium nigrescens* Viv. proved to be cyanogenic. All the remaining species are acyanogenic or low cyanogenic. In the genus *Lotus* there are both acyanogenic and high-cyanogenic species.

2. Within the *T. repens* L. species Polish varieties and wild populations contain a negligible per cent of cyanogenic plants.

3. In the *Lotus corniculatus* L. species the varieties include much more high-cyanogenic plants than the wild populations. This allows the conclusion that selection of plants in respect to the presence of the compounds in point is indispensable in the breeding of varieties.

REFERENCES

Blaim H., 1975. Pam. puł. 62: 141-147.
Corkill L., 1940. N.Z.J. Sci. Tech. 22, 2B: 65-67.
Daday H., 1954. Nature 174, 4428: 521.
Daday H., 1955. Brit. Grassland Soc. J. 10, 3: 266-274.
Dawson C. D. R., 1941. J. of Genetics 42, 112: 49-73.
Foulds W., and Grime J. P., 1972. Heredity 28: 143-146.
Gibson P. B., Barnett D. W. and Gillingham J. T., 1972. Crop Sci. 12 (5): 708-709.
Grant W. F., Sidhu B. S., 1967. Can. J. Bot. 45: 639.
Jones D. A., 1962. Nature 193 (4820): 1109-1110.
Jones D. A., 1968. Heredity 23: 453-455.
Jones D. A., 1972. Genetica 43 (3): 394-406.
Jones D. A., 1973. Heredity 30 (3): 381-386.
Phillips R. L., 1968. Crop Sci. 8 (1): 123-124.

Występowanie glukozydów cyjanogennych w rodzajach Lotus i Trifolium

Streszczenie

Określono częstotliwość występowania roślin cyjanogennych w 48 gatunkach *Trifolium*, 12 gatunkach *Lotus*, w dzikich populacjach oraz odmianach gatunku *T. repens* L. i gatunku *L. corniculatus* L. W rodzaju *Trifolium* tylko gatunek *T. nigrescens* Viv. okazał się wysokocyjanogenny, pozostałe gatunki są niecyjanogenne lub niskocyjanogenne. W gatunku *T. repens* L. odmiany i dzikie populacje zawierają znikomy procent roślin cyjanogennych. Rodzaj *Lotus* zawiera gatunki zarówno wysokocyjanogenne, jak i niecyjanogenne. W gatunku *L. corniculatus* odmiany zawierają znacznie więcej roślin wysokocyjanogennych niż dzikie populacje. Wydaje się więc, że hodowla odmian komonicy poszła w nieodpowiednim kierunku z powodu braku selekcji roślin na obecność omawianych związków.