Variability of cyanogenic glucoside content in white clover plants

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White clover (*Trifolium repens* L.) belongs to the group of plants containing cyanogenic glucosides. Noxious hydrocyanic acid (HCN) is liberated in the digestive tract of livestock during hydrolysis of cyanogenic glucosides. In the process of breeding it is necessary to monitor the content of these compounds which should not exceed 50 mg/100 g dry matter in clover plants.

During the period 1998–2001 we tested the contents of cyanogenic glucosides in the white clover varieties under development and in the wild ecotypes involved in the breeding process. We estimated the variation of these compounds in relation to individual cuts, variety and ecotype, as well as variability between individual plants within the same variety or ecotype. The lowest content of HCN 8.7–21.0 mg/100 g dry matter was found to accumulate in the herbage of the 1st cut, while in the subsequent cuts, especially in the herbage of the 3rd–4th cuts, its content increased to 10.2–44.8 mg/100 g dry matter. While investigating HCN contents in individual plants, a greater variability of these compounds was determined between individual plants of the same variety or ecotype rather than between the ecotypes studied. This makes it feasible to select individuals with a low content of cyanogenic glucosides and to include them in the breeding process. Individual parts of clover plants tend to accumulate different concentrations of cyanogenic glucosides. Higher concentrations are likely to accumulate in leaves (34.0 mg/100 g), stems, petioles and pedicles (19.0 mg/100 g). The white clover studied varied in morphological characteristics and biological traits. No significant correlation was determined between clover leaf size and cyanogenic glucoside content.

Key words: clover, breeding, cyanogenic glucosides

INTRODUCTION

Quite a few species are known in the world to accumulate high contents of cyanogenic glucosides. Such plants are encountered in *Leguminosae, Poaceae* and *Linaceae* families. White clover (*Trifolium repens* L.) is also attributed to the plant species containing cyanogenic glucosides, which are hydrolised in the digestive tract of livestock, resulting in the liberation of noxious hydrocyanic acid (HCN) [1].

Scientific aspects of white clover cyanogenesis phenomenon were described 60 years ago in New Zeland [2]. Synthesis of cyanogenic glucosides is genetically encoded and depends on the dominant allele *Ac* (*ac* – plants without HCN). Production of glucosides is controlled by the enzyme linamarase. Participation of another dominant allele, *Li*, localised in different chromosomes (glucoside loci) is necessary for the synthesis of this enzyme. A dramatic variation in the degree of cyanogenesis of white clover determined in later tests is confirmed by a high polymorphism [3, 4]. With respect to this character, stable and unstable clover genotypes were distinguished [5], more straightforward HCN content determination methods were elaborated [6].

In general, the content of cyanogenic glucosides in clover is not a constant index and is dependent on many factors such as geographical position, temperature regime, plant genotype and others [7]. Varieties with a low HCN content were identified, its variation at various clover growth stages was determined, effects of mineral nitrogen on HCN formation were estimated at the Lithuanian Institute of Agriculture. Clover genotypes with an increased HCN content were less infested by aphids [8].

The objective of the present study was to measure the levels of cyanogenic glucosides in the herbage of individual cuts of the registered white clover variety

'Sūduviai' and wild populations, to estimate the variability of these substances in individual plants of the same variety or ecotype, as well as to assess the morphological characteristics and biological traits.

MATERIALS AND METHODS

White clover is grown in the fields of the six-course crop rotation of LIA Grass Breeding Department. The soils of the experimental site are described as calcareous gleyic medium-heavy drained brown soil with the following characteristics of the plough layer: pH 7.2–7.5, humus content 1.9–2.2%, total nitrogen 0.14–0.16%, P_2O_5 201–270, K_2O 101–175 mg/kg soil. Clover is sown without a cover crop in June, using the narrow-row method (15 cm). Our test material was the clover variety 'Sūduviai', local populations collected during 1995–2000 in different geographical locations practically untouched by human activities, and the developed polycross hybrids.

The content of cyanogenic glucosides was determined by the mercurymetric method. The morphological characteristics and biological traits of clover were assessed according to the UPOV methodology [9]. The assessment is based on 1–9, 3–7 or 4–6 point systems, 1–3–4 being very low and low value of the character, 5 – medium, 6–7–9 – a very high and high value of the character. The obtained data were processed by analysis of variance and other statistical methods [10].

The experiments were carried out during the period 1998–2001, when the weather conditions were diverse. The vegetative growth seasons of 1998, 2000 and 2001 were rainy and the temperatures were close to many years' average. An exception was the 1999 growing season when there was a shortage of moisture throughout the whole growing season, often accompanied by a high air temperature. The spring of 2000 was also droughty.

RESULTS AND DISCUSSION

Our experimental evidence suggests that the investigated white clover differs in morphological characteristics and biological traits (Table 1). Compared with the standard variety, clover Nos. 1365 and 1369 and the polycross hybrid No. 1415 were distinguished for earliness. In terms of size of vegetative parts, clover collected in Bartkūniškis (No. 1370) and the polycross hybrid No. 1415 were equivalent to the standard. The above-mentioned clover No. 1370 and polycross hybrid No. 1416 were similar to the variety 'Sūduviai' in terms of earliness. The other wild populations and the polycross hybrid No. 1412 had finer leaves, thinner petioles and stolons.

The contents of cyanogenic glucosides in white clover herbage of different cuts were found to be different (Table 2). A lower content of these compounds was identified in the herbage of the 1st–2nd cuts. In the standard variety 'Sūduviai' the content of cyanogenic glucosides varied within 13.2–25.5, in wild populations 8.7–21.6 and in polycross hybrids 9.7–20.7 mg/100 g dry matter. The content of cyanogenic glucosides in the herbage of later cuts increases, especially in wild populations. The HCN content in the herbage of the 4th cut of wild population No. 1343 is close to a permissible level. It is reported in literature that white clover varieties that accumulate no less than 30 mg/100 g of HCN are subject to more severe attacks of pests and viral diseases [11]. Our experimental findings suggest the same. The population No. 1343 was more resistant to clover rot, its resistance was estimated by 1 point, and that of the standard by 3 points. Higher HCN contents accumulated in the herbage of this population could determine its better resistance to clover rot. The other wild populations accumulated lower HCN contents in the herbage of the last cut compared with the plants of the variety 'Sūduviai'. In

Table 1. **Assessment data of white clover morphological characteristics and biological traits (in points), 2000–2002**

Catalogue No.	Origin	Characteristics					
		Leaf				Stem: thickness of stolon	Time of flowering
		Length of central leaflet	Width of central leaflet	Length of petiole	Thickness of petiole		
415	Sūduviai, standard	5	5	5	5	5	5
1343	Ignalina district Šuminai village	3	3	4	3	3	5
1365	Kėdainiai district Burveliai village	3	3	4	3	3	3
1369	Kėdainiai district Plinkaigalis	3	3	4	3	3	3
1370	Kėdainiai distr. Bartkūniškis	5	5	5	5	5	5
1412	Polycross hybrid	3	3	4	3	3	5
1415	Polycross hybrid	5	5	4	5	5	3
1416	Polycross hybrid	5	5	5	5	5	5

Table 2. **Contents of cyanogenic glucosides (mg/100 g dry matter) in white clover herbage of different cuts, 2000–2001**

Catalogue No.	Cut				Average	
	I	II	III	IV	2000	2001
415	13.2–21.0	15.3–25.5	21.8–29.6	21.6–36.6	26.9	24.4
1343	8.7–16.7	14.3–21.6	17.5–36.2	23.6–44.8	21.3	24.5
1365	8.7–15.5	11.0–15.6	13.3–41.4	20.0–24.2	14.3	23.2
1369	12.9–18.3	13.0–19.4	13.3–25.3	16.4–28.6	15.6	17.9
1370	12.8–15.6	16.4–18.1	17.9–32.4	15.0–24.8	18.0	20.3
1412	9.7–16.0	15.1–19.0	16.2–34.5	10.2–18.0	12.8	21.9
1415	12.9–15.5	10.5–19.8	15.7–37.7	21.2–29.5	17.2	23.6
1416	12.2–15.3	11.5–20.7	18.6–41.4	27.3–29.8	17.4	26.8
LSD_{05}	2.40	1.95	4.77	5.94		

separate years polycross hybrids accumulate higher HCN contents in the herbage of the 3rd–4th cuts compared with the standard variety. Towards autumn, also structural changes occur in the sward of white clover – the number of inflorescences declines and leaves account for the largest share in the sward.

While investigating the distribution of cyanogenic glucosides in different parts of plants of the standard clover variety, the highest HCN contents (34 mg/100 g dry matter) were found in clover leaves, suggesting that its most intensive synthesis occurs here. HCN content identified in inflorescences reached 23, in stems 19, in petioles and pedicels and stems 19, in roots 13 mg/100 g dry matter.

An increase in the content of cyanogenic glucosides in the herbage of later 3rd–4th cuts is apparently related to the changes in clover structural elements. Synthesis of cyanogenic glucosides also depends on the characteristics of the genotype. The content of cyanogenic glucosides in the herbage of all cuts of wild population No. 1369 was lower than in the standard variety 'Sūduviai', the other investigated wild populations accumulated a lower content of cyanogenic glucosides in the herbage of the 1st–2nd cuts, however, in the herbage of the 3rd–4th cuts of some ecotypes the content of cyanogenic glucosides was higher as compared with the standard variety. In the developed polycross hybrids, except for the herbage of the 3rd cut, a the content of cyanogenic hybrids was lower than in the variety 'Sūduviai'.

The content of cyanogenic glucosides varied within the course of a day. The lowest contents of these compounds were found during the daytime from 10a.m. to 6p.m. An increase in HCN content was observed during the darker part of the day, with its maximum (46 mg/100 g dry matter) at 6 a.m. The contents of cyanogenic glucosides was compared in the herbage of the first cut in different years. The weather conditions for clover development were most favourable in 1998 and 2001. In 1998, when there was an excess of moisture, white clover produced a high yield in which higher contents of cyanogenic glucosides were determined. Due to a long drought in the spring of 2000 clover performed poorly, the yield was low and cyanogenic glucoside content was twice as low as in the yield of 1998. The genotypic effect of white clover on the production of cyanogenic glucosides was more pronounced that year. The coefficient of variation of this index was 20.7 and in the other experimental years 9.3–13.7%. Similar results were also obtained by other authors [4, 7].

The variation of cyanogenic glucoside content in the white clover accessions studied in the 1st–3rd cuts was not high, the coefficient of variation was 12.9–16.8%. During the 4th cut higher differences in cyanogenic glucoside content were revealed between the variety 'Sūduviai', wild populations and polycross hybrids; the variation coefficient was 27.7%. Investigation of cyanogenic glucoside contents in individual plants of the same variety or population showed that the variation of this parameter was higher, the variation coefficient being 14.1–52.4%. At the same time, between the variety 'Sūduviai' and other numbers this parameter was 12.9%. Variation in cyanogenic glucoside content in the individual plants of the standard variety ranged within 11.9–21.3 mg/100 g matter. Individual plants of the wild population No. 1353 accumulated rather different contents of cyanogenic glucosides, allowing to select individuals with a low content of these compounds and to include them into the further breeding process. The importance of wild clover in plant breeding has been pointed out by many foreign researchers. Experiments conducted in Norway suggest that wild populations collected from various localities of the country are valuable not only for routine but also for lawn grass breeding [12].

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CIANOGENINIŲ GLIUKOZIDŲ KIEKIO KINTAMUMAS BALTŲJŲ DOBILŲ AUGALUOSE

S a n t r a u k a

Baltųjų dobilų (*Trifolium repens* L.) sudėtyje yra cianogeninių gliukozidų, kuriems hidrolizuojantis gyvulių virškinamajame trakte išsiskiria nuodinga ciano vandenilio rūgštis (HCN). Vykdant selekcinį darbą būtina kontroliuoti šių junginių kiekį dobilų augaluose, jis neturėtų būti didesnis kaip 50 mg/100 g sausosios medžiagos.

1998–2001 m. tirtas cianogeninių gliukozidų kiekis baltųjų dobilų kuriamose veislėse ir į selekcinį procesą įtrauktose laukinėse populiacijose. Įvertinta šių junginių kaita priklausomai nuo augalų pjūties, veislės bei populiacijos, varijavimas tarp atskirų tos pačios veislės ar ekotipo augalų. Nustatyta, kad baltieji dobilai mažiausiai cianogeninių gliukozidų sukaupia pirmos pjūties žolėje – 8,7–21,0, vėlesnėje, ypač 3–4 pjūties, žolėje jų kiekis padidėja iki 10,2–44,8 mg/100 g sausosios medžiagos. Tiriant HCN kiekį atskiruose augaluose, didesnis šių junginių varijavimas nustatytas tarp atskirų tos pačios veislės ar populiacijos augalų negu tarp tirtų populiacijų. Tai sudaro galimybę atrinkti mažai cianogeninių gliukozidų turinčius individus ir juos įtraukti į selekcinį procesą. Atskiros baltųjų dobilų dalys nevienodai kaupia cianogeninius gliukozidus, daugiau jų lapuose – 34,0, stiebuose, lapkočiuose ir žiedkočiuose – 19,0 mg/100 g. Tirti baltieji dobilai skiriasi savo morfologiniais požymiais ir biologinėmis savybėmis. Tarp dobilų lapų stambumo ir juose esančio cianogeninių gliukozidų kiekio patikimas ryšys nenustatytas.