BIOLOGIJA. 2007. Vol. 53. No. 3. P. 12–15

Wild populations of *Dactylis polygama* H. for the formation of genetic collection and breeding

G. Dabkevičienė,

V. Paplauskienė,

P. Tarakanovas,

N. Lemežienė,

A. Liatukienė

Lithuanian Institute of Agriculture,
LT-58344 Dotnuva-Akademija,
Lithuania
E-mail: genetik@lzi.lt

Much effort has been made recently to expand the range of species suitable for modern farming. More efficient utilisation of the available genetic resources is one of the ways to achieve this purpose. Much attention has been drawn to *Dactylis polygama* H. which is a promising species for forage. The species has not yet been domesticated.

The present study was designed to estimate the feasibility of wild *D. polygama* H. population inclusion in breeding programmes. With this end in view, we compared traits of *D. polygama* H. population and widely grown cocksfoot (*D. glomerata* L.) by the conventional agrobiological assessment method (in points) and estimated polymorphism by the DNA fingerprint method.

D. polygama H. population was found to consist of plants differing in ploidy, therefore they were divided into two forms: diploid (2n = 14) and tetraploid (2n = 28). Comparison of their agrobiological traits with those of the *D. glomerata* L. variety 'Asta' (2n = 28) revealed that wild populations produced a lower herbage yield and were later-maturing but accumulated higher contents of crude protein and water-soluble carbohydrates and were characterised by better digestibility properties.

Comparison of traits of *D. polygama* H. populations suggests that the tetraploid plants surpass the diploid form in a number of characteristics (leaf width, regrowth rate in spring, plant height and herbage yield, the number of inflorescences, protein content). The diploid form accumulated less protein and lower contents of all fibre fractions. It is noteworthy that the diploid population had a lower content of fibre and a higher content of water-soluble carbohydrates which have a positive effect on dry matter digestibility.

Although PCR was performed with seven simple sequence repeat primers, only the GO3, GO7, UBC824 primers were suitable for genomic DNA amplification. In DNA fingerprints, there were produced 23 fragments, of them 8 (34.8%) were polymorphic. The fragment sizes covered the 400–1500 bp range. Individuals of the tetraploid form showed no genetic diversity, whereas DNA fingerprints of diploid plants varied – primers GO7 and UBC824 generated 4-type DNA fingerprints. There were also DNA fragments specific to diploid and tetraploid forms of *D. polygama* H. plants.

Key words: *Dactylis polygama* H., ploidy, agromorphological traits, ISSR fingerprinting, genetic diversity

INTRODUCTION

Much effort has been recently made to expand the range of species suitable for modern farming, and attention has been drawn to a more effective utilisation of genetic resources. Attempts have been made to domesticate wild clover and lucerne species [1]. For a number of years wild ecotypes have been successfully used as initial material in the breeding of perennial forage grasses [2–4].

Cocksfoot (*Dactylis* spp.) is a valuable perennial forage grass. Widely grown in Lithuania, cocksfoot *Dactylis glomerata* L., alongside its positive characteristics (high herbage yield, excellent regrowth, suitability for cutting and grazing), has some drawbacks (tends to rapidly become woody, to lose nutritive properties and to form tussocks). To have an effective sward conveyor, it is necessary to use grass varieties differing in the rhythm of development. Researchers from various countries have noticed a promising forage-type species *Dactylis polygama* H. It is characterised by the traits that breeders find attractive: late maturity, better and more persistent forage value indicators, lower aggressiveness (does not form tussocs), suitability for growing in mixtures with legumes [3, 5]. In Lithuania this grass species is not common. During expeditions arranged over the period 1996–2002 in natural habitats in connection with the genetic resources conservation programme, a few accessions of *Dactylis polygama* H. were collected and studies of its wild populations were started. Not only studies of morphological-physiological characteristics but also biochemical-molecular methods are currently being used to reveal plant diversity. Our experimental objective was to compare the agrobiological traits of *Dactylis polygama* H. populations with those of *Dactylis glomerata* L. populations, using a conventional agro-biological assessment method and quality indicators; to estimate the genetic differences of

Dactylis polygama H. wild ecotype by the DNA fingerprinting technique; to supplement the cocksfoot genetic collection with promising breeding material.

MATERIALS AND METHODS

The population of *D. polygama* chosen for investigations was found in 1999 in Trakai district, in an old Užtrakis park. For morphological analyses, clover was grown in an experimental nursery with a 50 × 50 cm nutritional area, 30 plants per plot. The cocksfoot was tested for 10–14 morphological or biological traits. Assessment in points was done using the standards developed by the International Plant Genetic Resources Institute (IBPGR): a 1–9 point scale, where 1, 2, 3 – very low or low, 4, 5, 6 – medium, 7, 8, 9 – very high value of the trait [6]. Plants of the test populations were compared with those of the *D. glomerata* L. registered variety 'Asta' which was chosen as a control.

The contents of crude protein, water-soluble carbohydrates, crude fibre, neutral detergent fibre, dry matter digestibility were measured with a NIRS-6500 near-infrared spectrometer. For this purpose, all samples were put in small ring cups 4.7 cm in diameter and scanned with a monochromator equipped with a spinning module. Reflection spectra (log 1 / R) from 400 to 2500 nm were recorded within a range of 2 nm. Equations developed at the LIA were used for the analysis of the spectra scanned and for determination of chemical composition [7].

DNA was extracted from young leaves of 15 plants from the populations, using the Doyle and Doyle protocol [8]. Polymerase chain reactions (PCR) were carried out in a 25 µl volume in an Eppendorf Master Cycler Gradient thermocycler. Amplification products were analysed in 1.5% agarose gel and electrophoresis was carried out in 1 × TAE buffer. GeneRuler™ DNA Ladder Mix (Fermentas) was used as the DNA fragment size marker. The gels were analysed in UV light by staining with ethidium bromide. PCR was done using seven simple repeat sequence primers: GO3-$(TCC)_5$GT, GO7-$(GAA)_5$CG, UBC824-$(TC)_8$G, 78H-AC$(GACA)_4$, UBC827-$(AC)_8$G, 104H-$(GACA)_4$GT, UBC825-$(AC)_8$T.

RESULTS AND DISCUSSION

D. polygama H. plants grown from seed were different. Cytological chromosome analysis showed that the plants of the population differed in ploidy: part of the plants had 14 chromosomes and the rest had 28 chromosomes in somatic cells. Based on the total agrobiological traits and chromosome number, the specimens of the ecotype were divided into two populations: the 4n form comprised tetraploid plants characterised by a higher productivity (more luxuriant, taller-stemmed, wider-leaved, etc.), and the 2n form included diploid, less productive individuals. Similar findings confirming that wild cocksfoot ecotypes contain plants differing in ploidy have also been reported by other researchers [9, 10]. *D. glomerata* H. plants formed wider leaves and taller stems, which resulted in a higher productivity than that of *Dactylis polygama* H. plants (Table 1). However, according to many other traits (leaf length, bunch density and growth habit, inflorescence formation indicators) *D. polygama* H. populations were similar to or surpassed the plants of *D. glomerata* L. variety 'Asta'. It is noteworthy that slower regrowth rate in spring is specific to *D. polygama* H. populations. As a result, the 2n form started flowering 8 days later and the 4n form 4 days later than the variety 'Asta'.

D. polygama H. plants were superior to those of *D. glomerata* L. variety 'Asta' also in terms of quality: they accumulated higher contents of crude protein and water-soluble carbohydrates, were characterised by a better digestibility because of a lower fibre content (Table 2).

Comparison of the agrobiological traits of both *D. polygama* H. populations suggests that the 4n form of plants surpassed the 2n form by a number of indicators: leaf width, regrowth rate in spring, plant height, herbage yield, and the number of inflorescences. The diploid form plants of *Dactylis polygama* H. are much shorter and therefore better suited for growing in mixtures with legumes. Specimens of 2n form *Dactylis polygama* H. accumulated a lower protein content and lower levels of all fibre fractions. Notably, the diploid form contained more water-soluble carbohydrates which have a positive effect on digestibility.

Our experimental results are congruent with those of other researchers. R. Linder et al. have reported that distinct differences in agrobiological traits and ploidy are also specific to *D. glomerata* L. natural ecotypes growing in the north-west of Spain [11]. V. Mika et al. [12] indicates that *D. polygama* H. populations mature seven days later than those of *D. glomerata* L., have a good feeding value, are well-adapted to growing in the

Table 1. **Description of agromorphological traits (in points) of *Dactylis* spp. populations**

Traits	Variety, form		
	***D. glomerata* L. cv. 'Asta' (2n = 4x)**	***D. polygama* H. diploid form**	***D. polygama* H. tetraploid form**
Leaf width	6	3	4
Leaf length	6	7	6
Beginning of flowering	3	7	5
Inflorescence formation uniformity	5	5	6
Bunch density	5	7	7
Regrowth in spring	9	6	8
Height	8	4	7
Herbage yield of 1st cut	9	6	8
Bunch growth habit	5	7	7
Abundance of inflorescences	7	7	9
Number of spikelets per inflorescence	7	8	7

Table 2. **Forage quality characteristics of *Dactylis* spp. populations**

Variety, form	Quality characteristics				
	Crude protein g kg^{-1}	Crude fibre g kg^{-1}	Neutral detergent fibre g kg^{-1}	Water-soluble carbohydrates g kg^{-1}	Dry matter digestibility %
D. glomerata L. cv. 'Asta' (2n = 4x)	113	309	603	154	558
D. polygama H. diploid form	142	230	498	224	690
D. polygama H. tetraploid form	151	269	563	171	560
LSD_{01}	8.1	9.1	12.5	10.9	11.7

Table 3. **ISSR products generated in *D. polygama* H. forms**

Primer	Oligonucleotide sequence	Total number of fragments	Number of polymorphic fragments	Fragment size range bp	DNA profile type	Repeatability of DNA profile types	
						Tetraploid form	Diploid form
GO3	$(TCC)_5GT$	8	2	500–1500	I	–	100%
					II	100%	–
GO7	$(GAA)_5CG$	8	2	400–1030	I	100%	–
					II	–	62.5%
					III	–	37.5%
UBC824	$(TC)_8G$	7	4	450–1200	I	100%	–
					II	–	50.0%
					III	–	50.0%

Central European region and are perfectly suitable for forage production.

The DNA fingerprinting (ISSR) method was used for a more comprehensive analysis of *Dactylis polygama* H. diversity**.** PCR was performed with seven simple sequence repeat primers. The GO3, GO7, UBC824 primers were suitable for genomic DNA amplification. The other primers generated either indistinct DNA profiles or no fragments at all.

Amplification of DNA of the plants of both populations with primer GO3 produced seven fragments. The fragments in DNA profiles for both populations were situated in the same 500–500 bp range and differed only in the locus of two fragments: the fragment 700 bp was specific to the profiles of plants of the 2n form, while the 750 bp fragment was specific to 4n form plants.

The GO7 primer used for amplification revealed a greater polymorphism of genomic DNA (Table 3).

Although the same number of fragments was produced as with the GO3 primer, they composed DNA profiles of three types. The fragments were situated within a narrower 400–1030 bp range. All the 4n plants were represented by one DNA profile type (fragments 400, 500, 600, 800, 900, 1030 bp). A greater diversity was observed in the 2n form where two DNA profile types were identified (I – fragments 400, 500, 600, 700, 800, 900, 950, 1030 bp; 62.5% of individuals belonged to this type). The rest 37.5% of plants were represented by DNA profile type II (fragments 400, 500, 600, 700, 800, 900, 1030 bp). DNA fingerprints of the tetraploid form plants had no 700 bp fragment which is specific to diploid plants.

The size of DNA fragments amplified by UBC824 primer ranged from 450 to 1200 bp. Like in previous research, 4n plants were not characterised by polymorphism, DNA fingerprints were identical according to the distribution of fragments (450, 550, 650, 700, 900 and 1030 bp). Plants of the 2n form were different. DNA fragments were situated in the two profile types (I – 550, 650, 900, 1030, 1200 bp and II – 550, 650, 1030 1200 bp), each profile type including 50.0% of the individuals. Plants of both populations had a specific fragment in the genomic DNA fingerprints: 4n form – 450 bp, 2n form – 1200 bp.

Our experiments showed that plants of the *DACTYLIS polygama* H. population are characterised by diversity which manifests itself by a different ploidy and DNA fragment composition. A possible reason for this phenomenon is heterozygosity of the forms and crosspollination. R. Kolliker with co-authors suggest that the great diversity of *Dactylis* spp. cultivars enabled this species to survive and adapt in the course of evolution [14]. Research evidence on cocksfoot polymorphisms is rather limited in the world's scientific literature. On assaying DNA of 57 natural *D. glomerata* L. populations by the RAPD method with 12 primers, there were produced 125 fragments of which 32% were polymorphic [9]. A similar level of polymorphism, by the ISSR method, was identified in our test where 34.8% of DNA fragments were found to be polymorphic. Although the forms of different ploidy differed by a small number of markers, we succeeded in identifying unique markers specific to the plants of tetraploid and diploid forms. It is noteworthy that plants of the 2n form were characterised by a higher polymorphism than tetraploids plants. Czech researchers made a spectrum analysis of phenolic acids in different species of the genus *Dactylis* and found that a higher polymorphism was characteristic of diploid but not tetraploid *Dactylis polygama* H. cultivars [5].

Our findings are relevant while searching for possibilities to include *Dactylis polygama* H. populations into breeding programmes. For a more comprehensive assessment of species polymorphism, it is necessary to expand the volume of research – to test and compare various wild populations and to look for correlations between DNA fingerprints and agromorphological traits.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support for this programme provided by the Lithuanian State Science and Studies Foundation (grant T-62/05) and the Lithuanian State Research Programme 'Genofondas'.

Received 27 July 2006
Accepted 24 April 2007

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MIŠKINĖS ŠUNAŽOLĖS (*DACTYLIS POLYGAMA* H.) LAUKINĖS POPULIACIJOS PANAUDOJIMAS GENETINĖS KOLEKCIJOS SUDARYMUI IR SELEKCIJAI

Santrauka

Pastaruoju metu siekiama praplėsti moderniam ūkininkavimui tinkančių rūšių asortimentą. Tam tikslinga efektyviau panaudoti turimus genetinius išteklius. Atkreiptas dėmesys į perspektyvią pašarui tinkančią rūšį – miškinę šunažolę (*Dactylis polygama* H.), kuri dar nėra sukultūrinta.

Šiuo darbu buvo siekiama įvertinti laukinės *D. polygama* H. populiacijos įtraukimo į selekcijos programas galimybę. Tam tradiciniu agrobiologiniu vertinimo balais metodu buvo palyginti *D. polygama* H. populiacijos ir plačiai auginamos paprastosios šunažolės (*D. glomerata* L.) augalų požymiai, DNR atspaudų (ISSR) metodu įvertinta genetinė įvairovė.

Nustatyta, kad *D. polygama* H. populiaciją sudaro skirtingo ploidiškumo augalai, todėl jie buvo padalyti į dvi formas – diploidinę (2n = 14) ir tetraploidinę (2n = 28). Lyginant jų agrobiologinius požymius su *D. glomerata* L. veislės 'Asta' (2n = 28) augalais nustatyta, kad laukinė populiacija išaugino mažesnį žolės derlių ir buvo vėlyvesnė, tačiau sukaupė didesnį žalių baltymų bei vandenyje tirpių angliavandenių kiekį, pasižymėjo geresnėmis virškinamumo savybėmis.

Lyginant tarpusavyje *D. polygama* H. formų požymius matyti, kad tetraploidinės formos augalai pirmavo pagal eilę rodiklių (lapų plotį, atžėlimo pavasarį tempą, augalo aukštį ir žolės derlių, žiedynų skaičių, baltymų kiekį). Diploidinės formos individai sukaupė mažiau baltymų, juose nustatyti mažesni visų ląstelienos frakcijų kiekiai. Pažymėtina, kad diploidinės formos augaluose rasti mažesni ląstelienos ir didesni vandenyje tirpių angliavandenių kiekiai turėjo teigiamą reikšmę ir sausųjų medžiagų virškinamumui.

PGR buvo atlikta su 7 paprastųjų pasikartojančių sekų pradmenimis. Nustatyta, kad genominės DNR amplifikavimui tiko tik trys pradmenys: GO3, GO7, UBC824. DNR atspauduose iš viso buvo gauti 23 fragmentai, kurių 8 (34,8%) buvo polimorfiški. Fragmentų dydžiai išsidėsto 400–1500 bp intervale. Tetraploidiniai individai genetiniais skirtumais nepasižymėjo, tuo tarpu diploidinės formos augalų DNR atspaudai buvo įvairūs – GO7 ir UBC824 pradmenys generavo keturių tipų DNR atspaudus. Taip pat pavyko nustatyti DNR fragmentus, specifiškai ženklinančius diploidinių ir tetraploidinių *D. polygama* H. formų augalus. 750 bp ir 1200 bp žymenys (panaudojus atitinkamai GO3, GO7 ir UBC824 pradmenis) būdingi 2n formos augalams, 450 bp ir 750 bp žymenys (panaudojus atitinkamai UBC824 ir GO3 pradmenis) specifiškai ženklina 4n formos augalus.