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Production of slender cocksfoot (*Dactylis polygama* H.) tetraploid populations and their assessment for agromorphological characteristics

Giedrė DABKEVIČIENĖ, Vilma KEMEŠYTĖ, Nijolė LEMEŽIENĖ, Bronislava BUTKUTĖ

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: giedre@lzi.lt

Abstract

Some attempts have been made recently to expand the range of perennial grasses tailored to the requirements of modern farming. Attention has been drawn to the forage plant species that have not been widely used so far. Apart from the common cocksfoot (*Dactylis glomerata* L.), which is traditionally used as a forage, another cocksfoot species – slender cocksfoot (*Dactylis polygama* H.) can be found in natural habitats in Lithuania. The plants of this species are characterised by some attractive traits: late maturity, better and more stable indicators of feeding value, and lesser aggressiveness (do not form tussocks). The genus *Dactylis* includes diploid (2n = 2x = 14) and tetraploid (2n = 4x = 28) species. The tetraploid *D. glomerata* varieties exhibit better agromorphological and quality characteristics. The current work was aimed to develop more productive tetraploid populations of slender cocksfoot and to assess them for agromorphological traits and compare them with diploid populations. The most effective method of tetraploid populations' development was proved to be colchicine treatment of meristems of young inflorescences by maintaining them on a nutrient medium supplemented with 0.2% colchicine for 96 h. Depending on the genotype, the tetraploid yield ranged from 16.7% to 100%. A comparison of averaged biomass qualitative and quantitative indicators of diploid and tetraploid populations showed the tetraploid populations of *D. polygama* to be more, albeit negligibly, productive and of better quality.

Key words: agromorphological characteristics, *Dactylis* spp., polyploidy, tetraploid.

Introduction

With a dramatic increase in the global consumption over the past few decades, plant breeders and geneticists have been continually challenged to produce competitive varieties, meeting contemporary demands. Organic farming and environmental protection standards also prompt expansion of the range of perennial grasses, suitable for modern farming. As a result, attention has been turned to perennial plant species that have not been extensively used so far. Cocksfoot (*Dactylis* spp.) is a valuable perennial grass. Widely grown in Lithuania, cocksfoot (*D. glomerata* L.) alongside its positive traits (high herbage yield, excellent re-growth, suitability for cutting and grazing), has some shortcomings (tends to rapidly become woody, lose nutritive properties and form tussocks). To achieve an effective grassland conveyor, it is essential that grass varieties differing in development rate are used. Another cocksfoot species *D. polygama* can be found in Lithuanian natural habitats. The plants of this species are characterised by the following attractive properties: late maturity, better and more stable indicators of feeding value, lesser aggressiveness (do not form tussocks) (Mika et al., 2003). This species can be grown in mixtures with legumes – *Lotus corniculatus*, *Trifolium pratense*, *Medicago* spp., and grasses – *Festuca* spp. *pratinsis*, *Lolium perenne.* Moreover, the species is recommended to be used as a component in turf grass mixtures intended for shaded park lawns and for planting in between rows in fruit tree nurseries (Mika et al., 2000). *D. polygama* variety 'Tosca' has been developed in Slovakia. There are no varieties of this species registered in Lithuania.

The genus *Dactylis* includes diploid (2n = 2x = 14) and tetraploid (2n = 4x = 28) species, with tetraploid ones being more numerous. According to Míka et al. (2002), there are several diploid (2n = 2x = 14) subspecies: ssp. aschersoniana, ssp. *galiciana*, ssp. *lusitanica*. *D. polygama* has originated from ssp. *aschersoniana*.

The seed accessions of *D. polygama* under study were collected in natural habitats in Lithuania and Russia (Kaliningrad region) during the expeditions conducted by the Institute of Agriculture. Having screened *D. polygama* plants for ploidy, diploid populations were selected and polyploidization work was initiated. Polyploidy, as one of the most efficient breeding methods of *Poaceae* forage grasses, has not lost its relevance in today's plant breeding (Bennett, 2004). Autopolyploidy of *Poaceae* grasses has been singled out as an especially useful technique used to increase the productivity of forage grasses. Induced polyploids are produced: 1) conventional method, by treating seeds, seedlings, stems, and other parts with

polyploidy inducing agents; 2) spontaneous method, in callus and suspension cultures (due to karyotype instability, regenerants with different ploidy level – somaclonal variants – are produced); 3) using polyploidy inducing agents in *in vitro* cultures (by treating callus, meristems and embryos, protoplasts, anthers and ovaries, etc. with polyploidy inducing agents). It is especially efficient to carry out polyploidization in the structures obtained from young organs characterised by high morphogenetic power – young inflorescences, apical points, and embryos (Hansen, Andersen, 1996; Jakše et al., 2003). Polyploidization *in vitro* systems is being successfully used in the breeding of horticultural, energy, ornamental and medicinal plants. Various organs and tissues: callus and microshoots, cotyledons, embryos, protoplasts are treated with polyploidy inducing agents (Binsfeld et al., 2000; Stanys et al., 2004; Glowacka et al., 2010; Lin et al., 2010). Little has been known about cocksfoot polyploidization so far (Borrill, 1978).

The current research work was aimed to develop tetraploid populations of *Dactylis polygama*, to assess them for morphological traits and to compare with diploid populations. With this research we expect to supplement the genetic collection of cocksfoot with promising breeding accessions.

Material and methods

Research objects – 4 ecotypes of *Dactylis polygama*, collected in natural habitats in Lithuania and Kaliningrad region (Russia). Preliminary results of populations' screening showed: population 2751 to be high-yielding, very late, 2456 – high-yielding, early, 2892 – short-growing, late, 2746 – high-yielding, early. Cocksfoot polyploidization was performed using a conventional, colchicine-treatment method (treatment of seeds and seedlings with colchicine) and an *in vitro* method (treatment of young meristems with colchicine and microcloning in a sterile culture) (Pašakinskienė, 2000) (Table 1).

Table 1. Polyploidization regimes of *Dactylis polygama*

Treatment	Colchicine concentration %	Treatment time h
Soaking of air-dried seeds in the solution	0.2	4
Soaking of swollen seeds in the solution	0.2	4
Soaking of seedlings (3–8 mm) in the solution	0.1	4
Keeping of meristems of young inflorescences on medium (1st method)	0.2	96
Soaking of meristems of young inflorescences in the solution (2nd method)	0.5	7

Colchicine treatment of air-dried seeds. The seeds were treated with 0.2% colchicine for 24 h at a temperature of 28ºC. After rinsing in distilled water (3 times for 10 min), the seeds were transferred into the soil in cassettes and were kept in a greenhouse at 18–20ºC.

Colchicine treatment of swollen seeds. The seeds were placed in Petri plates on filter paper moistened with distilled water and kept for 24 h in a thermostat at 25ºC. The swollen seeds were poured with 0.2% colchicine solution and kept for 4 hours at 28ºC. Colchicine treatment was interrupted by rinsing the seeds in distilled water (3 times for 10 min).

Colchicine treatment of seedlings. The seeds were germinated for 4–5 days at 25ºC on moistened filter paper in Petri plates. The germinated seeds with 5–10 mm sprouts were transferred into test tubes, poured with 0.1% colchicine and kept for 4 hours at 28ºC. The seeds were rinsed in distilled water (3 times for 10 min) and transferred into the soil in cassettes and were kept in a greenhouse at 18–20ºC. In all the three cases, at the 4–5 leaf stage the plants were transferred into pots. After the plants had tilled, they were checked for ploidy level by establishing the number of chromosomes in the rootlets (Karp, 1991). The selected tetraploids were kept for 2–3 months in a low-temperature (4ºC) chamber in order to induce formation of generative stems.

Polyploid production in vitro culture of young inflorescences. In this study, we used 20–80 mm young shoots (3–4 organogenesis stage according to BBCH scale) collected in May from the plants growing in the field. The shoots were washed for 1 h with running water, and then soaked for 10 min in sodium hypochlorite solution. For this purpose we used 50% ACE solution (commercial ACE contains 5–15% of sodium hypochlorite). Using a preparation needle, inflorescences were excised from the shoots washed with sterile-water for 20 min. The inflorescences were repeatedly sterilized with diocid (1 C2H5OHgCl:2 C21H37ClN) for 5 min (then rinsed with sterile water (3 times for 10 min) (1st method), or soaked in colchicine solution (2nd method). When applying the 1st method, explants (inflorescences) were cut into 5 mm pieces and planted on a Linsmaier and Skoog (LS) nutrient medium in test tubes. For three days the explants were cultivated at 24ºC in the dark, and then transferred into the light (9000 lx). Seven days after planting, Petri plates with explants were placed for 48 h into a refrigerator (2–4ºC) for synchronization of cell division. Having removed the plates from the refrigerator, the explants were transferred onto LS supplemented with colchicine for 96 h (25–28ºC, darkness). Colchicine treatment was discontinued after transferring the explants into test tubes on LS without colchicine. When applying the 2nd method, after low-temperature treatment, the explants of young inflorescences were poured with sterile colchicine solution and kept in it for 7 h (28ºC, darkness). Then the explants rinsed with sterile water (3 times for 10 min) were planted on the LS medium, one explant per test tube.

A separate experiment was set up to determine the effect of *meristem size on colchicine treatment efficacy.* Mersistems of two lengths (0.5–1 cm and 2–3 cm) of population No. 2751 were used. The following two methods of colchicine treatment were employed: meristems were kept on 0.4% medium for 20 h (1st method) and soaked in 0.5% solution for 20 h (2nd method).

The colchicine-treated (both methods) explants of young inflorescences were kept in a cultivation room for 2 months (at 24°C, photoperiod 16 h, 9000 lx). Regeneration frequency was recorded. The regenerants were planted on the LS medium, then were transferred

into the soil and were cultivated in the greenhouse (at 18–20ºC). After the plants had rooted, their ploidy was checked by establishing the number of chromosomes in the rootlets (Karp, 1991). The selected tetraploids were transferred into a low-temperature chamber (4ºC) in order to induce formation of generative stems. We used the LS nutrient medium having reduced salt content to 2/3 of the rate, and having added 7 g of agar, 30 g of sucrose, 0.2 mg indole-3-acetic acid (IAA) and 0.25 mg kinetin, 1 mg thiamine, 0.1 mg nicotinic acid; and 0.1 mg of piridoxin per 1 litre. The nutrient medium was sterilized by autoclaving 120ºC under 1 bar pressure for 30 min. Prior to autoclaving, the acidity of the medium pH = 5.6–5.8 was determined with a pH-meter. Colchicine was sterilized by filtering through bactericidal Millipore filters (pore size 0.5 µ).

Assessment of Dactylis polygama tetraploid populations for agrobiological traits. In 2009, the seedlings grown from the seeds collected from the plants of the tetraploid populations 2751, 2892, 2746 and 2880 were planted in an experimental field at 50 × 25 cm distances in June. In 2010, the overwintered plants of the tetraploid populations were compared with those of diploid populations (2 n). The main agrobiological characteristics were estimated: over winter survival %, earliness (recorded at the beginning of heading and when 50% of plants are flowering), plant height at inflorescence emergence cm, dry matter (DM) yield per plant (g), seed yield per plant (g). A near infrared spectrometer (NIR) systems model 6500 (Perstorp Analytical, USA) (Butkutė et al., 2003) was used to measure the contents of neutral detergent fibre (NDF), crude fibre (CF), crude protein (CP), water-soluble carbohydrates (WSC), dry matter digestibility (DMD) and carbon to nitrogen ratio (C:N). Lignin was determined by the Van Soest fibre fractionation method and the total nitrogen and carbon concentrations by the Dumas method (Faithfull, 2002).

Statistical analysis of data was performed using the software *ANOVA*, *STAT* from the *SELEKCIJA* and *IRRISTAT* package (Tarakanovas, Raudonius, 2003).

Results and discussion

Development of Dactylis polygama tetraploid forms. The first polyploids of *Poaceaea* plants were developed with the aid of colchicine back in 1939. Later on, other polyploidy inducing agents such as acenapthtene, amyprofos-methyl, oryzalin, trifluralin and others were started to be used for the production of polyploids (Hansen, Andersen, 1996); however, up till now colchicine has been considered as one of the most efficient reagents used to induce tetraploids. Since colchicine treatment of adult plants is complicated, it is a common practice to treat various plant organs and tissue: seeds, apical points, coleoptiles, tillering point (Слесаравиюс, 1992). In our experiment, we employed three conventional colchicine-treatment methods for the production of tetraploids: air-dried and swollen seeds as well as germinated seeds with 3–8 mm coleoptiles of *D. polygama* ecotype 2456 2n were treated with colchicine solution. In the control treatment (without colchicines treatment of seeds), the seed germination rate was 77.1%. Under the effect of colchicine, cocksfoot survival rate in the soil declined by 13.6–42.7%. Germinated seeds with 3–8 mm copeoptiles were most sensitive to toxic effects of colchicine, which resulted in as low as 34.4% survival rate of explants; however, the tetraploid yield in this treatment amounted to 26.2% (Table 2).

Table 2. Comparison of efficiency of conventional colchicine-treatment methods

Explants and polyploidization method	Number of treated explants	Plants grown		Tetraploids produced		
		number	%	number	% from the number of plants grown	% from the number of explants treated
Control	144	111	77.1	0	–	–
Air-dried seeds (0.2% colchicine, 4 h)	192	122	63.5	0	–	–
Swollen seeds (0.2% colchicine, 4 h)	192	110	57.3	1	0.8	0.5
Seeds with 3–8 mm coleoptiles (0.1% colchicine)	122	42	34.4	11	26.2	9.0
LSD_{05}			6.50		3.29	4.21

It is known that plants of different species exhibit a different response to colchicine treatment. It has been noted that colchicine treatment of swollen or germinated seeds in most cases results in a higher tetraploid induction rate both for *Poaceae* and *Fabaceae* plants (Joshi, Verma, 2004). Literature data suggest that in colchicine treatment studies on *Lolium* spp. and *Festuca* spp. the highest number of tetraploids was produced having treated air-dried seeds with 0.1% colchicine solution for 24 h and swollen seed with 0.3% colchicine solution for 2 h. Tetraploids accounted for 63.6–42.9% of the plants that survived in the soil (Dapkienė et al., 1999). Having treated *Bromus inermis* shoots with 0.2% colchicine + 2% dimethyl sulfoxide solution, only 14.3% of the plants grown were tetraploids (Слесаравиюс, 1992). Colchicine treatment of shoots of woody plants of *Acacia* spp. resulted in 18–29% tetraploid yield. Kulkarni and Borse (2010) reported that colchicine treatment of either pre-soaked seeds or the shoot tips of young seedlings resulted in as low as 0.3–1.0% tetraploid yield. In our study, only colchicine treatment of germinated seeds yielded an enough positive result. Other polyploidization methods were ineffective. With the recent rapid developments in biotechnologies, plant polyploidization is often combined with in vitro methods.

Alishah and Bagherieh-Najjar (2008) indicated treatment of germinated seeds with 4–7 mm hypocotyls of *Gossypium* spp. with 0.9% colchicine to be more effective than embryo treatment because of high death rate of embryos and infection risks. The efficiency

of the *in vitro* methods was determined by analysing the changes in multiplication coefficient value and tetraploid yield, expressed in percent (Table 3). Young inflorescence meristems were colchicine-treated using two methods: 1st – by keeping them on a nutrient medium supplemented with 0.2% colchicine for 96 h, 2nd – by soaking them in 0.5% colchicine solution for 7 h. Having treated meristems with colchicine, the value of multiplication coefficient declined by on average 2.9 times, compared with the control. The meristems of ecotype 2892 were most sensitive to colchicine treatment (multiplication coefficient was on average 2.3). The meristems of ecotype 2881 exhibited the least response to colchicine treatment, the multiplication coefficient was close to that of the control treatment and amounted to 5.8. The 1st colchicine-treatment method proved to be more effective for all ecotypes tested. It yielded 2.5 times more tetraploids. Analysis of tetraploid yield data revealed a different response of the tested ecotypes to the polyploidization methods applied. Having used meristem soaking in 0.5% colchicine solution, ecotypes 2746 and 2892 did not induce any tetraploids. Using the 1st polyploidization method, the highest tetraploid yield (100% and 72.7%) was obtained having colchicine-treated meristems of ecotypes 2881 and 2746, while the lowest tetraploid yield (16.7%) was recorded for ecotype 2892 meristemas.

Table 3. Comparison of efficiency of *in vitro* polyploidization methods of *Dactylis polygama*

Ecotype	Meristem colchicine-treatment method	Number of meristems treated	Multiplication coefficient	Tetraploid yield %	
				from the number of regenerants produced per test tube	from the number of regenerants that survived in the soil
2892	control	15	4.9	–	–
	1st, in 0.2% medium for 96 h	26	1.9	8.0	16.7
	2nd, 0.5% soaked for 7 h	4	2.7	0.0	0.0
2746	control	15	6.2	–	–
	1st, in 0.2% medium for 96 h	18	2.3	38.1	72.7
	2nd, 0.5% soaked for 7 h	10	5.0	0.0	0.0
2751	control	15	3.4	–	–
	1st, in 0.2% medium for 96 h	14	2.7	18.8	50.0
	2nd, 0.5% soaked for 7 h	33	2.7	17.8	22.9
2456	control	15	6.8	–	–
	1st , in 0.2% medium for 96 h	6	7.0	42.9	100.0
	2nd, 0.5% soaked for 7 h	19	4.6	43.5	71.4
LSD_{05}				3.66	3.52

It was interesting to find that tetraploid yield depended on the size of colchicine-treated inflorescence meristems (Table 4). Research was done on the meristems 0.5–1.0 cm and 2–3 cm in length of ecotype 2751. Colchicine treatment of less developed meristems both when soaking them in 0.5% solution for 20 h and when keeping on a nutrient medium supplemented with 0.4% colchicine for 20 h resulted in a 2.0-fold lower tetraploid yield. A comparison between the two colchicine-treatment methods showed the both meristem treatment techniques to produce a relatively similar efficiency: tetraploid yields were 17.6–11.5% and 28.1–31.0%, i.e. varied within the error range. We succeeded in producing a total of 63 tetraploid individuals of *D. polygama*.

Table 4. The effect of the size of young inflorescence meristems on polyploidization efficiency (population 2751)

Meristem colchicine treatment method	Number of meristems treated	Multiplication coefficient	Tetraploid output %	
			from the number of regenerants per test tube	from the number of regenerants that survived in the soil
2nd, 0.5–1 cm soaked in 0.5% solution for 20 h	23	3.1	13.0	17.6
2nd, 2–3 cm soaked in 0.5% solution for 20 h	43	2.4	21.9	28.1
1st, 0.5–1 cm kept on 0.4% medium for 20 h	27	1.8	11.1	11.5
1st, 2–3 cm kept on 0.4% medium for 20 h	47	1.8	29.4	31.3
LSD_{05}			4.55	5.71

Agrobiological and quality indicators of diploid and tetraploid populations of Dactylis polygama. All our tested populations of *D. polygama* were characterised by a high over winter survival rate (93.6–100%) (Table 5). Maceira et al. (1993) and Míka et al. (2002) have reported that tetraploid populations of *D. glomerata* tend to show better overwintering and winter-hardiness.

While establishing swards it is beneficial to use varieties of different earliness and in this way ensure the conveyor of use of forage grasses. Averaged data showed that the dates of beginning of inflorescence emergence and mass flowering coincided for all *D. polygama* populations tested. However, analysis of indicators of individual populations showed that the population of both ploidy groups 2751 and tetraploid population 2456 were 6 days' later-maturing. These findings agree with those of one the foremost researchers of *Dactylis* spp. Míka et al. (2002), who has maintained that *D. polygama* populations are of later maturity than those of *D. glomerata* by 7 days. It is noteworthy that *D. polygama* populations were characterised by a slower re-growth rate in spring. Later-maturing cocksfoot varieties are suitable for mixtures

Table 5. Comparison of agrobiological indicators in the plants of *Dactylis polygama* populations

Population	Over winter survival rate %	Beginning of inflorescence emergence / mass inflorescence emergence	Plant height cm	Seed yield per plant g	Dry matter (DM) yield per plant g
2746 2n	100	21 05 / 02 06	50.3	1.66	47.1
2746 4n	100	20 05 / 02 06	68.7	2.13	57.0
2751 2n	100	30 05 / 10 06	62.3	4.09	37.1
2751 4n	93.8	25 05 / 12 06	69.0	1.58	72.9
2892 2n	93.8	27 05 / 10 06	78.3	4.98	74.0
2892 4n	93.8	25 05 / 06 06	80.0	6.05	76.9
2456 2n	100	20 05 / 03 06	94.0	1.11	105.5
2456 4n	100	25 05 / 08 06	99.0	1.96	109.5
LSD_{05}			34.855	2.954	59.376

with clover and lucerne, bird's foot trefoil, while earlier-maturing varieties are better suited for pastures. The plants of the populations 2456 2n and 4n had the tallest stems, while those of 2746 2n and 4n had one of the shortest stems. Averaged data indicated that tetraploid *D. polygama* produced 7.98 cm taller stems. The data of our previous comparative research on *D. polygama* wild population and *D. glomerata* variety 'Asta' evidenced that *D. glomerata* plants produced wider leaves and taller stems as a result of which this species was more productive than *D. polygama*. However, according to many other characteristics (leaf length, bunch density and growth habit, inflorescence formation indicators) *D. polygama* populations were similar or superior to those of *D. glomerata* variety 'Asta' (Dabkevičienė et al., 2007). DM content is one of the major indicators of productivity. It varied from 37.1 to 109.5 g plant^{-1}. Population 2456 was noted for the highest DM yield – its diploid and tetraploid plants produced a DM yield of 105.5 and 109.5 g, respectively. Moreover, the plants of this population were the tallest. More marked differences between diploid and tetraploid plants were established for populations 2746 and 2751 – tetraploid plants accumulated 1.2 and 2.0-fold more biomass, respectively. Comparison of diploid and tetraploid populations' quantitative parameters (Table 6) showed that the plants of tetraploid populations accumulated a greater, though only slightly, DM content (13.2 g).

Table 6. Comparison of agrobiological and quality indicators of *Dactylis polygama* plants of different ploidy

Characteristics	Diploid populations		Tetraploid populations	
	mean	CV %	mean	CV %
Dry matter (DM), g plant^{-1}	65.90 ± 15.32	46.46	79.10 ± 11.01	27.86
Height, cm plant^{-1}	71.21 ± 9.51	26.72	79.18 ± 7.11	17.97
Seed yield, g plant^{-1}	2.92 ± 0.93	63.11	2.90 ± 1.04	69.73
Crude protein (CP)	11.52 ± 0.68	11.76	12.88 ± 0.63	9.77
Water-soluble carbohydrates (WSC)	11.43 ± 1.46	25.56	11.48 ± 0.77	13.45
Crude fibre (CF)	29.31 ± 1.50	10.27	23.15 ± 6.95	60.02
Dry matter digestibility (DMD)	56.53 ± 4.31	15.25	65.05 ± 3.55	11.81
Neutral detergent fibre (NDF)	61.88 ± 2.27	7.33	59.98 ± 18.88	6.27
Lignin	3.30 ± 0.33	19.3	3.09 ± 0.22	14.48
Carbon to nitrogen ratio (C:N)	25.28 ± 1.64	13.1	22.40 ± 1.13	10.07

CV % – coefficient of variation

Researchers investigating *D. glomerata* diploid and tetraploid populations reported a great genetic similarity between them. This fact provides an explanation why the differences in morphological traits are often negligible. Comparison of diploid *D. polygama* and tetraploid *D. glomerata* plants revealed that ploidy level had a direct effect on productivity (Lindner, Garcia, 1997; Míka et al., 2002). Another important productivity indicator is seed yield. Averaged data showed that both diploid and tetraploid populations produced the same seed yield (2.9 g plant^{-1}) (Table 2). The differences between populations show up while analysing indicators of individual populations. The plants of both ploidy forms of population 2892 were distinguished for the seed yield, amounting to 4.98 and 6.05 g plant^{-1}, respectively. High seed set was recorded for diploid population 2751 (4.09 g plant^{-1}). The plants of both ploidy forms of population 2456, which were noted for high DM yield, produced a low seed yield of 1.11 and 1.96 g, respectively (Table 5). The value of plants is also determined by biomass quality. Good quality forage should contain 14–17% protein. For many *Poaceae* grasses this indicator does not exceed 120.0 g kg^{-1} (Butkutė et al., 2003). Their data evidenced that according to CP content *D. glomerata* was comparable with meadow fescue and common timothy – 112–118 g kg^{-1}. Among *D. polygama* populations, tetraploid population 2746 stood out by CP content amounting to 14.6 %, while in other populations the value of this indicator varied from 9.56% to 12.8% (Table 7). Averaged data showed that the plants of tetraploid populations accumulated slightly greater CP content (Table 6). Both for diploid and tetraploid populations seed yield was the most variable indicator, the CV% value amounted to 63.11 and 69.73, respectively. Contents of CP and NDF were the most stable indicators, the CV% value ranged from 6.27 to 11.76.

WSC are readily processed and assimilated by an animal organism. Moreover, they are important in silage fermentation process. The highest WSC contents are accumulated by perennial ryegrass, while meadow

grass, common timothy and cocksfoot accumulate similar WSC contents. In our study WSC contents varied from 7.53% (population 2456 2n) to 14.6% (population 2892 2n). There were no significant differences in WSC contents between diploid and tetraploid populations.

Table 7. Quality indicators of *Dactylis polygama* biomass

Population	C:N	NDF	Lignin	CF	DMD	WSC	CP
2746 2n	30.0	63.9	3.28	33.2	52.9	12.0	9.56
2746 4n	19.4	57.0	3.08	27.1	63.9	12.2	14.6
2751 2n	22.6	61.2	2.73	28.6	61.8	11.6	12.6
2751 4n	22.3	56.5	2.87	26.9	66.3	12.6	12.8
2892 2n	24.9	55.9	2.97	27.3	65.2	14.6	11.7
2892 4n	24.8	62.6	2.82	29.5	59.8	11.9	11.6
2456 2n	23.6	66.5	4.23	36.1	46.2	7.53	12.2
2456 4n	23.1	63.8	3.72	33.5	50.2	9.20	12.5
LSD_{05}	4.20	4.81	1.74	4.53	4.82	3.14	3.23

C:N – carbon to nitrogen ratio, NDF – neutral detergent fibre, CF – crude fibre, DMD – dry matter digestibility, WSC – water-soluble carbohydrates, CP – crude protein

Of all forage grasses, *D. glomerata* is characterised by the lowest digestibility. Míka et al. (2002) found that *D. polygama* plants are characterised by better feeding value. Our previous research results indicated that *D. polygama* plants were superior to those of *D. glomerata* variety 'Asta' in terms of quality: they accumulated greater CP and WSC contents and exhibited better digestibility because they contained less fibre (Dabkevičienė et al., 2007). In this trial, the populations of *D. glomerata* differed in digestibility. Low digestibility was recorded for the population of both ploidy forms 2456 (46.2–52.9%) and diploid population 2746. For the rest of the populations this indicator was considerably higher (59.8–66.3%). Higher ploidy level had a marked effect on digestibility. Fibre is a major component of plant cell walls. It consists of cellulose, hemicelluloses and lignin. Crude fibre (lignin and cellulose) is cell wall fraction which is not degraded by strong acids and alkali. Enzymes of cattle microflora are able to break down cellulose while lignin impedes fibre digestibility. NDF is neutral detergent fibre (lignin, cellulose and hemicellulose). Previous comparative research on cocksfoot (Dabkevičienė et al., 2007) showed that *D. polygama* accumulated lower CF and NDF contents than *D. glomerata*. In the present study, the highest CF, NDF and lignin contents were accumulated by diploid 2746 and both (2n and 4n) 2456 populations. These populations were also characterised by the poorest DMD. Averaged data indicated that tetraploid populations were more valuable in terms of fibre content, i.e. the contained less fibre. According to carbon to nitrogen ratio, almost all populations tested were suitable for biogas production, the C:N did not exceed 30 (optimal carbon to nitrogen ratio in biomass intended for biogas production ranges from 10 to 30) (Cotana, Giraldi, 2007).

The summarised DM yield and quality indicators of individual populations of *D. polygama* distinguished three populations: 2746 4n, 2751 4n, 2892 2n. Their plants produced relatively high DM yield (57.0, 72.9 and 74.0 g plant^{-1}) and a seed yield of 2.13, 1.58 and 4.98 g plant^{-1}, respectively. These populations were noted for good quality – accumulated low fibre contents (57.0, 56.5 and 55.9 % DM – including lignin 3.08; 2.87 and 2.97 % DM), and relatively high contents of WSC (12.2, 12.6 and 14.6 % DM) and CP (14.6, 12.6 and 12.8 % DM). Although the plants of populations 2456 produced the highest DM yield, their biomass quality indicators were significantly inferior to those of other populations. This might have resulted from the fact that the latter population was earlier-maturing and at the sampling time was more woody (had a higher fibre content). With aging, cocksfoot DM yield increases and quality indicators decline (Berg, Hill, 1989).

Conclusions

1. *Dactylis polygama* ecotypes exhibited a different response to different polyploidization methods: tetraploid yield ranged from 0.0% to 100%. It is best to use explants that have reached later stages of organogenesis: sprouted seeds with 3–8 cm-long coleoptiles or 2–3 cm-long young inflorescences (tetraploid yield – 28.1–31.3%).

2. To produce cocksfoot polyploids, it is expedient to treat meristems with colchicine by maintaining them on a nutrient medium supplemented with 0.2% colchicine for 96 h. Averaged data showed that the yield of tetraploids was 2.5-fold higher than when soaking them in 0.5% solution for 7 h.

3. Comparison of the averaged data of biomass agrobiological and quality indicators of diploid and tetraploid populations showed tetraploid populations to be slightly more productive and have a better quality.

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Miškinės šunažolės (*Dactylis polygama* H.) tetraploidinių populiacijų kūrimas ir jų agromorfologinių savybių vertinimas

G. Dabkevičienė, V. Kemešytė, N. Lemežienė, B. Butkutė

Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

Santrauka

Pastaruoju metu siekiama išplėsti daugiamečių žolių, tinkančių moderniam ūkininkavimui, asortimentą. Atkreiptas dėmesys į iki šiol plačiai nenaudotas pašarinių augalų rūšis. Be tradiciškai pašarui naudojamos paprastosios šunažolės (*Dactylis glomerata* L.), Lietuvoje natūraliose augavietėse galima aptikti ir kitą rūšį – miškinę šunažolę (*Dactylis polygama* H.). Šios rūšies augalai pasižymi geromis ūkinėmis savybėmis: vėlyvumu, geresniais ir ilgiau išliekančiais pašarinės vertės rodikliais, mažesniu agresyvumu (nesudaro kupstų). *Dactylis* gentyje aptinkamos diploidinės (2n = 2x = 14) ir tetraploidinės (2n = 4x = 28) rūšys. Paprastosios šunažolės (*D. glomerata* L.) tetraploidinės veislės pasižymi geresnėmis agromorfologinėmis ir kokybės savybėmis.
Tyrimo metu siekta sukurti didesnio produktyvumo tetraploidines miškinės šunažolės populiacijas, įvertinti jų agromorfologinius požymius, palyginti su diploidiniais analogais. Nustatyta, kad efektyviausias būdas sukurti tetraploidines populiacijas yra jaunų žiedynų meristemų veikimas kolchicinu, jas 96 val. laikant ant maitinamosios terpės su 0,2 % kolchicino priedu. Priklausomai nuo genotipo, tetraploidų išeiga siekė 16,7–100 %. Palyginus diploidinių bei tetraploidinių populiacijų biomasės vidutinius kiekybinius ir kokybinius rodiklius nustatyta, kad tetraploidinės miškinės šunažolės populiacijos, nors ir nežymiai, yra produktyvesnės ir geresnės kokybės.

Reikšminiai žodžiai: agromorfologinės savybės, *Dactylis* spp., poliploidija, tetraploidas.