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FISH ASSESSMENT OF RIBOSOMAL DNA SITES IN THE CHROMOSOME SETS OF *LOLIUM, FESTUCA* AND *FESTULOLIUM*

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Abstract

Ribosomal DNA (rDNA) sites were mapped by fluorescent *in situ* hybridization (FISH) in mitotic chromosomes of perennial ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), meadow fescue (*Festuca pratensis*), and two *Festulolium* hybrid cultivars. Hybridization sites of 18S-5.8S-26S and 5S rDNA probes were counted in the sampled plants of diploid and tetraploid cultivars. The number of 18S-5.8S-26S rDNA (pTA71) sites was found to be variable in *Lolium* cultivars, and the variation was higher in tetraploids than in diploids. The mean number for diploids was 6.64 and 5.88, and for tetraploids 12.65 and 12.39 for *L. perenne* and *L. mutiflorum,* respectively. The cultivar 'Verseka' was distinguished by low number, 10.8 on average, of 18S-5.8S-26S sites in comparison to other tetraploid cultivars. The number of 5S rDNA was found to be quite stable, especially in the diploid *Lolium* cultivars where two sites were always detected. In *Festuca* plants, both 18S-5.8S-26S and 5S rDNA site numbers and positions were found to be consistent, whereas in *Festulolium*, the range of pTA71 sites was quite high, especially in the cultivar 'Pūga' where 6 to14 sites were detected (10.18 mean).

Key words: FISH, ribosomal DNA, *Lolium, Festuca, Festulolium.*

Introduction

Ryegrasses (*Lolium*) have been grown in Lithuania for a long time. Perennial ryegrass (*L. perenne*) breeding was started in Lithuania in 1926. It is one of the most valuable forage grasses valued for its productivity and forage quality. Seven Lithuanian cultivars of perennial ryegrass have been created: 'Veja' developed in 1946, 'Sodrė' (1973), 'Žvilgė' (1978), 'Verseka' (1987), 'Elena' (1988), 'Alduva' (1991) and 'Raminta' (1998) /Nekrošas, Kemešytė, 2007/. However, *Lolium* grasses lack resistance during harsh winters, especially in Northern countries. Their close relatives from fescue (*Festuca*) genus have lower nutritious value and digestibility, but they are much better adapted and tolerant to abiotic stress /Humphreys, 2004/. The hybrids of these two grasses, *Festulolium*, combine their best qualities. *Lolium* and *Festuca* species hybridize easily and their chromosomes pair and recombine extensively undergoing meiosis in the hybrids. One of the ways to put together the traits of *Lolium* and *Festuca* is amphiploidy when whole genomes are combined together /Humphreys et al., 2003; Nekrošas, Kemešytė, 2007 /. Since intergeneric hybrids between diploid *L. multiflorum*, *L. perenne* and *F. pratensis* are completely male sterile, it is more appropriate to use tetraploid

forms for production of *Festulolium* hybrids. Many cultivars of allotetraploid hybrids *Lolium multiflorum* (4x) × *Festuca pratensis* (4x) have been developed and some of *F. pratensis* (4x) × *L. perenne* (4x) have been produced as well. The best cultivars were created from reciprocal *L. multiflorum* × *F. pratensis* and *F. pratensis* × *L. multiflorum* hybrids: 'Perun', 'Achilles', 'Perseus' and HŽ14DK in Czech Republic /Kopecky et al., 2006/, 'Punia' and 'Pūga' in Lithuania /Nekrošas, Kemešytė, 2007/, 'Felopa', 'Sulino', 'Rakopan' and 'Agula' in Poland /Zwierzykowski, 2004/. New cultivar 'Vėtra' was produced by crossing *L. multiflorum* and *F. arundinacea* species /Nekrošas, Kemešytė, 2007/.

Fluorescent *in situ* hybridization (FISH) is a useful technique to reveal genetic constitution and species' genomic relationships in hybrid plants. The ribosomal DNA (rDNA) probes are widely used in FISH applications. In genus *Hordeum*, the use of 5S and 18S-5.8S-25S rDNA probes was applied for investigating karyotype evolution and phylogeny of the genus /Taketa et al., 2005/. These probes can also be used for chromosome identification in *Festulolium* plants /Kosmala et al., 2006/. In our previous studies, the numbers of 18S-5.8S-25S rDNA sites, i. e. chromosomal sites containing 18S-5.8S-25S rDNA (pTA71 probe) repeats have been counted in *Festulolium* cultivars 'Punia' and 'Rakopan' /Lideikytė et al., 2006/. Previously, the numbers of rDNA sites were also characterised in *Lolium* species and in *Festuca pratensis* plants, however this was done by analysing only a small number of plants from each species /Thomas et al., 1996, Thomas et al., 1997/. A 5S rDNA-specific probe was used in FISH studies with other plants, e. g. *Pseudotsuga menziesii* /Amarasinghe, Carlson, 1998/ and *Nicotiana rustica* /Matyasek et al., 2003/.

In this research, FISH technique using ribosomal DNA probes was used for the purpose to find and compare the patterns of rDNA locations in diploid and tetraploid cultivars of perennial ryegrass (*L. perenne*), Italian ryegrass (*L. multiflorum*), two new *Festulolium* hybrid cultivars, and meadow fescue (*F. pratensis*) cultivars. These results could add some knew knowledge about DNA changes and recombination in plant hybrids.

Materials and Methods

Plant material. 1. 8 tetraploid *Lolium perenne* (2n = 4x = 28) cultivars: 'Acento', 'Alduva', 'Aligator', 'Raminta', 'Sodrė', 'Verseka', 'Žvilgė' and 'Elena' and 4 diploid *L. perenne* (2n = 2x = 14) cultivars 'Rastro', 'Limes', 'Veja' and 'Vincent' were selected for this study. In addition, 2 diploid *Lolium multiflorum* cultivars (2n = 2x = 14) 'Adin' and 'Bellem', and 4 tetraploid cultivars (2n = 4x = 28) 'Atos', 'Catalpa', 'Lotos' and 'Meroa' were taken. From each cultivar, 2–5 plants were used for the study.

2. *Festuca pratensis* cultivars: Lithuanian diploid (2n = 2x = 14) 'Dotnuva', 'Kaita', and 'Sigita' (Lithuania) and tetraploid (2n = 4x = 28) 'Raskila' and diploid Norwegian 'Loken' and 'Fure'.

3. Lithuanian × *Festulolium braunii* cultivar 'Pūga' (*L. multiflorum* × *F. pratensis,* 2n = 4x = 28) and × *Festulolium* 'Vėtra' (*L. multiflorum* × *F. arundinacea*, 2n = 4x = 28).

Chromosome preparation. Mitotic chromosomes from root-tips were prepared on objective slides after pre-treatment in ice-cold water for 24 h, followed by fixation in

1:3 acetic acid-ethanol. The roots were softened in a mixture of 0.1 % pectolyase Y-23 and 0.1 % cellulase R-10, and squashed in 45 % acetic acid.

Probes. The pTA71 plasmid containing wheat 18S-5.8S-26S ribosomal DNA repeat /Gerlach, Bedbrook, 1979/ was cleaved with restriction enzyme EcoRI to release the ribosomal DNA sequence and labelled with fluorescein-12-dUTP (Fermentas) or rhodamine-11-dUTP (Roche) by nick translation method. 5S ribosomal DNA probe was made by PCR using primers 5SF (5'-GGATGGGTGACCTCCCGGGAAGTCC-3') and 5SR (5'-CGCTTAACTGCGGAGTTCTGATGGG-3') /Yang et al., 1998/. PCR reaction was set using DyNAzyme DNA polymerase (FinnZymes). A *L. perenne* and *F. pratensis* DNA was taken as templates. Following PCR cycles were used: 94 °C – 4 min., then 35 cycles of 94 °C – 1 min., 55 °C – 30 s and 72 °C – 1 min. The final cycle was 4 min. of 72 °C.

In situ hybridization. Slides were soaked in 45 % acetic acid for 5 min at RT and for 3 min at 48–50 °C. Denaturation of nuclear DNA was performed at 70 °C in 70 % deionized formamide for 2 min., followed by dehydration with cold ethanol series (70 %, 90 % and 100 %), 2 min. each and air-drying. Slides were incubated at 37 °C with 25 µl of denatured (10 min. at 70 °C) hybridization mix (2 µg DNA probe, 60 % formamide, 25 % dextran sulphate, 10 % 20 × SSC, 0.0025 % SDS solution) for 16 h in a moist chamber. After hybridization, slides were washed in 20 % formamide in 0.1 × SSC twice for 5 min. at 42 °C, and 3 times for 3 min. in 2 × SSC at 42 °C. Slides were mounted with Vectashield antifade and DAPI (4.6-diamidino-2-phenylindole) for counterstaining of DNA.

Analysis of hybridization signal. Hybridization signals were analysed by filters' set off Nikon Eclipse E800 fluorescence microscope. Three filter sets were used for detection as follows: 1) DAPI (excitation – 330–380 nm; beam – 400 nm; barrier – 420 nm); 2) rhodamine (excitation – 510–560 nm; beam – 575 nm; barrier – 590 nm); 3) fluorescein (excitation – 450–490 nm; beam – 505 nm; barrier – 520 nm). Photographs were taken by Pixera Penguin digital 600 CL camera. For processing colour pictures, Image Pro-Discovery 4.5 and Adobe Photoshop Elements were used.

Statistics. Microsoft Excel 2003 was used for statistical processing of the data. P-value was calculated for rDNA sites and chromosome numbers in *Festulolium* cultivars 'Pūga' and 'Vėtra'.

Results and Discussion

Ryegrasses and fescues. In our study we analyzed the numbers of ribosomal DNA sites in *L. perenne, L. multiflorum* and *F. pratensis* cultivars. For the study, 2 to 5 plants were taken from each cultivar. All *L. perenne, L. multiflorum* and *F. pratensis* plants from each cultivar were expected to have the same numbers and distributions of rDNA sites. In the previous study in 1996, H. M. Thomas et al. have shown that diploid *L. perenne* plants usually have seven 18S-5.8S-26S rDNA sites, while the number of 18S-5.8S-26S rDNA sites in *L. multiflorum* is six. Both species were found to have two 5S rDNA sites, on the short arms of the same chromosomes as 18S-5.8S-26S sites /Thomas et al., 1996/. Diploid *F. pratensis* plants were shown to have two 18S-5.8S-26S sites and two 5S rDNA sites that are found on separate chromosomes from 18S-5.8S-26S sites /Thomas et al., 1997/. We expected to find the same tendencies in our study.

This was true in *F. pratensis* cultivars where all diploid plants investigated had two 18S-5.8S-26S sites and two 5S rDNA sites – all present on the different chromosomes (Figure b). Respectively, a tetraploid *F. pratensis* cultivar 'Raskila' had four 18S-5.8S-26S and four 5S rDNA sites.

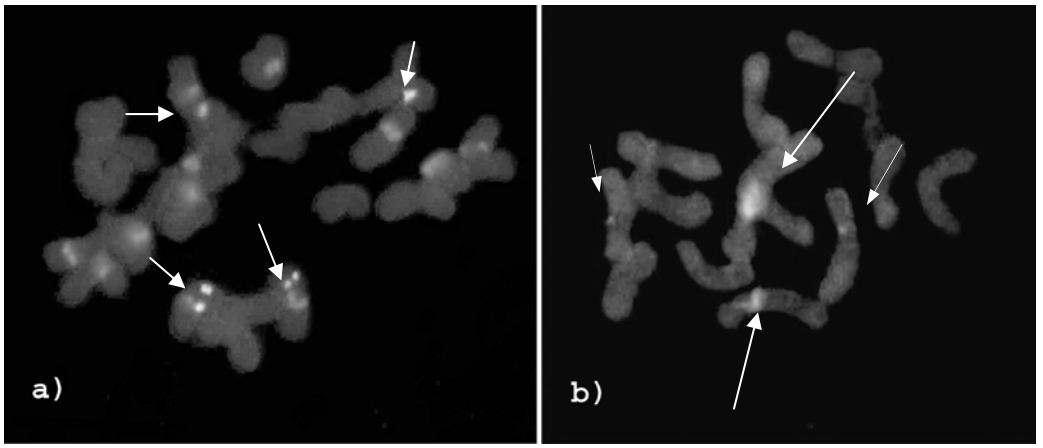

Figure. a) metaphase plate of tetraploid *L. perenne*, cultivar 'Raminta' (2n = 4x = 28). 12 large bright loci are 18S-5.8S-26S (pTA71 probe) sites (not arrowed), and 4 smaller brighter loci (arrowed) are 5S rDNA sites; b) metaphase plate of *F. pratensis*, cultivar 'Kaita' (2n = 14). Two large bright loci are 18S-5.8S-26S sites (large arrows), and two small bright loci – 5S rDNA sites (small arrows)

Paveikslas. *a) L. perenne augalo veislės 'Raminta' (2n = 4x = 28) metafazinė plokštelė. Didelės šviesios sritys yra 18S-5.8S-26S (pTA71) saitai (nėra rodyklių), ryškios mažesnės sritys (parodytos rodyklėmis) – 5S rDNR saitai; b) F. pratensis augalo veislės 'Kaita' metafazinė plokštelė (2n = 14). Dvi didelės ryškios sritys yra 18S-5.8S-26S saitai (didelės rodyklės), du maži šviesūs taškai – 5S rDNR saitai (mažos rodyklės)*

However, for *Lolium* cultivars, the results were different. It is shown in table 1 that numbers of 18S-5.8S-26S sites varied in the plants of all cultivars. As diploid *L. perenne* cultivars were shown to have seven 18S-5.8S-26S sites (Thomas et al., 1996), tetraploid cultivars were expected to have fourteen, but not all plants had exactly the same numbers. In diploid cultivars, the number of 18S-5.8S-26S sites varied from 6 to 8, the average was 7.0 in 'Veja' and 'Vincent', and the smallest average numbers of these sites were found in 'Limes' and 'Rastro' – 6.4. Similar tendency was recorded in tetraploid cultivars. From 9 to 16 18S-5.8S-26S sites were found, and the average was usually less than 14. An example of rDNA site pattern in tetraploid *L. perenne* is present in figure 1a represented by FISH on chromosome set of Lithuanian cultivar 'Raminta' plant. Cultivar 'Verseka' had the smallest number of 18S-5.8S-26S sites – 10.8 on average, and the highest number was found in 'Aligator' – 13.8.

We investigated few plants of diploid *L. multiflorum*. In four plants of cultivar 'Bellem', one plant had only five 18S-5.8S-26S sites (Table 1). Diploid *L. multiflorum* plants were shown to have six 18S-5.8S-26S sites /Thomas et al., 1996/, so tetraploid plants were expected to have twelve. We have also investigated four tetraploid

L. multiflorum cultivars and although the number of plants was not large, there was some difference between them. The plants had from 11 to 14 18S-5.8S-26S sites, and the average was slightly higher than 12.

Table 1. Numbers of 18S-5.8S-26S and 5S rDNA sites in the metaphase chromosomes in the plants of ryegrass (*Lolium*) and fescue (*Festuca*) cultivars
1 lentelė. *18S-5.8S-26S ir 5S rDNR saitų skaičiai įvairių veislių svidrių (Lolium) ir eraičinų (Festuca) augalų chromosomose*

Cultivar *Veislė*	Number of plants *Augalų skaičius*	Number of 18S-5.8S-26S rDNA sites *18S-5.8S-26S rDNR saitų skaičius*		Number of 5S rDNA sites *rDNR saitų skaičius*	Number of chromosomes with both 18S-5.8S-26S and 5S rDNA sites *Chromosomų, turinčių ir 18S-5.8S-26S, ir 5S rDNR saitus, skaičius*	
		Range *Ribos*	Mean *Vidurkis*		Range *Ribos*	Mean *Vidurkis*
L. perenne						
2X ʻLimes’	5	6–7	6.4	2	2	2.0
ʻRastro’	5	6–8	6.4	2	1–2	1.6
ʻVeja’	5	6–8	7.0	2	1–2	1.8
ʻVincent’	4	6–8	6.75	2	1–2	1.75
Total / Mean	19	6–8	6.64	2	1–2	1.79
4X ʻAcento’	5	11–13	12.4	4	4	4.0
ʻAlduva’	5	9–14	11.6	4	2–4	3.0
ʻAligator’	5	12–15	13.8	4–5	3–5	3.8
ʻRaminta’	5	12–16	13.6	4	3–4	3.4
ʻŽvilgė’	5	11–16	13.2	4	4	3.8
ʻElena’	5	11–14	12.6	4	3–4	3.8
ʻVerseka’	5	9–13	10.8	4	2–4	3.0
ʻSodrė’	5	12–14	13.2	4	4	4.0
Total / Mean	40	9–16	12.65	4–5	2–5	3.6
L. multiflorum						
2X ʻBellem’	4	5–6	5.75	2	2	2.00
ʻAdin’	2	6	6.00	2	2	2.00
Total / Mean	6	5–6	5.88	2	2	2.00
4X ʻCatalpa’	3	11–14	12.33	3–4	3–4	3.67
ʻLotos’	2	12–13	12.50	4–5	4–5	4.50
ʻMeroa’	3	12–13	12.33	4	4	4.00
Total / Mean	8	11–14	12.39	3–5	3–5	4.06
F. pratensis						
2X ʻDotnuva’	2	2	2	2	0	0
ʻFure’	1	2	2	2	0	0
ʻKaita’	5	2	2	2	0	0
ʻLoken’	1	2	2	2	0	0
ʻSigita’	5	2	2	2	0	0
Total / Mean	14	2	2	2	0	0
4X ʻPetra’	2	4	4	4	0	0
ʻRaskila’	2	4	4	4	0	0
Total / Mean	4	4	4	4	0	0

As for 5S rDNA sites, their number was found the same as expected – 2 in diploid cultivars and 4 in tetraploids. All except two tetraploid plants had 4 5S rDNA sites: there was one *L. perenne* plant of cultivar 'Aligator' and one *L. multiflorum* plant of cultivar 'Lotos' that had 5 5S rDNA sites (Table 1). However, the locations of these sites were different in *L. perenne* plants. In diploid *Lolium* species, both 5S rDNA sites are expected to be in the same chromosomes as 18S-5.8S-26S sites /Kopecky et al., 2006, Thomas et al., 1996/, but it was not all true for our plants. There was only one diploid cultivar 'Limes' and 2 tetraploid cultivars – 'Acento' and 'Sodrė', where all investigated plants had all 5S rDNA sites on the same chromosomes as pTA71 sites. In other diploid cultivars, there were some plants that had one chromosome with both 18S-5.8S-26S and 5S rDNA site, one chromosome with only 18S-5.8S-26S site and one with only 5S rDNA site. In other tetraploid cultivars, there were some plants that had only 2 or 3 chromosomes with both 18S-5.8S-26S and 5S rDNA sites; other 5S rDNA and 18S-5.8S-26S sites were found on the separate chromosomes.

These results show that the number of 18S-5.8S-26S sites is not strictly determined in the plants of *Lolium* cultivars, although 5S rDNA site number remains conservative in almost every plant. However, the location 5S rDNA is found to be variable – some plants may have chromosomes resembling *F. pratensis* chromosome pattern in this aspect. In *F. pratensis*, the situation is different – both 18S-5.8S-26S and 5S rDNA site numbers and location remain conservative in all investigated cultivars. As some cultivars have different numbers of rDNA sites, they could serve as markers in them, such as cultivar 'Verseka' that has a relatively small number of 18S-5.8S-26S sites and all 5S rDNA sites are located on the chromosomes together with 18S-5.8S-26S, and 'Aligator', having the greatest number of 18S-5.8S-26S sites of these cultivars. In some of *L. multiflorum* cultivars only 2–3 plants have been investigated, so findings in *L. mutiflorum* require further verification by a wider analysis in the future.

***Festulolium* cultivars 'Pūga' and 'Vėtra'.** We investigated 18S-5.8S-26S rDNA (pTA71 probe) sites in two new Lithuanian *Festulolium* cultivars – × *Festulolium* (*L. multiflorum* × *F. arundinacea*, 2n = 4x = 28).

We have already done such research with another two × *Festulolium braunii* cultivars – 'Punia' and 'Rakopan' /Lideikytė et al., 2006, Lideikytė, Pašakinskienė, 2007/, and no significant differences were detected in 18S-5.8S-26S site numbers of these cultivars. We aimed to compare rDNA site distribution in the chromosomes of plants of new cultivars 'Pūga' and 'Vėtra' with the ones of 'Punia' and 'Rakopan'.

Twenty two 'Pūga' plants and twenty four 'Vėtra' plants were investigated for 18S-5.8S-26S site numbers. Their mean 18S-5.8S-26S site number was different from that in 'Punia' and 'Rakopan' plants: the mean number of 18S-5.8S-26S sites in 'Punia' and 'Rakopan' cultivars was close to 9 /Lideikytė et al., 2006, Lideikytė, Pašakinskienė, 2007/, whereas both in 'Pūga' and 'Vėtra' it was higher: 10.18 and 12.25, respectively (Table 2). The difference was significant between these two cultivars ($P < 0.05$). 'Pūga' plants had a wider range of rDNA site number: from 6 to 14. In 'Vėtra' cultivar, there were some plants that had as many as fifteen 18S-5.8S-26S sites, although theoretically, tetraploid *L. multiflorum* plants should have 12, as diploid plants were shown to have 6 /Thomas et al., 1997/. As *L. multiflorum* has more 18S-5.8S-26S sites than *F. pratensis* or *F. arundinacea*, hybrids having more *Lolium* chromosomes are expected to have more

18S-5.8S-26S sites. We have found by genomic *in situ* hybridization (GISH) studies that in ʻPūgaʼ plants, there are more *Festuca* chromosomes and more recombinant chromosomes having *Festuca* DNA fragments than in ʻVėtraʼ plants (Table 3). This could be a reason why ʻVėtraʼ plants have more 18S-5.8S-26S sites than ʻPūgaʼ plants. It could also be the same reason why these two cultivars have more 18S-5.8S-26S sites than ʻPuniaʼ and ʻRakopanʼ plants /Lideikytė, Pašakinskienė, 2007/.

Table 2. Numbers of 18S-5.8S-26S (pTA71 probe) sites in the metaphase chromosomes of *Festulolium* cultivar plants
2 lentelė. *18S-5.8S-26S (pTA71 zondas) saitų skaičius Festulolium veislių augalų chromosomose*

Cultivar *Veislė*	Number of plants *Augalų skaičius*	Number of 18S-5.8S-26S rDNA sites *18S-5.8S-26S rDNR saitų skaičius*	
		Range *Ribos*	Mean *Vidurkis*
ʻPūgaʼ (*L. multiflorum* × *F. pratensis,* 2n = 4x = 28)	22	6–14	10.18
ʻVėtraʼ (*L. multiflorum* × *F. arundinacea*, 2n = 4x = 28)	24	10–15	12.25

Table 3. Numbers of fescue, ryegrass and recombinant chromosomes in *Festulolium* cultivars ʻPūgaʼ and ʻVėtraʼ
3 lentelė. *Grynų eraičino ir svidrės bei rekombinantinių chromosomų skaičius Festulolium veislių ʻPūgaʼ ir ʻVėtraʼ augaluose*

Cultivar *Veislė*	Number of plants *Tirtų augalų skaičius*	Number of fescue chromosomes *Eraičino chrom. skaičius*		Number of ryegrass chromosomes *Svidrės chrom. skaičius*		Number of recombinant chromosomes *Rekombinantinių chrom. skaičius*		Total number of chromosomes *Bendras chromosomų skaičius*	
		Range *Ribos*	Mean *Vidurkis*	Range *Ribos*	Mean *Vidurkis*	Range *Ribos*	Mean *Vidurkis*	Range *Ribos*	Mean *Vidurkis*
ʻPūgaʼ (*L. multiflorum* × *F. pratensis,* 2n = 4x = 28)	22	0–5	1.68	6–25	17.18	2–19	8.73	26–28	27.55
ʻVėtraʼ (*L. multiflorum* × *F. arundinacea*, 2n = 4x = 28)	24	0–3	0.46	10–26	19.46	2–16	7.79	27–28	27.79

Conclusions

1. The numbers and positions of 18S-5.8S-26S (pTA71 probe) and 5S rDNA were found to be stable in the chromosome sets of diploid (2 and 2) and tetraploid (4 and 4) *F. pratensis* plants.

2. *L. perenne* and *L. multiflorum* plants were found to have a variable number of 18S-5.8S-26S rDNA sites, even in the same cultivar. Numbers of 5S rDNA sites seem to be more stable – 2 in diploids and 4 in tetraploids, although in several tetraploid plants the number of 5S rDNA sites was 5.

3. In most *Lolium* cultivars, there were some plants that had 5S and 18S-5.8S-26S rDNA sites on separate chromosomes – a trait previously found only in meadow fescue genome. These plants usually had 1–2 chromosomes with only 5S rDNA site.

4. *Festulolium* cultivars have a wide range of 18S-5.8S-26S rDNA site number, and the average number in *Festulolium* cultivar 'Vėtra' (*L. multiflorum* × *F. arundinacea*, 2n = 4x = 28) was higher than that in cultivar 'Pūga' (*L. multiflorum* × *F. pratensis,* 2n = 4x = 28), 12.25 and 10.18, respectively.

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RIBOSOMINĖS DNR SAITŲ NUSTATYMAS *FISH* METODU *LOLIUM, FESTUCA* IR *FESTULOLIUM* CHROMOSOMOSE

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Santrauka

Fluorescencinės *in situ* hibridizacijos (FISH) būdu buvo pažymėti ribosominės DNR saitai daugiametės svidrės (*L. perenne*), gausiažiedės svidrės (*L. multiflorum*) ir tikrojo eraičino (*F. pratensis*) mitotinėse metafazinėse chromosomose, taip pat ir dviejose *Festulolium* hibridų veislėse. Diploidinių ir tetraploidinių veislių augaluose buvo nustatyti 18S-5.8S-26S ir 5S rDNR saitai. 18S-5.8S-26S rDNR (ženklinta pTA71 zondu) saitų skaičius *Lolium* veislių augaluose tetraploiduose varijavo labiau nei diploiduose. Vidutinis 18S-5.8S-26S saitų skaičius *L. perenne* augaluose buvo 6.64 diploiduose ir 12.65 tetraploiduose, o *L. multiflorum* – 5.58 diploiduose ir 12.39 tetraploiduose. Veislė ‘Verseka’ išsiskyrė mažu 18S-5.8S-26S saitų skaičiumi, palyginti su kitomis veislėmis – vidutiniškai 10.8. 5S rDNR skaičius buvo gana stabilus, ypač diploidinių veislių. Eraičinuose 18S-5.8S-26S bei 5S saitų skaičius ir jų padėtis buvo pastovūs, o *Festulolium* augaluose 18S-5.8S-26S saitų skaičiai varijavo gana smarkiai, ypač veislės ‘Pūga’ augaluose, kuriuose buvo nustatyta nuo 6 iki 14 saitų (vidutiniškai 10,18).

Reikšminiai žodžiai: FISH, ribosominė DNR, *Lolium*, *Festuca*, *Festulolium*.