Genomic Constitution of *Festulolium* Varieties

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Introduction Hybrids between species of ryegrass (*Lolium*) and fescue (*Festuca*) combine useful agronomical characteristics such as rapid establishment from seed and fodder quality from ryegrass and tolerance against abiotic and biotic stressses from fescue. The superior potential of hybrids has stimulated breeding programs generating so called *Festulolium* varieties. While the varieties have been evaluated extensively for their agronomic characteristics, little information is publicly available on their genomic constitution. The aim of our study was to analyse genomic constitution of a representative set of commercially available European *Festulolium* cultivars. To do this, we have employed genomic *in situ* hybridization (GISH).

Materials and methods Four Czech cultivars ('Hykor', 'Felina', 'Korina' and 'Lesana', all 2n=6x=42) were of *L. multiflorum* x *F. arundinacea* origin backcrossed to *F. arundinacea*. Three other Czech cultivars 'Perun', 'Achilles' and 'Perseus', two Polish cultivars ('Felopa' and 'Rakopan'), one Lithuanian cultivar ('Punia') and one German cultivar ('Paulita') originated from crosses between *L. multiflorum* x *F. pratensis* and were all tetraploid (2n=4x=28). Preparation of mitotic metaphase plates and GISH was done according to Masoudi-Nejad *et al*. (2002). In all experiments, DNA of *L. multiflorum* was labelled with digoxigenin and used as a probe; genomic DNA of the *Festuca* species was used to block hybridization of common repetitive DNA sequences. The probe to block ratio was 1:150 with minor deviations. The detection of the hybridization signal was with the Anti-DIG-FITC conjugate; counterstaining was with propidium iodide (PI).

Results GISH is based on hybridization of species-specific DNA sequences in a probe labelled by fluorochrome with DNA of chromosomes fixed on microscopic slide. The method facilitates characterization of hybrids by the number of parental chromosomes, number of translocated chromosomes, number of translocations, number of break points, and total amount of chromatin of both parents. In this study, we screened 25 plants per cultivar, on average. In *L. multiflorum* x *F. arundinacea* hexaploid hybrids, a relatively stable number of parental chromosomes as well as some translocated chromosomes were observed. All *L. multiflorum* x *F. pratensis* hybrids showed large numbers of translocated chromosomes indicating frequent recombinations of both parental genomes. Proportions of parental chromatins varied from cultivar to cultivar.

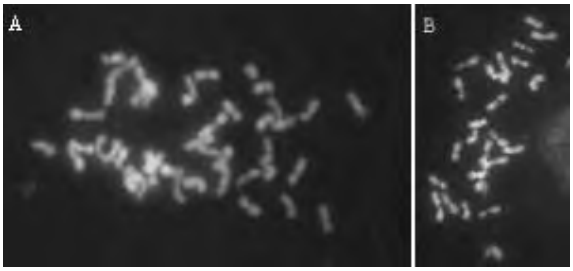

Figure 1 Mitotic metaphase plates of *Festulolium* cultivars after GISH. Genomic DNA of *L. multiflorum* was used as a probe after labelling with FITC (light grey colour); genomic DNA of *F. pratensis* and *F. arundinacea* were used as blocks, respectively. Chromosomes were counterstained by PI (dark grey colour). [A] A representative example of mitotic chromosomes of 'Hykor' with a large number of introgressions of *L. multiflorum* into *F. arundinacea*. [B] A representative example of mitotic chromosomes of 'Perun' with a large number of translocations.

Conclusions The results of our study demonstrate the value of genomic *in situ* hybridization for rapid and relatively cheap screening of commercially available *Festulolium* hybrids for their genomic constitution and for the detection of chromosome translocations. The analyses revealed that none of the commercially available *Festulolium* cultivars contained equal proportions of ryegrass and fescue genomes. Individual cultivars were characterized by a specific ratio of parental chromatin and the number of chromosomal translocations.

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Reference

Masoudi-Nejad, A., S. Nasuda, R.A. McIntosh, and T.R. Endo (2002). Transfer of rye chromosome segments to wheat by a gametocidal system. Chromosome Res. 10:349-357.