Introgression Breeding for Improvement of Winter Hardiness in *Lolium /Festuca* Complex Using Androgenenesis

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Introduction Intergeneric hybrids between closely related *Lolium* and *Festuca* species are used to broaden the gene pool and provide plant breeders with options to combine complementary traits to develop robust but high quality grass varieties. Androgenesis was found to be an effective procedure for selecting *Lolium-Festuca* genotypes comprising gene combinations rarely or never recovered by conventional backcross breeding programs. Here we describe the optimisation of androgenesis in *Lolium perenne* x *Festuca pratensis*. The male fertility and freezing tolerance of the *Festulolium* microspore-derived progenies were analysed and these progenies were also analysed by using genomic *in situ* hybridisation (GISH). The object of this study is to initiate introgression breeding for the improvement of winter hardiness in *Lolium /Festuca* complex.

Materials and methods Genotypes of *Lolium perenne* x *Festuca pratensis* (*Festulolium* hybrid), 'Prior', 'Bx350' and 'Bx351'were investigated in this study. PG-96 (Guo *et al.,* 1999) with 2 mgl^{-1} 2,4-D, 0.5 mgl^{-1} kinetin was used as embryo (calli) induction media. Calli were transferred to the solid medium 190-2 (Wang & Hu, 1984) supplemented with 0.1 mgl^{-1} 2,4-D, 1.5 mgl^{-1} kinetin for green plants regeneration. The ploidy level of androgenic progenies was analysed by Partec CAII flow cytometry (Münster, Germany) with DAPI staining. GISH was carried out according to the method described by Mizukami *et al*. (1998) with some modification. Androgenic-derived plants were grown outdoor for natural hardening during autumn. Crown tissues each genotype were analysed for freezing tolerance cooled to -17^{o}C. Male fertility was measured by staining pollen with 1% acetocarmine and counting the frequency of stainable pollen grains.

Results The calli and green plants were obtained from all three accessions, but the genotype responses differed; accessions 'Bx350' and 'Bx351' were more active than 'Prior' in androgenesis. Among microspore-derived progenies the diploids were dominant (68.2%). High levels of chromosome pairing and recombination were observed by GISH due to close homology between genomes of *L. perenne* and *F. pratensis*. These androgenic-derived *Festulolium* progenies showed a wide range of variation in freezing tolerance, 19 progenies (6.5%) exceeding that in the *F. pratensis* cv. 'Tomosakae'. More than 60% of flowing progenies produced dehiscing anthers with pollen stainability ranging from 5% to 85% in all three accessions (Table 1). The diploid progenies with both freezing tolerance and fertility potential have been crossed with *L. perenne.*

Table 1 Pollen fertility in amphidiploid *Festulolium* anther-derived progenies. F & PF, fertile and partial fertile, with pollen stainability ranging from 5% to 85%. MS, male sterile, no pollen or very few pollen (<5%) stained

B x 350				B x 351				Prior			
2n=2x=14		2n=4x=28		2n=2x=14		2n=4x=28		2n=2x=14		2n=4x=28	
F+PF	MS	F+PF	MS	F+PF	MS	F+PF	MS	F+PF	MS	F+PF	MS
25	12	8	9	43	19	11	3	26	11	4	4
46.3%	22.2%	14.8%	16.7%	56.6%	25.0%	14.5%	3.9%	57.8%	24.4%	8.9%	8.9%

Conclusions High frequency androgenesis in *L. perenne* x *F. pratensis* was established. The diploid microspore-derived progenies with both freezing tolerance and fertility potential are promising to introduce winter hardiness of *F. pratensis* to *L. perenne* by backcrossing with *L. perenne* as introgression breeding.

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