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Meiosis and fertility of reciprocal triploid hybrids of *Lolium multiflorum* with *Festuca pratensis*

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Abstract. Diploid and tetraploid forms of *Lolium multiflorum* and *Festuca pratensis* were crossed under controlled conditions and after embryo rescue all four combinations of autoallotriploid hybrids were obtained. Male and female fertility and chromosome pairing at metaphase I of meiosis were studied in several plants from each hybrid combination. The hybrids with two genomes of *L. multiflorum* and one of *F. pratensis* (genomic formulae LmLmFp and FpLmLm) were male and female fertile while the hybrids with two genomes of *F. pratensis* and one of *L. multiflorum* had a reduced fertility (FpFpLm) or were completely sterile (LmFpFp). Chromosome pairing at metaphase I varied among hybrid combinations depending on their genomic composition. LmLmFp and FpLmLm hybrids had similar patterns of pairing ($1.83_{I} + 5.29_{II} + 2.85_{III}$ and $2.22_{I} + 5.22_{II} + 2.75_{III}$, respectively) but they differed from those of FpFpLm ($3.65_{I} + 4.65_{II} + 2.68_{III}$) and especially from LmFpFp ($4.78_{I} + 5.87_{II} + 1.49_{III}$). Conventional analysis of meiosis failed to explain the differences in chromosome behaviour and fertility/sterility levels between the autoallotriploid hybrids with two *Lolium* or two *Festuca* genomes.

Key words: autoallotriploids, fertility, *Festuca pratensis*, *Lolium-Festuca* hybrids, *Lolium multiflorum*, meiosis.

Introduction

The genera *Lolium* (ryegrass) and *Festuca* (fescue) include most of the major temperate forage grasses. Various species within these genera offer a range of complementary agronomic traits, such as the high nutritive value of ryegrasses and the persistency and stress tolerance of fescues, which can be combined in intergeneric hybrids and exploited in the development of new, improved cultivars.

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Italian ryegrass (*Lolium multiflorum* Lam., 2n=2x=14 and 2n=4x=28) and meadow fescue (*Festuca pratensis* Huds., 2n=2x=14 and 2n=4x=28) can hybridize readily at different ploidy levels, producing diploid, triploid or tetraploid hybrids (see JAUHAR 1993 and ZWIERZYKOWSKI 1996 – review papers). Diploid hybrids exhibit full chromosome pairing at metaphase I of meiosis but are completely sterile (JAUHAR 1975, WERNER 1983). Triploid and tetraploid hybrids, in most cases, are both male and female fertile (JAUHAR 1975, ZWIERZYKOWSKI, RYBCZYŃSKI 1981, ZWIERZYKOWSKI 1987). The ease of hybridization and extensive chromosome pairing suggest that these species must be closely related. On the other hand, the sterility of diploid hybrids and especially the ease with which chromosomes of *L. multiflorum* and *F. pratensis* can be distinguished using genomic in situ hybridization (THOMAS et al. 1994, PASAKINSKIENE et al. 1998, ZWIERZYKOWSKI et al. 1998, 1999) suggest that the relationship cannot be particularly close.

Triploid hybrids (2n=3x=21) between diploid and autotetraploid forms of *L. multiflorum* and *F. pratensis* have been produced before (e.g. SULINOWSKI 1967, GROBER et al. 1974, JAUHAR 1975, ZWIERZYKOWSKI 1987, HUMPHREYS, THOROGOOD 1993, THOMAS et al. 1994). In most cases, these triploids were obtained by crossing colchicine-induced autotetraploid *L. multiflorum* with diploid *F. pratensis*. Such hybrids, which are usually partly male and female fertile, are currently being used in introgression programmes aiming at transfers of chromatin from *F. pratensis* into the *L. multiflorum* germplasm (e.g. HUMPHREYS, THOROGOOD 1993, THOMAS et al. 1994, ZWIERZYKOWSKI et al. 1999). Apart from the practical value, triploid F_1 hybrids combining parental genomes in various proportions are of interest in theoretical studies as they offer an opportunity to observe chromosome associations depending on different genome doses and genetic background.

This article presents data on meiotic chromosome pairing in triploid F_1 hybrids from all four possible cross combinations between diploid and tetraploid *L. multiflorum* and *F. pratensis*. As far as we know, this is the first simultaneous study of all possible autoallotriploid hybrid types of these species.

Material and methods

Reciprocal, triploid F_1 hybrids from all four possible cross combinations of diploid and tetraploid *L. multiflorum* and *F. pratensis* were produced by crossing the diploid (2n=2x=14) cv. Tur or autotetraploid (2n=4x=28) cvs. Lotos or Mitos of *L. multiflorum* (genomic formulae LmLm and LmLmLmLm, respectively) with diploid cvs. Skawa or Skra and the autotetraploid cv. Westa of *F. pratensis* (genomic formulae FpFp and FpFpFpFp, respectively). In each cross (plant × plant) different individuals of *Lolium* and *Festuca* cultivars were used. Immature embryos were removed 14-18 days after pollination and cultured on a B5 medium

(GAMBORG et al. 1968). All four triploid hybrid types presented here were produced simultaneously in the year 1995. The hybrids had the following genomic formulae:

1) *L. multiflorum* (4x) × *F. pratensis* (2x) = LmLmFp
2) *F. pratensis* (2x) × *L. multiflorum* (4x) = FpLmLm
3) *F. pratensis* (4x) × *L. multiflorum* (2x) = FpFpLm
4) *L. multiflorum* (2x) × *F. pratensis* (4x) = LmFpFp

Male fertility of the hybrids was tested as pollen stainability in a mixture of acetocarmine and glycerine on a microscopic slide. Random samples of 300 pollen grains per plant were scored for stainability. Female fertility was expressed as a percentage of seed set under open pollination in relation to the total number of florets in an inflorescence studied. Three randomly collected inflorescences per plant were scored.

For the observations of meiosis, inflorescences were fixed in a mixture of ethanol and glacial acetic acid (3 : 1). The anthers were stained and squashed in 2% acetoorcein and the frequencies of various chromosome associations at metaphase I (MI) were scored in 30 pollen mother cells (PMC) per plant (except for 20 cells of one plant). For a comparison of different hybrids, mean values and standard errors were calculated for the frequencies of chromosome configurations and chiasmata.

Results

The crossability of various diploid and tetraploid plants of *L. multiflorum* and *F. pratensis* was high and fairly large numbers of hybrids were produced (Table 1). In the combinations LmLmFp, FpLmLm and LmFpFp, 117, 201 and 40 plants were obtained, respectively. The FpFpLm combination showed a lower effective crossability, with only eight hybrids obtained. Reciprocal crosses have shown that crossability was higher when diploids were used as mother plants.

The fertility of the autoallotriploids clearly depended on their genomic constitution (Table 2). LmLmFp and FpLmLm plants had good male fertility (the mean

Table 1. Seed set and number of F_1 hybrids obtained from reciprocal crosses between diploid and tetraploid forms of *L. multiflorum* and *F. pratensis*

Cross combination	No. of crosses	No. of flowers pollinated	Seed set		F_1 plants obtained	
			number[a]	%	number[b]	%
Lm (4x) × Fp (2x)	7	1321	602	45.8	117	8.6
Fp (2x) × (Lm (4x)	6	1740	627	36.0	201	11.6
Fp (4x) × Lm (2x)	3	509	171	33.6	8	1.6
Lm (2x) × Fp (4x)	2	354	173	48.9	40	11.3

[a] Number of embryos cultured
[b] In relation to total number of pollinated flowers

pollen stainability was 62.6% and 67.8%, respectively) and seed set under open pollination ranging from 9.8% to 17.5%. The hybrids with two Fp genomes were clearly less fertile: the FpFpLm combination had only partly dehiscent or completely indehiscent anthers (those with dehiscent anthers had 40.0% of pollen stainability, while those with indehiscent anthers 0-6.5%) and only sporadically set single seeds. LmFpFp plants were completely male and female sterile.

Table 2. Pollen stainability and seed set in autoallotriploid hybrids of *L. multiflorum* with *F. pratensis*

Hybrid combination	Pollen stainability (%)		Seed set (%)	
	range	mean	range	mean
Lm (4x) × Fp (2x)	17.5 - 85.0 [a]	62.6	0.1 - 26.7	9.8
Fp (2x) × Lm (4x)	22.5 - 90.5 [b]	67.8	0.4 - 34.4	17.5
Fp (4x) × Lm (2x)	18.5 - 64.5 [c]	40.0	plants with dehiscent anthers sporadically set seeds	
Lm (2x) × Fp (4x)	sterile [d]			

[a, b] All F_1 plants of both combinations had dehiscent anthers
[c] Five F_1 plants had partly dehiscent anthers and three plants had indehiscent anthers
[d] All F_1 plants had indehiscent anthers

All F_1 hybrids studied had 21 chromosomes. Occasionally, B chromosomes were also present. Chromosome pairing at MI and chiasma frequencies were studied in four or six plants per combination (Table 3). All hybrids showed relatively high numbers of bivalents, most of which were ring-shaped. The remaining chromosomes formed trivalents, mostly frying-pan–shaped (with some triradial, chain-shaped, bird-cage–shaped and ring-shaped ones) or remained unpaired. Sporadically, single quadrivalents were also observed. Mean paired arms frequencies were markedly higher in the hybrids with two doses of *Lolium* and one dose of *Festuca* genomes (LmLmFp and FpLmLm; on average 18.91 and 18.35 paired arms per cell, respectively) than in those combining diploid sets of *Festuca* chromosomes with a haploid *Lolium* complement (FpFpLm and LmFpFp; 16.93 and 15.59 per cell, respectively). The higher overall pairing level was evident in the lower average numbers of univalents and higher numbers of bivalents and trivalents (Table 1). In each pair of hybrids, those with the cytoplasm of the single-genome parent (FpLmLm and LmFpFp) had markedly lower chiasma frequencies than their reciprocal hybrids with the cytoplasm of the double-genome parent (LmLmFp and FpFpLm, respectively).

Depending on genomic formulae, hybrids with two Lm genomes – LmLmFp and FpLmLm – had similar patterns of chromosome pairing ($1.83_I + 5.29_{II} + 2.85_{III}$ and $2.22_I + 5.22_{II} + 2.75_{III}$, respectively) but differed from those with two Fp genomes. These groups–FpFpLm and LmFpFp ($3.65_I + 4.65_{II} + 2.68_{III}$ and $4.78_I +$

Table 3. Chromosome pairing at metaphase I of meiosis in autoallotriploid hybrids of *L. multiflorum* with *F. pratensis*

Genomic constitution	No. of hybrids studied	No. of cells studied	Chromosome configurations mean ± SE (range)				Chiasmata per cell mean ± S.E. (range)
			I ± S.E.	II ± S.E.	III ± S.E.	IV	
Lm Lm Fp	6	180	1.83 ± 0.238 (0-6)	5.29 ± 0.074 (2-9)	2.85 ± 0.106 (0-5)	0.01 (0-1)	18.91 ± 0.251 (15-21)
Fp Lm Lm	6	180	2.22 ± 0.238 (0-6)	5.22 ± 0.328 (1-10)	2.75 ± 0.238 (0-6)	0.03 (0-1)	18.35 ± 0.227 (14-21)
Fp Fp Lm	4	120	3.65 ± 0.658 (0-7)	4.65 ± 0.232 (1-8)	2.68 ± 0.373 (0-5)	–	16.93 ± 0.660 (13-20)
Lm Fp Fp	6	170	4.78 ± 0.417 (0-7)	5.87 ± 0.115 (2-9)	1.49 ± 0.157 (0-4)	0.01 (0-1)	15.59 ± 0.327 (12-20)

$5.87_{II} + 1.49_{III}$, respectively) – differed also from one another in respect of each chromosome configuration. The proportion of the frying-pan–shaped trivalents was higher in the first group (91.6% and 90.9%, respectively) than in the second one (83.2% and 83.1%, respectively). No cells with configuration 7_{III} were observed.

Discussion

Our results clearly show that in reciprocal, triploid *Lolium-Festuca* hybrids, chromosome pairing and fertility depend on the proportions of parental genomes present and on the cytoplasm. The effect of double Lm genome on chromosome pairing as well as on fertility in triploid *Lolium-Festuca* hybrids has already been reported (HUMPHREYS, THOROGOOD 1993, HUMPHREYS et al. 1998, CANTER et al. 1999). In our experiment, among all four cross combinations a marked influence of a double dose of the *Lolium* genome was observed. Hybrids with two *L. multiflorum* genomes had higher male and female fertility and higher average numbers of chromosome arms paired per cell than the hybrids with two *F. pratensis* genomes. Male fertility of LmLmFp and FpLmLm hybrids was similar (on average 62.6% and 67.8%, respectively); these hybrids were also partly female fertile. Hybrids with two Fp genomes had less chromosome pairing and were less fertile. Most of the FpFpLm hybrids were sterile; several plants with partly dehiscent anthers averaged 40.0% pollen stainability. The reciprocal combination was completely male and female sterile.

Some autoallotriploid hybrids of *L. multiflorum*, *L. perenne* and *F. pratensis* were studied by JAUHAR (1975). In the FpLmLm hybrids pollen stainability was 43.8%, while the LmFpFp hybrids had indehiscent anthers. No female fertility data were reported. Autoallotriploids of *L. perenne* with *F. pratensis* showed similar fertility patterns (ESSAD 1968, GYMER, WHITTINGTON 1975, JAUHAR 1975), i.e. higher fertility of hybrids with two *Lolium* genomes.

Similarly to our observations, JAUHAR (1975) reported higher chromosome pairing in the presence of two *Lolium* genomes and one *Festuca* genome than in the reverse combinations. In his study the mean chromosome configuration in FpLmLm hybrids was $3.01_I + 3.56_{II} + 3.61_{III}$ and in LmFpFp hybrids it was $4.71_I + 4.84_{II} + 2.18_{III}$ per cell. The most common trivalent type was also frying-pan–shaped. Although in the type FpLmLm a maximum of 7_{III} was found, it was not in LmFpFp plants. JAUHAR'S analysis of autoallotriploids with *L. perenne* revealed similar relations. The patterns of chromosome configurations were $2.67_I + 3.04_{II} + 3.95_{III}$ for FpLpLp plants and $4.87_I + 5.03_{II} + 1.96_{III}$ for LpFpFp plants. Thus, the influence of a double *Lolium* genome was visible. Mean frequencies of trivalents observed in our work correspond very well to those reported by ESSAD (1968) but slightly differ from the data reported by GYMER and WHITTINGTON (1975) who demonstrated a larger extent of homoeologous

chromosome pairing. In all three papers cited, the influence of genomic constitution on trivalent performance in *L. perenne* × *F. pratensis* reciprocal auto-allotriploids was also shown.

Chiasma frequencies in JAUHAR'S FpLmLm plants were lower (15.60 compared with 18.35 per cell) and trivalent frequencies were higher (3.61 compared with 2.75) than in ours. In his LmFpFp hybrids higher trivalent frequencies (2.18 compared with 1.49) were accompanied by higher chiasma frequencies (17.20 compared with 15.59).

Studying intergeneric hybrids of *L. multiflorum* (4x) with *F. donax* (2x) and *F. drymeja* (2x), MORGAN (1990) found unexpected low trivalent frequencies (0.20 and 0.20 per cell) whereas bivalent (7.48 and 6.93 per cell, respectively) and chiasma frequencies (15.32 and 13.28 per cell, respectively) were rather high. Observations in triploid hybrids *L. perenne* (2x) × *F. mairei* (4x) (CAO et al. 2000) also revealed low trivalent (mean 0.57 per cell) and high bivalent (7.19) frequencies. A comparison of those results with frequencies for triploid hybrids of *L. multiflorum* and *L. perenne* with *F. pratensis* shows that chromosome configurations – particularly trivalent frequency – reflect to some extent the relationships among species and the extent of their divergence within the *Lolium-Festuca* complex. However, according to new suggestions, meiotic MI pairing of chromosomes in hybrids cannot be taken as a true index of phylogenetic relationships (SEBERG, PETERSEN 1998 and references therein). Chromosome pairing may be influenced by many factors, such as genome-specific genetic control, cytoplasmic genes and chromosome rearrangements. Nevertheless, an approach to studying chromosome differentiation in two species by analysing chromosome pairing in all types of F_1 hybrids showed the importance of well-chosen cross combinations in research and breeding programmes.

It can be assumed with a high degree of confidence that barring duplications in the haploid genomes of the parents of a wide hybrid, each pairing event in a diploid hybrid (as evidenced by a chiasma at MI) indicates homoeologous association. However, as discussed by KIMBER and ALONSO (1981), homoeologous pairing in diploid hybrids is a poor indicator of the level of chromosome affinity. Such an affinity can only be assessed under conditions of competition for pairing partners, that is, in polyploid hybrids. Unfortunately, in the triploid hybrids such as those studied here, conventional analyses of chromosome pairing do not have the desired resolution to determine the exact extent of homo- vs homoeologous pairing. Here, only the trivalent frequencies can be used as a reliable measure of homoeologous affinity. Observable frequency of quadrivalents, clear deficits of univalents and the extent of homoeologous recombination frequency observed among the progeny of similar hybrids (ZWIERZYKOWSKI et al. 1999) suggest that this may not be a very precise measure and that in fact a proportion of bivalents must involve homoeologues. Nevertheless, taking into account the apparent limitations of the technique, it is evident that the amount of homoeologous pairing in the triploid *Lolium-Festuca* hybrids depends to some extent on at least two

identifiable factors: the dosages of each genome present and the cytoplasm (ZWIERZYKOWSKI et al. 1999). Simultaneous observation of all four possible combinations suggests a role of the cytoplasm in chromosome pairing. In two reciprocal crosses, there was somewhat more pairing and, therefore, presumably more homoeologous pairing, when the cytoplasm was of the species with two nuclear genomes present (e.g. LmLmFp versus FpLmLm); a nuclear genome present in a single dose and in alien cytoplasm was always at a disadvantage in competition for pairing. Pairing initiation and pairing partner exchanges in these hybrids need to be studied in greater detail using high-resolution methods, probably GISH analyses. Such studies have been recently made in two autoallotriploid hybrids: *L. perenne/F. pratensis* (KING et al. 1999) and *L. perenne/F. mairei* (CAO et al. 2000). THOMAS et al. (1994), PASAKINSKIENE et al. (1998) and ZWIERZYKOWSKI et al. (1999) showed that in spite of similarity between *L. multiflorum* and *F. pratensis* genomes the two species are sufficiently divergent to be distinguished readily by GISH performed on mitotic chromosomes. GISH analyses of meiosis in *L. multiflorum/ F. pratensis* triploids are planned in the near future.

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