Use of Flow Cytometer in Breeding Festuca x Lolium Hybrids

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USE OF FLOW CYTOMETER IN BREEDING FESTUCA X LOLIUM HYBRIDS.

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ABSTRACT

Ploidy in various generic *Lolium - Festuca* progenies were assessed by flow cytometry and compared to conventional chromosome counting. In non-segregating triploid and tetraploid progenies, the cytometer estimated so accurately the level of plant ploidy that chromosomes counting would be no use. In segregant progenies, all the tetraploid plants according to the cytometer had effectively 28 chromosomes. Among the triploid and pentaploid plants detected by the cytometer, 40% of the plants were tetraploid. They represented only 29% of the progeny which must be counted.

The flow cytometry can be used in routine interspecific breeding programmes. Conventional chromosome counting should be used only for a few plants. Cytometry is more simple and less time consuming than chromosome counting.

KEYWORDS

Fescue, ryegrass, intergeneric hybrid, methodology, flow cytometry, ploidy determination, chromosme counting

INTRODUCTION

The *Lolium -Festuca* complex presents complementary traits which breeders attempt to associate into interspecific hybrid on *Festulolium* to produce persistent, drought tolerant plants having high nutritive value (Thomas and Humphreys, 1991). At Lusignan, different interspecific hybrids from Italian ryegrass X tall fescue crosses were backcrossed into diploid and tetraploid ryegrass (Ghesquière *et al.*, 1991). At each generation, the determination of the ploidy level requires counting the number of chromosomes from microscopic preparations of root tips in metaphase I. This technique is time consuming and depends on the number of dividing cells per root. Flow cytometry based on measurement of the DNA content of interphase nuclei was applied to plant ploidy determination (Marie and Spencer, 1993). The interest of flow cytometry was compared with usual chromosome counting in introgressed *Lolium - Festuca* progenies which ploidy level was segregrating.

MATERIAL AND METHODS

Varieties of 2x and 4x *Lolium multiflorum* , *L. perenne*, the spontaneous tall fescue *Festuca arundinacea* var. *glaucescens* (2n = 4x = 28) and the cultivated tall fescue *Festuca arundinacea* var. *genuina* (2n = 6x =42) were used as control. Two F1 hybrids were analyzed : *L. multiflorum* (2n = 4x = 28) X *F. arundinacea* var. *glaucescens* (2n = 4x = 28) (L4F4) and *L. multiflorum* (2n = 4x = 28) X *F. arundinacea* var. *genuina* (2n = 6x = 42) (L4F6). Fifty one and thirty plants from the first backcross L4F4 into 2x *L. multiflorum* and *L. perenne* (BC1) respectively and 95 progenies of BC1 L4F4 into 4x *L. multiflorum* were studied. Moreover, 78 BC1 plants from L4F6 into 4x *L. perenne* (Lp4) were analyzed.

Chromosomes were counted in root meristems according to Humphreys technique (1989). For cytometry, crude samples were chopped with a razor blade in 2ml of buffer (Tris HCl 0.1 M pH 7, MgCl2 2mM, NaCl 0,1M, Triton 0.05%) and 20 µg of Hoescht. The DNA quantity was determinated by flow cytometer (Partec CA II). First, rapeseed was used as internal reference, then 2x L *multiflorum* (cv Tribune) was used as external reference every 5 samples. The fluorescence intensity was read following logarithmic amplification in which 2C DNA content differed from 4C DNA content by 36 channels.

RESULTS AND DISCUSSION

The cytometer was calibrated in a way that 2C DNA of 2x *L multiflorum* occurred at the channel 100, nevertheless, the peak corresponding to 4C DNA content were present because of cells in G2 phase. Little variations were detected between the different varieties of *L. multiflorum* and *L. perenne*. As expected, 4x *L. multiflorum* showed a dominant 2C population at channel 136. No difference was observed between 4x *L. multiflorum* and *L. perenne*. The G1 peak of *Festuca arundinacea* var. *glaucescens* (2n = 4x) and the one of *Festuca arundinacea* var. *genuina* (2n = 6x) appeared on channel 136 and 156 respectively. As already shown by Thomas (1981) and Seal (1983), the difference between 2C DNA content of 4x *Lolium* and 4x *F. glaucescens* was very small. The 4x (L4F4) hybrid and the 5x (L4F6) hybrids showed a 2C DNA population at the channel 136 and at the channel 148, respectively. Then, cytometry was applied on different back-crossed progenies (Figure 1). L4F4 hybrids could be used in backcross into either 2x or 4x *Lolium*. Among the 95 BC1 progenies into 4x *Lolium*, the 2C DNA peak ranged from the channel 129 to the channel 143 (134.75+/-4.22). More than 85% of the individuals were included between the 132 and 138 channels. Despite the recorded variations, all the plants which number of chromosomes were counted had the expected number of chromosomes. In the first backcross into 2x *L. multiflorum* or *L. perenne,* 2C DNA quantities were the same. The 2C DNA peak ranged from channel 113 to channel 125 (120.15+/- 3.7). Here again, all the counted plants had 21 chromosomes. In the 78 individuals resulting from backross of L4F6 hybrids into 4x *L. perenne*, the cytometer allowed to separate three 2C DNA populations : 71% were presumed at the 4x level (135.8 +/- 5.62), 21% at 5x the level (148.3+/-2.47) and 8% at the 3x level (122.7 +/-2.94) (Figure 1). Forty of the 78 BC1 progenies were chromosome counted and the result confirmed given by the cytometer. The chromosome counting confirmed that all the 4x plants established by the cytometer had all 28 chromosomes. Nevertheless, within the triploid group and the pentaploid group characterized by the cytometer, 67% and 37% of the plants were 3x and 5x respectively. The cytometer appeared therefore to be accurate enough for estimating the 4x level, i.e. 71% of the progeny. However, 29% would be require to be chromosome counted to confirm that 5.4% and 7.8% are 3x and 5x individuals.

Flow cytometry method is exceptionally rapid compared to chromosome counting. More than one hundred plants can be analyzed per day (10 times more than chromosome counting). Nevertheless, sample preparation needs to be improved to reduce error standard deviation and to get more accuracy in the determination of ploidy level. Cytometry can easily take place in a routine screening of *Lolium-Festuca* progenies. In homogenous progenies as BC1of 4x hybrids into 4x *Lolium*, flow cytometry was accurate enough to replace chromosome counting. In segregant progenies, cytometry allowed to separate 4x plants from 3x and 5x plants reducing to 29% the number of plants to be chromosome counted in order to detect 15.8% of 4x plants which were missed by the cytometer.

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Figure 1
Distribution of different backcrossing progenies of *Lolium* X *Festuca* hybrids in function of their channel obtained by flow cytometry. Cumulated results were presented for 2x *L. multiflorum* and 2x *L. perenne*.