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Chromosome painting to locate genes for drought resistance transferred from *Festuca arundinacea* into *Lolium multiflorum*

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Genomic *in situ* hybridization (GISH) on mitotic chromosome preparations of two diploid *Lolium multiflorum*-like drought-resistant plants derived from a *L. multiflorum* × *Festuca arundinacea* hybrid is described. With *F. arundinacea* DNA as probe, each introgression line was found to carry a single *Festuca* recombinant chromosome segment. In both plants, the introgressed *Festuca* chromosome segment was on the long arm of chromosome 2 of the *L. multiflorum*-like hybrid derivative. A DNA probe of *F. pratensis*, which is one of the progenitor species of *F. arundinacea*, hybridized with the introgressed *F. arundinacea* chromosome segment. It is likely that genes that confer drought resistance have been transferred from the *F. pratensis* subgenome of *F. arundinacea* to *L. multiflorum*. It follows that the *F. pratensis* chromosome in *F. arundinacea* which is homoeologous to chromosome 2 in *L. multiflorum* carries genes for drought resistance.

Keywords: chromosome 2, drought resistance, genomic *in situ* hybridization, introgression lines, *Lolium–Festuca*.

Introduction

Within the *Lolium/Festuca* complex the combination of *Festuca arundinacea* and *Lolium multiflorum* has been claimed to offer the greatest number of complementary characters (Breese *et al.*, 1981). *Festuca arundinacea* has a wider distribution than *L. multiflorum* with superior persistency, and better ability to withstand extremes of temperature and water availability. However, compared with *Lolium* it has poor seedling vigour, slower establishment and lower nutritive value. *Lolium* and *Festuca* spp. may readily be hybridized and thus there are opportunities for gene transfer between the two genera. Indeed, a backcross breeding programme for the effective transfer of *F. arundinacea* genes into *L. multiflorum* has been developed (Humphreys, 1989). This involved the use of pentaploid hybrids formed by the hybridization of synthetic autotetraploid *L. multiflorum* ($2n = 4x = 28$) and *F. arundinacea* ($2n = 6x = 42$).

Diploid *Lolium*-like derivatives ($2n = 2x = 14$) of this backcrossing programme were assessed for drought resistance (Humphreys & Thomas, 1993). A low frequency (3 per cent) of the plants was shown to have drought resistance equivalent to that of *F. arundinacea*. Moreover, the drought resistance was shown to be inherited as it was maintained in subsequent generations (Thomas *et al.*, 1995).

The success in confirming the phylogeny of *F. arundinacea* by Humphreys *et al.* (1995) using genomic *in situ* hybridization (GISH) provides the means whereby the origin of any introgressed chromosome segment of *F. arundinacea* can be determined. The hexaploid *Festuca* species was shown to have been derived from the hybridization of diploid *F. pratensis* and tetraploid *F. glaucescens*. Chromosomes and chromosome segments of the progenitor *Festuca* species can be distinguished both from each other and from *Lolium* chromosomes (Humphreys, 1995).

This paper describes GISH using DNA from *F. arundinacea* and one of its progenitors, *F. pratensis*, as probe on mitotic chromosome preparations of two drought-resistant *L. multiflorum*-like plants derived from the backcross breeding programme described above. The location of alien *Festuca* introgressions and their possible origins within the genome of *F. arundinacea* is discussed.

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Materials and methods

The backcross breeding programme was described by Humphreys (1989) and by Humphreys & Thomas (1993) and is summarized in Fig. 1.

Two drought-resistant *L. multiflorum* introgression lines, 86/3/20 and 103/10/29, were randomly selected from the above backcrossing programme. The two drought-resistant lines were derived from different *Lolium* and BC1 parents.

GISH was carried out on mitotic chromosome preparations of the two drought-resistant *L. multiflorum* introgression lines, 86/3/20 and 103/10/29. The procedure was as described by Thomas *et al.* (1994). Total genomic DNA of *F. arundinacea*, and *F. pratensis* labelled with rhodamine-dUTP, was used as probe at 100 ng. per slide. Sonicated *L. multiflorum* DNA at ×40 probe concentration (4 μg per slide) was used as blocker in the hybridization mixture to enhance species-specific fescue DNA hybridization on the chromosome preparations. All preparations were counterstained with DAPI. Images of the introgressed fescue chromosome segments were captured digitally, in monochrome, with a CoolView CCD camera and Apple Macintosh computer using software by Improvision. The monochrome rhodamine images were given false red colours. They were then combined with images made separately using DAPI as counterstain which in turn was given a false blue colour.

Results

The two drought-resistant introgression lines, 86/3/20 and 103/10/29 are both diploid ($2n = 2x = 14$). Each contained a single introgressed *Festuca* chromosome segment (Fig. 2). The size of the introgressed chromosome segments differed between the two drought-resistant lines with 103/10/29 containing a larger fescue segment than 86/3/20. Despite the difference in size of the introgressed segments, they would both appear to overlap

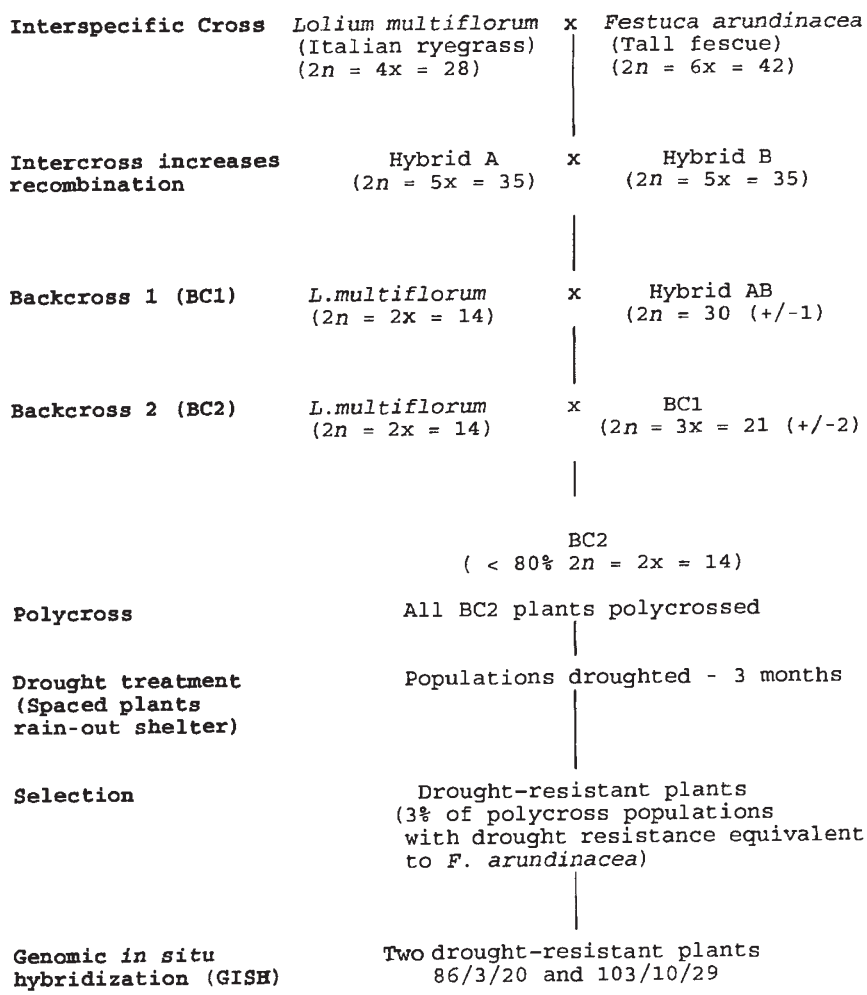

Fig. 1 The breeding programme for the transfer of drought resistance from *Festuca arundinacea* to *Lolium multiflorum*.

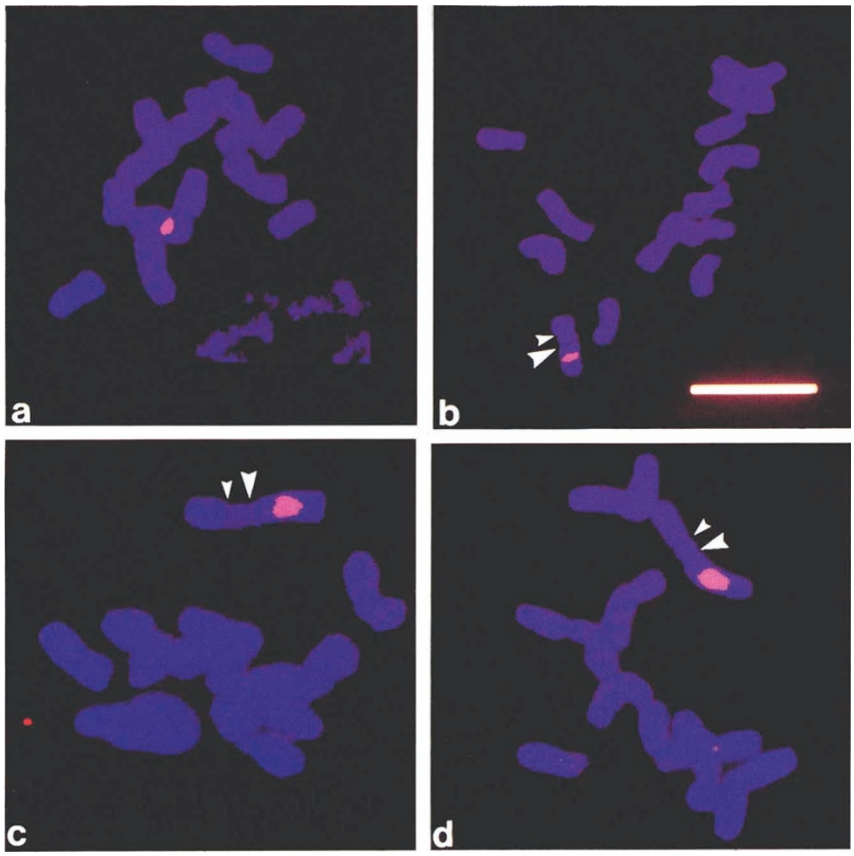

Fig. 2 Genomic *in situ* hybridization (GISH) on mitotic chromosome preparations of drought-resistant *L. multiflorum* (2x) lines 86/3/20 and 103/10/29 ($2n = 2x = 14$) derived from a *Lolium multiflorum* (4x) × *Festuca arundinacea* (6x) hybrid. (a) 86/3/20 with *F. arundinacea* and (b) with *F. pratensis* total genomic DNA as probe; (c) 103/10/29 with *F. arundinacea* and (d) *F. pratensis* total genomic DNA as probe. Cells (b) and (d) are incomplete cells chosen to illustrate the introgressed chromosome segment on chromosome 2. Primary (large arrowhead) and secondary (small arrowhead) constrictions on *L. multiflorum* chromosome 2 are indicated. Bar = 10 μm.

on the same *L. multiflorum* chromosome. In both cases the introgressed fescue segment was interstitially located on the long arm of satellite chromosome 2 (Lewis *et al.*, 1980). As the introgressed *Festuca* chromosome segment in both drought-resistant selection lines had flanking *Lolium* DNA on either side, at least two previous recombination events must have occurred in both involving the *L. multiflorum* chromosome 2. Despite the two introgression lines being derived from different BC1 parents, it is possible that they originated following only one interspecific recombination event early in the backcross breeding programme. In this case, the smaller introgressed *Festuca* chromosome segment seen in line 86/3/20 would have arisen following recombination with a complete *L. multiflorum* chromosome 2 at a site on the original introgressed *Festuca* chromosome segment observed in 103/10/29.

Introgression line 86/3/20

A narrow introgressed *Festuca* chromosome segment was seen in Fig. 2(a) when probed with total genomic DNA probe of *F. arundinacea*. The chromosome orientation was such that the *L. multiflorum* chromosome carrying the introgressed segment could not be identified. However, when a mitotic chromosome preparation of the same genotype was probed with a total genomic DNA probe of *F. pratensis* (Fig. 2b), a single narrow *Festuca* segment was observed approximately centrally in the long arm of a large satellite chromosome identified by its gross morphology as chromosome 2.

Introgression line 103/10/29

A large introgressed *Festuca* chromosome segment is seen in Fig. 2(c) which has been probed with total genomic DNA probe of *F. arundinacea*. The introgressed chromosome segment occupies a central position on the long arm of a satellite chromosome, again identified as chromosome 2. The introgressed *Festuca* chromosome segment occupied approximately half of the long arm of chromosome 2. A mitotic chromosome preparation of the same genotype was subsequently probed with a total genomic DNA probe of *F. pratensis* (Fig. 2d). The introgressed *F. arundinacea* chromosome segment hybridized with the *F. pratensis* DNA probe.

Discussion

The results describe the characterization of two diploid drought-resistant *L. multiflorum* plants using GISH. Each *Lolium* plant was derived from a backcrossing programme involving as a starting point a pentaploid hybrid between *L. multiflorum* and *F. arundinacea*. Both drought-resistant lines carry a single introgressed *Festuca* chromosome segment. Despite differences in the size of the introgressed *Festuca* segment they apparently occupy overlapping positions on the long arm of a large satellite chromosome identified as chromosome 2 (see Lewis *et al.*, 1980).

Lewis *et al.* (1980) described how the isozyme marker glutamate oxaloacetate transaminase (GOT/3) was located on chromosome 2 of *L. multiflorum*. Current investigations by the Cytogenetics Group at IGER will confirm the precise position of the *GOT/3* locus. If the *GOT/3* locus is located on the long arm of chromosome 2 in *L. multiflorum*, then this locus could be used to assist the selection of genes for drought resistance in a breeding programme. The current development of molecular marker systems such as RFLPs and RAPDs in *Lolium* (Hayward *et al.*, 1994) has identified other marker loci linked to *GOT/3* and therefore also located on chromosome 2. As the numbers of these markers increase, it should be possible closely to tag and select genes for drought resistance in any introgression programme and exclude individuals with additional and possible deleterious *Lolium/Festuca* gene combinations.

The recent determination of the progenitors of hexaploid *F. arundinacea* using GISH (Humphreys *et al.*, 1995) confirmed other research workers' (e.g. Chandrasekharan & Thomas, 1971) conclusions that the polyploid species was derived from a hybrid between *F. pratensis* (2x) and *F. glaucescens* (4x). Genes which determine drought resistance in *F. arundinacea* may be found in all three subgenomes of the polyploid species. Humphreys & Ghesquière (1994) demonstrated using an isozyme marker, phosphoglucoisomerase (PGI/2), that it was possible to obtain recombinants in *L. multiflorum* from each of the three subgenomes which make up *F. arundinacea*. However, recombinants between the PGI/2-labelled chromosome of one subgenome of *F. arundinacea* (found to be *F. pratensis* (Humphreys, 1995)) and its homoeologous *L. multiflorum* partner were obtained at twice the frequency of those involving *Lolium* and the other two *Festuca* genomes.

The higher frequency of recombination between *L. multiflorum* and the *F. pratensis* genome of *F. arundinacea* compared with that obtained from the *F. glaucescens* genomes, makes it more likely that the drought resistance transferred from the hexaploid fescue species was derived from the *F. pratensis* subgenome of *F. arundinacea*. The detection of the introgressed *Festuca* chromosome segments in the two drought-resistant plants, by *F. pratensis* labelled total genomic DNA probe, would appear to support this conclusion. Humphreys (1995) found GISH between DNA of *F. pratensis* and *F. glaucescens* to be very species-specific with little or no cross-hybridization between the DNA of the two *Festuca* species. It is unlikely that a total genomic DNA probe of *F. pratensis* would hybridize with the introgressed *F. arundinacea* chromosome segments if they were derived from either of the *F. glaucescens* subgenomes of the hexaploid *Festuca* species.

The genotypes of 86/3/24 and 103/10/29, with the exception of the recombinant *Festuca* chromosome segments present on chromosome 2, appear identical to that of *L. multiflorum*. Both introgression lines have drought resistance which is significantly greater (Humphreys & Thomas, 1993) than that found in cultivars Tribune and RvP which were used as recurrent *L. multiflorum* parents in the backcross breeding programme. We propose that *Festuca* genes present on chromosome 2 in the two introgression lines have led to the increased drought resistance of *L. multiflorum*. A test-cross between the introgression lines and *L. multiflorum* will be required to confirm whether our expectations are valid. The effect of presence or absence of the introgressed *Festuca* genes and their relationship with drought resistance in *L. multiflorum* will then be confirmed.

We further propose that the chromosome of *F. pratensis* in *F. arundinacea* which is homoeologous to chromosome 2 in *L. multiflorum* is likely to carry genes for drought resistance.

Most *Lolium/Festuca* PGI/2 recombinants recovered in the backcrossing programme between *L. multiflorum* and the pentaploid hybrid between *L. multiflorum* and *F. arundinacea* involved the *F. pratensis* genome (Humphreys & Ghesquière, 1994). However, interspecific recombination between *F. pratensis* and *L. multiflorum* at the *PGI/2* locus at around 10 per cent is only half that obtained when *L. multiflorum* is backcrossed onto the triploid hybrid between *L. multiflorum* (4x) and *F. pratensis* (2x) (Humphreys, unpublished data). If the genes for drought resistance found in the *F. pratensis* subgenome of *F. arundinacea* are conserved in natural populations of *F. pratensis* (2x), then it may well be that introgression of drought resistance genes from *Festuca* spp. into *Lolium* may be better

accomplished directly by hybridizing *L. multiflorum* with *F. pratensis* rather than with the polyploid fescue.

Although many genes on different *Festuca* chromosomes may determine complex physiological processes which convey characters such as drought or cold resistance, certain genes will presumably have greater influence. By the identification and selection of the major drought or cold resistance determining genes in *Festuca* spp., the major components of these systems may be transferred into *Lolium* without undue loss of desirable traits in the host germplasm.

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