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# REGENERATION OF SOMATIC HYBRIDS BETWEEN *FESTUCA ARUNDINACEA* VAR. *GLAUDESCENS* AND *LOLIUM MULTIFLORUM* LAM.

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## ABSTRACT

Protoplasts derived from ten suspension cultures of tetraploid *Festuca arundinacea* var. *glaucescens* ( $2n = 4x = 28$ ) and diploid *Lolium multiflorum* Lam. ( $2n = 2x = 14$ ) were fused to produce somatic hybrids. Regenerated green plants in three fusion combinations were identified as somatic hybrids because they showed characters that were peculiar to both *Festuca arundinacea* var. *glaucescens* (hairs on auricle) and *Lolium multiflorum* Lam. (root-fluorescence). Moreover, the hybrids also had a chromosome number of about forty two.

## KEYWORDS

*Festuca arundinacea* var. *glaucescens*, *Lolium multiflorum* Lam., somatic hybrids, root-fluorescence, symmetric fusion, tall fescue, Italian ryegrass

## INTRODUCTION

Intergeneric hybrids between *Festuca* spp. and *Lolium* spp. have been obtained by conventional hybridization (Thomas and Humphreys, 1991; Zwierzykowski, 1996) and by cell fusion (Takamizo et al., 1991; Spangenberg et al., 1994), providing a wide range of variation. Usually, hybrids between *Festuca arundinacea* var. *glaucescens* and *Lolium multiflorum* Lam. can be rescued by embryo culture (Jadas-Hecart et al., 1991; Cao et al., 1994). In order to produce  $F_2$  seeds by hybridization breeding, however, it would be desirable that crossing materials of *Lolium* spp. are tetraploid or doubling of chromosomes would be required in a situation where diploid of *Lolium* spp. are used as crossing material. In any case, because somatic hybrids are theoretically autopolyploid, treatment for doubling of chromosomes of somatic hybrids would not be necessary. In this paper we report the regeneration of somatic hybrids by symmetric fusion between tetraploid *Festuca arundinacea* var. *glaucescens* ( $2n = 4x = 28$ ) and diploid *Lolium multiflorum* Lam. ( $2n = 2x = 14$ ) using the select method of somatic hybrids for the fluorescence test.

## MATERIALS AND METHODS

For callus induction, immature embryos of tetraploid *Festuca arundinacea* var. *glaucescens* (FG) and shoot meristems with basal young leaves of diploid *Lolium multiflorum* Lam. cv. 'Nasuhikari' (NH) (gratefully received from the Laboratory of Pasture Plant Breeding, National Grassland Research Institute, Tochigi, Japan) were used as explants. Suspension/protoplast cultures and cell fusion were undertaken according to the methodology reported previously (Takamizo et al., 1990, 1991) with some modifications. Ten genotypes each of FG and NH were tested for symmetric fusion combinations and assessed after a few months for morphogenic response: green plant, albino shoot, callus and colony. Somatic hybrids were selected from the green plants using root-fluorescence after they had been cultured on filter paper placed over 1/2 MS hormone free medium for a week. Most of the selected plants were grown to maturity in the field and assessed for the presence of hairs on the auricle. Chromosome numbers on the root tips were also evaluated.

## RESULTS AND DISCUSSION

Of the ten fusion combinations regeneration occurred as follows: 1) fluorescent green plants from callus in three cases; 2) albino shoots in three cases; 3) callus in two cases; and 4) only a few colonies in two cases (Table 1).

In most cultivars of *Lolium multiflorum* Lam. root tracing of a majority of young plants shows fluorescence under ultra-violet light (Dales, 1953), and regeneration rate of green plants on protoplast culture tends to be low (Dalton, 1998). In *Festuca arundinacea* var. *glaucescens* which contains hairs on the auricle (Borrill, 1972), root tracing of a majority of young plants does not show fluorescence. Regenerated green plants in three fusion combinations: eleven plants in FG1 (+) NH18, two plants in FG2 (+) NH19 and thirty five plants in FG4 (+) NH21 (Table 1), were identified as somatic hybrids because they showed characters that were peculiar to both *Festuca arundinacea* var. *glaucescens* (hairs on auricle) and *Lolium multiflorum* Lam. (root-fluorescence). Moreover, the hybrids gave a total chromosome number of about forty two; indicative of the combination between *Festuca arundinacea* var. *glaucescens* ( $2n = 4x = 28$ ) and *Lolium multiflorum* Lam. ( $2n = 2x = 14$ ). Based on these observations, it is our conclusion that callus, suspension and protoplast cultures in *Festuca arundinacea* var. *glaucescens* could be established and somatic hybrids with diploid *Lolium multiflorum* Lam. regenerated.

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**Table 1**

Morphogenic response and number of somatic hybrids derived from ten fusion combinations between *Festuca arundinacea* var. *glaucescens* and *Lolium multiflorum* Lam.

Fusion combination <sup>z</sup>	Morphogenic response	Number of somatic hybrids
FG1 (+) NH18	fluorescent green plant	11
FG2 (+) NH19	fluorescent green plant	2
FG3 (+) NH20	albino shoot	0
FG4 (+) NH21	fluorescent green plant	35
FG5 (+) NH22	albino shoot	0
FG6 (+) NH23	colony <sup>y</sup>	0
FG7 (+) NH24	callus	0
FG8 (+) NH25	callus	0
FG9 (+) NH26	colony	0
FG10 (+) NH27	albino shoot	0

<sup>z</sup>FG was the suspension line of *Festuca arundinacea* var. *glaucescens*, and NH was the suspension line of *Lolium multiflorum* Lam. cv. 'Nasuhikari'.

<sup>y</sup>Only a few colonies were formed.