JARQ 28, 200-205 (1994)

Genetic Variation in Plant Regeneration from Callus Culture of Tall Fescue (*Festuca arundinacea* Schreb.) Cultivars

Tadashi TAKAMIZO, Nobuyuki FUKASE*, Yoshihiko SARUWATARI and Ken-ichi SUGINOBU*****

Department of Plant Breeding, National Grassland Research Institute
(Nishinasuno, Tochigi, 329-27 Japan)

Abstract

Calli were induced from immature embryos and mature seeds of tall fescue (*Festuca arundinacea* Schreb.) on Murashige and Skoog's medium supplemented with 2,4-D in order to analyze the genetic variation in morphogenic potential. There were significant differences among cultivars in terms of callus induction and plant regeneration from calli. Cultivars bred from Mediterranean ecotypes (Gloria, Maris Jebel and Maris Kasba) showed a low frequency of plant regeneration irrespective of the explants and low frequency of callus induction from immature embryos. Cultivars Manade, Forager and Yamanami showed a high frequency of plant regeneration from immature embryo-derived calli throughout the 3-year period. There was a significant correlation (r=0.815) between the frequency of callus induction and that of plant regeneration in immature embryo-derived calli, while no significant correlation (r=0.021) was observed between them in mature seed-derived calli.

Discipline: Biotechnology
Additional key words: immature embryo, 2,4-D

Introduction

In tall fescue (*Festuca arundinacea* Schreb.), which is an important temperate grass in pasture, plant regeneration from callus[4,6-8], suspension culture[10], and suspension culture-derived protoplasts[3,11] has been already reported. In those studies, however, only a limited number of cultivars were used and cultivar differences in terms of their morphogenic potential were not examined.

The objective of this study was to analyze the morphogenic differences of cultivars in tall fescue callus cultures derived from both immature embryos and mature seeds.

Materials and methods

Immature embryos dissected from open-pollinated spikelets about 2 weeks after anthesis and dehusked whole mature seeds were used

Present address:
* Yamagata Agricultural Extension Office (Yamagata, 990 Japan)
** Forage Crop Breeding and Seed Research Institute Inc. (Nishinasuno, Tochigi, 329-27 Japan)
*** Hokkaido National Agricultural Experiment Station (Hitsujigaoka, Sapporo, 062 Japan)

as explants for callus induction. Callus induction medium consisted of Murashige and Skoog's (MS) medium[9] supplemented with various levels (2 or 10 mg/*l* for immature embryo and 5 mg/*l* for mature seed) of 2,4-dichlorophenoxyacetic acid (2,4-D), 0.2 mg/*l* 6-benzylaminopurine, 100 mg/*l* casein hydrolysate, 3% sucrose, and 0.8% agar.

Ten immature embryos (1988, 1989 and 1991) derived from the same genotype grown in the field, or individual 10 mature seeds (1990) were placed on the callus induction medium in a 9 cm diameter petri dish and 5 dishes were used as replicates for each cultivar. All the dishes were incubated in the dark at 25°C for 1 to 2 months. Induced calli were then transferred to MS hormone-free medium and the number of calli developing shoot was scored. The frequencies of callus induction and plant regeneration were calculated by dividing the number of induced or regenerated calli by the number of total explants in a petri dish. Contaminated explants were omitted from the data. The data were analyzed with a complete randomized design.

Results

1) Callus cultures from immature embryos

Callus was readily induced from all the cultivars and the frequency of induction varied considerably (Table 1) among the cultivars tested. In 1989, Manade showed the highest frequency (88.6%), while Gloria showed the lowest (10.0%). In 1991, Forager showed the highest frequency (92.2%), while Demeter showed the lowest (5.0%). Manade, Forager and Nanryo showed a relatively high frequency of callus induction, while Gloria showed a very low frequency in both years, respectively. Demeter and Kentucky 31 showed a much lower frequency in 1991 than in 1989.

Varietal differences in the frequency of plant regeneration over the 3-year period are shown in Table 2. In 1988, Kentucky 31 showed the highest frequency (78.0%), while Maris Kasba showed the lowest (5.0%). In 1989, Manade showed the highest frequency, while Gloria showed the lowest (0%). In 1991, Forager showed the highest (60.1%) and Demeter showed the lowest (1.8%) frequency. Manade showed the highest frequency of plant regeneration (50.9%) and Gloria showed the lowest (8.2%) in the mean of 3 years.

Table 1. Cultivar differences in the frequency of callus induction in immature embryo-derived calli of tall fescue

Cultivar	1989	1991
Clarine	n.t.[1)]	38.3 efg[2)]
Demeter	75.0 ab	5.0 i
Forager	55.4 bc	92.2 a
Gloria	10.0 c	12.9 hi
Hokuryo	n.t.	61.0 cd
Jaguar	n.t.	39.6 efg
Kentucky 31	57.4 bc	16.3 hi
Lubrette	n.t.	37.8 eft
Ludion	n.t.	41.1 def
Luther	n.t.	53.9 cde
Manade	88.6 a	66.9 bc
Maris Jebel	n.t.	19.4 ghi
Maris Kasba	n.t.	38.7 efg
Nanryo	73.6 ab	68.9 bc
Southern Cross	39.3 c	32.0 efgh
Yamanami	43.6 c	86.7 ab
Means	55.3	44.4

1): Not tested.
2): Values with the same letter are not significantly different at 1% level.

2) Callus cultures from mature seeds

Clarine showed the highest frequency of callus induction (Table 3) while Demeter showed the lowest, Kentucky 31 showed the highest frequency of plant regeneration, while Gloria, Maris Jebel and Maris Kasba showed a low frequency or absence of plant regeneration, as was the case in the immature embryo-derived calli. Manade showed a low frequency of plant regeneration (5.4%) in contrast to the results obtained in immature embryo-derived calli.

Table 2. Cultivar differences in the frequency of plant regeneration from immature embryo-derived calli of tall fescue

Cultivar	1988	1989	1991	Means
Clarine	58.9 a[1)]	n.t.[2)]	21.7 bcde	40.3 abcd
Demeter	69.2 a	14.8 bc	1.8 f	30.7 bcd
Forager	66.2 a	14.4 bc	60.1 a	48.2 a
Gloria	n.t.	0.0 c	8.8 ef	8.2 f
Hokuryo	22.1 b	n.t.	37.0 b	28.6 bc
Jaguar	52.5 a	n.t.	20.1 cde	36.9 abcd
Kentucky 31	78.0 a	25.8 ab	7.9 ef	37.1 abcd
Lubrette	53.1 a	n.t.	26.1 bcd	39.6 abcd
Ludion	55.6 a	n.t.	14.0 def	34.8 abcd
Luther	64.6 a	n.t.	31.8 bc	44.0 abc
Manade	64.8 a	45.3 a	36.8 b	50.9 a
Maris Jebel	15.0 b	n.t.	11.2 def	13.1 def
Maris Kasba	5.0 b	n.t.	16.0 cdef	11.6 ef
Nanryo	70.8 a	18.6 bc	27.1 bcd	43.0 abc
Southern Cross	55.7 a	29.1 ab	13.6 def	34.7 abcd
Yamanami	69.1 a	27.1 ab	39.4 b	46.3 ab
Means	59.0	21.9	23.3	37.3

1): Values with the same letter are not significantly different at 1% level.
2): Not tested.

Table 3. Cultivar differences in the frequency of callus induction and plant regeneration from mature seed-derived calli of tall fescue (1990)

Cultivar	Frequency of callus induction (%)	Frequency of plant regeneration (%)
Clarine	94.4[1)]	11.3 bcde[2)]
Demeter	24.2	3.7 def
Fawn	51.3	12.2 bcd
Gloria	50.0	1.7 ef
Hokuryo	68.6	13.3 bcd
Kentucky 31	60.8	23.7 a
Manade	60.3	5.4 cdef
Maris Jebel	67.7	0.0 f
Maris Kasba	86.9	0.0 f
Nanryo	59.3	18.9 ab
Southern Cross	44.4	0.0 f
Yamanami	47.3	20.6 ab
Means	60.1	8.4

1): Statistical analysis was not carried out.
2): Values with the same letter are not significantly different at 1% level.

Two types of calli were observed for their appearance, e.g. watery, non-embryogenic (Plate 1a) and compact, embryogenic (Plate 1b) types. The latter type displayed a large number of shoot-like structures still in the callus induction medium and shoot elongation occurred

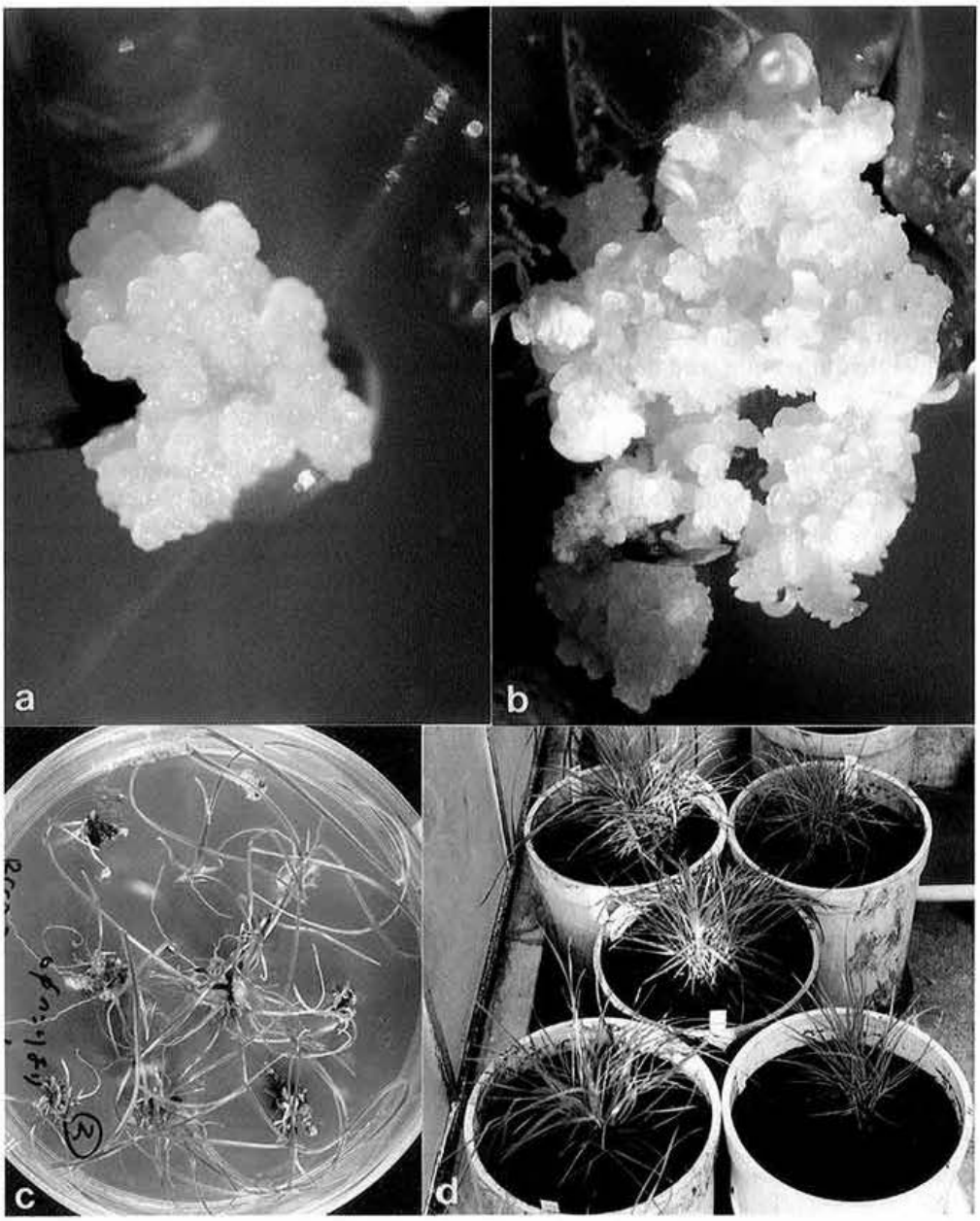

Plate 1. Plant regeneration from mature seed-derived callus culture of tall fescue
a: Non-embryogenic callus,
b: Embryogenic callus,
c: Plant regeneration from embryogenic callus,
d: Regenerated plants in pots.

rapidly (Plate 1c) upon the transfer to the regeneration medium. However, this classification of calli was not rigid and there was often an intermediate or mixed type. Regenerated plantlets could be easily transferred to pots (Plate 1d).

3) Correlation between the frequency of callus induction and plant regeneration

Correlation between the frequency of callus induction and plant regeneration in immature embryo-derived calli was significant ($r = 0.815$) (Fig. 1), while that in mature seed-derived calli was not significant ($r = 0.021$) (Fig. 2).

4) Effect of 2,4-D level

Effect of 2,4-D level on the frequency of callus induction was not significant in 1989, but a higher frequency of callus induction was observed at 2 mg/*l* than 10 mg/*l* in 1991 (data not shown). Effect of 2,4-D on plant regeneration varied with the experimental year. There was no interaction between cultivar and 2,4-D in both the frequency of callus induction and plant regeneration.

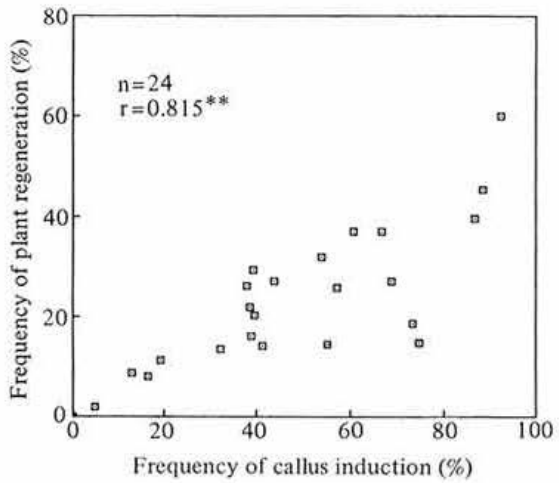

Fig. 1. Correlation between the frequency of callus induction and plant regeneration in immature embryo-derived calli of tall fescue
Each spot represents the mean of 1989 and 1991.

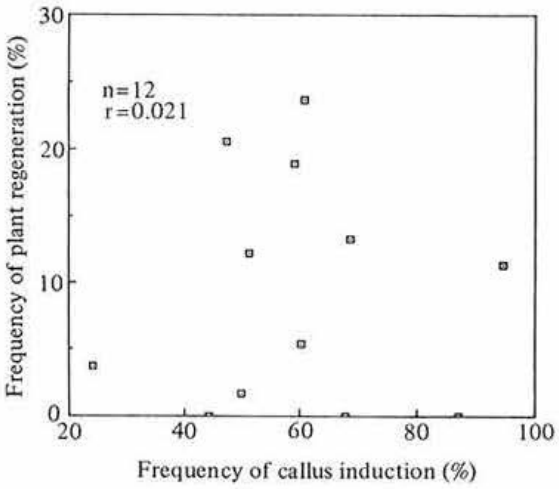

Fig. 2. Correlation between the frequency of callus induction and plant regeneration in mature seed-derived calli of tall fescue (1990)

Discussion

This study clearly showed that cultivars differed in the frequency of callus induction and plant regeneration from calli in tall fescue, and that considerable fluctuations were observed depending on the experimental year in some cultivars. Cultivars like Maris Kasba, Maris Jebel and Gloria showed a low frequency of callus induction and plant regeneration throughout the study. These cultivars originated from Mediterranean ecotypes which are genetically distant from European cultivars and show meiotic instability when crossed with them[5]. Similarly, it has been reported that in indica rice cultivars plant regeneration from root-derived calli is more difficult than in japonica rice cultivars[1].

As the immature embryos used in this study were derived from an open-pollinated population, the cultivar differences may have been masked by unknown pollen donor. Nevertheless, cultivars like Forager, Yamanami and Nanryo showed a relatively high frequency of regeneration irrespective of the year and

explants. These cultivars thus seem to have a stable morphogenic potential.

Effect of the level of 2,4-D on callus induction and plant regeneration was not clear. Creemers-Molenaar et al.[2] (1988) examined the effect of the 2,4-D level on the formation of embryogenic callus in perennial ryegrass and did not detect any difference between 2.5 mg/*l* and 15 mg/*l*.

The mean frequency of plant regeneration from immature embryo-derived calli (37.3%) was higher than that from mature seed-derived calli (8.4%). In fact, a larger number of non-morphogenic watery calli (Plate 1a) were produced from mature seeds than from immature embryos (data not shown). The infrequent development of shoots from watery calli may partly account for the fact that the correlation between the frequency of callus induction and plant regeneration from immature embryo-derived calli was highly significant while that from mature seed-derived calli was not significant (Figs. 1, 2). However, when mature seeds are used as explants, the effect of seasonal variations can be neglected. The lower frequency of induction of regenerable calli can be compensated by the increase in the number of seeds plated. The embryogenic calli derived from mature seeds in this study could be used as a source for suspension culture in which fertile plants were regenerated from protoplasts (data not shown). They could be further used for somatic hybridization[12] and genetic transformation[13] in tall fescue.

References

1) Abe, T. & Futsuhara, Y. (1984): Varietal difference of plant regeneration from root callus tissues in rice. *Jpn. J. Breed.*, **34**, 147–155.
2) Creemers-Molenaar, J. et al. (1988): The effect of 2,4-dichlorophenoxyacetic acid and donor plant environment on plant regeneration from immature inflorescence-derived callus of *Lolium perenne* L. and *Lolium multiflorum* L. *Plant Sci.*, **57**, 165–172.
3) Dalton, S.J. (1988): Plant regeneration from cell suspension protoplast of *Festuca arundinacea* Schreb. (tall fescue), *Lolium perenne* L. (perennial ryegrass). *J. Plant Physiol.*, **132**, 170–175.
4) Eizenga, G.C. & Dahleen, L. S. (1990): Callus production, regeneration and evaluation of plants from cultured inflorescence of tall fescue (*Festuca arundinacea* Schreb.). *Plant Cell Tissue Organ Cult.*, **22**, 7–15.
5) Evans, G. M., Asay, K., H. & Jenkins, R. G. (1973): Meiotic irregularities in hybrids between diverse genotypes of tall fescue (*Festuca arundinacea* Schreb.). *Crop Sci.*, **13**, 376–379.
6) Kasperbauer, M. J., Buckner, R. C. & Springer, W. D. (1980): Haploid plants by anther-panicle culture of tall fescue. *Crop Sci.*, **20**, 103–106.
7) Kasperbauer, M. J. & Eizenga, G. C. (1985): Tall fescue doubled haploids via tissue culture and plant regeneration. *Crop Sci.*, **25**, 1091–1095.
8) Lowe, K. W. & Conger, B. V. (1979): Root and shoot formation from callus culture of tall fescue. *Crop Sci.*, **19**, 397–400.
9) Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, **15**, 473–497.
10) Rajoelina, S. R., Albert, G. & Planchon, C. (1990): Continuous plant regeneration from established embryogenic cell suspension cultures of Italian ryegrass and tall fescue. *Plant Breed.*, **104**, 265–271.
11) Takamizo, T., Suginobu, K. & Ohsugi, R. (1990): Plant regeneration from suspension culture derived protoplasts of tall fescue (*Festuca arundinacea* Schreb.) of a single genotype. *Plant Sci.*, **72**, 125–131.
12) Takamizo, T. et al. (1991): Intergeneric hybridization in Gramineae: somatic hybrid plants between tall fescue (*Festuca arundinacea* Schreb.) and Italian ryegrass (*Lolium multiflorum* Lam.). *Mol. Gen. Genet.*, **231**, 1–6.
13) Wang, Z.-Y. et al. (1992): Transgenic plants of tall fescue (*Festuca arundinacea* Schreb.) obtained by direct gene transfer to protoplasts. *Bio/Technology*, **10**, 691–696.

(Received for publication, Feb. 24, 1994)