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Full Length Research Paper

Comparison of callus induction and plant regenerationfrom twenty tall fescue varieties

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Tall fescue (*Festuca arundinacea* Schreb.) is an important grass species both as turf and forage. In this study, 20 varieties of tall fescue were used to investigate the effects of genetic variations in tissue culture response. Plant genotype had a significant effect both on callus formation and plant regeneration in tall fescue. The forage type tall fescue varieties readily formed callus than turf type tall fescue varieties. Of all the forage varieties studied, Jesup, Greenmaster, Kentucky-31 and Fawn not only formed callus at a higher frequency but also had a high regeneration when compared with other varieties. These results can be used not only to provide additional improvements in the plant regeneration frequency from transgenic callus, but also useful for molecular breeding of tall fescue through genetic transformation.

Key words: Tall fescue, callus, plant regeneration, genotype.

INTRODUCTION

Tall fescue (*Festuca arundinacea* Schreb.) is one of the perennial grass species belonging to graminaceous family which is frequently developed and used as lawns for soil preservation in parks and golf courses (Buckner et al., 1979) and in forming grazing grasslands, etc (Cho et al., 1999) because of its excellent adaptability in diverse soil types. However, tall fescue has the disadvantages of rough leaves and low palatability by livestock. Furthermore, due to abnormal atmospheric temperature and climate warming, problems of poor growth and low permanence are occurring in tall fescue which is a cold-temperate grass that grows well in cool climate (Fieser and Vanzant, 2004). To address these issues, studies on tall fescue breeding are actively conducted with emphasis on digestion rate enhancement and environmental disease resistance (Choi et al., 2010).

However, breeding new variety by conventional means is laborious and time consuming. Further, all agronomic traits cannot be achieved in a single breeding (Van Wijk et al., 1993). To complement these problems, recently, studies on the development of new tall fescue varieties using molecular breeding (Dong and Qu, 2005; Chen et al., 2003; Bettany et al., 2003) have been actively conducted. Plant *in vitro* culture shows great variances by grasses of all species to tissue culture conditions such as explant tissues, carbon sources, the types and concentrations of plant growth regulators, additives added to culture media and culture environments (Somer et al., 2003; Zhang and Rouf Mian, 2003). In particular, callus induction and plant regeneration are greatly affected by different genotypes being cultured (Lee et al., 2004).Consequently, this study was intended to culture calluses induced from mature seeds of 20 tall fescue varieties held by Grassland and Forages Division, National Institute of Animal Science, Rural Development Administration (RDA) as genetic resources and examine plant regeneration efficiency of the calluses in order to utilize the results as basic data for developing new tall fescue varieties through transformations of useful genes.

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MATERIALS AND METHODS

Mature seeds of 13 forage-type tall fescue varieties (Kentucky-31,

Fawn, Au Triumph, Enforcer, Hokuryo, Martin, Penngrazer, Phyter, Southern Cross, Stef, Yamanami, Greenmaster and Jesup) and 7 turf-type tall fescue varieties (Amigo, Montauk, Mustang, Pixie, OFI-B1, Quest and Tar Heel) retained by Grassland and Forages Division, National Institute of Animal Science, RDA as genetic resources were used. Seed coats were removed from mature seeds while stirring for 20 min in a 50% H_2SO_4 solution. Then, the mature seeds were washed at least 10 times with sterile water and sterilized in accordance with the method of Lee et al. (2007). After removing the seed coats of the mature seeds, the seeds were surface sterilized, washed three times with sterile water and then again surface sterilized by adding a 5% (v/v) sodium hypochlorite solution and stirring for 30 min. The sterilized seeds were washed with sterile water at least three times and moved to sterilized filter paper to completely remove moisture before they were placed on callus induction medium.

Embryogenic callus induction

To induce calluses from the total 250 mature seeds, the callus induction medium (Lee et al., 2007) was made by adding 3 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid), 0.1 mg/L BA (6-benzyladenine), 1 g/L casein hydrolysate, 1 mg/L thiamin-HCl, 100 mg/L myo-inositol, 500 mg/L L-proline, 30 g/L sucrose and 3 g/L gelrite to the MS (Murashige and Skoog, 1962) basal medium and the pH was adjusted to 5.7 before autoclaving. The sterilized seeds were placed directly on the culture medium, and cultured for four weeks in a growth room at 24 ± 2°C under continuous dark. Callus forming rates by variety were indicated by the ratios of the numbers of induced calluses to the numbers of seeds sown in percentages, and the fresh-weight of the calluses and the forms of the induced calluses were examined.

Plant regeneration

The plant regeneration culture medium (Lee et al., 2007) was used to regenerate plants from the mature seed-derived calluses. It was modified by adding 1 mg/L 2,4-D, 3 mg/L BA, 1 g/L casein hydrolysate, 1 mg/L thiamin-HCl, 100 mg/L myo-inositol, 500 mg/L L-proline, 30 g/L sucrose and 3 g/L gelrite to the N6 basic culture medium (Chu et al., 1975). In order to examine plant regeneration efficiency by variety, in accordance with the method of Lee et al. (2004), 4 weeks old embryogenic calluses were transfer to the new regeneration culture medium and cultured for three weeks under a condition of 24 ± 2°C, 16 h light/8 h dark.

Then, the calluses were subcultured on a new culture medium having the same composition and maintained on it for six weeks for shoot induction. Shoots reaching at least 2 cm were regarded as individuals. From this, regeneration rates were indicated by the ratio of the numbers of calluses forming shoots to the numbers of transplanted calluses.

RESULTS AND DISCUSSION

Callus induction efficiency

In order to examine the differences in callus induction efficiency among the 20 tall fescue varieties, 13 forage-type varieties and 7 turf-type varieties were used to compare embryogenic callus induction rates (Table 1). Callus began to form in all the varieties at different frequencies after four days.

Among the seeds cultured for four weeks, the forage-type seeds generally showed higher callus induction rates. Of the 13 forage-type varieties, those that showed high callus induction efficiency of at least 50% were Jesup, Greenmaster, Kentucky-31 and Fawn variety with efficiency values of 69.6, 62, 55.6 and 50%, respectively. On the other hand, Au Triumph and Martin varieties showed low frequency of callus induction with 23.2 and 27.6%, respectively. In the case of the turf-types, no significant difference in callus induction efficiency was shown among the varieties even though with lower efficiency when compared with the forage-types.

This could have been due to the differences in genetic culture abilities possessed by individual varieties and similar differences in culture efficiency by variety have been reported in turf-type tall fescue variety culture (Bai and Qu, 2000) and orchard grass culture (Lee et al., 2004). Different genotypes have different levels of endogenous phytohormones, which could be responsible for their variable response to the exogenous hormones present in culture medium.

Callus characteristics

The types and tissue forms of calluses induced from mature seeds greatly affect plant regeneration efficiency (Lee et al., 2004). The characteristics of calluses induced from the 20 tall fescue varieties were examined individually and the results are summarized in Table 2.

Although, the fresh-weight of calluses showed similar trends in most of the varieties, the tissue density of induced calluses (Figure 1) showed considerable differences among the varieties (Table 2). Varieties with higher callus induction rates formed more calluses that denser tissues and excellent regeneration abilities.

Plant regeneration efficiency

The calluses induced from the mature seeds were cultured for four weeks and moved to a regeneration medium for six weeks under 24 ± 2°C and 16/8 h photoperiod. Then, the regeneration frequency for each variety was recorded (Table 3). High plant regeneration rates exceeding 50% were shown by four forage-type varieties including Greenmaster, Kentucky-31, Jesup and Fawn with 53, 52, 51 and 50.5% respectively in order of precedence. Most of the turf-type varieties had regeneration efficiency of less than 25%. Further investigation on their culture efficiency should be systematically examined in relation to plant growth regulators and other additives added to culture media during *in vitro* culture.

As with callus culture, the tall fescue varieties showed considerable differences in plant regeneration efficiency among them. Regenerated plants were further cultured in a rooting medium composed of 1/2 MS to develop into complete plants. Varieties with generally high callus induction rates showed excellent regeneration efficiency,

Table 1. Response of different tall fescue varieties to callus induction. Dehusked mature seeds were placed on the callus induction medium and cultured for 4 weeks.

Variety	Type	Number of explant	Total number of explants forming callus	Callus induction frequency (%)
Kentucky-31	Forage	250	139	55.6
Fawn	Forage	250	125	50
Au Triumph	Forage	250	58	23.2
Enforcer	Forage	250	77	30.8
Hokuryo	Forage	250	98	39.2
Martin	Forage	250	69	27.6
Penngrazer	Forage	250	87	34.8
Phyter	Forage	250	75	30
Southern Cross	Forage	250	83	33.2
Stef	Forage	250	92	36.8
Yamanami	Forage	250	85	34
Greenmaster	Forage	250	155	62
Jesup	Forage	250	174	69.6
Amigo	Turf	250	52	20.8
Montauk	Turf	250	48	19.2
Mustang	Turf	250	67	26.8
Pixie	Turf	250	55	22
OFI-B1	Turf	250	49	19.6
Quest	Turf	250	65	26
Tar Heel	Turf	250	63	25.2

Table 2. Callus morphology of different tall fescue variety on callus induction medium.

Variety	Type	Callus fresh weight (mg)	Quality	Type
Kentucky-31	Forage	68.7 ± 4.2	+++++	Compact
Fawn	Forage	67.0 ± 2.0	+++++	Compact
Au Triumph	Forage	74.0 ± 6.1	+++	Soft
Enforcer	Forage	71.3 ± 5.5	+++	Compact
Hokuryo	Forage	71.7 ± 3.8	++++	Compact
Martin	Forage	65.3 ± 7.2	+++	Compact
Penngrazer	Forage	64.7 ± 7.0	+++	Compact
Phyter	Forage	64.0 ± 5.6	+++	Friable
Southern Cross	Forage	70.0 ± 5.0	+++	Compact
Stef	Forage	68.3 ± 3.5	+++	Compact
Yamanami	Forage	69.0 ± 1.7	+++	Friable
Greenmaster	Forage	70.3 ± 1.5	+++++	Compact
Jesup	Forage	70.3 ± 4.7	+++++	Compact
Amigo	Turf	68.7 ± 3.5	+++	Compact
Montauk	Turf	56.0 ± 2.6	++++	Compact

Table 2.Contd

Mustang	Turf	58.0 ± 4.6	+++	Friable
Pixie	Turf	63.7 ± 4.0	++++	Compact
OFI-B1	Turf	63.0 ± 5.0	+++	Friable
Quest	Turf	61.0 ± 6.6	++++	Compact
Tar Heel	Turf	61.7 ± 3.5	+++	Friable

Quality: + = poor, +++ = average, +++++ = best.

Figure 1. Different types of callus of tall fescue. A, Embryogenic callus (+++++); B and C represents the non-embryogenic type (+++) of callus of Greenmaster variety, respectively. + = Poor, +++ = Average, +++++ = Best.

Table 3. Effect of varieties on plant regeneration from mature seed-derived callus culture of tall fescue. The calli were transferred to the plant regeneration medium and cultured for 6 weeks.

Variety	Type	Number of calli transferred[a]	Plant regeneration (%)
Kentucky-31	Forage	200	52
Fawn	Forage	200	50.5
Au Triumph	Forage	200	18
Enforcer	Forage	200	26
Hokuryo	Forage	200	42
Martin	Forage	200	20
Penngrazer	Forage	200	21
Phyter	Forage	200	16
Southern Cross	Forage	200	39
Stef	Forage	200	34
Yamanami	Forage	200	25.5
Greenmaster	Forage	200	53
Jesup	Forage	200	51
Amigo	Turf	200	21
Montauk	Turf	200	10
Mustang	Turf	200	19
Pixie	Turf	200	21
OFI-B1	Turf	200	24
Quest	Turf	200	25
Tar Heel	Turf	200	23.5

therefore, it could be said that Greenmaster, Kentucky-31, Jesup and Fawn are suitable for developing new tall fescue varieties by genetic transformation.

Differences in culture efficiency among varieties in forage-type grasses' mature seeds were also reported in perennial rye grass culture (Wang et al., 1993), Italian rye grass culture (Rim et al., 2000) etc. It is assumed that these differences among varieties are mostly attributable to differences between the genotypes of mother plants (Vasil and Vasil, 1984).

In order to establish optimum tissue culture conditions of varieties using 13 forage-type tall fescue varieties and 7 turf-type tall fescue varieties, the rates of embryogenic callus induction from mature seeds and plant regeneration efficiency from calluses were separately examined. The highest callus induction rate from the seeds cultured for four weeks was shown by Jesup followed by Greenmaster, Kentucky-31 and Fawn in order of precedence and high plant regeneration frequencies exceeding 50% were shown by Greenmaster, Kentucky-31, Jesup and Fawn in order of precedence. In general, varieties with high frequency of callus induction had high plant regeneration rates. The tall fescue varieties that have excellent callus induction efficiency and plant regeneration rates may be usefully in developing new tall fescue varieties through direct introduction of useful genes.

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REFERENCES

Bai Y, Qu R (2000). An evaluation on callus induction and plant regeneration of 25 turf-type tall fescue (*Festuca arundinacea* Schreb.) cultivars. Grass Forage Sci. 55: 326-330.

Bettany AJE, Dalton SJ, Timms E, Manderyck B, Dhanoa MS, Morris P (2003). *Agrobacterium tumefaciens*-mediated transformation of *Festuca arundinacea* (Schreb.) and *Lolium multiflorum* (Lam.). Plant Cell Rep. 21: 437-444.

Buckner RC, Powell JB, Frakes RV (1979). Historical development. In: Buckner RC, Bush LP (eds) Tall Fescue. Agronomy, 20: 1-8.

Choi GJ, Lim YC, Ji HC, Kim KY, Park HS, Seo S, Moon CS, Kim DH, Lee SH (2010). A stress-tolerant and high-yielding tall fescue new variety, 'Greenmaster'. J. Kor. Grassl. Forage Sci. 30(3): 199-204.

Cho MJ, Ha CD, Lemaux PG (2000). Production of transgenic tall fescue and red fescue plants by particle bombardment of highly regenerated tissues. Plant Cell Rep. 19: 1084-1089.

Chen L, Auh CK, Dowling P, Bell J, Chen F, Hopkins A, Dixon RA, Wang ZY (2003). Improved forage digestibility of tall fescue (*Festuca arundinacea*) by transgenic down-regulation of cinnamyl alcohol dehydrogenase. Plant Biotechnol. J. 1: 437-449.

Chu CC, Wang CS, Sun CC, Hsu C, Yin KC, Chu CY, Bi FY (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Scienta Sinica. 18: 659-666.

Dong S, Qu R (2005). High efficiency transformation of tall fescue with *Agrobacterium tumefaciens*. Plant Sci. 168: 1453-1458.

Fieser BG, Vanzant ES (2004). Interactions between supplement energy source and tall fescue hay maturity on forage utilization by beef steers. J. Anim. Sci. 82: 307-318.

Lee S-H, Lee D-G, Lee B-H (2004). Effects of medium supplements on seed-derived callus culture and regeneration of orchardgrass. J. Kor. Crop Sci. 49: 232-236.

Lee S-H, Ahsan N, Lee K-W, Kim D-H, Lee D-G, Kwak S-S, Kwon S-Y, Kim TH, Lee B-H (2007). Simultaneous overexpression of both CuZn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. J. Plant Physiol. 164: 1626-1638.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15: 473-497.

Rim YW, Kim KY, Choi GJ, Sung BR, Shin JS (2000). Callus induction from seeds of Italian ryegrass and plant regeneration. J. Kor. Grassland Sci. 20: 25-30.

Van Wijk AJP, Boonman JG, Rumball W (1993). Achievements and prospectives in the breeding of forage grasses and legumes. In: Baker MJ (eds), Grasslands for our world, SIR, Wellington, pp. 116-120.

Vasil V, Vasil IK (1984). Induction and maintanance of embryogenic callus cultures of Gramineae. In: Vasil IK(eds), Cell culture and somatic cell genetics of plants, Vol 1, Academic Press, Orlando, pp. 36-42.

Wang ZY, Nagel J, Potrykus I, Spangenberg G (1993). Plants from cell suspension-derived protoplasts in *Lolium* species. Plant Sci. 94: 179-193.

Somers DA, Samac DA, Olhoft PM (2003). Recent advances in legume transformation. Plant Physiol. 131: 892-899.

Zhang Y, Rouf Mian MA (2003). Functional genomics in forage and turf-present status and future prospects. Afr. J. Biotech. 12: 521-527.