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Field performance of transgenic tall fescue (*Festuca arundinacea* Schreb.) plants and their progenies

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Abstract Tall fescue (*Festuca arundinacea* Schreb.) is a hexaploid, outcrossing grass species widely used for forage and turf purposes. Transgenic tall fescue plants were generated by biolistic transformation of embryogenic cell suspension cultures that were derived from single genotypes of widely used cultivar Kentucky-31. Primary transgenics from two genotypes, their corresponding regenerants from the same genotypes and control seed-derived plants were transferred to the field and evaluated for 2 years. Progenies of these three classes of plants were obtained and evaluated together with seed-derived plants in a second field experiment. The agronomic characteristics evaluated were: heading date, anthesis date, height, growth habit, number of reproductive tillers, seed yield and biomass. The agronomic performance of the primary transgenics and regenerants was generally inferior to that of the seed-derived plants, with primary transgenics having fewer tillers and a lower seed yield. However, no major differences between the progenies of transgenics and the progenies of seed-derived plants were found for the agronomic traits evaluated. Primary transgenics and regenerants from the same genotype were more uniform than plants from seeds. Progenies of transgenics performed similarly to progenies of the regenerants. The addition of a selectable marker gene in the plant genome seems to have had little effect on the agronomic performance of the regenerated plants. No indication of weediness of the transgenic tall fescue plants was observed. Our results indicate that outcrossing grass plants generated through transgenic approaches can be incorporated into forage breeding programs.

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Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is worldwide the most important forage species of the *Festuca* genus (Sleper and West 1996). It is widely grown in southern Europe and is the predominant cool season perennial grass species in the USA, commonly used in pastures, lawns, sports fields, highway medians and roadsides (Barnes 1990). Tall fescue is a polyploid ($2n = 6x = 42$), wind-pollinated species with a high degree of self-incompatibility. This makes breeding management difficult and selection schemes complex, resulting in slow breeding progress, especially for traits with low heritability (Barnes 1990; Stadelmann et al. 1999).

Since forage production is generally a low-cash-input system, the most economical way to deliver advanced technology to farmers and ranchers is through the genetic improvement of cultivars (Wang et al. 2001a). Biotechnological approaches have the potential to complement or accelerate conventional breeding by extending the range of sources from which genetic information may be obtained, thus offering new opportunities for molecular breeding (Spangenberg et al. 1998; Wang et al. 2001a). There has been considerable interest in manipulating tall fescue by genetic transformation in the past decade. Transgenic tall fescue plants were generated by direct gene transfer to protoplasts (Ha et al. 1992; Wang et al. 1992; Dalton et al. 1995) and microprojectile bombardment (Spangenberg et al. 1995a; Kuai et al. 1999; Cho et al. 2000; Wang et al. 2001b). However, to assess the suitability of biotechnological approaches in grass improvement programs, the agronomic performance of transgenic plants and their progenies must first be investigated. Moreover, such experiments must be carried out in the field to test the plants under conditions relevant to plant breeding (Stadelmann et al. 1998b). To date, there

are no reports on agronomic evaluations of transgenic forage grass plants under field conditions.

Transgenic plants of tall fescue were obtained by the biolistic transformation of embryogenic suspension cells. Transgenic plants, cell suspension- and seed-derived tall fescue plants were subsequently transferred to the field. The aim of the study reported here was to comparatively evaluate the agronomic performance of transgenic and non-transgenic control plants as well as that of their half-sib families of this important outcrossing forage species.

Materials and methods

Plant regeneration from embryogenic suspension cultures

An efficient plant regeneration system was established based on the use of single genotype-derived embryogenic suspension cultures (Wang et al. 1994, 1995). Sterilized seeds/caryopses of the most widely cultivated tall fescue cultivar, Kentucky-31, were used as explants to induce callus. Embryogenic calli derived from single seeds/caryopses (representing individual genotypes) were individually transferred to liquid culture medium to establish single genotype-derived cell suspension cultures (Wang et al. 1994, 1995). Plants regenerated from two cell suspensions (i.e. two genotypes) were used in the experiment. The set of plants regenerated from cell suspension one was named CG1, and the set of plants from cell suspension two was named CG2.

Generation of transgenic tall fescue plants

Cell clusters from the same two suspension cultures used for plant regeneration were used as direct targets for biolistic transformation to generate transgenic plants (Wang et al. 2003). A chimeric hygromycin phosphotransferase (*hph*) gene, which renders transformed cells resistant to hygromycin, was used as the selectable marker gene (Bilang et al. 1991). A chimeric β-glucuronidase (*gusA*) gene was co-transformed with the *hph* gene (Wang et al. 2003). Hygromycin-resistant calli were obtained after microprojectile bombardment of suspension cells and subsequent selection in the presence of hygromycin (Wang et al. 2003). Transgenic tall fescue plants were regenerated from the hygromycin-resistant calli and later transferred to the greenhouse and the field. The set of primary transgenics obtained from cell suspension one was named TG1, and the set of plants from cell suspension two was named TG2.

Molecular characterization of transgenic plants

Total genomic DNA was isolated from freeze-dried leaf material from greenhouse-grown plants. Isolation of genomic DNA, gel electrophoresis and DNA blotting were carried out following standard protocols (Lichtenstein and Draper 1985; Sambrook et al. 1989). Hybridization probes (*hph*) were [^{32}P]-dCTP-labeled using the RadPrime DNA Labeling System (Invitrogen no. 18428-011, Carlsbad, Calif.), and the unincorporated nucleotides were removed by passing through the ProbeQuant G-50 micro-columns (Amersham Pharmacia no. 27-5335-01, Piscataway, N.J.). Southern hybridizations were performed using the QuikHyb hybridization solution (Stratagene no. 201221, La Jolla, Calif.) according to the manufacturer's specifications.

Field evaluation of primary transgenics, primary regenerants and seed-derived plants

The experimental area was located in Ardmore, Oklahoma, USA. The mean annual temperature and rainfall of the experimental site were 17.7 °C and 912 mm, respectively. In October 1999, two sets of primary transgenics (TG1 and TG2), each containing eight plants, two sets of cell suspension derived plants (CG1 and CG2), each containing eight plants and 16 seed-derived plants were transferred to the field under USDA regulations. The plant-to-plant and row-to-row spacing were 0.61 m and 0.61 m, respectively. The experimental design was a randomized complete block with three replications. The plants were replicated by vegetative propagation from tillers. The investigated plants were surrounded by border plants. Weeds around the plots were controlled by herbicide; weeds in the rows were removed by hand.

In spring 2000 and 2001, phenology, morphology and fertility of primary regenerants, primary transgenics and seed-derived plants were measured on individual plants. Heading date was recorded as the day of year (day) when the tips of three panicles were visible. Likewise, anthesis date was recorded when anthers were protruded from at least three panicles. Growth habit was visually scored on a 1–9 scale, with 1 being prostrate and 9 being erect. Plant height was measured on the first three stems on which anthers appeared. The number of reproductive tillers was determined at seed harvest. Open-pollinated seeds were harvested, dried at 50 °C for 48 h, threshed, sieved and cleaned. The residue from each plant was collected after seed harvest and dried at 50 °C for at least 72 h. The harvested residue was combined with debris from seed cleaning to represent biomass.

Field evaluation of the progenies of the primary transgenics, primary regenerants and seed-derived plants

Seeds harvested from individual mother plants in spring 2000 and basic seed of tall fescue cv. Kentucky-31 were sown in peat pots in the greenhouse at the end of August 2000. Due to the lack of seed set of most of the primary transgenic plants of genotype 2 (TG2), only progenies from primary transgenic plants of genotype 1 (TG1) and corresponding regenerants (CG1), as well as seed-derived plants, were included in the field test. The experiment included the following material: seven half-sib families from TG1 (HS-TG1), three half-sib families from CG1 (HS-CG1), three half-sib families from seed-derived plants (HS-SeedP), and a population from basic seed of Kentucky-31 (SeedP). A diagram illustrating the origin and terminology of different plants and half-sib families used in the experiment is shown in Fig. 1.

The established plants were transferred to the field at the end of October 2000. The experimental design was a randomized complete block with three replications, each containing 14 plots of ten plants. Rows were spaced 0.6 m apart with 0.3 m between plants within rows. Each plot consisted of one half-sib family or a population from the basic seed. Agronomic traits were measured in spring 2001, as described above for the primary transgenics and regenerants.

Containment of the transgenic material

In order to ensure the containment of the transgenic material, we followed a strict protocol during the entire experimental period to fulfill the performance standard for field trials under USDA notification. Special care was taken to make certain there was no inadvertent mixing or environmental release of the transgenic material. A well-contained vehicle was used to transport experimental material from the greenhouse to the field and from field to the laboratory. All field plants were recorded and monitored regularly. A strong electric fence was built around the experimental area to prevent animals from entering the test site. All plants were planted and harvested by hand. Equipment was only used for seed cleaning, and this was done in the laboratory. The equipment was

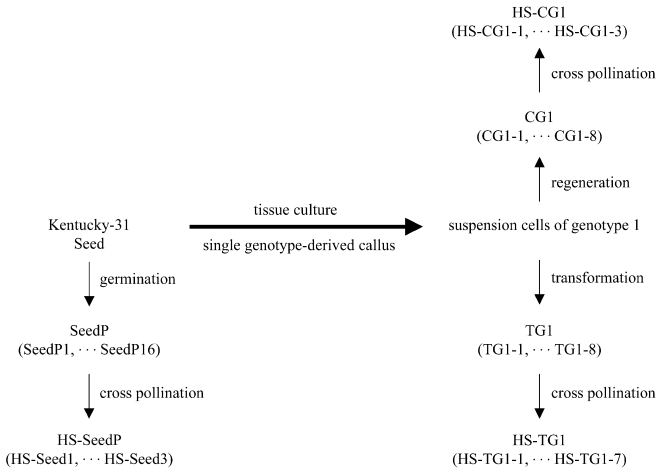

Fig. 1 Origin and terminology of different plants and half-sib families of genotype 1 used for the field test. *CG1* Plants regenerated from cell suspension cultures of genotype 1, *HS-CG1* half-sib families of CG1, *TG1* primary transgenic plants obtained from genotype 1, *HS-TG1* half-sib families of TG1, *SeedP* plants grown from Kentucky-31 seed stock, *HS-SeedP* half-sib families of SeedP

inspected before and after the cleaning process. Any non-seed residues from the seed cleaning process were collected and autoclaved. After termination of the experiment, the plants in the field were killed by application of a glyphosate herbicide. The experimental field has been continuously monitored for volunteer plants, which are killed immediately.

Statistical analyses

Statistical analyses were carried out using the SAS statistical package (SAS Institute, Cary, N.C.). Analysis of variance, using the mixed model and PROC MIXED procedure, was performed to compare the means of different groups. Replication was considered a random effect, whereas group was considered a fixed effect. Differences were declared significant when $P < 0.05$. PROC FACTOR and varimax rotation were used for factor analysis.

Results

Generation and field evaluation of primary transgenics and regenerants

Highly regenerable cell suspension cultures (Fig. 2A) were established from embryogenic callus of single-seed origin. Green plants (Fig. 2B) regenerated from two of the established single genotype-derived cell suspensions, named CG1 and CG2, were transferred to the greenhouse and the field.

The suspension cells were plated on filter paper and bombarded with DNA-coated gold particles. Hygromycin-resistant calli were obtained after microprojectile bombardment and subsequent selection in the presence of 250 mg l^{-1} hygromycin (Fig. 2C). Primary transgenic tall fescue plants were regenerated from the hygromycin-resistant calli (Fig. 2D). Southern hybridization analysis confirmed the transgenic nature of the regenerated plants (Fig. 3). Hybridization signals corresponding to high-molecular-weight bands and the full-length *hph* gene were observed in the Southern analysis using digested and undigested genomic DNA samples, respectively (Fig. 3). This indicates the integration of complete transgene copies in the plant genome of the transformed plants. In addition to single-copy integration of transgenes, different hybridization patterns, including additional *hph*-hybridizing bands, were also observed for some samples (Fig. 3), indicating that multiple insertions of rearranged or partial copies of the chimeric *hph* gene occurred. Two sets of primary transgenic plants, designated TG1 and TG2, were transferred to the greenhouse and the field (Fig. 2E, F).

Among the agronomic traits evaluated during 2000 and 2001, only height and seed yield had significant genotype × year effect, thus the mean values of these two traits are listed separately for 2000 and 2001 in Table 1. For heading date, anthesis date, growth habit, tiller number and biomass, combined values of the 2 years are given (Table 1).

When compared with seed-derived plants, the primary transgenics (TG1 and TG2) and regenerants (CG1 and CG2) had significantly reduced seed yield, fewer repro-

Table 1 Means[a] and comparisons of key agronomic traits of primary transgenics, primary regenerants and seed-derived plants of tall fescue cv. Kentucky-31 in 2000 and 2001 (*doy* day of year)

Traits	Year	Source of plants[b]:				
		TG1	TG2	CG1	CG2	SeedP
Heading date (doy)	2000–2001	119a[b]	119a	118a	120a	118a
Anthesis date (doy)	2000–2001	125a,b	127a	124b	127a	124b
Height (cm)	2000	87.7a,b	81.9a	94.6c,d	93.1b,c	99.1d
Height (cm)	2001	76.2a	73.1a	78.8a	73.8a	93.1b
Growth habit	2000–2001	6.3a	6.4a	6.7a	6.7a	6.8a
Tiller number $plant^{-1}$	2000–2001	13a	4b	14a	9a,b	25c
Seed yield $plant^{-1}$ (g)	2000	3.8a	0.5b	3.4a	1.7b	6.6c
Seed yield $plant^{-1}$ (g)	2001	3.4a	0.6b	3.4a	2.0b	15.8c
Biomass $plant^{-1}$ (g)	2000–2001	200.2a,b	116.5a	223.3a,b	171.9a,b	267.2b

[a] Means within rows followed by the same letter are not significantly different ($P = 0.05$)
[b] TG1, Primary transgenic plants obtained from genotype 1; TG2, primary transgenic plants obtained from genotype 2; CG1, plants regenerated from cell suspension cultures of genotype 1; CG2, plants regenerated from cell suspension cultures of genotype 2; SeedP, plants grown from Kentucky-31 seed stock

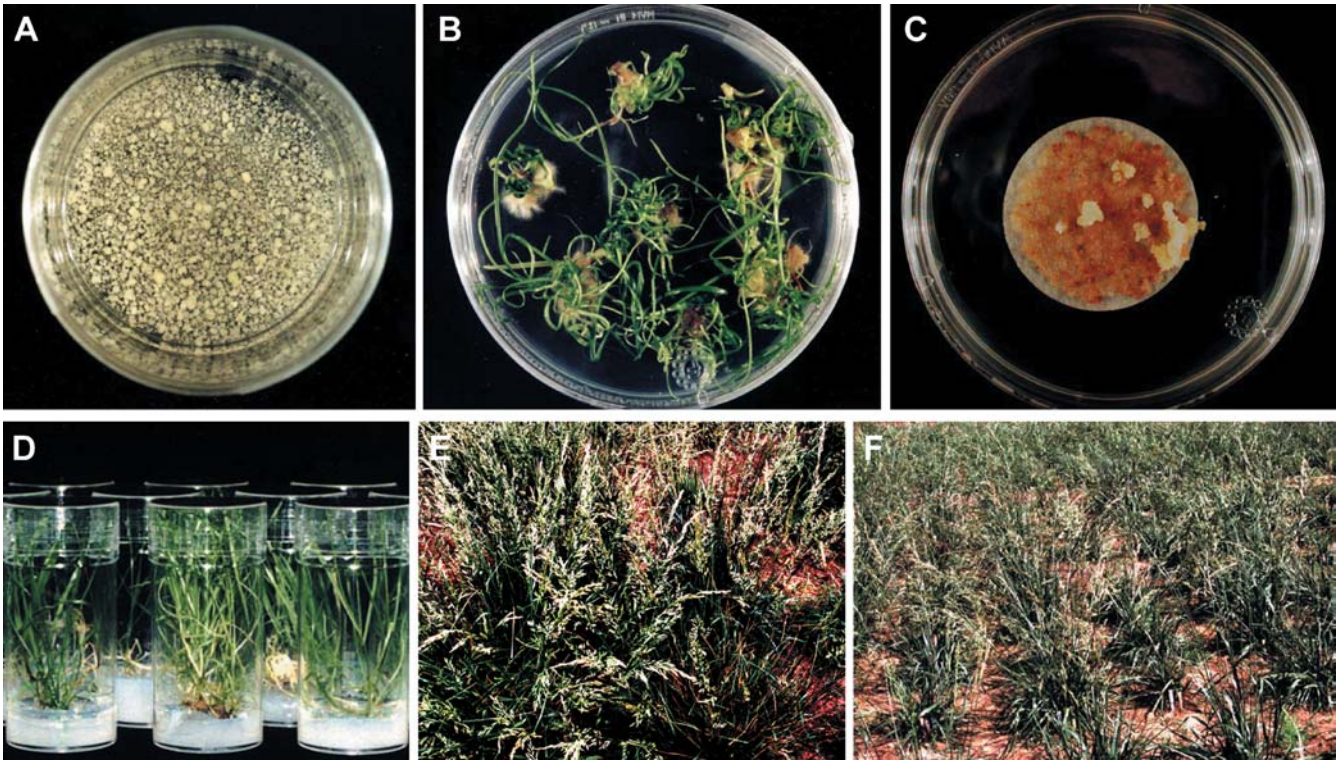

Fig. 2A–F Generation and field evaluation of transgenic tall fescue plants. **A** Single genotype-derived embryogenic cell suspension cultures established from embryogenic callus, **B** regeneration of green plantlets from plated embryogenic suspension cells, **C** Hygromycin-resistant calli obtained after microprojectile bombardment with *hph* gene and selection of bombarded cells on medium containing 250 mg l^{-1} hygromycin, **D** in vitro transgenic plants recovered from hygromycin-resistant calli, **E** primary transgenics, regenerants and seed-derived plants growing in the field, **F** progenies of transgenics, regenerants and seed-derived plants growing in the field

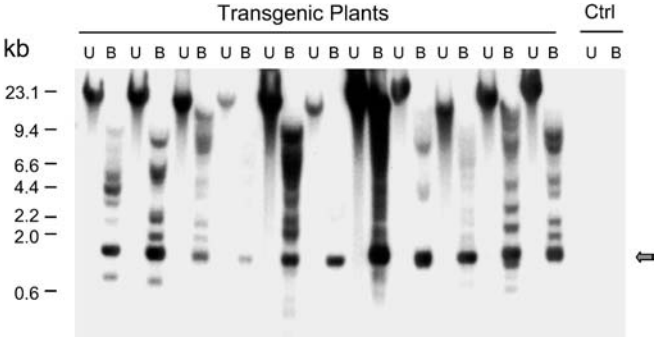

Fig. 3 Southern blot hybridization analysis of transgenic tall fescue plants using hygromycin phosphotransferase gene *hph* probe. *U* Undigested genomic DNA, *B* genomic DNA digested with *Bam*HI, which cut out the *hph* coding sequences in the plasmid used, *Ctrl* untransformed plants serving as control. *Arrow* indicates the expected size of the *hph* coding sequences after *Bam*HI digestion and Southern hybridization

ductive tillers and reduced height, with the exception that CG1 was similar in height to seed-derived plants in 2000. No difference was found among the sets of plants for heading date and growth habit (Table 1). Transgenics from genotype 1 (TG1) performed similarly to regenerants from the same genotype (CG1) for most of the traits evaluated, including anthesis date, tiller number, seed yield and biomass. Likewise, transgenics from genotype 2 (TG2) performed similarly to the corresponding regenerants (CG2) with regard to anthesis date, tiller number, seed yield and biomass (Table 1). Plants from genotype 1 (TG1 and CG1) had more tillers, greater seed yield and flowered earlier than plants from genotype 2 (TG2 and CG2) (Table 1). Plants from genotype 1 were more similar to seed-derived plants in anthesis date and biomass than to plants from genotype 2, indicating a possible 'background' effect – a genotype effect on agronomic performance independent of possible regeneration or transgene effects.

Factor analysis after varimax rotation revealed three common factors explaining 93.8% of the total variance based on the eigenvalue, indicating that most of the information had been retained after the analysis (Table 2). Factor 1, which consisted of the major productivity characteristics including tiller number, seed yield and biomass, accounted for the largest portion (42.4%) of the observed variation. Factor 2 represented the phenological traits, including heading date and anthesis date. Factor 3 involved two morphological traits, height and growth habit (Table 2). A scatter diagram of the tested plants based on the first and second vectors from the factor analysis, explaining 71.1% of the total variability, is

Table 2 Loadings of rotated factors for key agronomic traits of primary transgenics, primary regenerants and seed-derived plants of tall fescue cv. Kentucky-31 (*doy* day of year)

Traits	Commu-nality	Factor 1	Factor 2	Factor 3
Heading date (doy)	0.973	0.000	0.978	–0.103
Anthesis date (doy)	0.976	–0.119	0.969	–0.143
Height (cm)	0.999	0.492	–0.290	0.718
Growth habit	0.999	0.061	–0.109	0.990
Tiller number plant^{-1}	0.987	0.979	–0.116	0.105
Seed yield plant^{-1} (g)	0.991	0.983	–0.047	0.106
Biomass plant^{-1} (g)	0.997	0.887	0.016	0.171
Eigenvalue		2.970	2.006	1.580
Proportion (%)		42.4	28.7	22.6
Cumulative (%)		42.4	71.1	93.7

shown in Figure 4. A trend from negative to positive, corresponding to plants from genotype 2 (TG2 and CG2), genotype 1 (TG1 and CG1) and seed-derived plants was revealed by factor 1 (Fig. 4). This indicates that most of the plants from genotype 2 had fewer tillers, a lower seed yield and less biomass than plants from genotype 1, whereas plants from genotype 1 were inferior in these traits when compared with seed-derived plants. Most of the transgenics and regenerants from genotype 1 could be grouped together; similarly, most of the transgenics and regenerants from genotype 2 could also be grouped together (Fig. 4). With respect to factor 2, more transgenics and regenerants were located in the negative sector than were the seed-derived plants, with only a few plants from genotypes 1 and 2 having late heading and anthesis dates (Fig. 4).

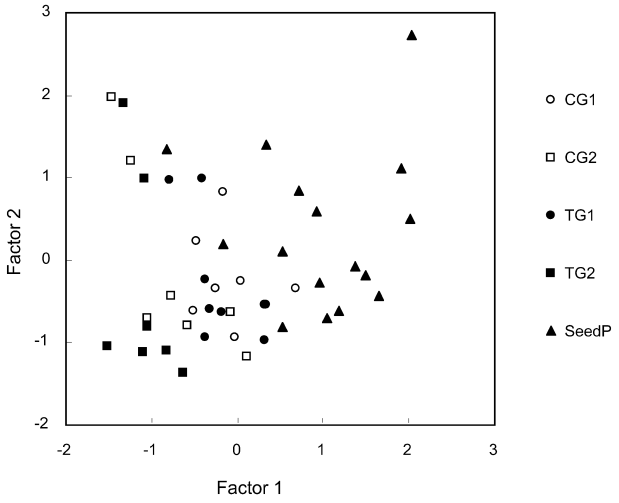

Fig. 4 Scatter diagram of primary transgenics, primary regenerants and seed-derived plants based on factor analysis. *TG1* Primary transgenic plants obtained from genotype 1, *TG2* primary transgenic plants obtained from genotype 2, *CG1* plants regenerated from cell suspension cultures of genotype 1, *CG2* plants regenerated from cell suspension cultures of genotype 2, *SeedP* plants grown from Kentucky-31 seed stock

Field evaluation of progenies of transgenics, regenerants and seed-derived plants

Half-sib families of transgenics (HS-TG1), regenerants (HS-CG1) and seed-derived plants (HS-SeedP) did not differ significantly for heading date, anthesis date, tiller number, seed yield or biomass (Table 3). When compared with plants from the original seed stock, the half-sib families had earlier heading and anthesis dates (Table 3). Half-sib families of transgenics had longer stems and a more erect growth habit than the other groups of plants (Table 3).

The varimax rotation factor analysis assigned the evaluated traits to three common factors, explaining 97.4% of the total variance (Table 4). The composition of the three factors was similar to that of the primary transgenics and regenerants. The main characteristics in factor 1 were tiller number, seed yield and biomass, accounting for 40.9% of total variability (Table 4). Factor 2 consisted of the phenological traits, including heading date and anthesis date, and factor 3 involved two morphological traits, height and growth habit (Table 4). A scatter diagram of the progenies based on the first and second vectors of the factor analysis revealed that no clear

Table 3 Means[a] and comparisons of key agronomic traits of progenies of transgenics, regenerants and seed-derived plants of tall fescue cv. Kentucky-31 in 2001 (*doy* day of year)

Traits	Source of plants[b]:			
	HS-TG1	HS-CG1	HS-SeedP	SeedP
Heading date (doy)	130a	130a	126a	138b
Anthesis date (doy)	136a	136a	133a	144b
Height (cm)	77.7a	73.0a,b	72.5b	73.9a,b
Growth habit	6.1a	5.9a,b	5.0b	4.9b
Tiller number plant^{-1}	23a	23a	27a	24a
Seed yield plant^{-1} (g)	5.2a	5.3a	7.1a	5.0a
Biomass plant^{-1} (g)	147.2a	149.9a	165.5a	157.3a

[a] Means within rows followed by the same letter are not significantly different ($P = 0.05$)
[b] HS-TG1, Half-sib families of transgenic plants from genotype 1; HS-CG1, half-sib families of plants regenerated from cell suspension cultures of genotype 1; HS-SeedP, half-sib families of seed-grown plants; SeedP, plants grown from Kentucky-31 seed stock

Table 4 Loadings of rotated factors for key agronomic traits of progenies of transgenics, regenerants and seed-derived plants of tall fescue cv. Kentucky-31 (*doy* day of year)

Traits	Commu-nality	Factor 1	Factor 2	Factor 3
Heading date (doy)	0.998	–0.313	0.942	–0.099
Anthesis date (doy)	0.996	–0.342	0.932	–0.097
Height (cm)	0.999	0.012	–0.080	0.983
Growth habit	0.999	–0.341	–0.096	0.902
Tiller number plant^{-1}	0.996	0.943	–0.278	–0.099
Seed yield plant^{-1} (g)	0.987	0.901	–0.403	–0.065
Biomass plant^{-1} (g)	0.999	0.909	–0.268	–0.236
Eigenvalue		2.863	2.084	1.867
Proportion (%)		40.9	29.8	26.7
Cumulative (%)		40.9	70.7	97.4

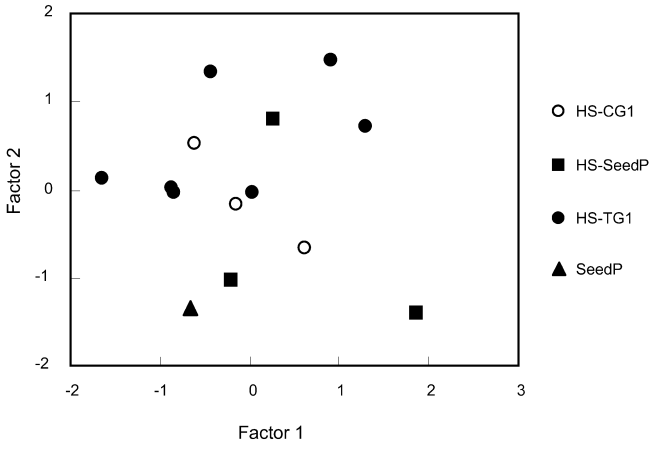

Fig. 5 Scatter diagram of progenies of transgenics, regenerants and seed-derived plants based on factor analysis. *HS-TG1* Half-sib families of transgenic plants from genotype 1, *HS-CG1* half-sib families of plants regenerated from cell suspension cultures of genotype 1, *HS-SeedP* half-sib families of seed-grown plants, *SeedP* plants grown from Kentucky-31 seed stock

grouping patterns could be identified based on the origin of the half-sib families (Fig. 5).

Discussion

In outcrossing forage species like tall fescue, individual seeds within a cultivar might represent different genotypes. The use of single genotype-derived cell suspension cultures in this study allowed the generation of transformants from defined genotypes. This made it possible to compare transgenics with not only seed-derived plants but also with plants regenerated from the same genotype.

The production of transgenic forage grasses have been reported for several important species, such as tall fescue (Ha et al. 1992; Wang et al. 1992; Dalton et al. 1995, 1998; Spangenberg et al. 1995a; Kuai et al. 1999; Cho et al. 2000; Wang et al. 2001b), perennial ryegrass (Spangenberg et al. 1995b; Dalton et al. 1998, 1999; Altpeter et al. 2000), Italian ryegrass (Wang et al. 1997; Ye et al. 1997; Dalton et al. 1998, 1999), Kentucky bluegrass (Ha et al. 2001), orchardgrass (Horn et al. 1988; Denchev et al. 1997; Cho et al. 2001) and switchgrass (Richards et al. 2001). In many of these cases, the cell cultures used for transformation were from a mixture of genotypes, and the transgenics were not of single genotype origin. Since most of the forage species mentioned above require vernalization to flower, the majority of the reports only showed the generation of primary transgenics with no progeny recovered. To date, no information is available on field evaluation of any transgenic forage grass species.

The agronomic performance of the primary transgenics and regenerants was generally inferior to that of the seed-derived plants, especially when major traits, such as seed yield, were considered. Factor analysis showed plants from the same genotype were more uniform than plants from seeds. This is not surprising since seed-derived plants would be expected to differ at a number of loci. Genotype differences were observed in this study, with both transgenic and regenerated plants from genotype 1 performing better than plants from genotype 2.

The addition of a selectable marker gene in the plant genome seems to have little effect on the agronomic performance of the regenerated plants, since the performance of the transgenics was very similar to that of the corresponding regenerants from the same genotype. It should be noted that the selectable marker gene was only used for the selection of transformants. Selection pressure was only applied during the tissue culture stage in the laboratory, whereas no selection pressure was applied or naturally available in the field.

Although great differences were observed between primary transgenics and seed-derived plants, no major difference concerning the key agronomic traits evaluated was found between the progenies of transgenics and the progenies of seed-derived plants. In addition, progenies of the transgenics performed similarly to progenies of the regenerants. Some of the phenotypic variation (mainly less vigorous growth) observed in the primary transgenics and regenerants may be of epigenetic origin and thereby not heritable. It is also possible that the effects of genes responsible for the inferior performance were masked in the progenies. Low seed yield and weak growth of cell culture-derived plants have been reported in different grass species (Stadelmann et al. 1998a, b; Stadelmann et al. 1999), possibly due to carry-over effects of tissue culture or somaclonal variation. Results from the present research indicate that once seeds are obtained from the primary transgenic plants, normal agronomic performance of the progenies can be expected.

Transgenic plants did not show significant changes in morphology compared with non-transgenic plants. The range of variation of transgenic plants and their progenies was within the range of variation of the seed-derived plants. None of the transgenic plants showed the formation of stolons (or rhizomes) or an increase in shattering. Thus, there is no indication of weediness in the transgenic plants. No changes were observed concerning the incidence of pest, beneficial insects or pathogens in the transgenic plants. Therefore, there was no indication of environmental risks associated with the transgenic material tested.

In the last two decades, methodology pertaining to tissue culture and genetic transformation has been developed for several important forage and turf grasses. Potential targets have been identified, and novel germplasm is being generated by genetic engineering for the improvement of these species (Wang et al. 2001a). Biosafety evaluation and risk assessment of transgenic grasses under natural conditions have become an important issue. Field tests of transgenic plants carrying agronomic genes are an extremely important step in the biosafety evaluation process so as to verify that such plants will not pose environmental risks and will benefit agricultural production.

In summary, we present the first report of field tests involving transgenic plants of an important outcrossing forage grass species. The primary transgenic tall fescue plants had a lower productivity than the non-transgenic control plants. However, progenies from these transgenics showed an agronomic performance similar to that of the non-transgenic plants. This study provided evidence that plants generated through transgenic approaches can be incorporated into breeding programs. The knowledge gained from this study can be useful for agronomic evaluations of transgenic grasses with different agronomic genes.

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