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Field performance of cell-suspension-derived *Lolium perenne* L. regenerants and their progenies

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Abstract Two sets of plants (*Lb* and *Lc*), regenerated from different single-genotype-derived embryogenic suspension cultures of *Lolium perenne* cv Citadel, were evaluated for agronomic traits in a modified polycross design in the field. Seed from the primary regenerated plants was harvested to evaluate morphological and phenological traits of corresponding progenies in a replicated field experiment. When compared to seed-grown plants of the same cultivar, primary regenerants of the *Lb* set showed a significant delay in ear emergence and a more-erect growth habit, while primary regenerants from the *Lc* set showed a significantly higher seed yield. However, progenies of regenerated plants did not differ from those of seed-grown plants. Embryogenic suspension cells of *L. perenne* have the potential for producing fertile, well-performing, material which can be integrated into breeding programs.

Key words Field evaluation · *Lolium perenne* L. · Primary regenerants · Progenies · Somaclonal variation

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Introduction

Perennial ryegrass (*Lolium perenne* L.) is a key forage species for grassland agriculture in cool temperate climates throughout the world. Since it is an out-crossing species with a high degree of self-incompatibility, breeding management is difficult, and selection schemes are complex, thus resulting in slow breeding progress. The application of biotechnology to forage grass improvement has the potential for complementing and enhancing conventional breeding efforts (Spangenberg et al. 1995). However, a basic prerequisite for the application of some biotechnological methods for plant improvement is the efficient regeneration of true-to-type plants from cells that are amenable to genetic manipulations for a wide range of agronomically useful germplasm. Embryogenic suspension cells can be used as targets for biolistic™ transformation (Spangenberg et al. 1995) and are also a unique source of totipotent protoplasts for graminaceous monocot species. However, in order to assess the suitability of these approaches for application in improvement programs, the performance of regenerated plants and their progenies needs to be investigated. Moreover, such experiments have to be carried out in the field to test the plants under conditions relevant to plant breeding.

The aim of the present study was to test whether *L. perenne* plants, regenerated from embryogenic suspension culture (E.S.C.), maintain the agronomically important characters of the cultivar from which they originate. For this purpose, primary regenerants and their polycross progenies were evaluated in replicated field experiments together with seed-grown plants of the cultivar from which the E.S.Cs had been established. In addition, somaclonal variation was assessed by comparing different regenerants from the same single-seed-derived culture and their progenies.

Materials and methods

Plant regeneration

Tetraploid (2n = 4x = 28) *L. perenne* cv Citadel plants were regenerated from embryogenic suspension cultures as described by Wang et al. (1993). Calli obtained from two mature embryos were used for the initiation of two single-genotype-derived embryogenic suspension cultures (E.S.Cs), *Lb* and *Lc*, and maintained by a weekly subculture (Wang et al. 1993). Plantlets were regenerated from somatic embryos formed on the surface of suspension-cell-derived calli. The regenerated plants were transferred to soil and established in the greenhouse. The term 'set' refers to all plants originating from the same single genotype-derived embryogenic cell suspension. Each individual primary (R_0) plant, regenerated from the same genotype-derived embryogenic cell suspension, gave rise to one 'somaclone' after vegetative propagation.

Investigation of primary regenerants and seed-grown plants

The experimental area was located at the Swiss Federal Research Station for Agroecology and Agriculture (FAL), Zürich-Reckenholz (440 m above sea level), near Zürich in Switzerland. The soil at the experimental site was a fertile, eutric cambisol. In autumn 1993, three somaclones (R_0) of the *Lb* set, 16 somaclones (R_0) of the *Lc* set and 16 seed-grown genotypes (F_0) were planted in the field. Seed-grown plants had been raised from basic seed of *L. perenne* cv Citadel (Mommersteeg International B.V., AA Vlijmen, The Netherlands).

At the end of March 1994, three somaclones of the *Lb* set, five somaclones of the *Lc* set and five seed-grown plants were selected randomly, split into eight ramets and re-planted. The experimental design was three polycrosses (PC I, PC II, PC III), separated by intervals of at least 1.5 m. In each polycross, plants were planted randomly at a distance of 30 cm within four replications. PC I included five seed-grown genotypes and three somaclones of the *Lb* set, PC II consisted of the same five seed-grown genotypes and five somaclones of the *Lc* set, and PC III of three somaclones of the *Lb* set and five somaclones of the *Lc* set. The following parameters were investigated on each single parental plant: growth habit was visually scored by the angle formed by the imaginary line through the region of the greatest leaf density and the vertical, at the end of May. Date of ear emergence was recorded when the tips of three ears were visible. Before anthesis, each polycross was isolated with a plastic tunnel up to seed maturity. The length of the stem and the ear were measured on the first three stems with anthers appearing. Lodging was prevented by fixing generative tillers to a stick. At seed maturity, seed yield and the number of reproductive tillers were investigated. Polycross-pollinated seed was harvested by removing all the reproductive tillers when the first seeds were shedding. After drying in a greenhouse, the seed was threshed and sieved (1.8×1.8 mm) by hand and cleaned (Saugluftstufensichter Type 2, Kurt Pelz Saatmeister, Bonn, Germany). The number of seeds was counted using a seed-counter (Type 400, Elmor, Rothfluhstr. 12, CH-8702 Zollikon, Switzerland).

At planting, the surrounding area was sown with *Poa pratensis* cv Monopoly ($10\ g\,m^{-2}$) and cut frequently throughout the experiment. N fertilizer was applied after planting ($30\ kg\,ha^{-1}$ N). Dicotyledonous weeds were controlled by herbicides; monocotyledonous weeds within the plots were removed by hand.

Investigation of the progenies of regenerated and seed-grown plants

The experimental area was located at the research station of the Swiss Federal Institute of Technology, Eschikon (550 m above sea level), near Zürich in Switzerland. The soil at the site was a fertile, eutric cambisol.

At the end of August 1994, seed harvested on single mother plants and four populations from basic seed of *L. perenne* cv Citadel were sown in quick-pots. After 5 weeks in the greenhouse, the plants were transplanted to the field at the end of September in 1994. The experimental design was a completely randomized block with four replications, each containing 30 plots (70 cm apart) of 17 plants spaced 12.5 cm apart. Each plot, consisting of one single F_1 family or control population, had the following structure: two border plants; four plants for the investigation of morphology and fertility (same investigations as in the parental plants); four plants on which no measurements were made; four plants on which the shoot yield (0.05 m above cutting height) was harvested on 20 May and 23 June and dry matter determined (65°C, 48 h); three border plants.

At planting, *P. pratensis* cv Parade ($10\ g\,m^{-2}$) was sown between the plots and cut frequently throughout the experiment. N fertilizer was applied after planting ($30\ kg\,ha^{-1}$ N) and at a rate of $50\ kg\,ha^{-1}$ in April, May and July in 1995. P and K fertilizers ($100\ kg\,ha^{-1}$ P_2O_5 and $300\ kg\ ha^{-1}$ K_2O) were applied in late October in 1994.

Statistical analyses

Statistical analyses were carried out using the statistical SAS package (SAS Institute, Cary, N.C., USA). An analysis of variance, using the GLM procedure, was performed to compare the means of single groups.

For factor analysis and clustering, growth traits were averaged by parental genotype and somaclone with respect to the polycross and by corresponding F_1 families. These values were transformed to standard deviates (Sokal and Rohlf 1995). Factor analysis was applied using the varimax rotation method suggested by Kaiser (1958), and clustering was performed by the group-average method (Everitt 1993). Rotated factor values of 0.50 or greater were considered to be important in interpreting factor associations (Backhaus et al. 1996).

Results

Performance of primary regenerants and seed-grown genotypes

Most growth parameters of primary regenerants (R_0) varied significantly between the two sets of embryogenic suspension cultures (*Lb*, *Lc*) used for plant regeneration (Table 1, PC III). In comparison to seed-grown plants (100%), primary regenerants of the *Lb* set showed a significantly more-erect growth habit, a delay in ear emergence of 16 days, longer stems (107%) and a reduced seed yield per ear (80%) (Table 1, PC I). Primary regenerants of the *Lc* set, in comparison to seed-grown plants (100%), had significantly shorter stems (92%) and ears (93%), more reproductive tillers (185%) and seeds (182%), a higher seed yield per plant (165%), a lower seed yield per ear (85%) and lower 1000-seed weight (90%) (Table 1, PC II).

The varimax rotation factor analysis assigned the nine growth traits to three factors, accounting for 83% of the total variability (Table 2). Factor 1 involved all the seed production traits except for the seed yield per tiller. The contribution of 1000-seed weight to this factor was negative. Factor 2 was made up of the date

Table 1 Means and comparisons of growth parameters for primary regenerants and seed-grown plants of *L. perenne* cv Citadel grown in three polycrosses (PC I, PC II, PC III) in the field in 1994

Growth parameters		PC I		PC II		PC III	
		Set *Lb*	Seed-grown	Set *Lc*	Seed-grown	Set *Lb*	Set *Lc*
Growth habit[a]	[no]	8.4 a[b]	5.3 b	5.6 b	5.8 b	8.7 a	5.9 b
Ear emergence	[d.o.y.]	153 a	139 b	140 b	139 b	155 a	138 b
Length of stem	[cm]	94 a,b	88 c	86 c	93 b	99 a	85 c
Length of ear	[cm]	34.1 a,b	34.8 a,b	33.1 b	35.5 a	34.2 a,b	35.8 a
Reproductive tillers plant^{-1}	[no]	97 b,c	85 c,d	115 a,b	63 d	70 d	134 a
Seeds plant^{-1}	[no]	5148 b	5450 b	8387 a	4608 b	4040 b	8660 a
Seed yield plant^{-1}	[g]	16.5 b	17.3 b	23.2 a	14.1 b	12.7 b	23.2 a
Seed yield ear^{-1}	[mg]	162 d	202 b	199 b,c	233 a	170 c,d	173 b,c,d
1000-seed weight	[g]	3.22 a	3.19 a	2.79 b	3.10 a	3.07 a	2.65 c

[a] Visual scoring of the angle formed by the imaginary line through the region of the greatest leaf density and the vertical (1 = prostrate; 9 = erect)

[b] Means within rows followed by the same letter are not significantly different ($P = 0.05$) using Duncan's Multiple Range Test

Table 2 Varimax rotated scores for the three first factors of nine traits of regenerated and seed-grown *L. perenne* cv Citadel plants in 1994

Traits	Communalities	Factors		
		1	2	3
No. of reproductive tillers plant^{-1}	0.93	**0.96**	− 0.07	− 0.02
No. of seeds plant^{-1}	0.97	**0.95**	− 0.26	0.02
Seed yield plant^{-1}	0.92	**0.90**	− 0.29	0.16
1000-seed weight	0.61	− 0.66	0.00	0.42
Date of ear emergence	0.89	− 0.24	**0.91**	0.12
Growth habit	0.86	− 0.29	**0.88**	0.08
Seed yield tiller^{-1}	0.67	− 0.10	− 0.67	0.45
Length of stem	0.93	− 0.26	0.36	**0.86**
Length of ear	0.71	0.23	− 0.22	**0.78**
Variance explained by each factor		3.338	2.377	1.770

of ear emergence, the growth habit and – a negative contribution – the seed yield per tiller. Factor 3 consisted of two morphological traits, stem and ear length. Figure 1 shows the scatter diagram of the first and second vectors from factor analysis, explaining 63% of the observed variation. In the same diagram five main clusters, obtained after clustering at the 80% between-cluster variation level using the group-average method, are shown (Fig. 1). Both factor analysis and clustering grouped plants with respect to their origin: all somaclones of the *Lc* set were grouped in one cluster (3), separated from the seed-grown plants mainly by factor 1. The primary regenerants of the *Lb* set were separated from all other plants by a higher level of factor 2, and split into two groups (clusters 1 and 2) by both factors. The seed-grown plants were split into two clusters (4 and 5), characterised by negative values for both factors.

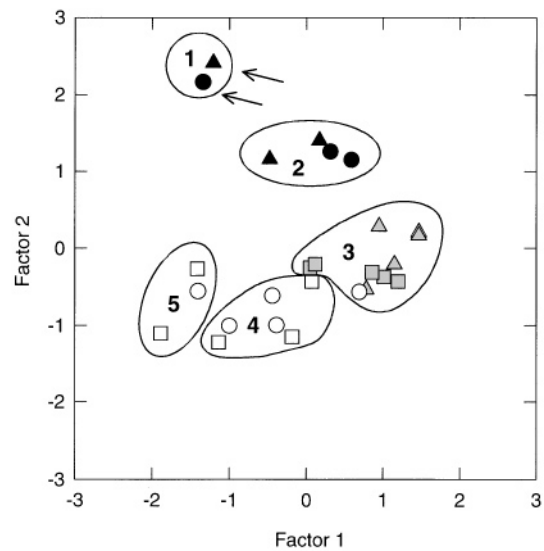

Fig. 1 Scatter diagram of factor analysis based on nine growth parameters for parental *L. perenne* cv Citadel plants. Somaclones of the *Lb* set from PC I (●) and PC III (▲); somaclones of the *Lc* set from PC II (■) and PC III (△); seed-grown genotypes from PC I (□) and of PC II (○). Numbers *1 to 5* indicate main clusters at the 80% between-cluster variation level. *Arrows* indicate one strongly deviating somaclone within the *Lb* set originating from two different PCs

Performance of the progenies of regenerated and seed-grown plants

The performance of progenies regarding growth parameters was more strongly influenced by the polycross

than by the origin of the mother plants within a polycross (Table 3). When pollinated with the same group of genotypes of seed-grown plants, the *Lb* set (in PC I) gave rise to more-erect, later-flowering progenies with longer stems than the *Lc* set (in PC II). These differences reflected those observed on the parental plants (Table 2). The most striking feature of progenies originating from PC III, containing only regenerated plants, was a reduced seed yield per ear and a reduced 1000-seed weight. In general, there was no significant difference between the progenies of regenerated plants and seed-grown plants within the same polycross. The only significant differences were a more-erect growth habit and a delay in ear emergence of progenies of the *Lb* set, as compared to progenies of the seed-grown plants (Table 3, PC I). Progenies of sets *Lb* and *Lc*, originating from PC III and involving only these two sets, did not differ at all. No difference was found between progenies of seed-grown plants from either PC I or PC II and the control plants, grown from basic seed material. This indicates that the five genotypes of seed-grown plants selected for use in PC I and PC II were a representative sample of the original cultivar Citadel. For the progenies of the seed-grown plants, there was no effect of the paternal polycross (Table 3, PC I/II).

Factor analysis of the progenies and the basic seed plants assigned the ten growth parameters into three factors, with two parameters present in two factors (Table 4). Factor 1 consisted of two morphological traits and four fertility parameters, which were all positively correlated. Factor 2 included the date of ear emergence and the growth habit, and – with a negative contribution – the 1000-seed weight and shoot yield. Factor 3 was made up of the number of reproductive tillers and shoot yield. Figure 2 shows the scatter diagram of the first and second vector of the factor analysis from all progenies based on the ten investigated growth parameters, as well as the five main clusters obtained at the 80% between-cluster variation level. In contrast to the primary regenerants, the progenies were not generally grouped according to their origin. Only progenies of PC III were clearly separated from the

Table 3 Means and comparisons of growth parameters for F_1 progenies of regenerated and seed-grown plants of *L. perenne* cv Citadel grown in three polycrosses (PC I, PC II, PC III) and plants grown from basic seed (control) in 1995

Growth parameters		PC I		PC II		PC III		Basic seed
		Set *Lb*	Seed-grown	Set *Lc*	Seed-grown	Set *Lb*	Set *Lc*	
Growth habit[a]	[no]	6.92 a[b]	6.40 b,c	6.25 c	6.25 c	6.84 a,b	6.80 a,b	6.42 b,c
Ear emergence	[d.o.y.]	148 b	143 c	144 c	143 c	151 a	149 a,b	144 c
Length of stem	[cm]	104 a	104 a	98 b,c	101 a,b	97 c	98 b,c	105 a
Length of ear	[cm]	29.9 a,b	29.8 a,b	29.3 a,b	29.1 a,b	28.6 a,b	28.6 b	30.5 a
Reproductive tillers plant^{-1}	[no]	40.5 a	36.4 a	36.6 a	36.6 a	40.4 a	39.9 a	40.8 a
Seeds plant^{-1}	[no]	1644 a	1724 a	1543 a	1645 a	1429 a	1463 a	1727 a
Seed yield plant^{-1}	[g]	5.7 a	6.1 a	5.1 a	5.8 a	4.6 a	4.7 a	6.1 a
Seed yield ear^{-1}	[mg]	139 a,b,c	166 a	136 b,c	156 a,b	105 d	112 c,d	143 a,b
1000-seed weight	[g]	3.36 a,b	3.48 a	3.24 b,c	3.42 a,b	3.14 c	3.14 c	3.37 a,b
Shoot yield plant^{-1}[c]	[g]	23.7 a,b	25.3 a	25.4 a	24.3 a,b	20.1 b	21.7 a,b	25.5 a

[a] Visual scoring of the angle formed by the imaginary line through the region of the greatest leaf density and the vertical (1 = prostrate; 9 = erect)
[b] Means within rows followed by the same letter are not significantly different ($P = 0.05$) using Duncan's Multiple Range Test
[c] Total of the first and second harvests in 1996

Table 4 Varimax rotated scores for the three first factors of ten traits of progenies of regenerated and seed-grown *L. perenne* cv Citadel plants and plants grown from basic seed (control) in 1995

Traits	Communalities	Factors		
		1	2	3
Length of stem	0.79	**0.88**	− 0.05	0.09
Seed yield tiller^{-1}	0.92	**0.87**	− 0.38	− 0.19
Seed yield plant^{-1}	0.92	**0.86**	− 0.25	0.34
No. of seeds plant^{-1}	0.87	**0.83**	− 0.17	0.39
Length of ear	0.65	**0.72**	− 0.17	0.31
1000-seed weight	0.79	**0.64**	− 0.60	0.14
Date of ear emergence	0.91	− 0.32	**0.88**	− 0.18
Growth habit	0.72	− 0.04	**0.79**	0.31
No. of reproductive tillers plant^{-1}	0.85	0.17	0.18	**0.89**
Shoot yield plant^{-1}	0.73	0.33	− **0.52**	**0.59**
Variance explained by each factor		4.129	2.323	1.693

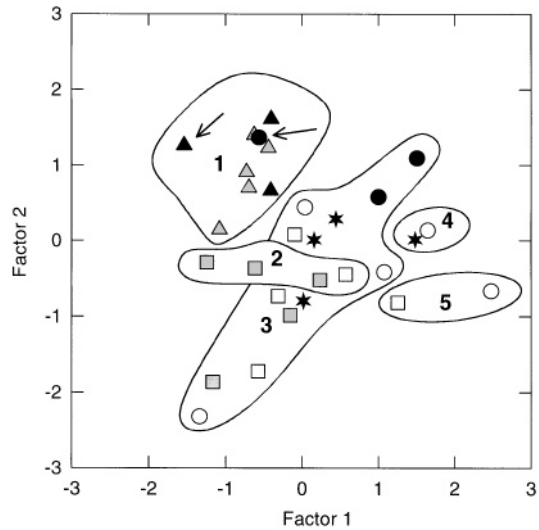

Fig. 2 Scatter diagram of factor analysis based on ten growth parameters for progenies of *L. perenne* cv Citadel plants. Somaclones of the *Lb* set from PC I (●) and PC III (▲); somaclones of the *Lc* set from PC II (▣) and PC III (△); seed-grown genotypes from PC I (○) and of PC II (□). Populations of basic seed plants (★). *Numbers 1 to 5* indicate main clusters at the 80% between-cluster variation level. *Arrows* indicate two F_1 families of one somaclone within the *Lb* set originating from two different PCs

other groups; they were located in the upper left of the scatterplot and grouped into a single cluster (cluster 1).

Assessment of somaclonal variation

In the *Lb* set, there was one somaclone which differed strongly from the other two somaclones (Fig. 1, indicated by two arrows) independent of the polycross where it was growing. This specific somaclone showed poorer fertility (factor 1) and a later phenology (factor 2) than the other somaclones. In the F_1 generation the corresponding progenies (Fig. 2, indicated by two arrows) still showed lower fertility (factor 1) than progenies from the respective polycrosses of the other somaclones. As a consequence, the progeny of this particular somaclone from PC I was grouped with progenies of PC III in cluster 1.

Discussion

Primary regenerants of the two investigated sets differed from each other and from seed-grown plants of the respective cultivar (Fig. 1, Table 1). The most distinct characteristics of the *Lb* set were a significantly more-erect growth habit and a pronounced delay in ear emergence when compared to both seed-grown plants and primary regenerants of the *Lc* set. A similar drift in inflorescence emergence has been reported for primary regenerants of *Hordeum vulgare* L. (Baillie et al. 1992) and for progenies from primary regenerants of *Triticum aestivum* L. and *Oryza sativa* L. (Qureshi et al. 1992; Mezencev et al. 1995; Yamagishi et al. 1997). The most distinct characteristic of primary regenerants of the *Lc* set compared to the other groups, was a significantly higher seed yield per plant. This higher seed-yield is exceptional. Several authors (Qureshi et al. 1992; Mezencev et al. 1995; Stadelmann et al. 1998) described a lower, or at best equal, seed yield of regenerated plants. However, the higher seed yield per plant of the *Lc* set was due to a higher tiller production ($r^2 = 0.90$; $P < 0.001$), whereas the other seed yield components (seed yield per ear, 1000-seed weight) were lower than for seed-grown plants (Table 1). The primary regenerants of both sets did not differ in the seed yield of the two respective polycrosses (Table 1). This indicates that the pollen-donor – either a seed-grown or a regenerated plant – had no effect on the seed yield of the primary regenerants.

The results obtained by evaluating single agronomic key traits are consistent with those obtained by factor and cluster analysis, thus demonstrating the distinct behaviour of the two sets (Fig. 1). The factor analysis indicated that plants can be sufficiently characterised by the main factors of ertility and phenology.

In our study, the performance of primary regenerants was similar or even superior to seed-grown plants, and thus, no negative correlation between high regeneration ability and agronomic performance was apparent. This is in contrast to reports by Orton (1984), Baillie et al. (1992) and Qureshi et al. (1992), in whose studies regenerated plants generally corresponded to the agronomically inferior types within the respective cultivar.

Differences in growth traits between progenies of regenerated and seed-grown plants within PC I and PC II were less distinct than between corresponding mother plants (Tables 1 and 3), as expected because of the introduction of within-family variation (Aastveit and Aastveit 1990). Only in PC I were differences between the regenerants and the seed-grown plants transmitted to the progenies (Table 3). The reason for the more distinct behaviour of the two groups from PC I is, that primary regenerants of the *Lb* set differed mainly in traits of high heritability, such as ear emergence and growth habit (Loos 1994), whereas primary regenerants of the *Lc* set differed in quantitative characteristics of low heritability such as seed yield (Aastveit and Aastveit 1990).

In contrast to the progenies of regenerated plants in PC I and PC II, the progenies of PC III were grouped closer, building a main cluster (cluster 1) at the 80% between-cluster variation level and separated in the upper left of the scatter diagram (Fig. 2). Such a close grouping was expected due to the isolated cross-pollination of only two parental sets: excluding somaclonal variation, all progenies of PC III belong to the same full-sib family. The differences between progenies of

PC III and the other progenies were mainly in an agronomically inferior direction, as progenies of PC III had a reduced seed yield (factor 1), a later ear emergence and a lower 1000-seed weight (factor 2). The results of key growth traits showed that the seed yield per ear was strongly reduced, when compared to corresponding progenies of the other polycrosses (Table 3). The lower seed yield per ear was related to the later phenology which resulted in a shortened period of time to reach maturity ($r^2 = -0.91$; $P < 0.001$). Due to the faster ripening, the 1000-seed weight was lower and, therefore, the seed yield per ear was reduced (Table 3). This is consistent with reports by Elgersma (1990) and Griffiths et al. (1978) who showed that the 1000-seed weight of *L. perenne* plants is positively correlated with earliness and negatively correlated with high temperature. The fact that progenies of the *Lc* set did not show a better tillering capacity, similar to their mother plants, suggests that the superior tillering of primary regenerants of the *Lc* set was due to carry over effects of the culture environment and were thus of an 'epigenic' nature (Karp 1991; Winicov 1996). This stresses the importance of an evaluation of progenies in order to differentiate between transient and heritable in vitro induced variation.

In our study, one somaclone of the *Lb* set deviated in an agronomically inferior direction: it had a delayed ear emergence, and fertility was lower. Somaclonal variation in an agronomically undesirable direction is well documented (Baillie et al. 1992; Qureshi et al. 1992; Mezencev et al. 1995; Stadelmann et al. 1998; Yamagishi et al. 1997). Regarding fertility, the phenotypic deviation of the primary regenerant was apparently transmitted to the F_1 generation (Fig. 2, indicated by two arrows). However, the differences in the date of ear emergence and growth habit observed in the primary regenerants within the *Lb* set (Fig. 1, factor 2) disappeared in the progenies (Fig. 2, factor 2). This suggests that the particularly late flowering of this specific somaclone was caused by epigenetic changes. It may have increased the rate of self-pollination, which in turn may have resulted in a reduced fertility of its progenies, without fertility *per se* being reduced genetically. This interpretation is supported by the fact that RAPD analysis did not show different banding patterns for the parental somaclones within the *Lb* set (Stadelmann et al. 1998).

In conclusion, primary regenerants of *L. perenne* differed from seed-grown plants of the same cultivar in morphological and phenological traits. However, with regard to fertility, they performed similarly or were even superior when compared to seed-grown plants. Moreover, progenies of regenerated plants showed very little difference to progenies of seed-grown plants. Phenotypic differences between somaclones of a single set were rare and more likely of an 'epigenic' nature than of a true genetic origin. The results suggest that embryogenic suspension cells of *L. perenne* have the potential for producing fertile and genetically stable material which performs well and maintains the characteristics of the original cultivar.

References

Aastveit AH, Aastveit K (1990) Theory and application of open-pollination and polycross in forage grass breeding. Theor Appl Genet 79:618–624

Backhaus K, Erichson B, Plinke W, Weiber R (1996) Multivariate Analysemethoden: eine anwendungsorientierte Einführung, 8th edn. Springer Verlag, Berlin Heidelberg New York

Baillie AMR, Rossnagel BG, Kartha KK (1992) Field evaluation of barley (*Hordeum vulgare* L.) genotypes derived from tissue culture. Can J Plant Sci 72:725–733

Elgersma A (1990) Genetic variation for seed yield in perennial ryegrass (*Lolium perenne* L.). Plant Breed 105:117–125

Everitt BS (1993) Cluster analysis. 3rd edn. Edward Arnold a division of Hodder and Stoughton, London Melbourne Auckland, pp 61–62

Griffiths DJ, Roberts HM, Bean EW, Lewis J, Pegler RAD, Carr AJH, Stoddart JL (1978) Principles of herbage seed production. 2nd edn. Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth UK, pp 24–34

Kaiser HF (1958) The varimax criterion for analytic rotation in factor analysis. Psychometrika 23:187–200

Karp A (1991) On the current understanding of somaclonal variation. In: Miflin BJ (ed) Oxford Surveys Plant Mol Cell Biol 7. Clarendon Press and Oxford University Press, UK, pp 1–58

Loos BP (1994) Morphological variation in Dutch perennial ryegrass (*Lolium perenne* L.) populations, in relation to environmental factors. Euphytica 74:97–107

Mezencev N, Clément G, Guiderdoni E (1995) Variation among progenies of diploid plants regenerated from haploid, microspore-derived cell-suspension protoplasts of rice (*Oryza sativa* L.). Plant Breed 114:149–154

Orton TJ (1984) Somaclonal variation: theoretical and practical considerations. In: Gustafson JP (ed) Gene manipulation in plant improvement. Plenum Press, New York, pp 427–468

Qureshi JA, Hucl P, Kartha KK (1992) Is somaclonal variation a reliable tool for spring wheat improvement? Euphytica 60:221–228

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. 3rd edn. WH Freeman and Company, New York

Spangenberg G, Wang ZY, Wu X, Nagel J, Potrykus I (1995) Transgenic perennial ryegrass (*Lolium perenne*) plants from microprojectile bombardment of embryogenic suspension cells. Plant Sci 108:209–217

Stadelmann FJ, Boller B, Spangenberg G, Kölliker R, Messerli M, Wang ZY, Nösberger J (1998) Fertility and growth in the field of *Lolium perenne* and *Festuca rubra* plants regenerated from suspension cultured cells and protoplasts. Plant Breed (in press)

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

Winicov I (1996) Characterization of rice (*Oryza sativa* L.) plants regenerated from salt-tolerant cell lines. Plant Sci 113:105–111

Yamagishi M, Itoh K, Koba T, Sukeiyko Y, Shimamoto K, Shimada T (1997) Characteristics of genetic variation in the progenies of protoplast-derived plants of rice, *Oryza sativa* cv Nipponbara. Theor Appl Genet 94:1–7