GENETIC SHIFT OF SEEDLING FLUORESCENCE IN RYEGRASS OVER SEED INCREASE GENERATIONS

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ABSTRACT

Seedling root fluorescence has generally been used to discriminate perennial ryegrass (*Lolium perenne* L.) from Italian ryegrass (*L. multiflorum* Lam.). The trait, however, has introgressed between the two species and breeders now determine fluorescence levels for new ryegrass cultivars. Our objective was to ascertain genetic change for fluorescence expression during generations of seed multiplication. Four ryegrass populations, differing in fluorescence levels, were increased three generations at each of three Oregon locations. Fluorescence levels were measured for each generation cycle at each location. Variation was present for fluorescence among locations within populations and for seed production generation within locations. One population, for example, initially at 10% fluorescence increased to 36% over three generations of seed multiplication at one location, but decreased to 8 and 2% at the other two locations. This large effect of location and seed generation on fluorescence expression must be examined and carefully considered when describing fluorescence levels of cultivars for seed certification.

KEYWORDS

Ryegrass, genetic shift, fluorescence, seed production, genotype X environment

INTRODUCTION

Since its discovery as a phenotypic marker, the seedling root fluorescence trait in ryegrass has been used to separate Italian from perennial ryegrass (Genter, 1929). Generally, roots of Italian ryegrass, growing on white filter paper, secrete a compound that fluoresces under ultraviolet light. Similarly cultured seedlings of perennial ryegrass typically do not. The pigment responsible is an alkaloid called annuloline (Axelrod and Belzile, 1958). However, fluorescent perennial ryegrass lines exist as well as non-fluorescent Italian ryegrass (Nilsson, 1930; Nitzsche, 1963; Nyquist , 1963; Okora et al., 1995).

The Association of Official Seed Analysts commented in their 1988 progress report that ryegrass breeding has recently resulted in varieties with atypical fluorescence patterns (AOSA, 1988). Plant breeders began documenting inherent fluorescence levels for new and existing ryegrass cultivars in 1991. By August 1995, fluorescence values of eighty-three perennial ryegrass cultivars and seven Italian ryegrasses were documented (U.S.D.A., 1995).

This study was initiated to obtain information needed to assist breeders in documenting fluorescence levels of developed cultivars. Our objectives were: 1. to determine if fluorescence expression remains stable through generations of seed increase, and 2. to determine if the environment of seed production affects fluorescence expression of ryegrass cultivars.

METHODS

Perennial ryegrass source material was derived from open pollinations between 'Palmer' and clones collected from old turfs in St. Louis, MO and Washington, D.C. Italian ryegrass source material was derived from 'Gulf' and 'Marshall', and diploid annual ryegrass accessions of unknown origin. Potential parents for four populations were chosen from the source materials based on evaluation of individual plants grown in a greenhouse at Tangent, OR during Spring 1991. In addition to seedling root fluorescence, the mature plant characters observed were time of heading, vegetative leaf vernation, presence or absence of floral awns, and glume length. These characteristics have been used to separate Italian and perennial ryegrass (Jung et al., 1996). The four populations were constructed to represent varying proportions of characteristics from the two species, and parental clones placed into each population were based on overlapping heading time (Table 1). Population 1 had ten parental clones and population 2 had nine, and were phenotypically perennial ryegrass with one and two parents being fluorescent variants, respectively. Population 3 represented an intermediate phenotype between perennial and Italian ryegrass. It was composed of ten parents; seven of which were fluorescent types. Population 4 was composed of six parents possessing Italian ryegrass phenotype with five being nonfluorescent variants based on an Italian ryegrass normal fluorescence pattern (Jung et al., 1996).

Three locations were chosen to increase seed of each population for three years. Each initial population (Syn 0) contained six ramets of each parent and were transplanted to isolated crossing blocks arranged in randomized complete block (RCB) designs at experiment stations of Oregon State University at Aurora, Corvallis, and Madras. Five parental clones among the populations were weak, producing little if any viable seed the first generation, and were discontinued from future seed increases at all sites. Three additional parental clones were discarded from further increase at the Madras location (Table 1). After harvest, seed was composited and maintained by maternal half-sib family throughout the study. Generation one (Syn 1) seed was harvested from each population at each location in June/July 1992. For succeeding generations, direct seeded rows were established, maintaining maternal half-sib family integrity and at equal seeding rates, at Corvallis and Aurora. Progressive generations of increase at Madras continued by establishing transplanted seedlings equally representing each half-sib family.

Seed of each population was increased separately within locations in isolated crossing blocks with half-sib families arranged in RCB designs of four replications. At all locations, seed composited by half-sib family from the Syn 1 (1992) harvest was sown for the Syn 2 generation and seed from the Syn 2 (1993) harvest was sown for the Syn 3 (1994) generation. Cereal grains attaining a height of 1.5 to 2.0m were used each year as a pollination barrier between populations at Corvallis and Aurora. A minimum of 70m physical distance was used yearly at Madras. Plots at Madras were irrigated weekly to maintain plant turgor and overall vigor. Supplemental irrigation was not necessary for the other two sites. Harvest determination for each half-sib family was made subjectively by judging ease of seed disarticulation.

As seed from each generation was harvested, it was dried at ambient field conditions, hand threshed, cleaned, weighed, and placed in a controlled environment seed storage room. Germination and fluorescence tests were conducted on seed of half-sib family lines for each population in each of the three years of increase. Seed tests were conducted twice following the Association of Official Seed Analysts (AOSA) guidelines (AOSA, 1994). Data were analyzed using the GLM procedure (SAS Institute, 1994). The statistical model considered populations, generations, and locations as fixed effects.

RESULTS AND DISCUSSION

Fluorescence level varied with generation and location of increase for each population. Differences among populations were expected since they were purposely assembled with different base levels of fluorescence. Except for population 3, fluorescence levels were highest from seed produced at Corvallis (Table 2).

There was significant location by generation interaction among populations for fluorescence expression. Fluorescence of seed produced at Corvallis increased over three generations. The Aurora and Madras sites showed no increase in fluorescence levels over generations. Rumball (1970) in New Zealand monitored fluorescence expression through seven generations of seed increase. He cited nearly a one percent

per generation increase in fluorescence for a ryegrass cultivar.

It is not clear why location of increase has such a large impact on fluorescence level. A possibility may be pollen contamination from a concentration of commercial seed production fields of Italian ryegrass in southern Willamette Valley represented by the Corvallis site. Effects from this pollen load may be higher than realized. An earlier experiment in Oregon assessing pollen contamination between fluorescent and non-fluorescent cultivars revealed that little out crossing occurs beyond 6m from the field border (Copeland and Hardin, 1970). The cereal pollination barriers used in this study were 7m at any minimum width. It may also be that some locations provide a greater selection advantage for fluorescing genotypes in a population. Further research is needed to document these concerns. In general, commercial seed production of ryegrass does not exhibit such pronounced genotype by location (G X L) effects. Initial fluorescence values for cultivars, however, are usually lower for perennial ryegrass compared with the perennial ryegrass populations of this study. An accurate survey needs to be conducted among commercial cultivars sown at different locations from common seed stock sources to provide further information on G X L effects. Large genotype X environment interaction effects for fluorescence expression must be examined and carefully considered when describing fluorescence levels of cultivars intended for seed certification.

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Table 1

Parental description of four ryegrass populations.

Parental designation	Leaf vernation[1]	Fluorescence expression	Greenhouse heading date[2]	Presence of awns	Glume length[3]
Population 1 (Constructed population base fluorescence = 10.00%)					
94-1	F	-	May 23	no	long
99-1	F	-	May 20	no	long
123-7**	F	-	June 1	no	long
I42-2	I	+	May 22	yes	medium
152-1	F	-	May 20	no	long
155-6	F	-	May 23	no	long
160-15*	F	-	May 24	no	long
171-5	F	-	May 20	no	long
176-2	F	-	May 24	no	long
179-8	F	-	June 1	no	long
Population 2 (Constructed population base fluorescence = 22.22%)					
94-2**	F	+	May 28	no	long
94-4*	F	-	June 1	no	long
123-3	F	+	May 28	no	long
152-8	F	-	May 31	no	long
155-2	F	-	May 25	no	long
160-4	F	-	May 22	no	long
168-5	F	-	May 28	no	long
176-1	F	-	May 28	no	long
179-2	F	-	June 1	no	long
Population 3 (Constructed population base fluorescence = 70.00%)					
14-4*	R	+	May 23	yes	short
26-8*	I	+	May 15	yes	short
34-2	F	-	May 21	no	long
43-2	I	+	May 14	yes	short
59-5	I	+	May 13	yes	short
106-4	I	-	May 12	yes	medium
106-13	R	-	May 13	yes	long
112-9	I	+	May 13	yes	medium
126-14*	R	+	May 28	yes	medium
142-10**	F	+	May 20	yes	long
Population 4 (Constructed population base fluorescence = 16.67%)					
228	F	+	May 28	yes	long
4867	R	-	May 13	yes	short
4948	R	-	May 31	yes	short
5149	R	-	May 31	yes	medium
5182	R	-	May 29	yes	short
5304	R	-	May 31	yes	short

[1] Leaf vernation was visually scored on expanding vegetative leaves: F=folded, R=rolled, I=intermediate

[2] Heading date was determined when one spike of the parental plant was emerged from boot.

[3] Glume length was subjectively evaluated relative to the total length of spikelet.

* Half-sib family lines dropped from further increase for each population at all sites because of poor seed production in 1992.

** Additional half-sib lines discarded from each population at the Madras location because of poor seed production in 1992.

Table 2

Fluorescence percentage of four ryegrass populations increased for three generations at three locations.

Population	Generation	Location: Aurora	Corvallis	Madras
		- - - - - - - - - - - - - - - - - % - - - - - - - - - - - - - - -		
1 (fluorescence base=10.00%)				
	Syn 1	3.98	15.69	14.74
	Syn 2	3.47	27.47	10.25
	Syn 3	2.04	36.42	8.44
	Mean	3.16a[†]	26.53b	11.15a
2 (fluorescence base=22.22%)				
	Syn 1	29.24	34.07	17.91
	Syn 2	26.61	46.98	24.27
	Syn 3	27.10	58.36	27.74
	Mean	27.65a	46.47b	23.31a
3 (fluorescence base=70.00%)				
	Syn 1	76.32	78.62	83.59
	Syn 2	77.84	83.57	87.09
	Syn 3	79.95	92.75	90.03
	Mean	78.04a	84.98a	86.90a
4 (fluorescence base=16.67%)				
	Syn 1	1.52	18.91	2.55
	Syn 2	0.13	23.57	2.38
	Syn 3	0.80	28.36	0.14
	Mean	0.82a	23.70b	1.69a

[†] Means with the same letter in a row are not significantly different using least significant