Inheritance of Phosphoglucosomerase in Fountain Grass

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Abstract. Starch gel electrophoresis was used to screen 10 enzyme systems for variation in fountain grass, *Pennisetum alopecuroides* (L.) Spreng. plants exhibiting four different growth habits: dwarf(d), mound(m), prostrate(p), and upright(u). Only phosphoglucosomerase (PGI; E.C. 5.3.1.9) was found to be polymorphic at one locus, PGI-2, and was expressed as two alleles, which appeared to be associated with growth habit. The dwarf form expressed one slow band (SS), the mound and prostrate forms exhibited one fast band (FF), and the upright form carried triple bands indicating a heterodimer (FS). Hybrids between FF and SS parents were detected as triple bands (FS). Three generations of progeny resulting from 16 crosses and selfs of these growth habits all followed the expected segregation ratios for typical Mendelian inheritance of this isozyme.

Fountain grass is an attractive ornamental, $2n = 18$ (Ono and Tateoka, 1953), typically 90–125 cm tall with many purple or mauve inflorescences that give the plants the appearance of a fountain in August and September in the northern temperate zone (Bailey, 1949; Meyer, 1975). Although its popularity has increased in the past 10 years, no records of any genetic improvement have been reported. Fountain grass is native over a large area from northern Manchuria (Kitagawa, 1979) southward to New South Wales in Australia (Wheeler et al., 1982), so it is likely that many forms and variants exist. In 1987, a breeding program for fountain grass was initiated at the Univ. of Minnesota with the goal of selecting new ornamental forms. Initial accessions revealed four growth habits, which were identified as dwarf, mound, prostrate and upright.

Isozymes offer several applications in studying germplasm collections, such as separation and classification of genotypes (Tanksley and Orton, 1983), plant identification (Cousineau and Donnelly, 1992), tagging of economic traits (Rick and Fobes, 1974), and studying phylogenetic relationships within a species (Tostain and Marchais, 1989). The purpose of this research was to use isozyme analysis to classify the variation in fountain grass germplasm and to determine isozyme inheritance.

Materials and Methods

Plants or seeds of fountain grass were acquired from several sources. Material received as plants was field grown in a Waukegan silt loam soil in St. Paul, Minn., or in a Hayden loam soil at the Univ. of Minnesota Landscape Arboretum in Chanhassen. Seeds were germinated in the greenhouse and seedlings transplanted to the field.

All plants were evaluated for variation by measuring 13 morphological characters. After 2 years, 4 growth habits—dwarf, mound, prostrate, and upright—were identified. Detailed descriptions of these growth habits and their inheritance have been reported (Meyer, 1993). The dwarf growth habit was obtained as bareroot plants from John Greenlee Nursery, Pomona, Calif., and

was labeled *Pennisetum alopecuroides* 'Weserbergland'. The mound and prostrate plants were received as seed (PI 90190, origin Korea) from the U.S. Dept. of Agriculture Plant Introduction Station Germplasm Laboratory in Experiment, Ga. The upright growth habit was received as bareroot plants from K. Bluemel Nursery, Baldwin, Md.

Four plants, one of each growth habit, were selected for use as parents and crossed in a complete diallel during August 1990 and repeated in August 1991.

Fountain grass, like other species of *Pennisetum*, is protogynous; the stigmas precede anther exertion and dehiscence by 2 to 5 days (Burton, 1974; Jauhar, 1981). Hybrids in pearl millet, *Pennisetum glaucum* (L.) R. Br., are made by taking advantage of this difference in anther and stigma emergence (Burton, 1991; Poehlman, 1986). Phenotypic variation and heterosis in progeny from fountain grass crosses evaluated in 1989 and 1990 confirmed controlled pollination had occurred without emasculation. Therefore, emasculation was not necessary to obtain the crosses reported in this paper. Inflorescences were bagged before stigma emergence. For the F_1 crosses, the appropriate pollen was applied when stigmas were observed to be receptive and after pollination the heads were immediately rebagged. For selfs, inflorescences were bagged before stigma emergence and not removed until the seed was harvested. About 1 month after pollination, the inflorescences were collected, and each was cleaned and weighed separately.

From the original diallel, the 12 F_1 and 4 S_1 lines of seed were germinated on moistened blotter paper and then transplanted to cell packs in the greenhouse. In late May 1991 and 1992, seedlings were field-planted in a Waukegan silt loam soil type with 1 x 1-m spacing in a randomized design by lines. The plants were maintained with normal cultural practices such as weeding, irrigation, and fertilization.

To obtain the second generation, all twelve F_1 and the four S_1 lines were selfed by bagging a minimum of five random heads per line before stigma emergence. At maturity, each inflorescence was collected and the seeds were cleaned and weighed. The following spring these seeds were germinated and the subsequent plants grown as outlined above.

The third generation was obtained by selfing all S_1 and S_2 lines by bagging a minimum of five random inflorescences prior to stigma emergence. Seeds were collected and cleaned as described above.

A total of 16 F_1 and S_1 progeny lines from 1990, 16 S_1 and S_2 selfed lines from 1991, and 13 S_2 and S_3 selfed lines (three lines

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Fig. 1. Interpretive drawing of enzyme systems that did not show variation in banding patterns among tested fountain grass growth habits.

failed to set seed) from 1992 were screened for isozyme variation and inheritance.

For electrophoresis, individual dry seeds were crushed with a glass rod in ceramic wells containing ≈ 0.1 ml of refrigerated extraction buffer as described by Leblanc and Pernes (1983). Young leaf tissue was used to screen the dwarf S_2 plants, otherwise seeds were used for isozyme analysis. The electrophoretic buffer, stain, and gel recipes were as described by Morden et al. (1987) and Cardy et al. (1983), but modified by using potato starch in place of corn starch. The extracts were absorbed onto paper wicks (2×11 mm, Whatman 3MM chromatography paper) that were loaded into the starch gels. Electrophoresis was conducted at 4°C at 17 W, ≈ 300 volts. After ≈ 4 h, the gels were sliced and stained.

Designation of isozymes was based on the migration distance from the origin of the extracts. The fastest migration (most anodal) enzyme was designated with the code F. Seed from the prostrate plant (S_1) was used as the control in most gels. Inheritance analyses were conducted using a χ^2 goodness-of-fit test assuming monogenic and codominant expression of alleles at a single locus.

Results and Discussion

Alcohol dehydrogenase (ADH; E.C.1.1.1.1), catalase (CAT; E.C.1.11. 1.6), esterase (EST; E.C.3.1.1), malate dehydrogenase (MDH; E.C.1.1.1.40), phosphogluconate dehydrogenase (PGD; E.C.1.1.1.44), and phosphoglucomutase (PGM; E.C.5.4.2.2) were monomorphic for all plants tested (Fig. 1). Acid phosphatase (ACP; E. C.3.1.3.2), glutamate oxaloacetate transaminase (GOT; E.C.2.6.1.1), and isocitrate dehydrogenase (IDH; E.C.1.1.1.42) were difficult to resolve and require further investigation.

PGI exhibited two zones of activity, the one closest to the anode labeled PGI-1, was monomorphic and often very light (Fig. 2); the second, furthest from the anode, labeled PGI-2, was polymorphic and represented two alleles (Fig. 2). The banding pattern of PGI-2 consisted of either one slow, one fast, or three bands, which suggests a dimeric enzyme structure (Fig. 3). In the original parents, seed from the dwarf plants expressed one slow band, SS; mound and prostrate S_1 seed exhibited one fast band, FF; and extracts from seeds of the upright plant formed a triple band, FS,

Fig. 2. PGI expression in fountain grass. PGI-1 and PGD were monomorphic, PGI-1 expression was weak. PGI-2 was expressed as one fast band, FF, one slow band SS, or as a triple band, FS. Lanes 1, 14, and 28 are controls, dry seed from the prostrate plant, expressing FF alleles; the other 25 lanes are extracts from young leaf tissue of putative selfs from a dwarf parent. Plants with the FS alleles in lanes 3, 8, 16, and 25 were not phenotypic dwarfs, all other 21 lanes show the expected SS alleles and were dwarfs.

Fig. 3. Banding pattern of PGI-2 in fountain grass. SS, one slow band, was first expressed in the plants with a dwarf growth habit; FF indicating one fast band, was exhibited by the prostrate and mound plants and FS, a heterodimer, with three bands, was expressed by the upright growth habit plants.

or a heterodimer, indicating the heterozygote, FS.

All 46 lines tested (3 lines could not be tested due to failure to set seed)—the F_1 crosses, S_1 , S_2 , and S_3 progeny—showed the expected segregation patterns for simple monogenic inheritance at the PGI-2 locus (Table 1). Intraspecific hybrids could be identified in some crosses. Heterozygous plants were selected, when possible, for screening in the second and third generations. All selfed and hybrid progeny from prostrate and mound parents formed one fast band (data not shown). In the upright growth habit (FS), seed from six plants over the two generations were screened. Only one carried the FF alleles, the other five were heterozygotes (FS), and their seed segregated in a 1:2:1 ratio (Table 1). High, yet acceptable ($P = 0.16$) levels of FS individuals were counted for the S_2 indicating the heterozygote maybe favored. The upright x dwarf (FS x SS) and dwarf x upright progeny segregated 1FS: 1SS and the S_1 and S_2 plants selected were heterozygotes with progeny that segregated 1 FF: 2 FS :1 SS. Prostrate x upright (FF x FS) and the reciprocal cross resulted in a 1 FF: 1 FS segregation in the F_1 . Seed of the S_1 either segregated 1 FF: 2 FS: 1 SS from a parent with FS alleles, or all progeny carried only FF alleles from a homozygous parent. In the upright x prostrate S_2 , 25 seed screened expressed only the F alleles, indicating a homozygous parent. Only two seed resulted in the prostrate x upright S_3 , not enough to verify segregation.

The prostrate x dwarf (FF x SS) cross and the reciprocal cross resulted in all FS individuals in the F_1 . S_1 and S_2 progeny followed 1:2:1 segregation indicating heterozygous parents. Progeny from the upright x mound (FS x FF) and the reciprocal cross segregated 1 FF : 1 FS as expected. All progeny screened in the S_1 and S_2 generations carried only the FF alleles, indicating homozygous parents. Eleven selfs could be detected along with 19 progeny in the mound x dwarf, FF x SS cross. Visual heterosis of these hybrid progeny was reported (Meyer, 1993) and was sufficient to ensure that only hybrids were selfed to obtain subsequent generations. All

individuals tested in the reciprocal cross, dwarf x mound were hybrids (FS). Segregation from a heterozygous parent was as expected, a 1:2:1 in the S_1 , and plants screened in the S_2 carried only FF alleles.

All S_1 progeny from the dwarf expressed only SS alleles. In the S_2 , 69 individuals were screened, all were expected to carry only SS alleles, but 5 were detected as FS. These five plants were identified as nondwarfs, or outcrosses (phenotypically distinct with coarse foliage and taller culms), in the S_2 population. In the zymogram for 25 of these putative selfed dwarfs, the four nondwarfs could be identified as heterodimers (Fig. 2). The remaining S_2 's were 30 dwarf plants that carried the two SS alleles. Although many in florescences were bagged, only six seed were obtained for the S_3 generation, too few to verify segregation.

PGI appears to be a dimeric enzyme in fountain grass. PGI-2, as analyzed here, corresponds to the cytosolic locus of this enzyme which has been confirmed in several plants including spinach (*Spinacia deracea*) (Schnarrenberger and Oeser, 1974), tomato (*Lycopersicon esculentum*) (Tanksley and Rick, 1980), pearl millet *Pennisetum glaucum* (Leblanc and Pernes, 1983), corn (*Zea mays*) (Goodman and Stuber, 1983), tepary bean (*Phaseolus acutifolius* var. *latifolius*) (Garvin et al., 1989), and guayule (*Parthenium argentatum*) (Hashemi et al., 1991). These examples reinforce Gottlieb's (1982) review that subunit structure, number of loci, and subcellular localization of isozymes, especially those catalyzing steps in primary metabolism such as PGI in glycolysis, are well conserved in plant species.

In six lines of progeny [dwarf x mound; dwarf x prostrate; dwarf x upright; mound x dwarf, (S_2) prostrate x dwarf, and upright selfed], there were high levels of heterozygotes (FS) although the ratios fit χ^2 expectations. Weeden and Wendel (1989) discuss skewed segregation ratios where isozyme loci were closely linked to genes or chromosomal segments exposed to strong selection pressures during gametophytic and postzygotic development. Mitton (1989) reported that at least some enzyme polymorphisms have a substantial impact on plant physiology and other reports suggest that heterozygous individuals can capture the highest fitness (Rainey et al., 1987) and maybe favored to compete and survive over homozygotes. The S allele maybe somehow linked or associated with a developmental disadvantage in the dwarf growth habit since low numbers of these individuals were detected. Dwarfism in pearl millet was reported as the result of the inability to use gibberellic acid (GA_3) (Krishna Rao et al., 1981).

Whether or not the fountain grass individuals with a FS genotype are superior in gametophytic development or have a higher level of fitness in direct response to the enzyme produced by this PGI heterodimer remains unknown.

In conclusion, fountain grass expressed polymorphism at only one locus studied, PGI-2. The inheritance for three generations at this locus followed typical Mendelian segregation.

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Table 1. Segregation and χ^2 statistic for inheritance of PGI-2 in fountain grass.

Cross	Observed progeny segregation			Expected test ratio	χ^2	P
	FF	FS	SS			
Upright, selfed, FS						
S ₁	14	17	10	1:2:1	1.96	0.38
S ₂ (FS) ²	13	39	11	1:2:1	3.69	0.16
S ₃ (FS)	9	13	6	1:2:1	0.78	0.68
Upright x dwarf, FS x SS						
F ₁	---	13	16	1:1	1.33	0.57
S ₁ (FS)	6	19	8	1:2:1	0.99	0.61
S ₂ (FS)	9	17	7	1:2:1	0.27	0.87
Dwarf x upright, SS x FS						
F ₁	---	28	22	1:1	0.72	0.40
S ₁ (FS)	9	25	6	1:2:1	2.95	0.23
S ₂ (FS)	3	7	3	1:2:1	0.07	0.96
Prostrate x upright, FF x FS						
F ₁	14	13	---	1:1	0.04	0.85
S ₁ (FF)	24	---	---	---	---	---
S ₂	2 seed	---	---	---	---	---
Upright x prostrate, FS x FF						
F ₁	22	20	---	1:1	0.09	0.75
S ₁ (FS)	7	24	8	1:2:1	2.11	0.35
S ₂ (FS)	5	---	---	---	---	---
Prostrate x dwarf, FF x SS						
F ₁	---	31	---	---	---	---
S ₁ (FS)	11	26	10	1:2:1	0.51	0.77
S ₂ (FS)	11	34	18	1:2:1	1.95	0.38
Dwarf x prostrate, SS x FF						
F ₁	---	29	---	---	---	---
S ₁ (FS)	13	23	5	1:2:1	3.64	0.16
S ₂ (FS)	13	28	9	1:2:1	1.36	0.51
Upright x mound, FS x FF						
F ₁	15	15	---	1:1	0.00	0.99
S ₁ (FF)	26	---	---	---	---	---
S ₂ (FF)	30	---	---	---	---	---
Mound x upright, FF x FS						
F ₁	24	26	---	1:1	0.08	0.77
S ₁ (FF)	37	---	---	---	---	---
S ₂ (FF)	25	---	---	---	---	---
Mound x dwarf, FF x SS						
F ₁	11	19	---	---	---	---
S ₁ (FS)	13	27	6	1:2:1	3.49	0.18
S ₂ (FF)	25	---	---	---	---	---
Dwarf x mound, SS x FF						
F ₁	---	27	---	---	---	---
S ₁ (FS)	15	28	7	1:2:1	3.28	0.19
S ₂ (FF)	10	---	---	---	---	---
Dwarf, selfed, SS						
S ₁	---	---	29	---	---	---
S ₂	---	---	34	---	---	---
S ₂ leaf samples	---	5	30	---	---	---
S ₃	6 seed					

²Parental genotype for PGI-2.

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