In Vitro Pollen Germination in Fountain Grass

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Fountain grass [*Pennisetum alopecuroides* (L.) Spreng.] is a bunch-type ornamental grass (Meyer, 1975). Except for chromosome number [$2n = 18$ (Ono and Tateoka, 1953)], little information is available concerning its breeding behavior. To our knowledge, there has been no effort to develop new cultivars. Breeding of fountain grass was initiated in 1987 at the Univ. of Minnesota. Preliminary research revealed highly variable seed set in self, open, and cross pollinations among and within accessions in the field and greenhouse.

Fountain grass is protogynous, which favors outcrossing (Burton, 1974). Although variation in seed set could be due to several factors, we observed variation in pollen viability as a potential factor. Pollen viability was easy to test by in vitro germination.

Poaceae pollen is trinucleate, which can make in vitro germination more complex and difficult (Mulcahy and Mulcahy, 1983). Pollen viability in the Poaceae and especially *Pennisetum* has been estimated by using vital stains (Simpson and Bashaw, 1969), in vitro germination and in vivo tube growth (Helsop-Harrison et al., 1984), and seed set (Cooper and Burton, 1965). Although the ultimate functional test should be fertilization as measured by seed set (Helsop-Harrison et al., 1984), incompatibility reactions and other phenomena may interfere with pollen function (Stanley and Linskens, 1974).

In vitro germination is considered the standard for testing viability of *Pennisetum* pollen, and although it may show false negatives, it is simpler than seed set correlation (Helsop-Harrison et al., 1984).

The objective of this research was to determine 1) if fountain grass pollen could be germinated in vitro, 2) whether germination differed among accessions, and 3) if germination decreased with selfing.

We found four growth habits in the fountain grass germplasm from several sources: 1) upright, which is commonly found in the trade; 2) dwarf, not as common, but also found in the trade; 3) mound; and 4) prostrate (Table 1). These four growth habits and their F_1 progenies

(selfs) were the source of pollen. Detailed descriptions of these forms, their inheritance patterns, and isozyme analyses have been reported (Meyer, 1993). Four plants, selected as typical for each growth habit, were grown in a Hayden loam soil at the Univ. of Minnesota Landscape Arboretum.

Pollen was collected from four parent plants, each representing a different growth habit. F_1 pollen samples were bulk collections from several plants within the full sib lines. Samples were collected by shaking inflorescences into glassine pollen bags about twice weekly at dehiscence, between 1000 and 1200 hr. Collections were made from early Aug. until mid-Sept. 1992. When dehiscence was delayed due to cool, wet weather, entire heads were taken indoors, where culms were placed in distilled water until the anthers dehisced.

Pollen was placed on a glass slide in a covered petri dish with filter paper moistened with distilled water for 20 to 30 min. Pollen was then dusted onto ≈ 0.01 ml of germination solution (25% sucrose and 100 ppm boric acid in distilled water) and sealed in a depression slide at room temperature (20 to 22°C). Use of lower concentrations of sucrose or boric acid, and failure to seal in moisture, resulted in poor germination (unpublished data). Fresh pollen also appeared to be critical for success.

Germination counts were made in ≈ 24 h. Pollen was placed on the germination solution in late morning and counts were taken the following morning. Germination had occurred, and a pollen grain was considered to be viable when the length of the pollen tube was equal to or longer than the diameter of the grain. A minimum of 300 grains per slide was counted.

Of the four parents, the upright growth habit had the most viable pollen (Table 1). A half sib of this plant produced pollen of similar viability. The mound form produced $\approx 50\%$ as

much viable pollen, the dwarf and prostrate types had the lowest viability.

In the F_1 generation, pollen germination from the prostrate and dwarf forms was similar to that of the parents. However, progeny of the upright and mound growth habits exhibited a significant decrease in viability. Although the F_1 mound plants were sampled >23 times, pollen viability was never $>10\%$, significantly less than the mound parent, the upright parent and F_1 , and the dwarf F_1 . F_1 mound pollen appeared to be a poor choice for use in crosses, and more pollen may be necessary for seed set. Because fountain grass is protogynous and appears to outcross readily, one generation of inbreeding may account for the detrimental effect on pollen germination in the upright and mound F_1 lines.

Samples varied widely in the parent and first generation of selfs; the cause of this variation is unknown. No physically damaged or shrunken grains were visible in any pollen samples.

In conclusion, in vitro germination can be used to determine pollen viability of fountain grass, but growth habits differed in pollen germination, i.e., viability. However, fountain grass appears to have sufficient pollen viability to be used in a breeding program, although wide variation was found within and among growth habits.

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Table 1. Pollen germination of four growth habits of fountain grass and their F_1 progeny.

Growth habit and source ^z	No. slides sampled ^y	Germination % mean	Range
Prostrate (U)	14	8 ab ^x	0-29
F_1 (self)	11	17 ab	0.1-28
Upright (B)	13	47 c	18-61
Upright (1/2 sibs)	18	44 bc	3-77
F_1	17	25 bc	6-58
Mound (U)	15	22 b	3-64
F_1	23	4 a	0.2-10
Dwarf (G)	16	12 ab	1-28
F_1	14	27 bc	3-66

^zU = U.S. Dept. of Agriculture Germplasm Lab., Experiment, Ga.; B = Kurt Bluemel Nursery, Baldwin, Md.; G = John Greenlee Nursery, Pomona, Calif.

^yEach slide represents a minimum count of 300 grains, 100 grains/field, three fields per slide.

^xMeans separation as determined by LSD test after square root transformation, at $P \leq 0.05$.

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