

Seed Production on Detached Culms of Pearl Millet x Elephantgrass Hexaploid Hybrids

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Abstract. Five nutrient solutions were evaluated in the greenhouse to determine which solutions would allow detached culms of *Pennisetum* to produce seed. The genotypes tested originated from the hybridization of *Pennisetum glaucum* L. (Pearl millet) x *P. pennisetum* Schum. (elephantgrass). The solutions were water, Hoagland's, sucrose, sucrose + hydroxyquinoline sulfate (HQ), and Hoagland's + sucrose + HQ. Neither the water nor the Hoagland's solution supported high seed set. Although the sucrose solution enhanced seed production, the seeds were low in weight and did not germinate well. The best nutrient solutions were 2% sucrose + 0.02% HQ or Hoagland's + 2% sucrose + 0.02% HQ. The four genotypes used differed substantially in seed production, but all produced seed, with germination >25%. This result indicates that the cut-culm technique is a possible way of getting recurrent restricted phenotypic selection seed in *Pennisetum* hexaploid hybrids.

Recurrent restricted phenotypic selection (RRPS) requires that male and female gametes are restricted to known sources at the time of pollination. A breeder may desire to use detached culms if freezing weather may hinder field crossing. Also, with the tall grasses, wind and other adverse field conditions make it more efficient to detach the seed heads before anthesis and place them in a nutrient solution until all the seeds can be harvested. Several grass species have successfully produced seed from detached culms or inflorescences (Burton, 1974; Grau, 1982; Keller, 1943; Wofford et al., 1986). The detached culm technique was successful with *Pennisetum* hybrids when panicles were placed in a solution containing sucrose at 20 g·liter⁻¹ and hydroxyquinoline sulfate (HQ) at 0.2 g·liter⁻¹ (Diz, 1994). There was a reduction in seeds per panicle and 100-seed weight in the excised panicles, which is typical on other grasses (Wofford et al., 1986). Nevertheless, adequate amounts of seed for breeding purposes were produced through this procedure (Diz and Schank, 1993).

Our objectives were to evaluate several nutrient solutions in the greenhouse and to

determine which solution would produce sufficient viable seed for breeding and testing using *P. glaucum* x *P. pennisetum* hybrids. The effect of plant genotype also was investigated.

Materials and Methods

Four genotypes of *Pennisetum glaucum* x *P. purpureum* were tested under greenhouse conditions. The genotypes selected (45, 128, 131, and 140) had the following characteris-

tics: intermediate to tall (3 to 4.5 m at maturity), many tillers (25 to 60), relatively early flowering (end of September to mid-October); and high seed production. All four genotypes had been used in a field defoliation experiment (Diz et al., 1995). Four nutrient solutions were compared to water. Three replications (two seed heads in each replication) were used in a randomized complete-block arrangement. Software used was SAS general linear model (SAS Institute, 1985) without transforming any of the data. Seed heads were collected from plants growing in the field in Aug. 1992. Panicles were cut with a stem length of ≈60 to 70 cm. The panicles were all at the stage before stigma exertion, where the panicles were only partially exerted from the sheath of the flag leaf. The leaf blades were stripped off to reduce transpiration, and the culms were placed immediately in a bucket containing water. The buckets with stems were taken to the greenhouse where the stems were recut to a length of 45 cm. Secondary panicles were removed by cutting the leaf sheath with a razor blade and removing them. At the time of making the new cut, stems were placed into one of the following solutions: 1) water only, 2) Hoagland's solution (Hoagland and Arnon, 1950), 3) sucrose only (2%); 4) sucrose (2%) + hydroxyquinoline sulfate (HQ) (0.02%), or 5) sucrose (2%) + HQ (0.02%) + Hoagland's solution.

Once the panicles started shedding pollen, they were shaken once or twice daily to enhance pollination. Stems of the panicles were maintained in the respective solutions for 3 to 4 weeks or until the seed was mature. The solutions were not refreshed after the start of the experiment, but the glass Erlenmeyer flasks were covered with aluminum foil to reduce fungal and algal growth. Since the panicles elongated in the solutions, stems were recut to

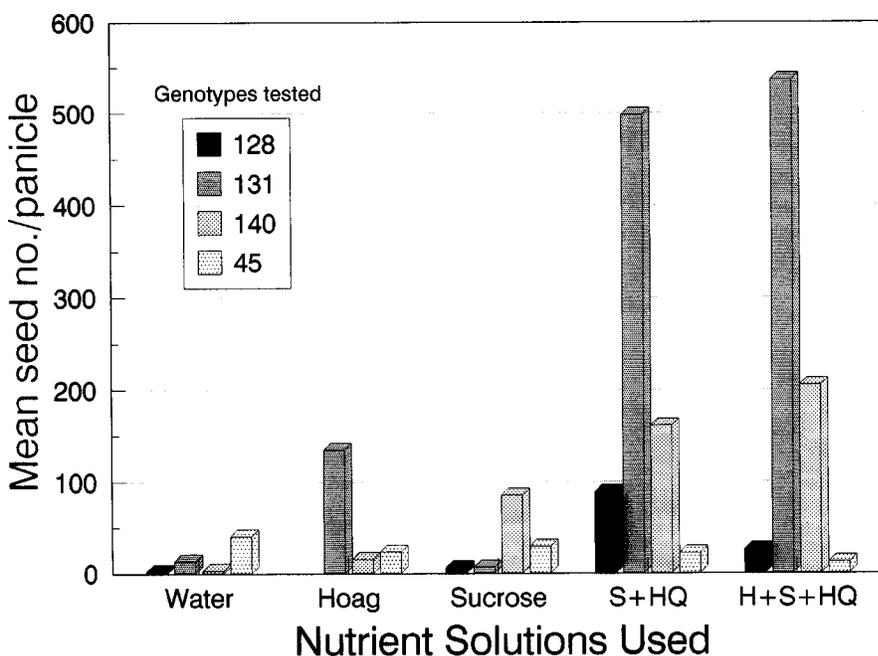


Fig. 1. Seed production in *Pennisetum* hexaploid hybrids using the detached culm method. Four genotypes (45, 128, 131, and 140) and five nutrient solutions were used. Hoag = Hoagland's; S + HQ = sucrose + hydroxyquinoline sulfate; H + S + HQ = Hoagland's + sucrose + hydroxyquinoline sulfate.

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50 to 60 cm at 7 days. Seeds were harvested from each group of two inflorescences and placed in paper bags for subsequent analysis. After collection, seed was dried for 1 week at 41C and then stored in an air-conditioned laboratory at 23C and 54% relative humidity.

Seeds were extracted from the inflorescences by using a pneumatic seed shucker (Ag Renewal, Weatherford, Okla.) To ensure that all seeds were extracted, a rubber-covered rubbing board was used to remove glumes, lemmas, and paleas from the caryopses. The caryopses from each treatment were weighed immediately after extraction, and the number of seeds was counted using an automatic seed counter (Count-a-pak; Chicago). Four weeks after seeds were extracted, germination tests of seed from each treatment were performed in a germinator at 27C.

Table 1. Main effects of nutrient treatments on seed characteristics.

Treatment	Seed/inflorescence		Germination (%)
	Wt (g)	Count	
Water	0.015 B ^z	14.4 B	2.1 B
Sucrose (S)	0.029 B	31.7 B	15.1 B
Hoagland's (H)	0.031 B	42.9 B	3.4 B
S + HQ ^y	0.309 A	192.5 A	38.8 A
H + S + HQ ^y	0.239 A	194.5 A	35.3 A

^zMean separation within a column by Duncan's multiple range test at $P < 0.05$.

^yHQ = hydroxyquinoline sulfate.

Results and Discussion

Seed set was unequal among the five nutrient treatments and among the four genotypes tested (Fig. 1). Water alone did not support high seed set and neither did the Hoagland's solution. Although the sucrose solution facilitated production of some seeds, the seeds were low in weight and did not germinate well. The best nutrient solutions were 2% sucrose + 0.02% HQ or Hoagland's + 2% sucrose + 0.02% HQ. Genotype 131 produced 10-fold more seeds than genotype 45. All of the genotypes produced some seed, with germination >35% in the HQ treatments (Table 1), which indicates that the cut-culm technique is a possible way of getting RRPS seed in *Pennisetum* hexaploid hybrids. There were no significant genotype \times treatment interactions, and none of the replications showed a significant F value (Table 2). Despite high cvs (116 to 225), differences existed. The cvs were high because we used few experimental units (two inflorescences per treatment) or possibly because of the high summer temperatures in the greenhouse.

This greenhouse experiment clearly showed that *Pennisetum* hybrids can produce the quantities of seed necessary to support RRPS breeding if sucrose (2%) and HQ (0.02%) are combined in the nutrient solution. With RRPS breeding, 100 to 500 seeds would be a sufficient quantity. The sucrose provides the necessary energy source required for seed production, and the HQ serves as a mold inhibitor,

which keeps the stems fresh for a longer period than sucrose solution without HQ, a well-documented result with cut flowers (Han et al., 1990; Marousky, 1971; Tjia et al., 1987). Our study also showed that the genotype of potential parents may influence the amount of seed produced. Three of the four genotypes produced seed with the Hoagland treatment. A preliminary screening for seed set using sucrose + HQ would ensure quantities of seed >100.

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Table 2. Analysis of variance tables for seed weight, seed count, and germination (nontransformed).

Source	df	Seed					
		Wt		Count		Germination (%)	
		F value	P > F	F value	P > F	F value	P > F
Model	21	1.99	0.031	2.45	0.008	2.74	0.003
Genotype (G)	3	4.03	0.014	5.56	0.003	3.02	0.417
Treatment (T)	4	2.93	0.033	3.61	0.014	7.44	0.0002
Replication	2	0.35	0.708	0.28	0.758	1.05	0.360
G \times T	12	1.44	0.190	1.65	0.119	1.39	0.2131