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Optimizing seed quality during germplasm regeneration in pearl millet

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Abstract

The effect of water stress during flowering and grain filling on seed longevity was studied in three pearl millet genotypes, ICTP-8202, ICTP-8203 and MBH-110. The seeds were produced by three pollination methods; open pollination, selfing (individual panicles enclosed in paper bags), and cluster bagging (panicles from 3–4 adjacent plants enclosed in a paper bag), stored in air-tight plastic bottles under ambient conditions (20–40 °C, 30–80% RH) and germination was tested at 12-month's intervals. The seeds lost germination completely after six years of storage in all treatments. Analysis of variance of the estimates of potential seed longevity (i.e. the seed lot constant K_i of the seed viability equation) showed significant effects of water stress and pollination method ($P < 0.01$). The interaction between irrigation treatment and method of pollination control was also significant ($P < 0.05$). Averaged over genotypes and pollination methods, potential longevity was greatest ($K_i = 2.8$) in the irrigated control, and averaged over genotypes and irrigation treatments, it was greatest ($K_i = 3.1$) in seeds produced by open pollination. The implications of these results were discussed in relation to germplasm seed production.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal grown in marginal areas with very low rainfall. The genebank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India holds the world collection of more than 21000 accessions of both cultivated and wild *Pennisetum* species. Most germplasm accessions are landraces, which are heterogeneous mixtures of genotypes and vulnerable to genetic shift during regeneration. The problem is further complicated by the protogynous and cross pollinating nature of the crop. Like in other outbreeding species, the genetic integrity of pearl millet landraces could be maintained by preventing contamination by foreign pollen or by isolating different genotypes through space (Breeze 1989). Some amount of genetic shift is inevitable with each regeneration. However, the frequency of regeneration could be minimized by maximizing seed longevity. Potential longevity of seeds depends on initial seed quality, which in turn is influenced by several factors such as crop management, seed production environment, maturity, and harvesting and drying practices (Kameswara Rao and Sastry 1998). The genebank curator should therefore adopt appropriate seed production methods that would minimize genetic shifts and maximize seed quality.

Pearl millet is mostly grown as a rain-fed crop and sown with the onset of rains in arid and semi-arid regions, hence deficiency in soil moisture during later stages of growth often impairs seed production. There are several reports on the effect of water stress during flowering and seed filling on the grain yield in pearl millet (Lahiri and Kharabanda 1965; Mahalakshmi et al. 1985, 1988), but information on seed quality and storage longevity is largely missing. In corn and soybean, water stress during seed maturation was reported to affect the seed quality (Cloninger et al. 1975; Vieira et al. 1992). Sorghum and finger millet

seeds produced with frequent irrigation (10–11 days) had better quality and storability than those produced with less frequent irrigation (Vanangamudi et al. 1990a, 1990b). In brassica, however, maximum potential longevity was greatest in seeds when irrigation was stopped 16 days after pollination compared to those from irrigated controls (Sinniah et al. 1998).

At ICRISAT, pearl millet landraces are maintained by cluster bagging (Appa Rao 1980) where panicles from 3 to 4 adjacent plants are enclosed in a single paper bag before anther dehiscence and an equal quantity of seed from each panicle is bulked at harvest to reconstitute the accession. However, genetic stocks are maintained by selfing i.e., covering individual panicles in parchment paper bags. In both these methods, the micro-climate around the panicles in the bags is likely to be different from that around open pollinated panicles or in isolation grow-outs. This could possibly influence initial seed quality and subsequent longevity. We report here the results of our study on the effect of water stress and different methods of germplasm maintenance on pearl millet seed longevity.

Materials and methods

Three methods of pollination (open, selfing and cluster bagging) and two levels of irrigation (irrigated control and stress imposed during flowering) were studied for their effect on seed longevity in three genotypes, ICTP-8202, ICTP-8203, and MBH-110. The experiment was laid out in a split-split plot design with three replications in the postrainy season in alfisols at ICRISAT, Patancheru, India, during 1991. Each genotype was planted in eight rows, each 4 m long, and spaced 75 cm apart. All plots were irrigated at 10 day intervals until flowering. While irrigation continued until maturity in the control, it was withheld immediately after 50% flowering in the plots sown for water stress treatments. Selfing was achieved by covering the individual panicles in paper bags, prior to anthesis. In cluster bagging, one panicle each from 3–4 adjacent plants was enclosed in a single paper bag. In each case, the seed was harvested at maturity, cleaned, dried under shade and stored in air-tight plastic bottles under ambient (20–40 °C, 30–80% RH) conditions. The moisture content of the seeds was determined by the high constant temperature oven method (International Seed Testing Association 1996a, 1996b) before storage. The initial germination was tested using 200 seeds, in four replicates each of 50 seeds. The seeds were plated on top of a moist filter paper (Whatman 181) in 9 cm Petri dishes and incubated at an alternating temperature of 20/30 °C (16/8 h). Germination counts were made 7 days and 14 days after incubation. Seedlings, which produced normal roots and shoots, were considered as germinated following the rules of the International Seed Testing Association (International Seed Testing Association 1996a, 1996b). Seed samples were drawn from storage and subsequent tests for germination were conducted every 12 months. The moisture content of the seeds stored for six years was also determined to assess changes, if any during storage.

Seeds of the same harvest conserved in the genebank at 4 °C and 20% RH were evaluated for microflora infection to draw inferences on observed longevity under ambient conditions. Fifty seeds, as five replicates of 10 seed each were sown on moist blotting paper lined in the lower lids of Petri dishes. The seeds were incubated for 7 days at 22 °C under 12 h Near Ultra Violet light cycle. Each seed was examined under a stereomicroscope for the presence of fungal and bacterial growth and percent seed infection was determined.

The relative humidity and temperature were recorded with the Dickson Temperature/Humidity Monitor to find the differences in the microenvironment around the panicles with the three methods of pollination control. The observations were recorded three times a day at 9.00, 13.00 and 16.00 h from three randomly selected areas in each plot.

Results and discussion

The moisture content of the seeds determined after six years of storage ranged between 9.4% and 10.6% in the various treatments, which was close to the initial moisture contents (≈10%) observed at the beginning of storage. The seeds deteriorated gradually and lost complete germination after six years, in all the treatments. However, differences were apparent among treatments, as seeds harvested from water stressed plots lost viability faster than those from irrigated plots, and seed produced by cluster bagging deteriorated faster than those obtained by selfing and open pollination (Figure 1). The seed survival curves were sigmoid, therefore the normal germination percentages were subjected to probit analysis, where regres-

sion was performed on transformed percentage germination against time in storage according to the equation,

$$v = K_i - p/\sigma,$$

where v is probit viability after p days of storage, K_i is the seed lot constant and σ is the standard deviation of seed deaths over time (Ellis and Roberts 1980). The potential longevity of the seeds was quantified by the value of the seed lot constant (K_i) which was provided by the intercept of the transformed seed survival curves. Within each genotype, the slopes of the survival curves ($1/\sigma$) were found to be similar ($P > 0.05$), therefore, the seed lot constants (K_i) were computed by constraining the survival curves to a common slope. Analysis of variance of the estimates of seed lot constants (K_i) showed significant differences between the two irrigation treatments and among the methods of pollination (Table 1). The interaction between irrigation treatment and the method of pollination control was also significant ($P < 0.05$). The differences between varieties although significant, their interaction with irrigation treatments and pollination method was insignificant ($P > 0.05$), indicating that the response of varieties to water stress and method of maintenance was similar. From irrigated plots, the potential longevity of seeds produced by open pollination was greatest ($K_i = 3.2$), followed by selfing ($K_i = 2.9$) and cluster bagging ($K_i = 2.3$). Among seeds harvested from water stressed plots, those produced by open pollination had the highest potential longevity ($K_i = 3.1$), compared to selfing ($K_i = 2.2$) and cluster bagging ($K_i = 1.3$). The differences in potential longevity were marginal for seeds obtained by open pollination from the irrigated and water stressed plots. However, differences in longevity of seeds produced by selfing and cluster bagging from irrigated and water stressed plots were significant ($P < 0.05$; Table 2). Averaged over genotypes and pollination methods, potential longevity of seeds harvested from irrigated plots was higher ($K_i = 2.8$) than that from water stressed plots ($K_i = 2.2$), and averaged over genotype and irrigation treatments, potential longevity of seeds was greatest ($K_i = 3.2$) in seeds produced from open pollinated panicles, followed by selfing ($K_i = 2.6$) and cluster bagging ($K_i = 1.8$). Among the three varieties used in these studies, differences in potential longevity of seeds were only marginal for the genotypes ICTP 8202 and MBH 110. Potential longevity of seeds of these two genotypes was significantly higher than in ICTP 8203 for all treatments ($P < 0.05$) (Table 2).

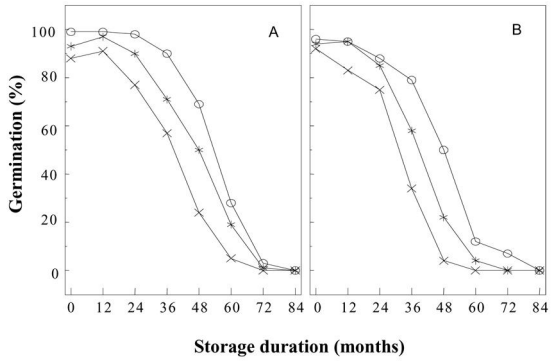

Figure 1. Survival curves (germination percentage plotted against time in storage, mean of three genotypes) in pearl millet seeds produced under (A) irrigated and (B) water stressed conditions using three pollination methods, (○) open pollination, (*) selfing and (×) cluster bagging.

The results clearly demonstrate that seed quality would be inferior when produced by cluster bagging under water stress, and highest when produced by open pollination under irrigated conditions. In cluster bagging, the microenvironment within the bag, characterized by high relative humidity, could have affected the quality of developing seeds. Enclosing several panicles in a single bag could increase transpiration, resulting in increased temperature and relative humidity within the bags, making it ideal for the growth of seed-borne pathogens. In selfing, since individual heads are enclosed in paper bags, the relative humidity within the bags is expected to be lower than that in cluster bagging, resulting in lesser microbial growth. In the open pollinated panicles, the possibility of humidity buildup around the panicles would be less because of air movement, with consequent reduction in microbial growth. Results from seed health tests corroborated these assumptions. A total of 20 fungi, belonging to 13 genera were found to be associated with the seeds. *Aspergillus* and *Penicillium* spp. were predominant on seeds produced by cluster bagging (Table 3) which in general, correlated with the observed loss of seed viability. The mean (±s.e.) relative humidity (%) and temperature (°C) were found to be higher with cluster bagging (51.2 ± 0.06 and 32.3 ± 0.05, respectively) and selfing (44.4 ± 0.09 and 31.6 ± 0.02, respectively) than in open pollination (37.0 ± 0.10 and 30.2 ± 0.02, respectively), which possibly favored increased microbial growth, consequently affecting initial seed quality in these treatments.

In contrast to the findings in brassica, where termi-

Table 1. Analysis of variance for potential longevity of pearl millet seeds produced by three methods of pollination under irrigated and water stressed conditions.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value
Genotype (G)	2	6.8362	63.4196	0.008**
Water stress (S)	1	5.0803	5.0803	0.003**
Pollination method (M)	2	16.8812	8.4406	< 0.001**
G × S	2	0.1908	0.0954	0.680
G × M	4	0.8637	0.2159	0.383
S × M	2	2.1825	1.0874	0.011*
G × S × M	4	0.3897	0.0974	0.742
Residual	24	3.2909	0.1371	

** Significant at 1%; * Significant at 5%.

Table 2. Potential longevity of pearl millet seeds produced by three methods of pollination under irrigated and water stressed conditions.

Treatment/Genotype	Potential longevity (K_i)		
	Open pollination	Selfing	Cluster bagging
Irrigated control			
ICTP-8202	3.53	3.03	2.64
ICTP-8203	2.66	2.45	2.00
MBH-110	3.50	3.23	2.11
Mean	3.23	2.90	2.25
Water stressed			
ICTP-8202	3.35	2.48	1.62
ICTP-8203	2.64	1.50	0.66
MBH-110	3.27	2.62	1.50
Mean	3.08	2.20	1.26

LSD (5%) = 0.74.

nal drought resulted in maximum seed quality, results from the present studies showed that water stress during flowering reduces potential seed longevity. In pearl millet, flowering and grain-filling stages were reported to be most sensitive periods to water stress (Lahiri and Kharabanda 1965; Mahalakshmi and Bidinger 1985). In the present study, water stress imposed during flowering may have impaired normal seed development, and resulted in inferior quality. Water stress has greater effect on longevity of seed produced by cluster bagging and selfing, than by open pollination. The high relative humidity during seed maturation might have increased the adverse effect of water deficit on initial seed quality. It is therefore evident that pre-harvest water management affects seed quality and subsequent longevity, and in order to have seeds of highest quality for conservation, water stress should be avoided while regenerating germplasm accessions. Regeneration should be done in locations with assured irrigation or the crop should be planted early to escape terminal drought under rainfed conditions. Based on the results of this experiment, it could be surmised that seeds with maximum potential

Table 3. Pathogens associated with pearl millet seeds produced by three methods of pollination under irrigated and water stressed conditions.

Pathogen	Frequency (%)																	
	ICTP-8202						ICTP-8203						MBH-110					
	Irrigated			Water stressed			Irrigated			Water stressed			Irrigated			Water stressed		
	OP	S	CB	OP	S	CB	OP	S	CB	OP	S	CB	OP	S	CB	OP	S	
Aspergillus spp.	10	4	30	8	8	8	12	6	36	18	40	48		2	2	2	4	4
Penicillium sp.	8	10	8	2	14	52	14	26	14	8	2	14			12			2
Alternaria spp.		2	4	2				4	2	2	2							
Cladosporium sp.	2							4		2	2							
Curvularia spp.			8	2	12	8	6	16	2	6	2	2			4		2	
Fusarium sp.	2			2		2	2			6							2	
Bipolaris sp.			2	2			2											
Nigrospora sp.		6	2	2	2		2	2		6						2		2
Epicoccum sp.								2										
Others	2	6			6			2						2	2			
Total	24	28	54	20	42	70	38	62	54	48	48	64	0	4	20	4	8	8

OP = Open pollination, S = Selfing, CB = Cluster bagging.

longevity could be harvested when pearl millet landraces are regenerated by spacial isolation, avoiding the use of any artificial barriers to prevent outcrossing. However, isolation becomes difficult when several hundred germplasm accessions have to be regenerated at one time. Although potential longevity of seeds produced by selfing was greater compared with cluster bagging, selfing cannot be used to maintain pearl millet landraces because of inbreeding depression. However, useful inferences could be drawn from the results, as sib-mating (pollination of one receptive panicle on each plant with a mixture of pollen from the accession) which also involves bagging individual panicles is likely to produce seeds similar in quality to selfing. Nevertheless, cluster bagging remains the most convenient and relatively less expensive method to maintain pearl millet landraces compared to sib-mating and it should be possible to improve seed quality by minimizing the bagging duration or use of different bagging material to improve aeration.

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