Germplasm conservation strategies – impact of conditioning on the viability of dry pearl millet seeds

DVSSR Sastry, HD Upadhyaya* and **CLL Gowda**

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

*Corresponding author: h.upadhyaya@cgiar.org

Citation: Sastry DVSSR, Upadhyaya HD and Gowda CLL. 2011. Germplasm conservation strategies – impact of conditioning on the viability of dry pearl millet seeds. Journal of SAT Agricultural Research 9.

Introduction

Seed deterioration is a continuous process and conservation of germplasm in ex-situ facilities necessitates techniques that prolong seed longevity. A combination of 3–7% seed moisture content (mc) and a storage temperature below 0°C is suitable for long-term preservation of orthodox seeds (FAO/IPGRI 1994). When seeds of different crops are dried to low moisture levels, there is a decrease in weight and volume and when large seeds dry too rapidly, the outside of the seed loses moisture more rapidly than the inside of the seed and it reduces in volume more quickly leading to cracking of the seed coat (Ellis et al. 1985). Internal drying stresses also increase susceptibility of seeds to mechanical injuries and the dryness of seeds is also critical to the occurrence of imbibition injury (Ellis et al. 1990). Imbibition injury occurs in standard germination tests of very dry seeds, even when the germination medium is of low osmotic potential. In addition to legume seeds, problems of imbibition injury have been encountered with forage legumes, cotton and sorghum (*Sorghum vulgare*) (Ellis et al. 1985). Imbibition injury to seeds depends on several factors such as seed maturation, age, mc and storage temperature (Powell and Matthews 1979, Tully et al. 1981, Taylor and Prusinski 1990). Imbibition injury to seeds is a potential problem for genebanks handling germplasm samples of very dry seeds especially in meeting the requirements for long-term storage. A critical mc below which a constant proportion of seeds fail to germinate as a result of imbibition injury and the susceptibility of very dry seeds to imbibition damage has been reported by Ellis (1987) and Ellis et al. (1990).

Need for humidification

The aim of humidification (Rao et al. 2006) is to increase seed mc slowly to 16–18% mc by absorption of water vapor instead of rapid imbibition of liquid water. Seeds at 8% mc and below, irrespective of species, should routinely be humidified before germination tests. Seed of some accessions at higher mc (8–12%) also benefit from humidification. The humidified seeds will age much more rapidly than the dry seeds and consequently some loss in viability may occur (Ellis et al. 1985) and a germination test is usually the best method of estimating seed viability.

In genebanks, when large numbers of accessions from base collections (long-term storage) are to be tested or monitored for germination, humidification of many such samples (sometimes in thousands) following the recommended humidification procedures is rather difficult and resource demanding. Considering these limitations an experiment was conducted using pearl millet (*Pennisetum glaucum*) germplasm seed accessions conserved in the genebank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.

The genebank maintains 22,211 accessions of pearl millet from 50 countries as active collection (medium-term conservation at 4°C and 30% RH) and as base collection (long-term conservation at –20°C) for present and future use. The collection represents wide range of diversity for different morphoagronomic characters including some important seed traits like shape, size and texture (Upadhyaya et al. 2007). Pearl millet seeds belong to orthodox seed nature based on their behavior before and during storage (Roberts 1973) and pearl millet germplasm seed lots could be safely dried to about 5% mc at 15°C and 15% RH using sorption type drier with secondary refrigeration (Sastry et al. 2003). The objective of this study is to assess the duration of humidification treatment for pearl millet germplasm seeds dried to lower moisture levels for storage and their influence on seed viability.

Materials and methods

A set of 30 accessions from the global pearl millet collection representing wide diversity for seed traits

(seed size, shape, 1000-seed weight and endosperm texture) was used for the study. Freshly harvested seed samples from 2005 postrainy season regeneration at ICRISAT, Patancheru were used for the study. Hand-threshed and cleaned seed samples were held in cloth bags under short-term storage conditions (25°C and 40% RH) to achieve equilibrium moisture levels (critical mc under these conditions) in all the samples for recording seed weight and before keeping the seed samples for drying. Test weight (g per 1000 seeds) of the accessions was recorded using a precision balance on a randomly drawn sample. For drying, about 200 g clean seed in each accession was collected in perforated muslin cloth bags which permit free flow of air during the drying period. These bags were held uniformly on shelves in a walk-in seed drier maintaining 15°C and 15% RH using sorption type drier with secondary refrigeration. Drying was continued until the seeds reached equilibrium moisture levels. Seed samples were drawn for moisture estimation following oven-dry method using two replicates each of 5 g (ISTA 1993). Separate samples of about 20 g seed were collected in paper bags with covers opened and equilibrated under ambient conditions (25°C and 50% RH) for 72 hours. Seed mc in these samples was estimated as described earlier. Dry seeds were humidified for 24 hours and 48 hours following standard procedures (Rao et al. 2006) before testing viability. Seed mc of the humidified samples was estimated based on the initial moisture values and changes in the weight of humidified seed and expressed on wet weight basis. Seed viability was recorded from a standard germination test on a random sample of 100 seeds (two replicates) following 'Between Paper' method (ISTA 1993). Data were analyzed using Genstat 9.1.

Results and discussion

Observations on seed mc and viability of pearl millet accessions under different periods of humidification are presented in Table 1. Test weight of the accessions ranged from 2.4 to 15.8 g representing wide diversity among the accessions used in this study. Seed drying in controlled environment was uniform and no significant differences were observed among the accessions. Seed mc of dry seeds ranged from 5 to 7% with a mean of 6.1%, a moisture level recommended for long-term conservation (FAO/IPGRI 1994).

Changes in seed mc among humidification treatments were significant. Humidification for 24 hours increased seed mc from 6.1 to 13.8% and further to 16.5% in 48 hours. Seed mc ranged from 12.8 to 15.1% among accessions after 24 hours and was 15.7–17.7% after 48 hours of humidification. These values were similar to the seed moisture levels recommended by several workers (Ellis et al. 1985) before germination testing. The accessions showing low and high mc are represented by wide range in 1000-seed weight (5.4–15.8 g) suggesting that the initial seed weight has no influence on moisture increase during humidification. In the ambient stored (72 hours) seed samples in paper bags, the mean seed moisture increased from 6.1 to 10.9% with a narrow mc range (10.2–11.4%).

The mean viability of dry seeds, humidified for 24 hours, 48 hours and ambient stored seeds was 94.7%, 92.8%, 90.2% and 94.6% respectively. The viability range across the treatments was 76–100%. Significant differences were observed for initial viability (dry seeds) among the tested accessions in all the treatments. The lowest viability was observed in IP 17989 in dry seeds as well as under 24-hour and 48-hour humidification treatments, while IP 14930 recorded highest viability in all the treatments. When dry seeds were tested for germination, 16 accessions had ≥95% viability and two accessions had <90% viability. There are several factors such as crop management and seed production environment contributing to initial seed quality including viability during regeneration (Kameswara Rao and Sastry 1998). The number of accessions losing viability gradually increased (18 accessions) with 24-hour humidification and further (28 accessions) with 48-hour duration compared to dry seeds. The increased seed moisture levels of 13.8% and 16.5% due to humidification thereby contributed negatively to the viability and vigor of pearl millet seeds. The deleterious effects were pronounced by increased number of abnormal seedlings and diseased/dead seeds as observed from the germination test. As mentioned earlier, humidified seeds age much more rapidly than dry seeds and consequently some loss in viability may occur (Ellis et al. 1985). The seed viability levels of 72-hour ambient stored seed samples were not significantly different from those of dry seeds and majority of the accessions had maintained the initial viability levels. Non-occurrence of hard seeds across the treatments was an important observation in this study and hence no benefits of humidification in pearl millet.

Conclusions

Diverse pearl millet seeds could be safely dried and germinated over wide seed moisture regimes (5–11%). Such processes save time, space and resources for handling large numbers of germplasm accessions during testing/monitoring seed viability. In genebanks, collecting a random sample from dry seeds for germination test is a regular process during packaging. These could be comfortably preserved for shorter periods under ambient conditions

Table 1. Seed moisture content (mc) and viability of pearl millet germplasm accessions under different conditions of humidification periods.

IP no.	1000-seed weight (g)	Seed mc (%)				Seed viability (%)			
		Dry seed	Humidified (24 h)	Humidified (48 h)	Ambient stored (72 h)	Dry seed	Humidified (24 h)	Humidified (48 h)	Ambient stored (72 h)
8151	8.3	5.6	14.1	16.7	10.7	92	94	90	94
8664	10.3	6.0	13.9	16.5	11.0	97	93	92	94
8864	5.4	5.7	13.0	15.7	10.5	98	97	96	97
11263	8.5	6.2	13.5	16.2	10.8	97	96	90	97
11349	13.1	6.6	14.0	16.6	10.8	97	97	95	99
12470	6.4	6.5	13.9	16.3	10.7	94	93	89	94
12619	8.1	5.7	13.8	16.4	10.8	99	97	95	99
12781	6.2	6.3	14.8	17.4	10.8	98	96	95	96
12890	11.5	6.5	14.7	16.8	10.6	96	92	94	98
13001	10.7	6.8	14.3	16.9	11.3	95	86	84	92
14605	9.9	6.0	13.0	15.7	11.2	92	90	87	92
14930	2.4	5.8	13.4	16.0	11.0	100	100	98	100
15117	7.3	6.3	13.2	15.9	11.0	93	93	90	95
15246	5.7	5.8	13.9	16.4	10.5	93	94	91	93
15248	6.7	5.8	14.1	16.8	11.0	91	91	88	92
15319	5.2	6.0	13.9	16.5	10.5	97	95	93	97
15345	9.8	6.3	15.1	17.7	10.5	94	95	94	97
15351	5.4	5.2	13.5	16.2	10.2	96	97	96	96
15379	5.5	6.2	13.3	16.2	10.9	93	93	89	96
15464	5.9	5.8	13.5	16.4	10.8	88	87	83	87
15593	13.9	6.0	14.4	16.9	11.3	93	89	87	91
15617	11.1	6.1	12.8	15.7	11.3	91	91	88	93
15663	9.3	7.0	13.7	16.4	11.3	96	97	90	94
15769	7.9	6.2	15.0	17.7	11.4	98	92	88	98
15922	15.8	6.5	13.0	15.7	11.4	97	95	94	96
15928	14.6	6.7	14.5	17.2	11.3	93	89	86	93
17981	9.8	5.2	13.8	16.8	10.7	98	94	94	95
17987	10.3	5.6	13.3	16.3	10.9	92	87	83	92
17989	7.0	5.0	14.3	17.2	10.7	87	78	76	87
20677	10.6	6.1	13.6	16.3	10.7	95	95	91	95
Range	2.4–15.8	5.0–7.0	12.8–15.1	15.7–17.7	10.2–11.4	87–100	78–100	76–98	87–100
Mean	8.7	6.1	13.8	16.5	10.9	94.7	92.8	90.2	94.6
SE±	0.571	0.086	0.111	0.102	0.058	0.578	0.803	0.875	0.574

for testing germination directly. However, for pearl millet seed stored at ultra dry moisture levels, there is a need to identify humidification requirements.

References

Ellis RH. 1987. Monitoring the viability of seed accessions. Plant Genetic Resources Newsletter 71:16–21.

Ellis RH, Hong TD and **Roberts EH.** 1985. Handbook of seed technology for genebanks. Volume I. Principles and methodology. Rome, Italy: IBPGR.

Ellis RH, Hong TD and **Roberts EH.** 1990. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibition damage. Seed Science and Technology 18:131–137.

FAO/IPGRI. 1994. Genebank standards. Rome, Italy: Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute.

ISTA. 1993. International rules for seed testing. Seed Science and Technology 21 (Supplement):1–288.

Kameswara Rao N and **Sastry DVSSR.** 1998. Seed quality considerations in germplasm regeneration. Pages 144–149 *in* Regeneration of seed crops and their wild relatives. Proceedings of a Consultation Meeting, 4–7 December 1995, ICRISAT, Hyderabad, India. Rome, Italy: International Plant Genetic Resources Institute.

Powell AA and **Matthews S.** 1979. The influence of testa conditions on the imbibition and vigour of pea seeds. Journal of Experimental Botany 30:193–197.

Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and **Larinde M.** 2006. Manual of seed handling in genebanks. Handbooks for Genebanks 8. Rome, Italy: Bioversity International.

Roberts EH. 1973. Predicting the storage life of seeds. Seed Science and Technology 1:499–514.

Sastry DVSSR, Kameswara Rao N and **Bramel PJ.** 2003. Seed drying under controlled environment for long-term conservation of germplasm. Seed Research 31(2):148–153.

Taylor AG and **Prusinski J.** 1990. The influence of seed moisture content and mechanical damage on germination, imbibitional chilling injury and leakage. Ann. Rep. Bean Improv. Cooper 33:143–144.

Tully RE, Musgrave ME and **Leopold AC.** 1981. The seed coat as a control of imbibitional chilling injury. Crop Science 21:312–317.

Upadhyaya HD, Reddy KN and **Gowda CLL.** 2007. Pearl millet germplasm at ICRISAT genebank – status and impact. Journal of SAT Agricultural Research 3. (ejournal.icrisat.org)