Jones, Oyatomi, Akinyele, Odiyi, Abberton and Hay (2020).
Seed Science and Technology, **48**, 3, 413-418.
https://doi.org/10.15258/sst.2020.48.3.10

Research Note

Effect of high temperature drying on seed longevity of Bambara groundnut (*Vigna subterranea*) accessions

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(Submitted May 2020; Accepted November 2020; Published online November 2020)
(Handling editor: A.A. Powell)

Abstract

In this paper, we considered the effects of different drying regimes on the subsequent longevity of Bambara groundnut seeds. Freshly harvested Bambara groundnut seeds from 27 genebank accessions were divided into five samples. One sample (control) was immediately dried at 17°C/15% RH and the other samples were dried at 45°C/35% RH for up to eight days, before transfer to 17°C/15% RH. After drying, seed moisture content was raised to 10.9% before packing the seeds in aluminium foil packets and placing at 45°C. Samples were removed at regular intervals for germination tests to compare seed longevity. Initial drying at the higher temperature resulted in a faster reduction in seed moisture content. The effect of the different drying regimes on subsequent longevity was not significant for most accessions. Nonetheless, the seed longevity of smaller-seeded accessions was perhaps enhanced by initial drying at a higher temperature; in contrast, initial drying at 17°C appeared to be the best drying treatment for larger-seeded accessions. Overall, the results suggest that initial drying at a higher temperature was beneficial for reducing processing time and for some accessions would be beneficial for seed longevity, but further work is required to understand for which seed lots.

Keywords: genebank, plant genetic resources, relative humidity, seed drying, seed longevity, storage environment, temperature

Experimental and discussion

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a self-pollinating annual plant that belongs to the Leguminosae family, sub-family Papilionoidae. It is the third most important grain legume in semi-arid Africa (Ocran *et al*., 1998). The seeds have high

nutritive value as they contain protein (19%), carbohydrate (63%) and fat (6.5%) (Goli, 1995). The seeds can be eaten fresh or boiled after drying and are consumed at different developmental stages. The immature seeds can be consumed fresh, boiled or grilled, or mixed with immature groundnuts or green maize (Bamshaiye *et al.*, 2011). It is resistant to high temperature and suitable for marginal soils where other leguminous crops cannot be grown (Yamaguchi, 1983). The plant is known for soil improvement because of nitrogen fixation and it makes very little demand on the soil (Baryeh, 2001). It is not prone to the risk of total harvest failure even in low and uncertain rainfall regions.

Seed longevity is an important agronomic factor in the preservation of seed physiological quality after harvest (Finch-Savage and Bassel, 2016) and maximising seed longevity is crucial for genetic resources conservation. The longevity of seeds in storage is influenced by the initial quality as well as storage conditions. In legumes, longevity is progressively acquired during seed maturation, from seed filling onwards (Zanakis *et al*., 1994; Verdier *et al*., 2013; Righetti *et al*., 2015; Leprince *et al*., 2017). Upon harvest, seed longevity is then dependent on the moisture content of the seeds, storage temperature (Ellis and Roberts, 1980) and, to a lesser extent, availability of oxygen (Ellis and Hong, 2007). The Genebank Standards advise storing seeds hermetically after drying at 5-20°C and 10-25% RH (FAO, 2014), however it has been reported that drying at a higher temperature results in increases in seed longevity under experimental storage conditions, at least for some species produced under tropical conditions (Whitehouse *et al*., 2015, 2018). The study described in this paper was conducted with the objective of determining the optimal drying temperature for subsequent seed longevity of Bambara groundnut seeds in storage.

This research was conducted at the seed processing laboratory of the genebank of the International Institute of Tropical Agriculture (7°30' N, 3°54' E), Ibadan, Nigeria (7°30' N, 3°54' E) using 27 accessions of Bambara groundnut obtained from the genebank. Seeds sampled from genebank storage were sown on 30 August 2017 into 10 × 10 m plots with inter-row spacing of 100 mm and intra-row spacing of 1 m. The planting depth was 5 mm with one seed per hole. The first irrigation was carried out one week after planting with subsequent irrigation every two weeks until senescence to prevent the pods from rotting. Fertilizer application (side dressing) was done two weeks after planting, using triple super phosphate produced by Elixir Garden Supplies (Morecambe, UK) at the rate of 5 ml per plant. Seeds were harvested on 10 February 2018. A small sample of freshly harvested seeds (pods) was hand-threshed and used to determine the moisture content at harvest with the aid of a Sinar Agripro seed moisture reader (Sinar Technology, Camberley, Surrey, UK). The harvest moisture content ranged between 8.0% (accession TVSu-1804) and 15.4% (TVSu-609).

Freshly harvested seeds (still in their pods) were divided into five samples. One sample per accession was dried at low temperature according to the normal genebank process, in a drying room at 17°C/15% RH, for 14 days. The remaining four samples were dried for 2, 4, 6 or 8 days at 45°C/35% RH and then transferred into the genebank drying room for 14 days for final equilibrium drying. Seeds placed directly into the drying room, gradually lost moisture over the 14 days, reaching a mean moisture content of 6.6% (figure 1). This is a little higher than the expected moisture content of Bambara groundnut seeds at equilibrium with these conditions (5.8%), calculated using the Seed Viability Constants module of the Seed Information Database (Royal Botanic Gardens Kew, 2020),

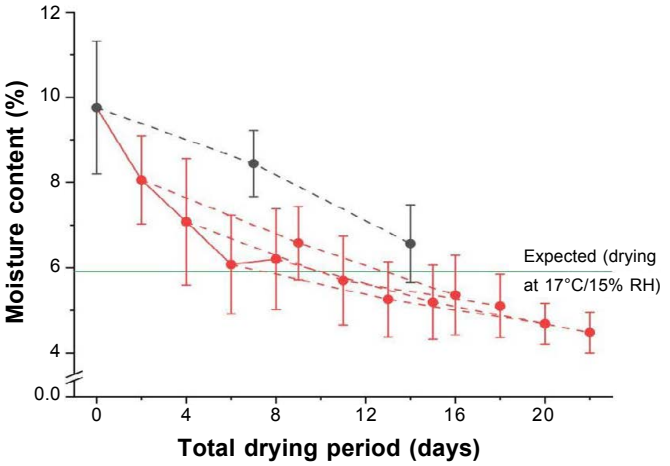

Figure 1. The moisture content of Bambara groundnut seeds during drying in the IITA genebank drying room at 17°C/15% RH (dashed line) or initially dried at 45°C/35% RH (solid line) for 2, 4, 6 or 8 days, before transfer to the drying room. Data shown are the mean of 27 accessions. The expected equilibrium moisture content of Bambara groundnut seeds is also shown (calculated using the Viability Constants tool of the Seed Information Database; Royal Botanic Gardens Kew, 2020).

based on a mean seed oil content for these accessions of 6.36% (s.d. 0.63, range 4.93-7.48%), determined using a Soxtec System HT2 fat extractor (Foss Analytical, Hilleroed Denmark)). Seeds initially dried at 45°C lost moisture more quickly, reaching 6.1% after six days; further drying at this temperature did not result in significant further change in moisture content. Upon transfer to the drying room, seed moisture content declined more gradually, but the final moisture content was consistently lower for seeds initially dried at higher temperature than for seeds only dried in the drying room. This suggests that initial drying at a higher temperature than the genebank currently uses is more efficient with respect to processing time.

After drying, seeds were sealed in aluminium foil bags and stored at 5°C for 16 days until the storage experiments commenced. Seed samples in their foil bags were allowed to equilibrate to laboratory temperature before opening. Each seed sample was weighed and transferred into individual net bags. Bags of seeds were placed over water in an incubator (Percival Scientific, Perry, Iowa, USA) set at 25°C until they reached the target moisture content of 10.9%. The moisture content of the seeds was estimated based on the change in weight of each sample, which was measured daily. Once each sample had reached the desired moisture content, each seed lot was divided into subsamples according to their drying treatment (accession × drying treatment) and sealed inside aluminium foil bags which were placed in an incubator at 45°C. One bag per treatment was removed on different days for up to 60 days for germination testing. For each germination test, three samples of 20 randomly selected seeds of each seed sample were sown on wet germination paper in a plastic germination box. Germination boxes were tightly closed and then transferred to a germination room where the temperature ranged between 25 and 30°C. Counts of germination were made on day-10 (first count) and day-15 (final count) after sowing.

Data were analysed using GenStat 20th Edition (VSN International Ltd., Hemel Hempsted, UK). The germination data from the seed storage experiments were subjected to probit analysis, thereby fitting the Ellis and Roberts (1980) viability equation:

$$v = K_i + \left(-\frac{1}{\sigma}\right) p \qquad \text{[eqn. 1]}$$

where v = seed lot viability in probits after p days in the storage environment; K_i = initial viability of the seed lot in probits (seed lot constant) and σ = time (days) taken for seed lot viability to drop by one probit ($-1/\sigma$ is the slope of the survival curve plotted using a probit percentage scale and is the parameter estimated in the probit analysis). The data for different drying treatments within each accession were analysed together, constraining first σ and then both σ and K_i to the same values among the different seed

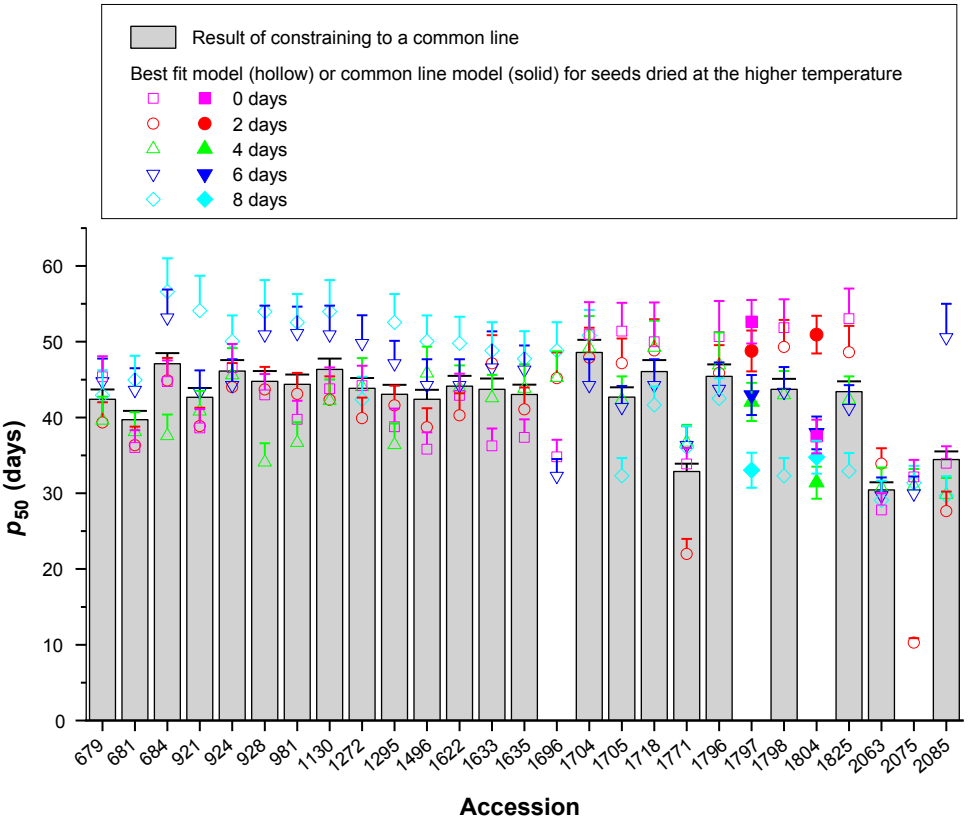

Figure 2. Estimates (± standard error) of the time for viability to fall to 50% (p_{50}) during experimental storage for Bambara groundnut seeds initially dried at 45°C for 0, 2, 4, 6 or 8 days. For the accessions for which it was possible to constrain the survival curves to a common line, p_{50} is represented by columns, with the result of the best-fit estimates represented by symbols. For those accessions where it was not possible to fit the data using a common line, the estimates shown are the result of fitting a best-fit or common slope model.

lots (drying treatments). An approximate *F*-test was used to determine whether either of these constrained models could be accepted without a significant increase in deviance. As part of the model fitting, the time (days) taken for germination to decline to 50%, p_{50}, was also estimated.

Seed longevity varied among accessions (figure 2). For 23 of the 27 accessions, it was possible to constrain the survival curves to a common line without a significant increase in residual deviance ($P > 0.05$). However, since the sampling intervals were not optimised and, further, some of the *F*-test results were only just significant (i.e. between $P = 0.05$-0.10), the best-fit estimates are also shown. For two accessions, TVu-1797 and -1804, it was possible to fit the data using a common slope model without a significant increase in residual deviance, while for the remaining two accessions, TVu-1696 and -2075, it was not possible to introduce any constraints into the model fitting, i.e. there were significant differences in both K_i and σ, and hence, p_{50}, depending on the drying treatment. Most of the accessions could be allocated to one of two groups according to which treatment resulted in the maximum p_{50} according to the best-fit model fitting: those for which initial drying at 45°C for eight days resulted in the highest estimate of p_{50} (e.g. TVu-921, -1130, -1496) and those for which immediate drying in the drying room (17°C) was best (e.g. TVu-1705, -1797, -1825). Although it appears that this sorting is according to accession number, accessions were not harvested sequentially and nor were the storage experiments conducted sequentially. There was however, a significant difference in the seed weight between the two groups, with those with a higher p_{50} after drying at the higher temperature more likely to have a lower seed weight (measured after final equilibrium drying) than those with a higher p_{50} after (one-tailed *t*-test carried out using GenStat: $P = 0.011$). The response did not appear to be related to the harvest moisture content (two tailed *t*-test using the same groupings as above: $P > 0.05$), as was seen in rice (Whitehouse *et al*., 2015, 2018). Given the variable response in Bambara groundnut, further investigations are required to understand the likelihood of a freshly-harvested seed lot responding positively or negatively to initial drying at a high temperature; whether the response is related to seed size; and whether other non-standard drying temperatures (i.e. >17°C) may be better than 45°C. However, there is perhaps a clear benefit of initial drying at a high temperature in relation to the length of time required to dry the seeds to an acceptable level for long-term genebank storage (figure 1; Justice and Bass, 1978). Thus, based on these and previous results, it may be useful to consider a two-step drying procedure to boost subsequent seed longevity in genebank storage and reduce the rate and hence cost and risks of regenerating accessions.

Acknowledgements

This work was undertaken as part of the Seed Quality Management activity of the CGIAR Genebank Platform. We would like to thank all funders who supported this research through their contributions to the CGIAR Trust Fund.

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