

# *Two-step drying of soya bean seed germplasm often improves subsequent storage longevity*

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# Two-step drying of soya bean seed germplasm often improves subsequent storage longevity

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**Abstract** It is recommended that seeds for genebank storage are dried to low moisture content at 10–25 °C, 10–15% RH, but in some crops, an initial warmer drying temperature may provide better longevity. Seeds of diverse accessions of soyabean (*Glycine max* (L.) Merr.) produced in three seasons and harvested shortly before or at harvest maturity were initially dried at 17, 30 or 40 °C with 15% RH and subsequently at 17 °C with 15% RH; seeds were fumigated either before or after initial drying. Seed longevity in hermetic storage at 45 °C with approximately 9% moisture content was affected by the initial drying treatments, but effects varied among treatment combinations and accessions. Seeds harvested before harvest maturity, at 10–14% moisture content, tended to benefit from warmer temperature drying.

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Fumigation before initial drying resulted in shorter longevity than fumigation afterwards for about 70% of harvested seed lots. Most treatment combinations across all experiments showed seed longevity was improved, often considerably, by 4 days' initial drying at 40 °C compared to drying at 17 °C throughout. Longevity was also improved in many treatment combinations (fewer than at 40 °C) by initial drying at 30 °C compared to 17 °C. In a minority of treatment combinations, seed longevity was reduced by drying at 40 or 30 °C compared to 17 °C. The research shows a potential benefit to subsequent seed longevity from initially drying soya bean seeds at 40 °C, rather than 10–25 °C, for most accessions, particularly those harvested early; this benefit may have been due to greater post-harvest maturation in the warmest regime.

**Keywords** Genebank · Plant genetic resources · Seed drying · Seed fumigation · Seed longevity · Soya bean

## Introduction

Conservation of plant genetic resources—cultivated plants, landraces and related wild species—by genebanks is vital for global research and development to improve crops, human nutrition, food security and promote sustainable agriculture. The role of genebanks is specifically mentioned in the United Nations Sustainable Development Goals: the aim

of Target 2.5 is to maintain the genetic diversity of seeds, cultivated plants and related wild species, through carefully managed seed and plant genebanks (UN 2015). The genebank of the International Institute of Tropical Agriculture (IITA) maintains a large and diverse collection of soya bean (*Glycine max* (L.) Merr.; Fabaceae) with 4948 unique samples from 237 countries, representing 70% of African cultivars and nearly half of the crop's global diversity (IITA 2024).

Soya bean is the most widely-grown legume in the world, the largest source of protein in animal feed, a significant source of protein for humans, a raw material for industrial purposes and the second largest source of vegetable oil (Abbey et al. 2001; Nout 2015; FAOSTAT 2021). As a legume, symbiotic dinitrogen fixation also gives it an advantage over cereal crops in terms of soil health and sustainability (Yusuf et al. 2008). The pods typically have one to three seeds per pod differing in shape, size and colour (Yang et al. 2024; Niu et al. 2013).

Seeds show orthodox seed storage behaviour, surviving desiccation to moisture contents as low as 5% without injury, thereby tolerating sub-zero temperatures. Furthermore, their longevity in storage is improved in a predictable way with reduction in moisture content and temperature (Ellis et al. 1982). The seeds survive well in air-dry storage but eventually die, their longevity (survival period) being dependent upon the storage environment (Ellis et al. 1982) and their initial quality (Zanakis et al. 1994). Therefore, it is critical to understand how to maximize seed quality before soya bean seeds enter storage.

The general procedures for seed storage in genebanks have been standardized for all orthodox species: seeds should be dried to low moisture content at 10–25% RH at 10–25 °C and then stored at  $-18 \pm 3$  °C for long-term storage of base collections or at 5–10 °C for medium-term storage of active collections (FAO 2014). In practice, many genebanks have drying rooms running at about 15% RH and 15 °C; that at IITA is set at 15–20% RH and 16–18 °C. This relatively low drying temperature was adopted to minimize the rate of ageing during the drying process, particularly for seeds harvested at high moisture content and/or species with seeds which are easily damaged by high-temperature drying (Cromarty et al. 1982).

In standard operations at the IITA genebank (Oyatomi et al. 2012), freshly-harvested soya bean

pods are threshed to extract the seeds, cleaned to remove debris, fumigated for 72 h using aluminium phosphide pellets to control insects, and then dried in the drying room, reaching approximately 6–8% moisture content (wet basis). They are then hermetically sealed in laminated aluminium foil packets for long-term storage at -20°C and medium-term storage at 5°C.

When maintaining large collections of single crops, it may be possible to optimize post-harvest processing—including drying—for the specific crop to maximize longevity and thereby reduce genebank costs and risks such as genetic drift during regeneration of seed accessions (Hay and Whitehouse 2017; Kameswara Rao et al. 2017). Several recent investigations of the influence of an initial higher temperature (30–45 °C) to dry seeds intended for genebank storage have reported a positive effect on subsequent seed longevity in rice (*Oryza sativa* L.) (Whitehouse et al. 2015, 2018a), cowpea (*Vigna unguiculata* (L.) Walp.) and soya bean (Whitehouse et al. 2018b), and Bambara groundnut (*Vigna subterranea* L.) (Jones et al. 2020). Similarly, Hartmann Filho et al. (2016) found that drying soya bean seeds at 40 °C improved shelf-life, whereas yet greater temperatures damaged seed quality. In some cases, the possible benefit to subsequent seed longevity from initial higher temperature drying may depend on other factors; for example, in rice it was only detected in accessions harvested at moisture contents above 16% (Whitehouse et al. 2018a).

In the research reported here, we tested the null hypotheses that neither the timing of seed harvest (during late seed maturation), nor the initial temperature of drying, nor timing of fumigation (a mandatory treatment at IITA) affects subsequent seed longevity. The specific objectives were to:

1. evaluate the effect of initial post-harvest drying temperature (17 °C, 30 °C or 40 °C all at 15–20% relative humidity) on subsequent storage longevity of seeds of diverse soya bean accessions;
2. determine if seed development stage at harvest affected the response of seed longevity to initial drying temperature; and
3. determine whether fumigating seed before or after initial drying affected subsequent longevity.

## Materials and methods

### Seed production and harvest

Seeds were produced at the experimental station of IITA ( $7^{\circ}30'N$ ,  $3^{\circ}54'E$ ), Ibadan, Nigeria. The investigation was carried out in three seasons because seed regeneration is carried out in wet and dry seasons

and in both the field and in pots in the nursery at IITA, whilst the pattern of development of potential seed longevity and its maximum varies with weather between years (e.g., Pieta Filho and Ellis 1991). Three accessions were common to all three seasons. The seeds sown of the respective accessions were sampled from the IITA genebank active collection. In season 1, seeds of four soya bean accessions (Table 1)

**Table 1** List of soya bean accessions from the genebank of the International Institute of Tropical Agriculture (IITA) used to study the effect of initial seed drying temperature on seed longevity

Accession TGm-	DOI <sup>1</sup>	Origin	Cultivar name
Season 1: sown 10 January 2019: seeds harvested before harvest maturity; FBD <sup>2</sup> , FAD <sup>2</sup>			
12	10.18730/M6FG	Liberia	IAC 74-2832
891	10.18730/M6RAK	Taiwan	–
1013	10.18730/M6VWP	Taiwan	–
1781	10.18730/M7J1W	USA	–
Season 2: sown 26 July 2019: seeds harvested before and at harvest maturity; FBD, FAD			
12	10.18730/M6FG~	Liberia	IAC 74-2832
38	10.18730/M6RAK	Taiwan	–
891	10.18730/M6VWP	Taiwan	–
1781	10.18730/M7J1W	USA	–
3978	10.18730/M7MYA	Indonesia	3035/AGS-112-11-4
Season 3: sown 13 November 2020: seeds harvested before and at harvest maturity; FAD			
12	10.18730/M6FG~	Liberia	IAC 74-2832
14	10.18730/M6FH\$	Nigeria	LAWOE
38	10.18730/M8PYS	Chile	–
63	10.18730/M6FV7	Chile	HAWKEYE
79	10.18730/M6FZB	Dominican Republic	BIENVILLE
115	10.18730/M6GFV	Nigeria	No.459
249	10.18730/M6HDM	USA	249-3/D66-866x(HILLxPI274454)
601	10.18730/M6KYV	Puerto Rico	NTUKACH-SUING
627	10.18730/M6M80	Indonesia	–
630	10.18730/M6MB3	Indonesia	–
713	10.18730/M6NXG	Indonesia	–
720	10.18730/M6P2N	Indonesia	–
800	10.18730/M6PQ5	USA	BLOOM P1135 589
942	10.18730/M6SNS	Nigeria	X2 529
949	10.18730/M6SW*	Nigeria	M98 SHIKA
968	10.18730/M6TFE	Taiwan	–
969	10.18730/M6TGF	Taiwan	–
1006	10.18730/M6VNF	Taiwan	–
1022	10.18730/M6W5Z	Taiwan	–
1781	10.18730/M7J1W	USA	–
1376	10.18730/M768N	USA	CLAY
1630	10.18730/M7E6G	USA	ROSE NON-POP
3978	10.18730/M7MYA	Indonesia	3035/AGS-112-11-4

<sup>1</sup>DOI for the Global Information System of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2014)

<sup>2</sup>Seeds fumigated before (FBD) or after (FAD) the initial drying treatment

were sown on 10 January 2019 in 10 m × 7.5 m (length × width) field plots (silty loam soil) at the rate of two seeds per hole (50 mm deep with 30 mm within- and 50 mm between-row spacing). Standard IITA production practices and routine plant protection measures were followed (Oyatomi et al. 2012). Fertilizer was applied two weeks after planting as a side dressing of 5 g P<sub>2</sub>O<sub>5</sub> (46% phosphorus) per plant stand (i.e., hole). Plots were irrigated immediately after sowing and twice a week thereafter. Samples of pods were harvested once on 24 April 2019 by hand when seed moisture content had declined to just below 20%. The harvest date was based on an estimate of moisture content from a sample of seeds using the low-constant-temperature-oven method (drying at 103 °C for 17 h; ISTA 2019).

In all three seasons, after harvesting by hand, the pods were first placed in labelled net bags. The total bulk of the freshly-harvested pods from each accession were weighed and sub-divided into separate sample net bags for each subsequent fumigation and drying treatment. Seed moisture content at harvest time was estimated using a moisture meter (Agripro 6095, SINAR Technology, Wokingham, RG41 1QW, UK, calibrated against values from the constant low-temperature-oven method; ISTA 2019).

In season 2, seeds of five accessions (three used in season 1; Table 1) were sown on 26 July 2019 into pots filled with sterile soil (silt loam texture). Six seeds were sown 50 mm deep per 5 L pot. Total super phosphate was applied at the rate of 5 g P<sub>2</sub>O<sub>5</sub> per pot, two weeks after planting as a side dressing, and pots irrigated twice each week. The plants were supported by stakes (iron rods) throughout the reproductive stage, from about four weeks after sowing. Pods were collected by hand on two dates; before (11 Nov 2020) and at harvest maturity (6 Dec 2020).

In season 3, seeds of 23 soya bean accessions (three used in season 1 and a further one from season 2; Table 1) were sown in field plots on 13 November 2020 following standard IITA production practices and routine plant protection measures as in season 1. The plots were irrigated three times each week until the end of the seed filling phase. Pods of each accession were harvested by hand on two dates, either shortly before (28 January) or at harvest maturity (10 February 2021).

## Fumigation and drying

Whole pods or seeds were fumigated in all three experiments, with its timing investigated in the first two, with samples fumigated before initial drying (FBD) or after initial drying (FAD). This mandatory treatment at IITA is at a set temperature and duration and so represents additional drying and/or storage. Hence, the potential impact of the relative timing of the fumigation treatment in the sequence of post-harvest processing on longevity was also investigated in the first two experiments. In the third experiment, seeds were fumigated after initial drying only (FAD). Fumigation followed IITA genebank's standard operating procedure: Phostoxine (D&D Holdings Inc., 153 Triangle Drive, Weyer Cave, VA 24486, USA), which contains 55% aluminium phosphide as the active ingredient, was applied in the fumigation room (20 m × 20 m) for 72 h at 25.7 °C with 51% RH.

The same three initial drying temperature treatments were used in all experiments: pods were dried within mesh bags in the genebank drying room (DR, 15–20 °C [hereafter, referred to as 17 °C] with 15–25% RH) for 7 days; or in controlled-temperature drying chambers (Heratherm™, Thermos Fisher Scientific, Inc., Waltham, USA) at either 28–30 °C with 15–30% RH or 39–40 °C with 15–30% RH for 4 days. These three drying regimes are referred to as 17, 30 or 40 °C, respectively, henceforth. After the different drying × fumigation treatment combinations (six in experiments 1 and 2; three in experiment 3), the pods were threshed by hand and all samples dried for a further 7 days in the drying room (as above). Seed moisture content was determined at each stage of post-harvest treatment using the moisture meter (as above).

After this final drying, seeds were cleaned by hand to remove broken or dirty seeds and the cleaned seeds of each treatment combination placed into separate, labeled, laminated aluminum foil packets (Moore and Buckle, St. Helens, UK) 0.13 m × 0.13 m (length × width) sealed using an Audionvac VMS 163 V tabletop chamber vacuum sealer (PAC Machinery Co., USA). These packets were stored temporarily at 5 °C for up to 2 weeks before beginning experimental storage.

### Seed survival in experimental storage

Seed storage survival was determined using a standard protocol (Newton et al. 2014; Hay et al. 2022). The packets of seeds were removed from medium-term storage and equilibrated to room temperature (25 °C) before opening. Each seed sample was first rehydrated (to minimize subsequent changes in moisture content during experimental storage) within net bags over water in a plastic container in an incubator at 25 °C with 51% RH (GR-66L; Percival Scientific, Inc., 505 Research Drive, Perry, USA) until they had equilibrated (6–10 days); change in seed moisture content over this period was frequently monitored using the moisture meter. Once this indicated that the desired moisture content of  $9.0 \pm 0.2\%$  had been achieved, the seeds were sealed inside laminated aluminium foil packets (as above) and left to equilibrate at room temperature (25–27 °C) for at least 24 h to ensure even moisture distribution among the seeds within each sample.

The seeds within each treatment combination were then subdivided into 16 subsamples of 60 seeds each and each subsample sealed inside laminated aluminium foil packets (0.13 m × 0.09 m, length × width). In each case, one packet was used to estimate initial ability to germinate while the remaining 15 packets were placed in the incubator at 45 °C. One packet per treatment was removed every 3 days between days 0–12 and every 6 days thereafter until day 42 and the seeds tested for ability to germinate.

### Seed germination testing

Ability to germinate normally (ISTA 2019) was used to assess seed viability with three replicates of 20 seeds dressed with 2 g of Mancozeb (a fungicide, active ingredient ethylene bisdithiocarbamate) and sown into plastic boxes (170 mm × 110 mm × 50 mm) containing 14 Seedburo K-24 germination paper sheets (also known as Versa-Pak 37236-007; Blue Ridge Tissue Corp, Lenoir, NC, USA), wetted with 30 mL distilled water. The boxes were placed in the germination room at 26 °C with 81% RH, either in

natural light or with a controlled photoperiod of 12 h light/12 h dark. The number of normal seedlings was scored after 6 and 10 days.

### Data analysis

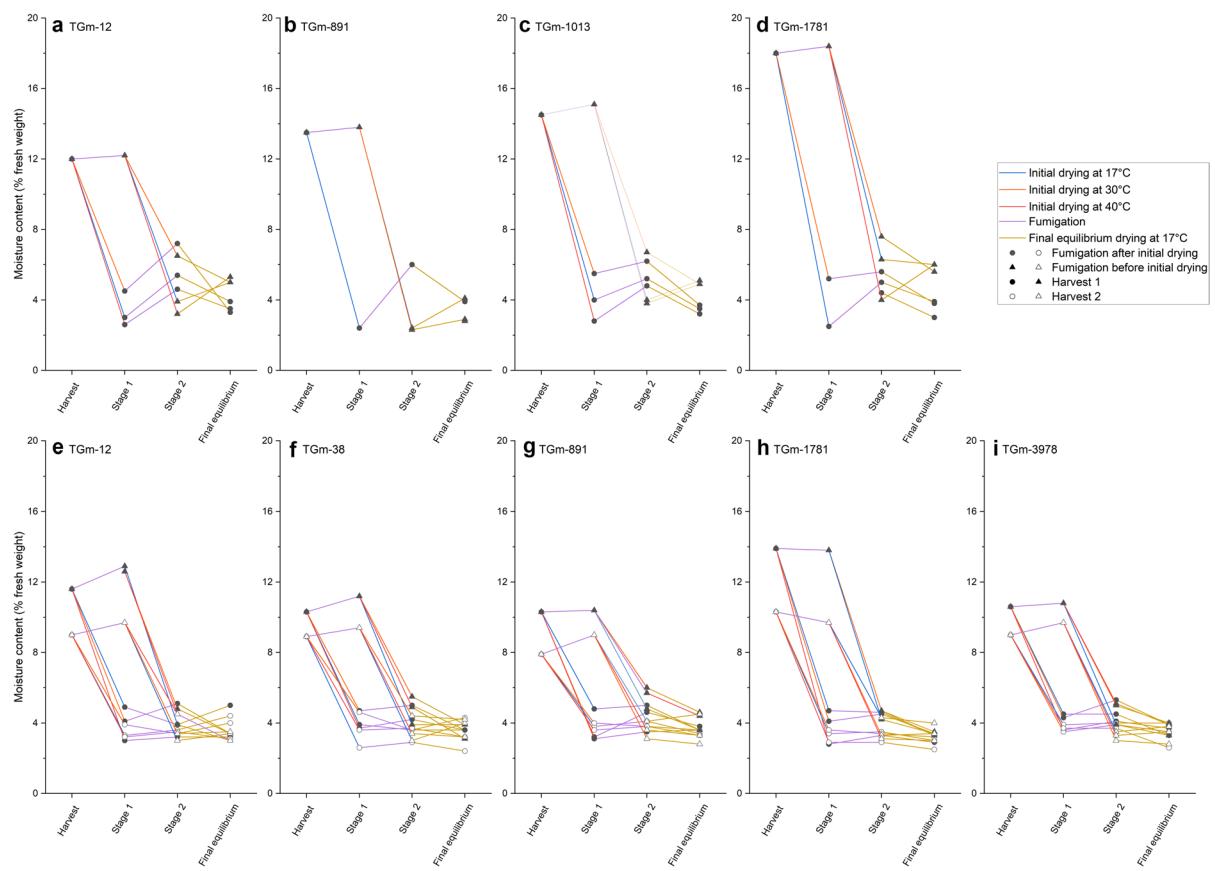
The observations for seed survival (ability to germinate) after different periods of hermetic storage for each treatment combination were subjected to probit analysis using GenStat Discovery 19th edition (VSN International Ltd., Oxford, UK), thereby fitting the viability equation (Ellis and Roberts 1980):

$$v = K_i - p/\sigma \quad (1)$$

where  $v$  is probit percentage viability (normal equivalent deviates) after  $p$  days in storage,  $K_i$  is a constant for the seed lot corresponding to the initial viability in probits, and  $\sigma$  is the standard deviation of the frequency distribution of seed deaths in time (days). Probit analysis was carried out for all the treatment combinations within each accession simultaneously, fitting full models (i.e., different estimates of  $K_i$  and  $\sigma$  for each treatment combination) and also reduced models in which the survival curves for all treatment combinations were constrained to first, a common slope ( $1/\sigma$ ), then a common intercept ( $K_i$ ) and lastly, a common line (common slope and intercept). Approximate F-tests ( $P < 0.05$ ) were used to compare models and determine the best one to accept. Estimates of the period of storage for viability to fall to 50% ( $p_{50} = K_i \times \sigma$ ) were calculated for the accepted model and subsequently the percentage change in  $p_{50}$  after initially drying at either 30 or 40 °C relative to drying at 17 °C.

## Results

Mean minimum and maximum temperatures during the period from peak flowering until harvest were similar in seasons 1 and 3 (approximately 23 and 33 °C, respectively) and relatively constant (Supplementary Fig. 1). In contrast, mean maximum temperature was < 30 °C for most of the seed development period in season 2, increasing to approximately 33 °C around the two harvest times, with a longer period of seed development than seasons 1 and 3.



**Fig. 1** Changes in the moisture content of soya bean seeds of different accessions (Table 1) harvested in seasons 1 (a–d) and 2 (e–i) and subjected to various drying (4 days at 30 or 40 °C or

7 days at 17 °C; then 17 °C) and fumigation treatments (either after or before initial drying). Note that some observations are obscured by coincidence of position (particularly in b)

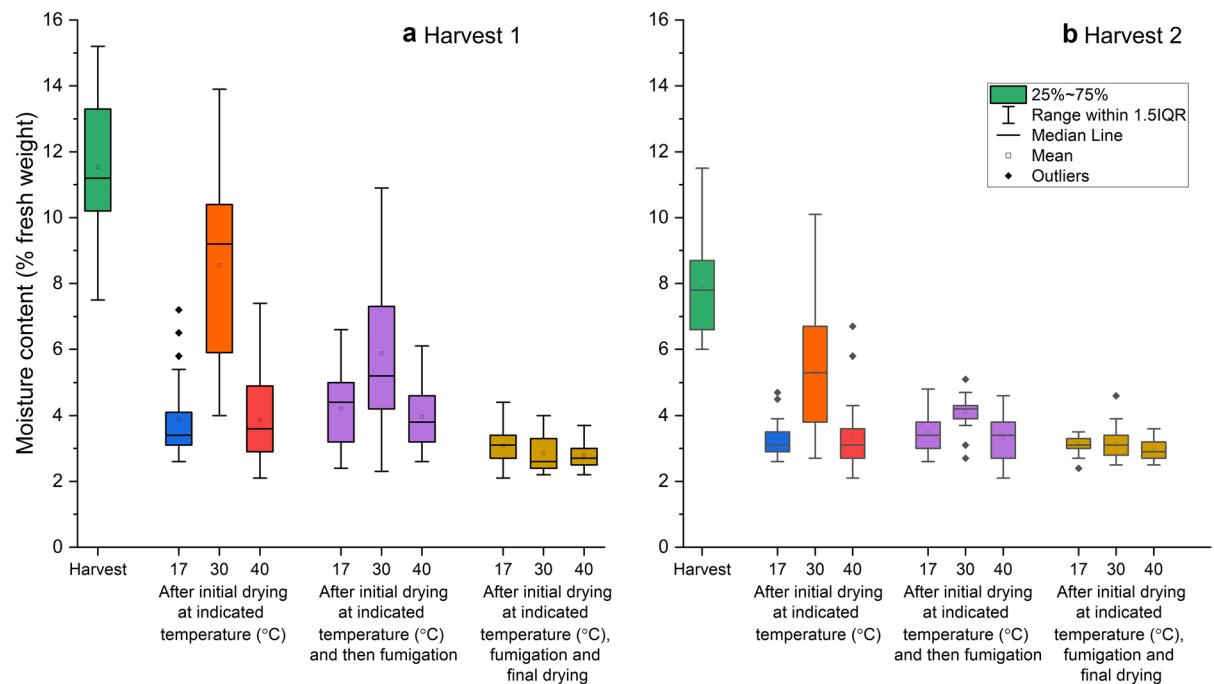
### Seed moisture content

Seeds were harvested at 12 to 18% moisture content in season 1 (Fig. 1a–d). Moisture content increased slightly above values at harvest for seeds fumigated before the initial drying treatments. Drying in all three regimes resulted in considerable loss in moisture, but fumigation after the initial drying treatments increased seed moisture content by around 2%. After final equilibrium drying, seed moisture content ranged from 3.0 to 3.8%.

Two harvests were made in season 2; the moisture content of seeds harvested before (harvest 1) and at harvest maturity (harvest 2) ranged between 10.3 and 13.9% and between 7.9 and 9.3%, respectively, among accessions (Fig. 1e–i). The changes in moisture content during post-harvest processing

were similar to those observed in season 1, and final moisture content after equilibrium drying ranged between 3 and 5%.

Seeds harvested before and around harvest maturity (harvests 1 and 2) in season 3 had mean moisture contents of  $11.6 \pm 1.8\%$  and  $7.7 \pm 1.2\%$ , respectively (Fig. 2). Seeds initially dried at 17 or 40 °C dried similarly and considerably. Moisture contents were reduced less from drying at 30 °C than from drying at 17 or 40 °C; seeds of some accessions took up moisture during this treatment. Consequently, seeds initially dried at 30 °C lost moisture during subsequent fumigation whereas seeds initially dried at 17 or 40 °C either maintained or increased their moisture content slightly. Mean seed moisture content after final equilibrium drying was  $3.1 \pm 0.4\%$  for all treatment combinations.



**Fig. 2** Boxplots showing changes in the mean seed moisture content of different soya bean accessions (Table 1) during post-harvest processing. Seeds were harvested on two occasions in

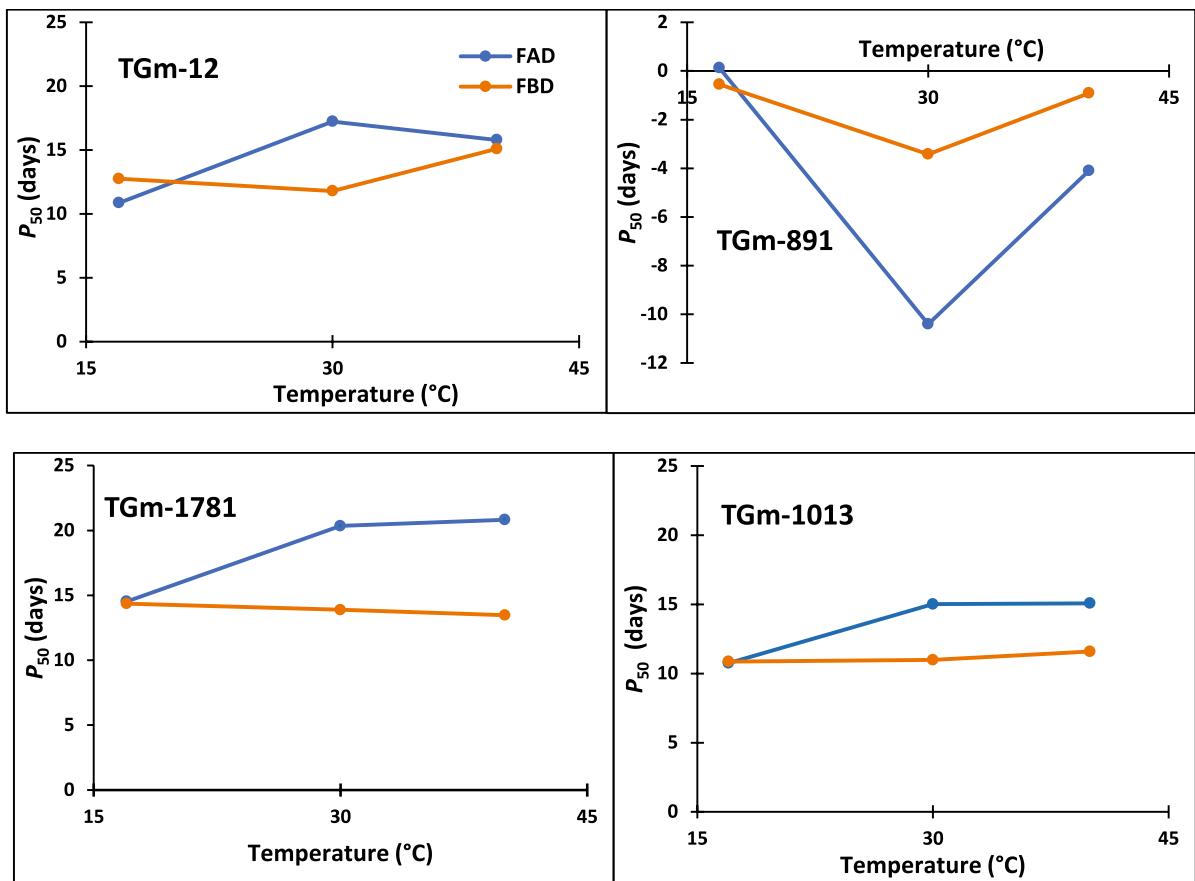
season 3 and fumigated after initial drying (FAD) at 17 for 7 days, or 30 or 40 °C for 4 days

#### Seed survival and longevity

Seed survival data during storage for all treatment combinations followed the expected cumulative normal distribution of seed deaths over time and Eq. 1 was fitted (Supplementary Figs. 2–5) in all but six combinations of harvest  $\times$  accession (season 3: TGm-630 and TGm-3978 from harvest 1; TGm-115, TGm-713, TGm-949 and TGm-800 from harvest 2). Seed survival curves could not be fitted in these six cases due to short longevity relative to the sampling interval or high variation in observations over the periods in storage. It was possible to constrain the survival curves for the various drying temperature  $\times$  fumigation timing treatment combinations within certain season  $\times$  accession  $\times$  harvest time combinations to a common slope ( $-\sigma^{-1}$ ) and/or common intercept ( $K_i$ ) (i.e. without a significant increase in residual deviance compared with independent estimates). For example, in TGm-1013 FAD common slopes were fitted, but in TGm-1781 FAD the slopes (and longevity) differed substantially amongst the three drying temperatures in season 1 (Supplementary Fig. 2).

In seasons 2 (Supplementary Fig. 3) and 3 (Supplementary Figs. 4 [harvest 1] and 5 [harvest 2]), all four possible types of variation in survival curves amongst the three temperatures were detected: no differences (common line), different intercepts but common slopes, different slopes but common intercepts, or different intercepts and different slopes. Estimates of longevity (period for viability to fall to 50%,  $p_{50}$ ) were obtained based on the model accepted by those analyses and compared amongst treatments.

Seeds from seasons 1 and 2 were fumigated either before or after initial seed drying at 17, 30, or 40 °C. In both these seasons, fumigation after initial seed drying provided greater longevity on average than fumigation before; one way ANOVA showed this advantage of fumigation after initial drying was significant in season 1 ( $F_{1,16}=4.9$ ,  $P<0.05$ ) but not in season 2 ( $F_{1,28}=1.0$ ,  $P>0.05$ ). In season 1, whether seeds were fumigated before or after initial drying at 17 °C did not affect subsequent longevity ( $p_{50}$ ) in experimental storage (Fig. 3). However, fumigation after initial seed drying at 30 °C provided greater longevity than fumigation before drying in three



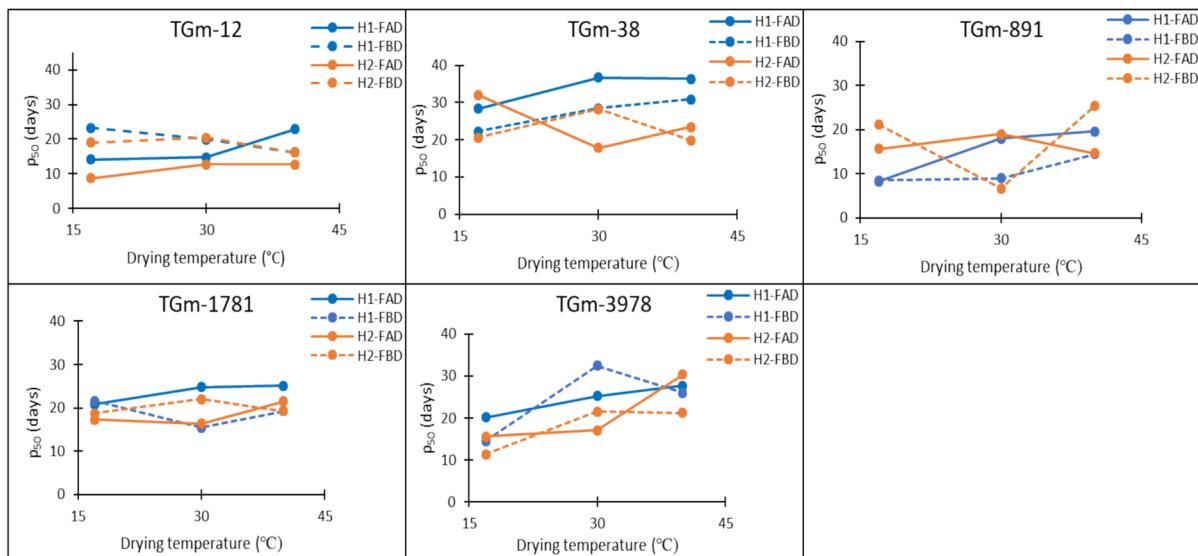
**Fig. 3** Effect of initial drying temperature (4 days at 30 or 40 °C or 7 days at 17 °C; then 17 °C) and fumigation before or after initial drying (FBD and FAD, respectively) on subsequent

soya bean seed longevity ( $P_{50}$ , period for viability to fall to 50%) in experimental storage at 45 °C with 9% moisture content in season 1. Seeds were harvested before harvest maturity

of the four accessions (TGm-891 was the exception) and similarly at 40 °C in two (TGm-12 and TGm-1781). Moreover, initial drying at 30 or 40 °C provided greater longevity than at 17 °C in three of the four accessions (again, TGm-891 was the exception). Seeds of accession TGm-891 showed poor viability before storage in all treatments (Supplementary Fig. 2) and differed in their response to the fumigation-drying treatments compared with the other accessions: drying at warmer temperatures and fumigating afterwards reduced longevity. In season 2, as in season 1, differences in longevity for seeds dried at 17 °C were relatively small between fumigation before (FBD) and after (FAD) initial drying for all five accessions and two harvest times (Fig. 4). The effect of FBD versus FAD with warmer drying temperature varied depending on harvest time, accession

and drying temperature. Whilst it was difficult to determine clear trends in season 2, fumigation after initial drying (FAD) at warmer temperatures tended to provide greater longevity, particularly in harvest 1 and for TGm-891. Hence, fumigation after initial drying (FAD) only was applied in the final season's investigations.

The effect of drying temperature varied somewhat in season 2 (Fig. 4). The longevity of seeds of all accessions from harvest 1 was improved by initial drying at 30 and/or 40 °C, compared with drying throughout at 17 °C, if seeds were fumigated after initial drying. In harvest 2, longevity in TGm-3978 was greater after initial drying at 40 °C than at either 17 or 30 °C for fumigation after drying, but in the other accessions longevity was either similar across the three drying temperatures or reduced in the two



**Fig. 4** Effect of initial drying temperature (4 days at 30 or 40 °C or 7 days at 17 °C; then 17 °C) and fumigation before or after initial drying (FBD and FAD, respectively) on subsequent soya bean seed longevity,  $p_{50}$  (period for viability to fall

to 50%) in experimental storage at 45 °C with 9.0% moisture content in season 2 for seeds harvested before harvest maturity (H1) or around harvest maturity (H2)

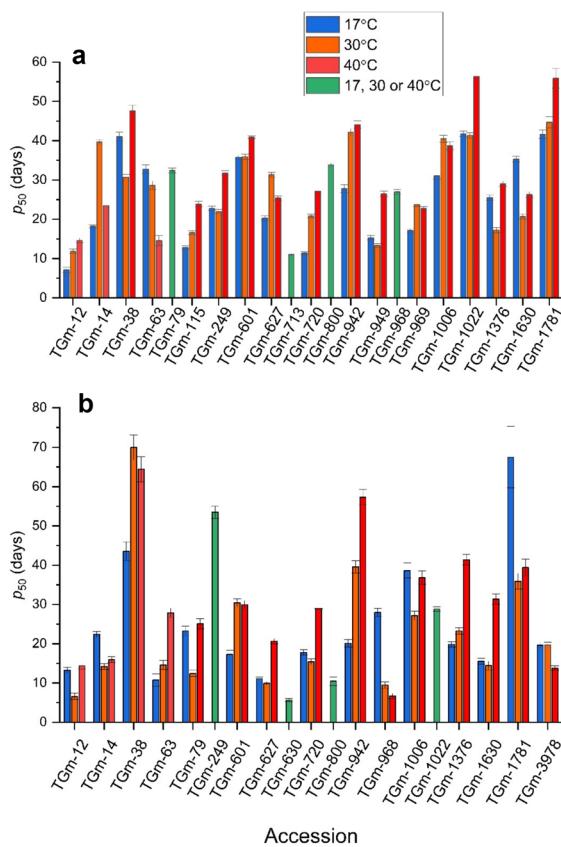
warmer regimes (TGm-38). The responses to drying temperature for seeds fumigated before initial drying were yet more inconsistent among the accessions and harvests varying from consistent improvement in longevity the warmer the drying temperature (e.g. TGm-38, harvest 1) to that at 30 °C being better than at 40 °C which in turn was greater than at 17 °C (e.g. TGm-3978, harvest 1), to a progressive decline in longevity from 17 to 40 °C (e.g. TGm-12, harvest 1).

In season 3, different patterns were observed amongst accessions in the estimates of  $p_{50}$  in response to the three initial drying temperatures (Fig. 5): no difference (one survival curve fitted to the data for all three drying temperatures);  $p_{50}$  following initial drying at 17 °C >  $p_{50}$  following initial drying at 30 °C >  $p_{50}$  following initial drying at 40 °C (17 °C > 30 °C > 40 °C); 17 °C > 40 °C > 30 °C; 30 °C > 40 °C > 17 °C; 40 °C > 30 °C > 17 °C; and 40 °C > 17 °C > 30 °C. Initial drying at 17 °C was optimal for longevity for more accessions from the second harvest (Fig. 5b) than from the first harvest (Fig. 5a), whereas initial drying at 30 or 40 °C was optimal for more accessions at the first than the second harvest; a good example of this contrast between the two harvests in the best initial drying temperature is shown by accession TGm-1781.

The relative change in  $p_{50}$  from initial drying at each of 30 or 40 °C compared with drying at 17 °C was calculated for all the seed lots produced from all three seasons (Fig. 6). The greatest relative improvement in  $p_{50}$  was 230.0% for seeds of TGm-942 from harvest 2 initially dried at 40 °C in season 3. The majority of the seed lots showed some improvement to longevity from initial drying at a higher temperature: 27 at both 30 and 40 °C (top right quadrant in Fig. 6); for 12 seed lots there was an improvement from initial drying at 40 °C but not at 30 °C (top left quadrant in Fig. 6); and for three seed lots initial drying at 30 but not at 40 °C improved longevity (bottom right quadrant in Fig. 6). In a minority of seed lots (c. 10), however, initial drying at both 30 and 40 °C reduced longevity compared to 17 °C (lower left quadrant in Fig. 6).

## Discussion

Optimizing genebank operations is important to ensure that crop biodiversity is conserved with maximum efficiency and effectiveness (Lusty et al. 2021; Wambugu et al. 2023). One part of this is to provide seeds of high initial quality and potential longevity



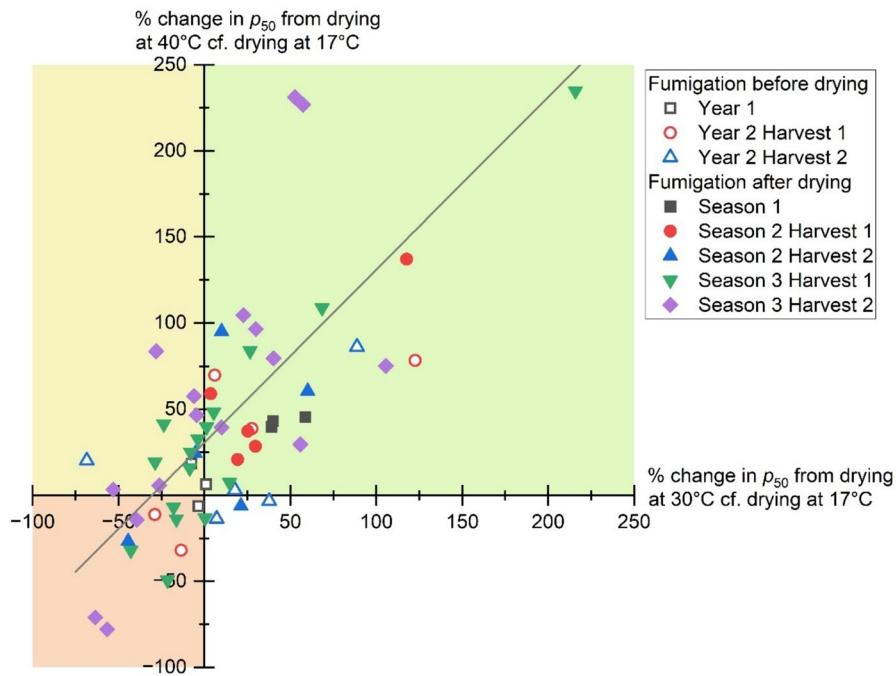
**Fig. 5** The effect of initial drying of soya bean seed for 7 days at 17 °C or 4 days at, 30, or 40 °C on the estimates of  $p_{50}$  at 45 °C with c. 9% moisture content for accessions harvested **a** before (H1: 28 Jan) or **b** close to harvest maturity (H2: 10 Feb) in season 3. Supplementary Figs. 4 and 5 provide the seed survival curves from which these estimates were obtained. Within each harvest, longevity did not differ among the three initial drying temperatures in several accessions (green histograms). No results are presented for accessions TGm-630 and TGm-3978 in A nor for TGm-115, TGm-713 and TGm-949 in B as seed survival curves could not be fitted (see text)

for long-term storage, because regeneration is often initiated due to poor seed viability. High seed viability upon receipt is also important to the end-users of genebank accessions. Factors that can affect longevity in storage include seed development environment, maturity at harvest, post-harvest handling processes and the delay before the seeds enter genebank storage (Probert et al. 2007; Hay et al. 2013; Ellis 2022). Seed drying is an essential part of post-harvest procedures, ensuring seeds are at low moisture content when packed for storage. It is currently recommended that seeds stored in genebanks are first dried

at 10–15% RH and 10–25 °C (FAO 2014). However, research in selected species has shown that a higher initial drying temperature may improve subsequent seed longevity (Whitehouse et al. 2015, 2018a; Jones et al. 2020), including soya bean (Whitehouse et al. 2018b). The potential benefits of drying at temperatures warmer than 10–25 °C were explored further here with diverse genotypes of soya bean harvested at different stages of maturity and with regard to the timing of fumigation relative to the initial drying treatment.

Fumigation is a standard operation for the IITA Genebank (Oyatomi et al. 2012) which aims to control insect pests in the seeds (Singh et al. 2003; Kameswara Rao et al. 2017). While routine in some genebanks, other genebanks do not fumigate their seed lots. The impact of a fumigation treatment on seed quality has not been systematically studied. In general (but not always), fumigation after, rather than before, initial drying was better for subsequent seed longevity (Figs. 3, 4). One possible reason for this is that there was little change in seed moisture content during fumigation before initial drying and hence seeds were held at a relatively high moisture content for longer (Figs. 1, 2) and so some ageing may have occurred. Another possibility, not mutually exclusive, is that the initial drying treatment before fumigation promoted further seed maturation *ex planta* if seeds were not fully mature when harvested (i.e. initial drying simulated natural maturation drying) since soya bean seed quality continues to improve in late maturation drying (Zanakis et al. 1994). Drying seeds in a low humidity environment, such as the IITA genebank drying room, will also reduce insect activity and may kill some insects (Kameswara Rao et al. 2017), though not their eggs (Hole et al. 1976). Thus, it is unclear how important fumigation treatments are in the genebank workflow. Other research is underway to address this question.

For many orthodox species, including soya bean, the end of the seed filling phase when maximum seed dry weight is attained, termed mass maturity (MM) (Ellis and Pieta-Filho 1992), is a critical point during seed development. Desiccation tolerance occurs before or around the time of MM in many species (e.g., barley [*Hordeum vulgare* L.]; Pieta Filho and Ellis 1991), but develops slightly later in soya bean (Zanakis et al. 1994). Potential seed longevity often continues to increase during the subsequent



**Fig. 6** Summary of the relative benefit or detriment to subsequent soybean seed storage longevity ( $p_{50}$  at 45 °C with c. 9% moisture content) from initial drying at warmer temperatures (4 days at 30 or 40 °C; then 17 °C with 15–25% RH) compared with 17 °C throughout. This figure is a co-plot of the relative improvement (%) in subsequent seed longevity from 4 days' initial drying at 40 °C cf. 17 °C throughout (y-axis; comparison between estimates of values expressed as percentage) versus relative improvement in seed longevity from 4 days' initial drying at 30 °C cf. 17 °C throughout (x-axis; comparison between estimates of  $p_{50}$  values expressed as percentage) for soya bean seeds harvested in three seasons (see Table 1). The fitted linear regression ( $R^2=0.55$ ) was significant ( $P<0.001$ ).

Seeds were harvested before (harvest 1) or around harvest maturity (HM) is reached (Zanakis et al. 1994; Sanhewe and Ellis 1996; Demir et al. 2002), but the relative timing and duration of the period whilst maximum longevity is maintained *in planta* can vary among crops, and potentially cultivars, and is highly dependent on the climatic conditions during seed development and maturation (Ellis 2019). In seasons 2 and 3, seeds were harvested well after mass maturity but shortly before harvest maturity or close to harvest maturity. Comparing longevity for fumigation after initial drying at 17 °C between the two harvests in season 2 showed that  $p_{50}$  was greater for the earlier harvest in two accessions but greater for the later harvest in the remaining three accessions (Fig. 4). Similar variation

maturation drying phase until harvest maturity (HM) is reached (Zanakis et al. 1994; Sanhewe and Ellis 1996; Demir et al. 2002), but the relative timing and duration of the period whilst maximum longevity is maintained *in planta* can vary among crops, and potentially cultivars, and is highly dependent on the climatic conditions during seed development and maturation (Ellis 2019). In seasons 2 and 3, seeds were harvested well after mass maturity but shortly before harvest maturity or close to harvest maturity. Comparing longevity for fumigation after initial drying at 17 °C between the two harvests in season 2 showed that  $p_{50}$  was greater for the earlier harvest in two accessions but greater for the later harvest in the remaining three accessions (Fig. 4). Similar variation

was detected amongst accessions in season 3. This variation tallies with the suggestion that the timing and duration of when maximum seed quality is achieved may both vary (Ellis 2019).

The limited drying at 30 °C for both harvests in season 3 (Fig. 2) was unexpected. Indeed, samples at both 17 and 40 °C dried further than at 30 °C. Temperature logging confirmed that the 30 °C temperature regime was maintained at the set value. Seed drying rate depends on seed factors (such as size and oil content) and on physical factors (difference between initial seed moisture content and the equilibrium value in the drying regime, temperature, the mass of seed, and the air flow rate) (Cromarty et al. 1982). One possibility is that air flow in the 30 °C incubator

was restricted, reducing seed drying rate. Whatever the cause, at the end of the full drying process (i.e., on removal from the drying room) seed moisture contents were similar across all three initial drying temperature treatments.

The relative effects of the three different drying temperatures on subsequent seed longevity were not consistent for all combinations of season, accession, harvest and relative timing of fumigation (Fig. 6). Nonetheless, the subsequent seed longevity of most of the seeds produced was improved by initial drying at 40 °C compared to drying at 17 °C throughout (symbols above the x-axis in Fig. 6). Moreover, this improvement was often substantial (more than 20% in most samples) and sometimes considerable (more than 100% in a minority). If seeds responded positively in terms of longevity to initial drying at 40 °C, they were more likely to also respond positively to initial drying at 30 °C (a value similar to ambient at IITA) compared to drying at 17 °C (symbols to the right of the y-axis in Fig. 6). Surki et al. (2012) and Hartmann Filho et al. (2016) have shown that drying at 50–80 °C is not safe for soya bean seeds as it can damage the embryo. Thus, 40 °C may be the maximum temperature suitable for drying soya bean seed.

We are not able to explain why a minority of samples provided greater longevity after drying at 17 °C cf. 30 or 40 °C (lower left quadrant in Fig. 6). The seven accessions which showed the greatest longevity following drying at 17 °C (in some but not all treatment combinations) were from the USA (three accessions), Chile (two), Liberia (one) and Taiwan (one) but it is unlikely that the response was related to their origin since other samples of these accessions benefitted from drying at warmer temperatures—as did other accessions from those four countries. Seed size can differ greatly between accessions, but the relative response of longevity to initial drying at higher temperatures was not correlated with seed size ( $P=0.09$ ).

In research on rice (Whitehouse et al. 2015, 2018a), soya bean (Whitehouse et al. 2018b) and Bambara groundnut (Jones et al. 2020), the extent to which seed longevity improved after initial warmer temperature drying depended on seed moisture content at harvest (indicative of the extent of maturation drying). For example, accessions of rice harvested at moisture contents  $>16\%$  benefitted progressively more from initial high temperature drying but drier seeds did not benefit (Whitehouse et al. 2018a). The

two greatest reductions in longevity from drying at 30 and 40 °C cf. 17 °C was for seeds collected at harvest maturity (lower left quadrant, Fig. 6 [season 3, harvest 2]) and initial drying at 17 °C was better than warmer temperatures for longevity for more accessions from the (drier) second harvest than the first in season 3 (Fig. 5). Whilst those particular results were compatible with the pattern reported by Whitehouse et al. (2018a), over the three experiments here, no significant correlations were detected between the moisture content of seeds at harvest and the relative response of longevity to initial drying at 40 cf. 17 °C (season 3,  $r=-0.19$ ,  $P=0.29$ ; season 2,  $r=-0.07$ ,  $P=0.76$ ) or to that at 30 cf. 17 °C (season 3,  $r=-0.02$ ,  $P=0.93$ ; season 2,  $r=-0.03$ ,  $P=0.90$ ).

This study involved many potential variables of interest during seed development and maturation, harvest, cleaning, drying, fumigation and finally storage, each of which might affect the results. Variation in the seed production environment and in genotype was accounted for by carrying out investigations in different seasons with many accessions. Estimates of seed moisture content from oven drying methods are typically subject to errors of  $c.\pm 0.5\%$  (from sampling as well as the determination itself). Applying the seed viability constants provided for soya bean (Ellis et al. 1982) suggests that a 0.5% difference above and below 9% moisture content would be expected to reduce seed longevity by 19% or increase it by 25%, respectively. Similarly, a 1 °C increase or reduction in seed storage temperature from 45 °C would be expected to reduce longevity by 16% or increase it by 18%, respectively. The procedure used here to raise seed moisture content and storing the seeds in the incubator at the same time were designed to minimize such errors but the comparatively large effect of a small difference in seed storage environment on seed longevity should be recognized when comparing treatments. Possible effects of all these particular sources of error (production environment, genotype and seed storage environment) were mitigated by comparing the results from all three seasons together (Fig. 6).

Many research institutes conserve plant agrobiodiversity in seed genebanks in different regions of the world (Hay and Probert 2013; Westengen et al. 2013; Walters and Pence 2021). Different regions provide different conditions for seed development and maturation when accessions are regenerated. Moreover, not

all species' seeds can be dried safely at the same temperature. Seed drying engineers have provided tables of "safe drying temperatures" for different species at different moisture contents for around a century or so which values vary from as low as 21 °C for very moist seeds of onion (*Allium cepa* L.) and cocksfoot (*Dactylis glomerata* L.), for example, to 70 °C for less moist seeds of certain cereals such as oat (*Avena sativa* L.) and rye (*Secale cereale* L.) (e.g. Roberts and Roberts 1972). We suggest it is somewhat misleading to think of the initial drying treatments imposed here solely as seed drying treatments. Rather, whilst the seeds did dry during all three treatments, this process was a critical part of the seed maturation programme and was able to continue to greater or lesser extents in many treatment combinations. In other words, the initial treatments (particularly where fumigation was postponed until afterwards) were combined maturation and drying treatments, in which post-harvest maturation was more likely in the warmer regimes. This tallies with the observation that heat shock proteins which are associated with subsequent longevity continue to accumulate until quite late in seed maturation (Sinniah et al. 1998).

Overall, the results from these experiments lead us to suggest that the IITA genebank maintains its current soya bean seed harvest and processing protocol (i.e., fumigation, then place in the current drying room at 15–20 °C with 15–25% RH) for seeds collected at or beyond harvest maturity. The protocol should be modified for seeds harvested slightly before harvest maturity so that the seeds are initially dried for up to 4 days at 40 °C, then fumigated, and then dried to equilibrium in the drying room. This protocol would appear to have substantial benefits in terms of subsequent seed longevity in storage, on average, thereby reducing the subsequent monitoring and regeneration workload at IITA. It may be a useful protocol for others drying soya bean seeds to low moisture contents to also consider.

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**Author contributions** All authors designed the study concept. OFS conducted the experiments and RHE, OAO, MTA and FRH were involved in the overall supervision of the research. OFS, FRH and RHE carried out data analysis and interpretations and drafted the manuscript; and all the authors have read and agreed to the final manuscript.

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**Data availability** Data is provided within the supplementary information files or from the authors upon request.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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