

Full Length Research Paper

Comparative studies of drying methods on the seed quality of interspecific NERICA rice varieties (*Oryza glaberrima* x *Oryza sativa*) and their parents

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Seed moisture content (MC) is a key component that determines storability of germplasm conserved in seed genebanks. The purpose of this research was to compare the efficiency of sun-, shade-, silica gel- and conventional room drying in terms of rice seed MC and viability using seeds of two interspecific progenies (NERICA 1 and NERICA 3) and their parents *Oryza glaberrima* and *O. sativa* varieties harvested 15 days before, at and 15 days after mass maturity. Sun drying most significantly reduced MC (4-5%) and was comparable with silica gel drying regardless of the variety tested and the maturity stage at harvest. Likewise, sun drying gave the best germination percentages followed by silica gel. Shade and room drying did not significantly lower MC and led to poor germination, especially when rice was harvested prematurely. Except for the *sativa* variety, harvesting prematurely resulted in no germination. In contrast, the initial germination percentages in all tested varieties improved as rice was harvested at or after mass maturity rather than 15 days earlier. Though comparable to sun drying, silica gel may not be readily available and affordable for resource-limited seed storage facilities. In this case sun drying was found an effective and affordable method for short-term storage, especially farm-saved seeds.

Key words: seed drying, seed storability, seed moisture content, germination percentages, rice.

INTRODUCTION

The rationale for drying seeds is to reduce their moisture content to a level, which prolongs longevity during storage in seed genebanks, and consequently increase the regeneration intervals. Regular regenerations of accessions are needed to ensure that the seeds stored in base collections do not fall below acceptable levels of viability and yet minimize the number of regeneration cycles to ensure that the genetic integrity of accessions is maintained. The regeneration intervals depend on the longevity of the seed in storage. For example, Harrington (1973) reported that seed longevity is doubled by each 1% reduction in moisture content. Cromarty et al. (1982)

and Ellis and Roberts (1991) suggested a curvilinear relationship between seed moisture content and the logarithm of longevity.

Many genebanks use the conventional drying room or desiccants such as silica gel to dry seeds. In many resource-limited African countries, either the required apparatus is missing or the power supply to run consistently the equipment is rather erratic and costly. Also, conventional methods such as desiccant drying or room drying of seed often do not reduce the water content of seeds sufficiently (Justice and Bass, 1978). Developing efficient and affordable low input drying procedures to reduce seed moisture content without causing damage to the seed tissue is a priority, especially for resource-limited seed bank curators in most national agricultural research systems in Africa. Also, the alternative of low-input drying methods is suggested to reduce

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Table 1. Analysis of variance of moisture content of seed of rice varieties harvested at different maturity stages using four drying methods.

Source of variation	Sum of squares ⁵	Degree of freedom	Mean Square	Computed F	Significance
Model*	2270.3	21	108.1	17.1	<0.0001
Variety	0.8	3	0.3	0.04	0.9880
Maturity	248.5	2	124.2	19.6	<0.0001
Method	2014.9	4	503.7	79.5	<0.0001
Variety*Method	6.2	12	0.5	0.08	1.0000
Error	240.7	38	6.3		
Corrected total	2511.1	59			

*R²=0.904⁵Type III of Proc GLM procedure(SAS).

the dependency on unreliable and expensive electricity supply for seed drying. Nevertheless, the effects of low input methods on seed quality need to be assessed. Seed maturity stage at harvest is another factor that may influence seed storage potential. Immature seeds are often sensitive to damage from desiccation to very low moisture contents (Ellis and Robert, 1991; Hong and Ellis, 1996). Delaying harvest in the field may also expose seeds to damage when they are dried to very low moisture contents.

While much research has focused on the optimal seed moisture content for storage (FAO/IPGRI, 1994), yet limited data exist on how to dry seeds. In the present research two low input drying regimes (sun-, and shade drying) were compared with the conventional desiccant- and room drying to determine the more efficient method for drying seeds of *Oryza glaberrima* and *Oryza sativa* parents and their two interspecific progenies (NERICA 1 and NERICA 3) harvested at three maturity stages; 15 days before, at and 15 days after mass maturity). Efficiency was evaluated against the lowest seed moisture content and highest germination percentages.

MATERIALS AND METHODS

Study site

The experiment was carried out at the main research station of the International Institute of Tropical Agriculture in Ibadan, Nigeria (210 masl, 2°34'E, 4°46'N) during the 2005 wet and dry seasons. The total rainfall during the field experiment period was 601 mm, while the average temperature and the average relative humidity (RH) were 25 °C and 83%, respectively.

Plant material

The most promising interspecific NERICA rice varieties (*Oryza glaberrima* x *Oryza sativa*) released or in the pipeline for release in Nigeria, namely WAB 450-I-B-P-38-HB (NERICA 1) and WAB 450-I-B-P-28-HB (NERICA 3), and their parents *O. glaberrima* steud (CG 14) and *O. sativa* L. (WAB 56-104) were grown (Table 1).

Seeds were obtained from the rice germplasm collection of the Genetic Resources Unit at the Africa Rice Center (WARDA).

The field experiment was conducted during the wet season (June-September) in three replicate blocks and seeds were harvested per block. The first block was harvested fifteen days before mass maturity (pre-maturity), the second block at mass maturity and the third block fifteen days after mass maturity (post-maturity). The maturity stage was defined according to the number of days to mass maturity of each variety, which was indicated in the technical fact sheet of the released varieties made available by the African Rice Initiative (ARI) project. For the parent varieties (CG 14 and WAB 56-104), the information was derived from the WARDA's Germplasm Information Sharing System (WAGIS) database. The pre- and post-maturity stages were then estimated at 80 and 110 days for NERICA 1 and NERICA 3 (15 days before and after mass maturity respectively), at 90 and 120 days for upland *O. sativa* japonica type WAB 56-104 (15 days before and after mass maturity respectively) and at 99 and 129 days for the African *glaberrima* rice (CG 14) (15 days interval).

Drying procedure

Shade, sun, and silica gel drying were compared with cold room drying. For each entry, four random samples of 50 g each of seeds were used for each drying treatment. For shade drying, seeds were laid on a black linen sheet and set under shade conditions where the temperature varied between 24 and 27 °C and the RH between 60 and 70% during the experiment.

For sun drying, samples were spread on concrete floors and set under sunbeams from 10 a.m. to 4 p.m. The temperature varied between 37 and 40 °C and the relative humidity ranged between 30 and 40%. At night, the seeds were sealed in aluminium foil and safely kept in a room. Also, when the bad weather conditions during the experiment period made it not possible to display seeds for drying every day, the seeds were sealed in aluminium foil and kept inside a room. The temperature in the room was around 27 °C and the relative humidity was below 50%.

Other samples were placed in desiccators that contained self-indicating silica gel (containing cobalt chloride) with a 1:1 (silica gel:seed) ratio by weight. Silica gel was replaced when 70% or less of the gel had changed its colour to pink (Fischler, 1993). On this basis, the silica gel was replaced each morning for the first two days, then every 2–3 days during the desiccation process. The temperature in the desiccators was maintained around 25 °C.

In the drying room, samples were placed in small open containers. The temperature inside the room was around 10 °C and the RH was around 40%. The seed moisture content (MC) was

Table 2. Mean time to drying (days) for rice seed after drying using four drying methods.

Maturity stage	Drying method	NERICA 1	WAB 56-104	NERICA 3	CG 14
15 days before mass maturity	shade	23	22	23	24
	sun	18	22	23	24
	Silica gel	23	22	22	22
	Cold room	24	23	23	21
Mass maturity	shade	19	18	17	20
	sun	18	18	18	18
	Silica gel	20	18	18	18
	Cold room	18	18	18	19
15 days after mass maturity	shade	11	12	12	12
	sun	11	12	12	12
	Silica gel	15	15	15	15
	Cold room	11	12	12	12

LSD_{0.05} (Drying method) = 0.8LSD_{0.05} (Variety) = 0.7LSD_{0.05}(Maturity) = 0.6

determined gravimetrically and expressed on a fresh weight basis. The dry weight was measured after heating seeds in the oven for 17 h at 103°C according to the International Seed Testing Association rules (ISTA, 1993). The weight of all replicates was monitored daily until no further change in weight was observed. The duration (number of days) after which constant weight was reached was recorded.

Seed viability

Seed viability was determined through germination tests before and after drying at the International Institute of Tropical Agriculture (IITA) research station laboratory in Ibadan, Nigeria. A completely randomised design with three replicates was used. The experimental unit was made of 100 seeds placed in a standard Petri dish on a moist germination paper. Germination paper in each Petri dish was watered with 5 ml distilled water. The Petri dish was placed in a standard in an incubator (THERMOSI, SR 3000, model EBV 200) at a constant temperature of 30°C for 7 days. It was assumed that the conditions affecting germination (temperature, relative humidity) are constant within the incubator. The number of germinated seeds was counted after 7 days for each entry. Germinated seedlings were recorded for each entry and the germination percentage computed.

Statistics

Data were analysed using the general linear model (GLM) of the SAS program (SAS 2001). The least significant difference of means (LSD) was computed at a probability level of 5% to discriminate between means.

RESULTS

Seeds moisture content

Seed moisture content (MC) varied largely according to the maturity stage of varieties at harvest ($p < 0.0001$) and was significantly affected by the drying method used

($p < 0.0001$), but not by the variety tested ($p = 0.98$) (Table 1). As expected, the initial seed moisture content (IMC) was generally higher before mass maturity than at and after mass maturity (Figure 1). When seeds, irrespective of the variety tested were allowed to dry under sun and in the desiccator, their MC was significantly lowered. The MC of seeds of all four varieties, after mass maturity did not change significantly regardless of the drying procedure used (Table 2). Room drying did not significantly lowered MC as recorded in the seeds before drying. Drying under sun was comparable with silica gel drying for all materials tested. In contrast, seed MC did not reduce significantly when seeds were dried under shade and room drying as compared with sun and silica gel drying (Figure 1). Both sun and silica gel drying methods allowed drying seeds to lower moisture contents than the other procedures, irrespective of the maturity stage of varieties at harvest. The MC of all the rice seeds, regardless of the varieties reached an average of 5–7% when dried over silica gel. Sun drying allowed lower MC of 4–5%, though the difference was not significant (LSD=2.6 at $p < 0.05$). Means of varieties, when averaged over maturity stages at harvest and drying methods showed no significant differences. On the other hand, delayed harvest significantly reduced seed MC, irrespective of the variety tested and the drying method used. However, this was true for shade and room drying, but not for sun and silica gel drying. So, it did not pay to leave over in the field the tested varieties if their seeds were to be sun or silica gel-dried.

Days to drying

Table 2 shows the number of days necessary to reach constant seed weight under the various drying conditions

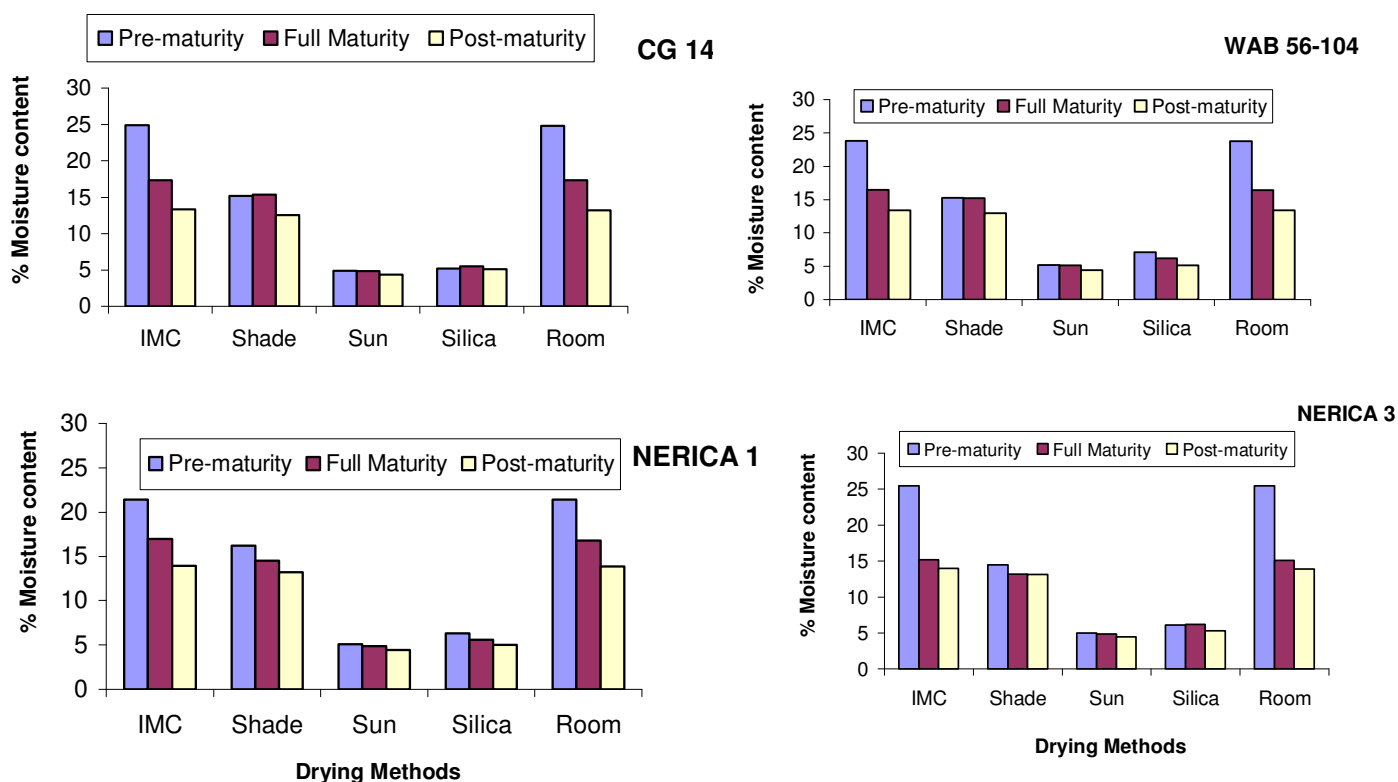


Figure 1. Percentage of moisture content of rice seeds before and after drying. IMC, initial moisture content (before drying). $LSD_{0.05}$ (Method), $LSD_{0.05}$ (Maturity).

tested. The drying time was found to vary depending largely ($p < 0.0001$) on the seed maturity stage at harvest and less on the drying method ($p = 0.006$) and variety ($p = 0.04$). Seeds harvested prematurely 15 days before or at mass maturity generally needed more time to reach constant MC (Table 2). The drying patterns for seeds using the various methods were rather similar within maturity stage of the variety at harvest. However, delayed maturity stage at harvest shortened the time to reach constant seed weight (Table 2). The results showed an average of 22, 18 and 12 days corresponding to the time needed to reach a constant seed weight when varieties were harvested 15 days before, at and 15 days after mass maturity, respectively.

Effect of drying procedure on seed viability

The effect of the drying procedure on seed viability was evaluated through germination tests (Table 3 and Figure 2). The percent germination of seed varied significantly between rice varieties ($p = 0.0006$), and was largely affected by the maturity stage at harvest ($p < 0.0001$) and the drying method used ($p = 0.002$) (Table 3). Except for the *O. sativa* parent variety (WAB 56-104), harvesting 15 days prematurely resulted in no germination of seeds of

the interspecific varieties (NERICA 1 and NERICA 3) and their *O. glaberrima* male parent (CG 14). It was observed that at any maturity stage or any drying method used CG 14 never reached more than 60% germination while WAB 56-104 and the progenies NERICAs recorded as high as 100% (Figure 2). With the exception of CG 14 the initial germination percentages improved as rice was harvested at mass maturity rather than 15 days earlier (0 to 40% germination for the NERICAs; 25 to 30% for WAB 56-104). The extra 15 days in the field caused the initial germination in CG 14 to reach 20% while this delay increased germination from 40 to 50% in NERICA1, 30 to 50% in WAB 56-104 and doubled it to 80% in NERICA 3. Drying by any method improved germination of the seeds harvested at any of the maturity stage studied. However, sun drying gave the best germination percentages at all maturity stages, in particular when the varieties were allowed an extra 15 days beyond the mass maturity date (Figure 2). Room drying led to poor seed germination, especially when rice was harvested 15 days before effective mass maturity.

DISCUSSION

Controversial reports consistently documented the impact

Table 3. Analysis of variance of percent germination of seed of rice varieties harvested at different maturity stages using four drying methods.

Source of variation	Sum of squares ^δ	Degrees of freedom	Mean Square	Computed F	Significance
Model*	49694.3	21	2366.4	5.6	<0.0001
Variety	9397.9	3	3112.7	7.3	0.0006
Maturity	30817.6	2	15408.8	36.2	<0.0001
Method	8950.7	4	2237.7	5.3	0.0018
Variety*method	758.5	12	63.2	0.2	0.9995
Error	16190.7	38	426.1		
Corrected total	65885.0	59			

*R²=0.854

^δType III of Proc GLM procedure(SAS).

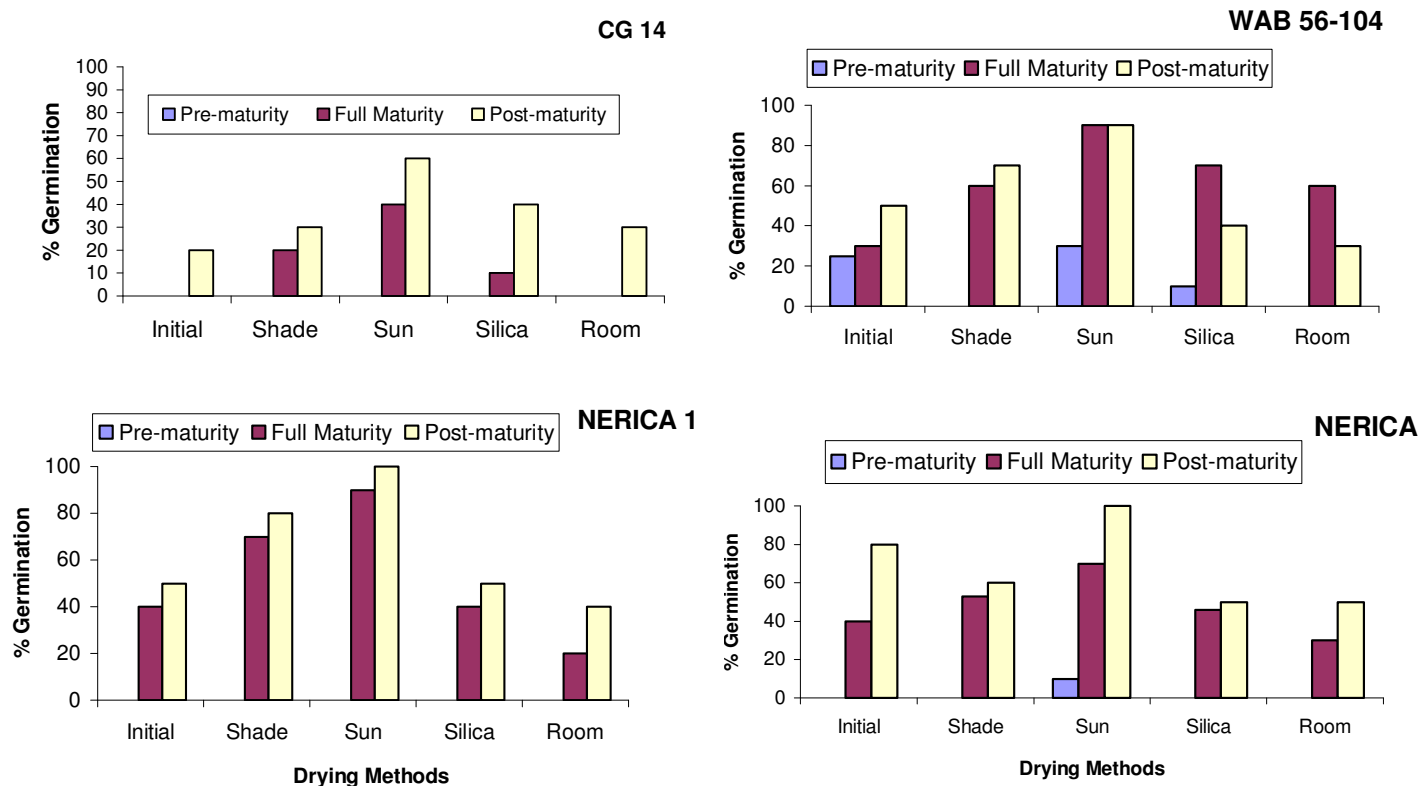


Figure 2. Percentage of germination of rice seeds before and after drying. Initial, before drying LSD_{0.05}(Method), LSD_{0.05}(Maturity)

on seed quality of the maturity stage at which seeds are harvested (Probert and Hay, 1999). For example, Harrington (1972) suggested that mass maturity may be the appropriate time for maximum seed viability and vigour. In contrast, Hay and Probert (1995), Kameswara Rao et al. (1991) and Pieta Filho and Ellis (1991) reported that seed quality continues to increase during the post-abscission phase. Monitoring the moisture content and dry weight of seeds during their development can often be useful in helping to decide

when to harvest seeds (Hong and Ellis, 1996). In the present study, the initial moisture content of seeds was variable and higher before mass maturity than at and after mass maturity (24.9 -17.3 -13.3% in CG 14, 21.4 -17.0-13.9% in NERICA 1, 25.5-15.2-14% in NERICA 3, 23.8-16.5-13.5% in WAB 56-104; the three values for each variety correspond to 15 days before, at and after mass maturity, respectively). The high initial moisture content recorded in the rice seeds before mass maturity was due to the premature harvest of the rice varieties (i.e.

15 days) considered in this study between the three maturity stages.

Comparison of the four drying methods indicated that shade and room drying did not significantly lower seed MC of any variety tested. During this study, the relative humidity (RH) recorded in the drying room was high (40%). This unusually high RH presumably led to a higher seed MC in comparison with drying under shade (Figure 1). Had RH been maintained at a lower level (10–15%), one would have expected that a more effective drying could have been achieved. Sun drying and silica gel drying were the most effective methods as they allowed seeds to be dried to as low as 4–5% and 5–7% MC, respectively.

Drying seeds under ambient RH and temperature is a common practice in many countries in Africa for small-scale seed drying (Probert and Hay, 1999), but the results obtained depend mainly on the season, location and species (Hong and Ellis, 1996). During the present study drying operations were carried out during the dry season in Ibadan, Nigeria. Achigan et al. (2004) studied the effects of contrasting drying procedures on maize and *Vigna* spp., and suggested the results to be very similar during both dry and wet seasons. It was reported that conventional drying methods may fail at times to reduce sufficiently seed MC to ensure long-term storage (Justice and Bass, 1978). Data in this study showed sun drying to be most effective in lowering rice seed MC. The high germination percentage of the dried seeds indicated that sun drying might not have affected initial seed quality even though further experimentations might be necessary to investigate the effect of sun drying on seed tissue integrity.

Silica gel drying using a 1:1 ratio (silica gel : seed) gave the next highest drying rate, compared with shade and conventional room drying, regardless of the variety tested and the maturity stage at harvest (Figure 1). This is consistent with other seed drying studies carried out using silica gel. Kong and Zhang (1998) dried *asparagus* bean seeds from 12 to 4% using silica gel with a ratio of 4:1 gel : seed. Seeds of two bean cultivars were dried for 50 days with silica gel in desiccators using a gel : seed ratio of 1:2 (Fischler, 1993). The same ratio was used by Zhang and Tao (1989) when drying bean seeds from 14 to 5% MC for 30–34 days. By using a higher silica gel to seed ratio, the frequent renewal of silica gel is reduced. Although silica gel is a very effective desiccant for drying seeds to very low MCs, many authors argued that the cost and labour involved in the daily regeneration of silica gel makes it a less practical method, as compared with oven drying or freeze drying (Hong and Ellis, 1996; Kong and Zhang, 1998). However, in situations where the supply of electricity is erratic and costly as witnessed in many countries in Africa, silica gel may be a viable option provided it is readily available cost effective.

This present study showed that all the drying methods

used improved germination of seeds harvested at different maturity stages. Previous studies indicated that desiccation tolerance is improved by slow drying or delayed drying (Hong and Ellis, 1997; Hay and Probert, 1995). One could speculate that the slow drying process allows seeds to continue to mature after the crop is harvested. In the case of the *O. glaberrima* variety (CG 14) and the interspecific NERICA progenies (*O. glaberrima* x *O. sativa*), the low initial germination percentages could be attributed to an innate dormancy that was subsequently lost after drying (Ellis et al., 1985). Guei et al. (2002) reported dormancy characteristics in the same *glaberrima* (CG 14) and NERICA varieties. Ellis et al. (1983) also demonstrated that germination of *O. glaberrima* seeds was improved after drying and attributed this to after-ripening. The results of the present study also showed that none of the drying methods used adversely affected seed viability. These observations were consistent for all methods and maturity stages studied.

In conclusion, this study identified sun- and silica gel drying methods as the most effective procedures in lowering seed moisture content and enhancing seed germination percentages in CG 14 (*O. glaberrima* steud), and WAB 56-104 (*O. sativa* L.) varieties and their progenies (NERICA 1 and NERICA 3) under the present experimental conditions. None of the four drying regimes used adversely affected seed quality, as measured by seed germination percentages. However, further research may be needed to study particularly the effect of the sun drying method on the rice seed tissue integrity. Data obtained in this study are of interest for short-term storage, including farm-saved seeds. From the germplasm conservation perspective, further studies are needed to determine the impact of the tested drying methods on the long term seed storability in seed genebanks. This was beyond the scope of the present work.

REFERENCES

- Cromarty AS, Ellis RH, Roberts EH. (1982). Handbooks for genebanks no. 1. The design of seed storage facilities for seed conservation. International Board for Plant Genetic Resources, Rome, Italy.
- Ellis RH, Roberts EH. (1991). The potential of ultra-dry storage of seeds for genetic conservation. University of Reading, UK.
- Ellis RH, Hong TD. (1994). Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). *Annals of Botany* 73: 501–506.
- Ellis RH, Hong TD, Roberts EH. (1983). Procedures for the safe removal of dormancy from rice seed. *Seed Science and Technology* 11: 77–112.
- Ellis RH, Hong TD, Roberts EH. (1985). Handbooks for genebanks no. 2. Handbook of seed technology for genebanks. Volume 1. Principles and methodology. International Board for Plant Genetic Resources, Rome, Italy.
- Ellis RH, Hong TD, Roberts EH. (1989). A comparison of low moisture content limit to the logarithmic relation between seed moisture and longevity in twelve species. *Annals of Botany* 63:601–611.

- FAO/IPGRI (1994). Genebank standards. Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute, Rome, Italy.
- Fischler M (1993). Bean germplasm conservation based on seed drying with silica gel and low moisture storage. Occasional publications series, no. 10.
- Guei RG, Adam A, Traore K (2002). Comparative studies of seed dormancy characteristics of two *Oryza* species and their progenies. *Seed Science and Technologies* 30: 499-505.
- Harrington JF (1972). Seed storage longevity. In: Kozlowski TT, editor. *Seed Biology*. Volume 3. Academic Press, New York, pp. 145-245.
- Harrington JF (1973). Problems of seed storage. In: Hydecker W, editor. *Seed Ecology*, Butterworths, London, pp. 251-264.
- Hay FR, Probert RJ. (1995). Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in fo xglove (*Digitalis purpurea* L.) *Annals of Botany* 76: 739-647.
- Hong TD, Ellis RH (1996). A protocol to determine seed storage behaviour. In: Engels JMM, Toll J, volume editors. IPGRI Technical Bulletin No. 1. International Plant Genetic Resources Institute, Rome, Italy.
- Hong TD, Ellis RH (1997). The effect of the initial rate of drying on the subsequent ability of immature seeds of Norway maple (*Acer platanoides* L.) to survive rapid desiccation. *Seed Science Research* 7: 41-45.
- Hu X, Zhang Y, Hu C, Tao M, Chen S (1998). A comparison of methods for drying seeds: vacuum freeze-drier versus silica gel. *Seed Science Research* 8(Suppl. 1): 29-33.
- ISTA (1993). International rules for seed testing. Rules 1993. *Seed Science and Technology*. 21(suppl.): 1-75.
- Justice OL, Bass LN. (1978). Principles and practices of seed storage. Agriculture handbook no. 506. VS Government Printing Office, Washington, DC, USA.
- Kameswara Rao N, Appa Rao S, Menghesa MH, Ellis RH (1991). Longevity of pearl millet (*Pennisetum glaucum* R. Br.) seeds harvested at different stages of maturity. *Annals of Applied Biology* 119: 19-103.
- Kong XH, Zhang HY (1998). The effects of ultra-dry methods and storage on vegetable seeds. *Seed Science Research* 8 (Suppl. 1): 41-45.
- Leopold AC, Vertucci CW (1989). Moisture as a regulator of physiological reaction in seeds. In: Stanwood PC, McDonald MB, editors. *Seed Moisture* (special publication no. 14). Crop Science Society of America, Madison, WI, USA, pp. 51-68.
- Pieta Filho C, Ellis RH (1991). The development of seed quality in spring barley in four environments. I. Germination and longevity. *Seed Science Research* 1: 163-177.
- Probert RJ, Hay FR (1999). Keeping seeds alive. In: Black M, Bewley JD, editors. *Seed Technology and its Biological Basis*, Chapter 11, pp. 376-409.
- SAS Institute (2001) The SAS system, version 8.2 for Windows. SAS Institute, Cary, North Carolina, USA.
- Walters C, Engels J (1998). The effects of storing seeds under extremely dry conditions. *Seed Science Research* 8 (Suppl. 1): 3-8.
- Walters C, Kameswara Rao N, Hu X (1998). Optimizing seed water content to improve longevity in *ex situ* genebanks. *Seed Science Research* 8(Suppl. 1): 3-8.
- Zhang XY, Tao KL (1989). Silica gel seed drying for germplasm conservation-practical guidelines. FAO/IBPGR. *Plant Genetic Resources Newsletter* 75/76: 1-5.