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Development of an efficient seed dormancy breaking method for African rice (*Oryza glaberrima*)

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Abstract

Seed dormancy is a significant factor limiting the conservation, evaluation, distribution and utilisation of African cultivated rice, *Oryza glaberrima*. In this study, 20 accessions from the AfricaRice genebank were grown at the AfricaRice Mbé station in Côte d'Ivoire, harvested manually, dried at 15% RH and 15°C, and stored at -20°C for one month before use. Seeds underwent dry heat treatment at 50 or 60°C for 2, 4, 7, 14, 21 or 28 days, or were treated with: 0.5, 1, 1.5 or 2 M KCl; 0.022, 0.112, 0.224 or 0.448 M HNO_3; 0.05, 0.1, 0.2, 0.4, or 0.5 M KH_2PO_4; and 2.89, 5.77 or 14.44 mM gibberellic acid (GA_3). Most treatments on intact seeds did not significantly increase germination, except for those treated at 50°C for 21 or 28 days. Dehulled seeds germinated effectively with all treatments except HNO_3 solutions. After validating the treatment on 287 *O. glaberrima* accessions, it was concluded that dry heat treatment at 50°C for 28 days effectively breaks dormancy. This protocol improves germination percentages of *O. glaberrima*, enhancing its use in various studies and aiding better decision-making in the management of *O. glaberrima* accessions in genebanks.

Keywords: African rice, dormancy, dry after-ripening, germination, gibberellic acid, heat, *Oryza glaberrima*

Introduction

Rice (*Oryza* spp.) is a staple food for more than half of the world's population (Pandey *et al*., 2010; Futakuchi *et al*., 2021). It is the third cereal crop in terms of production, after maize and wheat, with a world production of 518.2 million tonnes in 2021 (FAO, 2021). The African continent has the second largest rice consumption, after Asia (Saito *et al*., 2015; Futakuchi *et al*., 2021). In Africa, the share of rice in total cereal production has steadily increased from 9.3 to 17.8% between 1961 and 2018 (FAOSTAT, 2020). Paddy rice production in Sub-Saharan Africa was estimated at 26.5 million tons from a total of 11.95 million hectares of harvested area in 2018 (Ndindeng *et al*., 2021).

In West Africa, rice is a strategic commodity for food security. Rice consumption quickly expanded from the 1960s, driven by demographic growth, urbanisation and changes in consumer preferences (Mendez del Villar and Lançon, 2015; Sekiya *et al.*, 2020). The annual per capita rice consumption steadily increased from 10 kg in 1961 to 54 kg in 2017 (Osinski and Sylla, 2018). Annual rice consumption in Africa, per capita is projected to increase by about 5 kg over the period 2019–2028 (OECD/FAO, 2020). However, only 60% of consumer demand is met through local production and the rest is imported (Zenna *et al*., 2017). In the 2020/2021 trade year, Africa imported around 16.6 million metric tons of rice (Statista, 2023).

Although considerable efforts are increasingly being made to increase rice productivity worldwide, through the introduction of high-yielding varieties and the adoption of new cultivation practices, rice production in Africa is still subject to several biotic and abiotic constraints. Biotic stress is caused by bacteria, fungi, viruses, pathogenic nematodes, insect pests, weeds and invasion of parasitic plants (Ye *et al*., 2019). Regarding abiotic stress, drought remains one of the major factors that hinder plant growth or development (Nelson *et al*., 2014; Pandey and Shukla, 2015). Other factors such as soil salinity, acidity, iron and alumina toxicity, and phosphorus and zinc deficiency are all abiotic factors that hamper rice production (Sikirou *et al*., 2015; Fahimirad and Ghorbanpour, 2018; Pathak *et al.*, 2021).

Seed dormancy can be defined as an innate property of the seed that prevents germination even under suitable environmental conditions (Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is a complex trait, affected by multiple genes and environmental factors (Graeber *et al.*, 2014; Lu *et al*., 2018), and an important component of plant adaptation (Donohue *et al.*, 2005; Huang *et al.*, 2010). It is regulated by the antagonistic hormones, abscisic acid (ABA) and gibberellic acid (GA) (Finkelstein *et al.*, 2008; Shu *et al*., 2016; Née *et al.*, 2017a, b). Seed dormancy can be considered an undesirable trait by farmers, affecting crop establishment. Hence, selection to reduce levels of seed dormancy occurred during the rice domestication process and most cultivated plants germinate uniformly and rapidly after sowing, contrary to their wild ancestors (high dormant varieties/germplasms/accessions) (Kilian *et al.*, 2009). On the other hand, too low dormancy levels reduce seed quality for sowing and trigger pre-harvest germination, resulting in yield losses in cereals (Gubler *et al.*, 2005).

Seed dormancy in *Oryza* exhibits significant variation depending on the species and subspecies, reflecting the evolutionary and ecological adaptations of the genus. For example, differences in dormancy between different variety groups of *O. sativa* L. (Zhang *et al.*, 2020) highlight the influence of domestication and selection on this trait. Furthermore, the identification of over 150 quantitative trait loci (QTLs) associated with seed dormancy in *O. sativa* underscores the complexity of its genetic regulation (Zhang *et al.*, 2020). Comparative studies reveal that wild species such as *O. rufipogon* Griff. exhibit higher dormancy levels than cultivated species like *O. sativa* and *O. glaberrima* Steud. (Veasey *et al.* 2004). Interestingly, *O. glaberrima* displays stronger dormancy than *O. sativa*, as confirmed by Veasey *et al.* (2004) and Doku *et al.* (2016), possibly reflecting its adaptation to African environments and its role in traditional farming systems. These findings highlight the need for effective dormancy-breaking treatments tailored to the specific characteristics of *O. glaberrima.*

The Africa Rice Center (AfricaRice) genebank conserves 20,681 registered rice samples, representing five African indigenous wild species (*O. barthii* A.Chev., *O. longistaminata* A.Chev & Roehr., *O. eichingeri* Peter, *O. punctata* Kotschy ex Steud. and *O. brachyantha* A.Chev & Roehr.), as well as both modern and traditional *O. glaberrima* and *O. sativa* varieties (Ndjiondjop *et al.*, 2017). *O. glaberrima* accounts for approximately 17% (> 3,000 accessions) of the collection (Ndjiondjop *et al.*, 2022). An important process that is part of the genebank's routine operations is seed viability testing, usually through a germination test. To ensure that the germination results reflect the actual viability, it is essential that an effective dormancy breaking treatment is applied. For rice, different seed dormancy breaking treatments have been proposed, for example, removing the seed hull (palea and lemma), varying temperature, giving seed a dry heat treatment or applying chemicals (Doku *et al.*, 2016; Shiratsuchi *et al.*, 2017).

The AfricaRice genebank team have been placing *O. glaberrima* seeds at 50°C for five days to break dormancy. This allowed categorisation of the seed lots as: (*i*) weakly dormant; (*ii*) moderately dormant; and (*iii*) strongly dormant. However, this approach proved insufficient for consistently breaking dormancy across all accessions, as it did not result in high germination for many seeds. This limitation could be due to the variation in dormancy levels among accessions, which might require a more robust treatment to effectively break dormancy and achieve higher germination. The aim of the present study was to develop a more efficient treatment for routine use in viability testing *O. glaberrima* accessions. Specifically, the objective of this study was to (*i*) evaluate the extent of seed dormancy and its response to a variety of dormancy breaking treatments on 20 randomly selected *O. glaberrima* rice accessions (both intact and dehulled seeds); (*ii*) validate the best dormancy breaking treatment method on 287 accessions, representing the *O. glaberrima* mini-core collection.

Materials and methods

Fresh rice seed production, drying, packaging and conservation at -20°C.

Samples of seeds from 20 randomly selected accessions and 287 mini-core accessions of African rice (*Oryza glaberrima*) were taken from the AfricaRice genebank (table 1). Seeds were sown for multiplication in the field in May 2021 (irrigated lowland ecology) according to a randomised incomplete block design (RIBD) without repetition. Irrigation, weed management, fungicide/insecticide application, fertilizer application, rouging to eliminate off-types and other routine field cultural procedures were all undertaken as described in the rice knowledge bank website (http://www.knowledgebank.irri.org/step-by-step-production). Seeds were harvested when 80−85% reached maturity and seed moisture content was 18 ± 1% (determined using a Rice Flour Moisture Tester, SATAKE model MOISTEX SS7). Then, seeds were dried in the sun (August 2021), under a maximum temperature of 28.2°C and a minimum temperature of 21.7°C, to decrease their moisture content from 18 to 13 ± 1%. After threshing, cleaning, grading and labelling, seeds were dried further in a drying room where the conditions are controlled (15°C and 15% RH).

For the 20 random accessions, samples were packed in aluminium foil pouches according to the number of dormancy-breaking treatments to be tested. Seeds of the remaining accessions were also sealed inside aluminium foil pouches. Following Africa-Rice standard procedures, all the packages were stored at -20°C, in the long-term storage room until one month before use. Thereafter, packages were allowed to equilibrate at 2-5°C for approximately 48 hours, and then at room temperature (23 ± 2°C) before opening.

Seed viability and dormancy evaluation

Before proceeding with dormancy-breaking treatments, the viability of seeds from the 20 accessions was determined based on a tetrazolium test (ISTA, 2021). For each accession, 100 seeds were preconditioned by soaking in distilled water at room temperature (23 ± 2°C) for 18 hours and then, longitudinally dissected through the embryo and ¾ of the endosperm before being soaked in 1% tetrazolium solution for two hours at 25°C in the dark. After that, seeds were washed twice with distilled water. Seeds were scored as viable when the embryo was completely stained. Viability percentage of each accession was estimated based on the total number of seeds with the embryo completely stained divided by the total number of seeds tested.

Dormancy was assessed for all 307 accessions by germination of intact seeds in 90 mm-diameter Petri dishes, on filter paper moistened with distilled water, at room temperature (23 ± 2°C). Each accession was represented by three random samples of 100 seeds and germination was monitored daily over a period of 14 days. As also adopted by Shi *et al.* (2021), germination was considered here as emergence of the roots (root length ≥ 2.0 cm). Dormancy percentage of each accession was computed based on the total number of seeds not germinated divided by the total number of seeds sown. Seed lots with germination ≤ 25% were considered to be strongly dormant, whereas those with seed germination ≥ 50% were considered to be weakly dormant (Waheed *et al.*, 2012).

Dormancy-breaking treatments

Intact and dehulled seeds from each of the 20 accessions were used to test the effect of the different treatments described below. Three random samples, each consisting of 20 dehulled seeds and 20 intact seeds, were used for all treatments. Seeds of each accession were geminated as above and the number of intact and dehulled seeds germinated for each accession counted.

Heat treatments

Samples (intact and dehulled seeds from each accession) were incubated (dry) at 50°C or at 60°C, for 2, 4, 7, 14, 21 and 28 days in an oven (BINDER Machin). After that, seeds packages were allowed to equilibrate at room temperature (23 ± 2°C) during 12 hours before use. Both dehulled and intact seeds were germinated in Petri dishes under previous germination conditions described above.

Chemical treatments
Four chemical treatments at different concentrations were applied to intact and dehulled seeds: (*i*) 0.5, 1, 1.5 or 2 M KCl; (*ii*) 0.022, 0.112, 0.224 or 0.448 M HNO_3; (*iii*) 0.05, 0.1, 0.2, 0.4 or 0.5 M KH_2PO_4; (*iv*) 2.89, 5.77 or 14.44 mM gibberellic acid (GA_3). For each chemical and concentration tested, seeds were soaked for 24 hours and then washed twice with distilled water before germination in Petri dishes as described above.

Validation of the best treatment or protocol
Three random samples of 100 seeds from each of the 287 accessions collected from the *O. glaberrima* minicore set were treated with the best treatment identified from all previously tested treatments. Seeds were germinated in Petri dishes under the same germination conditions described above.

Statistical analysis
Data collected during the different treatments tested on the 20 accessions were imported into R program v4.0.3 (The R Development Core Team, 2011) and then visualised using the *ggplot2 R* package version 3.3.5 (Ginestet, 2011).

The germination data was coded into a binary format using the ≥ 50% accepted germination threshold for *Oryza glaberrima* species as applied by dormant Waheed *et al.* (2012) in *Oryza* genus. Accessions × pre-treatments with germination ≥ 50% were coded as 1 (successful germination) while accessions with < 50% germination were coded as 0 (unsuccessful germination). The data were then analysed in R software (version 4.4.0) using logistic regression with binomial error and logit function whereby the germination variable was fitted as the dependent variable with accessions and treatments as an independent variable (Olbana *et al.*, 2023). The predicted probability of a successful germination result for each accession is presented.

Results

Seed embryos for all *Oryza glaberrima* accessions seeds were stained red by 1% TZ solution, thus, all seeds were viable. For 16 of the 20 accessions, there was no germination, i.e. 100% dormancy. For accessions WAB0029199, WAB0029415, WAB0024666 and WAB0013353, dormancy percentages were 95, 95, 85 and 80%, respectively (table 1).

No germination was noted with intact and dehulled seeds not treated with the four chemical solutions. When intact seeds were treated with the different KH_2PO_4 solutions the germination percentage was less than 50% (figure 1). However, germination ranging from 18.25 to 57.25% for 0.5 and 0.05 M KH_2PO_4, respectively, was noted for dehulled seeds (averaged across different accessions). No effective germination was observed with intact seeds treated with different HNO_3 concentrations expect for the 0.224 M concentration (mean germination 10.5%; figure 1). For dehulled seeds, 0.022 M HNO_3 solution resulted in a mean germination percentage of 32% but no germination was observed with other HNO_3 concentrations. Different KCl solutions barely had any effect on overcoming the

Table 1. Passport information for the 20 accessions of *Oryza glaberrima* used for the dormancy-breaking experiments.

Accession number	Ecology	Biological status	Country of Origin	Dormancy (%)
WAB0029838	Rainfed Lowland	Traditional cultivar/landrace	Brazil	100
WAB0039053	Rainfed Lowland	Traditional cultivar/landrace	Burkina Faso	100
WAB0026193	Rainfed Lowland	Traditional cultivar/landrace	Nigeria	100
WAB0039052	Rainfed Lowland	Traditional cultivar/landrace	Burkina Faso	100
WAB0032322	Rainfed Lowland	Traditional cultivar/landrace	Nigeria	100
WAB0039066	Rainfed Lowland	Traditional cultivar/landrace	Burkina Faso	100
WAB0032311	Irrigated lowland	Traditional cultivar/landrace	Liberia	100
WAB0032309	Rainfed Lowland	Traditional cultivar/landrace	Liberia	100
WAB0011747	Shallow Forest Swamp	Traditional cultivar/landrace	Nigeria	100
WAB0029725	Rainfed Lowland	Traditional cultivar/landrace	Brazil	100
WAB0032272	Irrigated lowland	Traditional cultivar/landrace	Nigeria	100
WAB0001743	Upland	Traditional cultivar/landrace	Liberia	100
WAB0032570	Rainfed Lowland	Traditional cultivar/landrace	Brazil	100
WAB0032426	Irrigated lowland	Traditional cultivar/landrace	Liberia	100
WAB0029199	Upland	Traditional cultivar/landrace	Côte d'Ivoire	95
WAB0024666	Upland	Traditional cultivar/landrace	Côte d'Ivoire	85
WAB0029415	Irrigated lowland	Traditional cultivar/landrace	Guinea	95
WAB0013353	Irrigated lowland	Traditional cultivar/landrace	Guinea	80
WAB0032315	Rainfed Lowland	Traditional cultivar/landrace	Liberia	100
WAB0037616	Upland	Traditional cultivar/landrace	Senegal	100

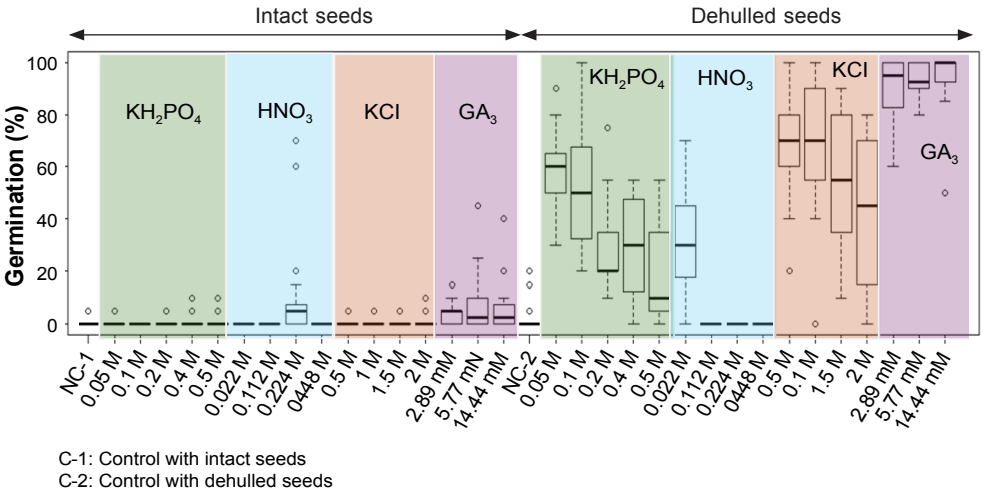

Figure 1. Germination of intact and dehulled seeds of 20 *Oryza glaberrima* rice accessions. Seeds were treated with different concentrations of KH_2PO_4, HNO_3, KCl or GA_3. The box-and-whisker plots displayed the distribution of germination percentage for the 20 accessions tested with each chemical treatment.

dormancy of intact seeds (figure 1), but when dehulled seeds were treated with the different KCl solutions, mean germination was 68, 66.50, 57.50 and 43% for 0.5, 1, 1.5 and 2 M KCl, respectively. Very low germination was observed for intact seeds treated with GA_3 (germination percentage $\leq 50\%$; figure 1) but GA_3 was effective in breaking dormancy of dehulled seeds. The mean germination values were 89.75, 93.75 and 94.25% for 2.89, 5.77 or 14.44 mM GA_3, respectively.

Intact seeds treated at 50 or 60°C for at least seven days showed some germination, but high levels of dormancy remained (figure 2). Treating intact seeds at 50°C for 14 days appeared to be more effective than treating at 60°C for the same length of time (64.75 vs. 52%). When intact seeds were heated at 50°C for more than 14 days, mean germination increased, e.g. from 86.75 to 90.5% after heating for 21 or 28 days, respectively.

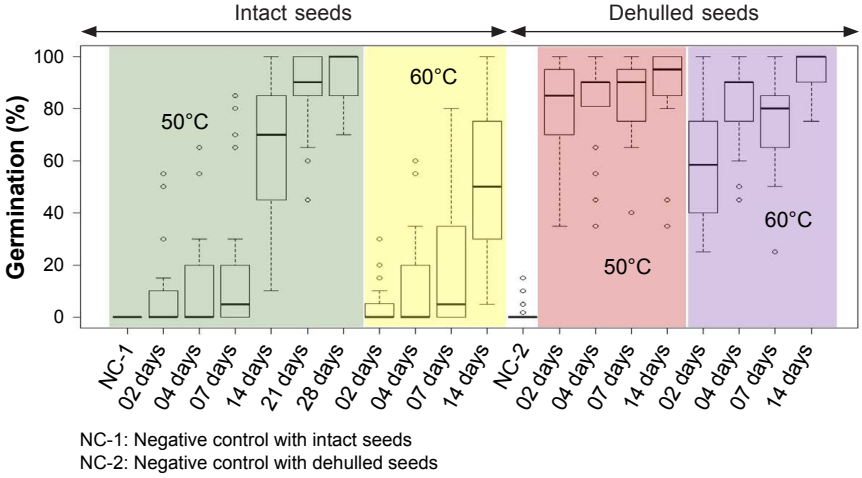

Figure 2. Germination percentages of intact and dehulled seeds evaluated for 20 rice accessions belonging to *Oryza glaberrima* (Steud). Intact seeds from each accession were placed at 50°C for 2, 4, 7, 14, 21 or 28 days, or at 60°C for 2, 4, 7 or 14 days; dehulled seeds were placed at 50°C for 2, 4, 7 or 14 days, or at 60°C for 2, 4, 7 or 14 days. The box-and-whisker plots show the distribution of germination percentage for the 20 accessions tested with each heat treatment.

Germination probability value was 0 for all 20 accessions (intact seeds) treated with all chemical solutions at different concentrations, except for two accessions (WAB0024666 and WAB0013353, values ≈ 0.52). Also, these two accessions showed a probability of $\geq 50\%$ germination with dry heat treatment at 60°C for 14 days (values $\approx$ 1). For intact seeds dried using heat at 50°C for 28 days, the predicted marginal means for the probability of $\geq 50\%$ germination were 1 for the 20 accessions. However, germination probability values were higher (≥ 0.85) for almost all dehulled seeds treated with various KCl and GA_3 concentrations, as well as with heat treatments at 50 and 60°C for 4 to 14 days (figure 3). Based on the results, it appears that heat treatment at 50°C for 28 days with intact seeds is effective in achieving $\geq 85\%$ germination for all 20 accessions (probability of 1).

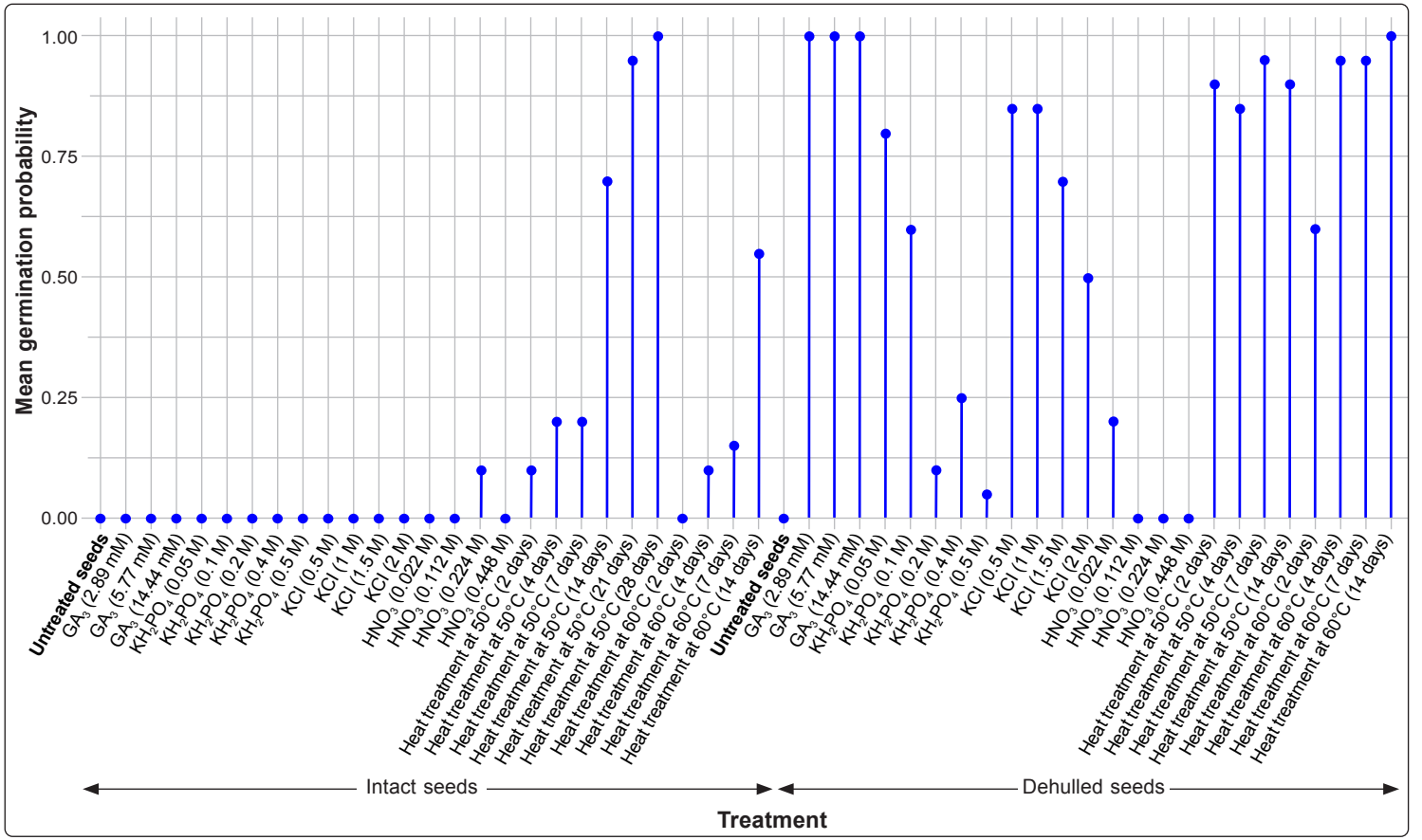

Figure 3. Effect of physical and chemical treatments on the mean germination probability of *Oryza glaberrima* seeds. Intact and dehulled seeds were subjected to various treatments, including dry heat treatment at 50°C or 60°C for 2 to 14 days, as well as chemical treatments with KCl, KH_2PO_4, HNO_3, and gibberellic acid (GA_3) at different concentrations. The mean germination probability was calculated for each treatment.

The mean germination of untreated seeds of 287 *O. glaberrima* accessions was 10.4% (figure 4A). More than 95% of the 287 accessions had less than 50% germination. After dry heat treatment at 50°C for 28 days, the overall mean germination for the 287 accessions was 90.01% (figure 4B).

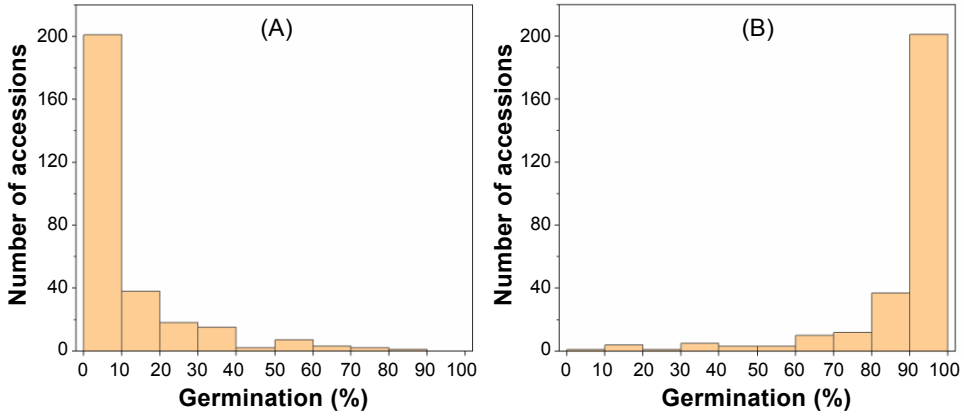

Figure 4. Summary of mean germination percentages of intact seeds estimated for the 287 accessions of rice belonging to *Oryza glaberrima* species. Data was collected from three repetitions of each accession. (A) intact seeds not heated before the germination test; (B) intact seeds heated in an oven at 50°C for 28 days before the germination test.

Discussion

In rice genebanks such as AfricaRice and IRRI where *O. glaberrima* germination tests are routinely performed to monitor the viability of (*i*) rice accessions stored in medium- and long-term storage, (*ii*) rice accessions to be distributed and (*iii*) newly acquired seeds, seed dormancy can be a very disadvantageous factor. For rice genebanks such as AfricaRice, seed dormancy can be a disadvantageous factor during both routine monitoring of the viability of *O. glaberrima* accessions in the genebank and also testing of newly acquired seeds.

This could result in misleading viability data, which can lead to erroneous management decisions. In addition, accurate measurement of seed viability is very important for genebanks as they must ensure the distribution of viable seed. Poor germination due to an inadequate dormancy breaking treatment could also lead to early scheduling of multiplication and/or seed stock regeneration activities. Rice seed dormancy can be a problem for farmers because (*i*) it affects the rapid and uniform germination, (*ii*) vigour of seeds during the early growth stages of the crop, thus reducing their productivity. (Tung and Serrano, 2011; Waheed *et al.*, 2012). Previous studies exposed that dormancy is higher in fresh seeds than those stored over time (Ilyas and Diarni, 2007; Bewley and Nonogaki, 2017). Kumar *et al.*, (2009) demonstrated that dormancy period in Asian Basmati 370 rice could be around 50 days after harvest. In the present study, only new fresh seeds that supposed will have a very high dormancy level were used.

Our seed viability testing results, performed using a 1% TZ solution, showed that all *O. glaberrima* seeds tested were viable, but none of them germinated in the control germination tests. These results demonstrated high percentages of dormancy in *O. glaberrima* accessions, indicating the need for proper dormancy breaking methods. Our findings are consistent with those of Kuya *et al.* (2019) who also stated that African rice *O. glaberrima* had stronger dormancy than *O. sativa*. This led them to place their samples at 50°C for five days and store them at room temperature for about six months after harvest, with the aim of breaking their dormancy before use. According to Waheed *et al.* (2012), a species is considered highly dormant when seed dormancy percentage is greater or equal to 75%. Compared to our results, the seed lots of all 20 accessions have a high dormancy (dormancy percentage $\geq 75\%$).

There was no significant germination when intact seeds were treated with the different KH_2PO_4 solutions (germination percentage $\leq 50\%$). Similar results were also found by Ros *et al.* (2000) in intact seedlings of *O. sativa* rice 'IR66'. However, a different result to ours was obtained by Bam *et al.* (2006) who noted a germination percentage around 94% with *O. sativa* 'IDSA 85', when the seeds were treated with 0.5% (w/v) KH_2PO_4 solution for 24 hours as in our study.

No significant germination was noted with intact and dehulled seeds treated with different HNO_3 concentrations. Yuningsih and Wahyuni (2015) reported that some dormancy breaking methods recommended by the International Seed Testing Association (ISTA) were not so effective in breaking dormancy. One of these methods was 1 M HNO_3 solution; this method was ineffective to break dormancy in Indonesian rice varieties, consistent with our results. Concerning dehulled seeds soaked in different HNO_3 concentration, we suspect that such concentrations (> 0.1 M HNO_3 for 24 hours soaking) could be fatal to the embryo in dehulled seeds, which is why no seeds germinated. Such suspicions were also formulated by Yuningsih and Wahyuni (2015). Our results showed that different KCl solutions tested barely had any effect on overcoming the dormancy of intact seeds. Bam *et al.* (2006) were able to efficiently break dormancy in 'IDSA 85' (89% germination) by soaking seeds in 0.5% (w/v) KCL solution for 24 hours. These results confirm the hypothesis that *O. glaberrima* seed dormancy level is higher than for *O. sativa*. Similar results to ours were also obtained by Brooks *et al.* (2020) who tried to improve upland rice seed germination and vigour under salinity and water stresses using KCL, KNO_3 and $MgCl_2$ solutions.

Very low germination was observed with intact seeds treated with GA_3. Compared to results reported by Waheed *et al.* (2012), germination percentage computed on intact seeds of the cultivated variety 'Swat-1' (*O. sativa*) is higher than those found in our study with intact seeds treated with the three different concentrations of GA_3 (55.9% versus 4.24 to 6.25%). However, GA_3 was effective in breaking dormancy of dehulled seeds. The average percentage germination obtained with dehulled seeds treated with GA_3 at 14.44 mM are slightly similar to those published by Mutinda *et al.* (2017) on intact seeds of *O. sativa* 'Basmati 370' also treated for 24 hours with a GA_3 solution at 14.44 mM (94.25 versus 95.7%). Several studies have reported the effectiveness of GA_3 solution in breaking dormancy in various cereals by promoting germination (Shalinee *et al.*, 2019; Wu and Shen, 2021; Ahmed and El Dessougi, 2023; Nedunchezhiyan *et al.*, 2023).

Intact *O. glaberrima* seeds heated at 50°C for 28 days showed high germination (90.5%) which is higher than those obtained by Hanumanthappa *et al.* (2016) and Waheed *et al.* (2012) on both wild and cultivated *O. sativa* accessions. Timple *et al.* (2018) also suggested that the dormancy of intact wild *Oryza* species seeds, including *O. barthii*, a wild rice species endemic to Africa and known as the progenitor of African cultivated rice, *O. glaberrima* (Bessho-Uehara *et al.*, 2017), can be effectively alleviated by dry heat treatment at 50°C and sowing directly in KNO_3, or by pre-soaking heat-treated seeds in GA_3 or H_2O_2 for 18 hours before soaking in distilled H_2O for a further 18 hours prior to sowing.

The mean germination of untreated seeds of 287 *O. glaberrima* accessions was very low confirming high levels of dormancy in fresh seeds of this mini-core germplasm (figure 4). According to the scale adopted by Waheed *et al*. (2012), the 287 accessions were grouped into three groups based on their dormancy level: (*i*) 254 accessions that were highly dormant (germination $\leq 25\%$); (*ii*) 20 accessions with medium dormancy (germination between 26 and 50%) and 13 accessions with low dormancy (germination $\geq 50\%$). This dormancy was effectively overcome by treating the seeds with dry heat treatment at 50°C for 28 days.

Conclusion

We strongly recommend that intact seeds of *Oryza glaberrima* be heated in an oven at 50°C for 28 days before sowing. For seeds with very high dormancy, heating at the same temperature for more than 28 days may yield better results. It provides a well-optimized protocol that can be used by rice genebanks, researchers and breeders, to efficiently break dormancy in *O. glaberrima* for its enhanced utilisation.

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