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SCREENING AFRICAN RICE (*O. glaberrima Steud.)* FOR TOLERANCE TO ABIOTIC STRESS. III. FLOODING

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SUMMARY

Flooding is an important abiotic stress affecting rice production in rainfed lowlands of Asia and Africa. Highly tolerant donor rice accessions are needed to breed improved flood-tolerant rice varieties that can increase the productivity of the rainfed lowland ecology. A large collection of African rice (*O. glaberrima*; >2000 accessions) was screened separately for tolerance to flooding at germination (anaerobic germination ability; AG), submergence and stagnant flooding stresses. The entire collection was first screened in preliminary evaluation and best performing accessions were further evaluated in advanced screening. In the preliminary screenings, most of the *O. glaberrima* accessions were found to have good levels of anaerobic germination ability and stagnant flooding tolerance but were sensitive to submergence stress. Based on preliminary screening; 43, 20, and 42 accessions were selected for tolerance to AG, submergence and stagnant flooding respectively. Results identified six *O. glaberrima* accessions (TOG 5505-A, TOG 5485, TOG 5980-A, TOG 8347, TOG 7252-A, TOG 16704) for AG. One accession (TOS 6454) was selected for submergence while four *O. glaberrima* accessions (IG 48, GERVEX 2674, TOG 7148 and IG 133) were selected for stagnant flooding. All selected accessions were similar or better than the best *O.*

sativa check. These accessions are potential sources of tolerance in breeding for flood tolerance in rice.

Key words: Anaerobic germination, *O. glaberrima*, *O. sativa*, stagnant flooding, submergence

Key findings: Promising accessions for breeding flood tolerant varieties were identified from the evaluation of two thousand and two accessions of *O. glaberrima* for tolerance of the three most important flood types affecting rice production in Asia and Africa: flooding at germination (AG), submergence at seedling stages and stagnant flooding.

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INTRODUCTION

Rice is cultivated under diverse ecologies, ranging from irrigated lowland, rainfed lowland, rainfed upland to deep water. Rainfed lowlands occupy about 32% (~52 M ha) of total rice area and account for about 19% of world rice production (GRISP, 2013). In Africa, rainfed lowland is the major rice growing ecosystem and occupies about 40% of rice area. Flooding is a major abiotic stress in rainfed lowlands. In Asia, more than 16 million hectares of rice are unfavourably affected by flooding (GRISP, 2013). For example, in Vietnam, floods in the year 2000 caused a total direct economic loss estimated at USD 289.8 million (Nguyen and James, 2013). India and Bangladesh make average annual loss in rice production of more than 4 million tons per year (IRRI, 2010a). Africa is highly vulnerable to climate change (IPCC 2014) but data is scarce on the effect of floods on rice production in Africa.The area affected by flooding is expected to increase due to climate change.

Iron toxicity is another abiotic stress prevalent in Africa; it is reported as a major constraint to rice production (Olaleye *et al.*, 2001; Gridley *et al.*, 2006). There is limited information on global rice area affected by iron toxicity and the annual losses in production due to this stress (Sikirou *et al.*, 2015). This is important in Africa as iron toxic soils are endemic and climate change is expected to increase the frequency of flooding globally. Currently, there is limited information on the ability of rice to germinate under flooded conditions in iron toxic soils.

The three important types of flooding stress in rainfed lowlands are flooding at germination, submergence at seedling stages and stagnant flooding (Manangkil *et al.*, 2008; Singh *et al.*, 2011). Flooding at germination occurs in rice areas where direct-seeding is practised. However, not all rice varieties are able to germinate under waterlogged conditions. This leads to significant economic loss to farmers. Most rainfed lowland rice farmers in Africa practice direct-seeding, often in poorly prepared fields, making their crop vulnerable to loss if flooding occurs during seed germination (AfricaRice, 2014). In submergence at the seedling stage, flood water completely covers the plants for a short term (usually 10-14 days). This can often lead to complete crop loss with devastating consequences on farmers' livelihood. Increasing water depth during flooding at the seedling stage inhibits production of basal tillers, reducing tiller number, which leads to decreased grain yield (Lockard, 1958 as cited by Ito *et al.*, 1999). Yield losses ranging from 10%-100% are experienced depending on age of the crop, depth, temperature, turbidity and turbulence of flood water (Setter *et al.*, 1997; Das *et al.*, 2009). Stagnant flooding occurs when flood water stagnates for most part of crop growth at a depth ranging from 20-50 cm. Stagnant flooding adversely affects rice yields as number of productive tillers is reduced (Sarkar, 2016).

In flood-prone ecology, rice farmers generally grow traditional rice varieties which can tolerate flooding; but these are usually poor yielding (Singh *et al.*, 2017). In recent years, remarkable progress has been made in breeding for seedling stage submergence tolerance. A gene for submergence tolerance (SUB1) has been isolated from an Indian rice cultivar FR13A and deployed via marker-aided breeding into several submergence susceptible rice varieties in Asia and Africa (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009; Bailey-Serres *et al.*, 2010; El-Hendawy *et al.*, 2011; Gonzaga *et al.*, 2016). The SUB1 gene is known to provide tolerance of submergence for up to two weeks. Recently, it has been realized that due to climate change, tolerance beyond two weeks is necessary as the quiescence strategy by which mega varieties with SUB1 survive submergence is not useful if water stagnates for more than 2-3 weeks (Sarkar *et al.*, 2006 as cited in Kuanar *et al.,* 2017). However, no significant progress has been made to further prolong tolerance beyond two weeks probably because there is no known suitable donor although new QTLs conferring submergence have been found (Septiningsih *et al.*, 2011; Gonzaga *et al.*, 2016; 2017).

Conversely, progress has been made to overcome germination stage flooding stress by exploiting anaerobic germination (AG) trait. Anaerobic germination is the ability of rice seeds to germinate, elongate and survive under flooded conditions (IRRI, 2010b). Few donors for AG have been identified. Using these donors, major QTLs have been mapped, two genes have been identified and gene-specific markers are being used in breeding (Ismail *et al.*, 2009; Angaji *et al.*, 2010; Septiningsih *et al.*, 2013; Baltazar *et al.*, 2014; Hsu and Tung, 2015; Kretzchmar *et al.*, 2015). However, to fully exploit this trait and to deploy it in a broad range of environments, further research is needed. Donors with stronger and stable expression of anaerobic germination are necessary for this.

In contrast to these two traits, progress in breeding for tolerance to stagnant flooding is limited. Very few rice varieties useful in breeding for stagnant flooding tolerance are known. Currently, breeding lines are selected by screening existing lines under stagnant flooding conditions ((Mallik *et al.*, 1995; Collard *et al.*, 2013; Vergara *et al.*, 2014; Sarkar, 2016; Sandhya *et al.*, 2017).

Thus, it is evident that there is a need to identify more donors for all the three types of flooding stresses. Presently, most flooding research work is focused on *Oryza sativa* and limited work is done to identify variation in other related species (Menguer *et al.* 2017).

Oryza glaberrima (2n = 24, AA) is a cultivated rice species that originated in Africa having evolved from the wild species *O. barthii* A. Chev. (Second, 1982). It yields less than *O. sativa* due to grain shattering and fewer branching of the panicles. *O. glaberrima* is adapted to the local production constraints in West and Central Africa (Sie *et al.*, 2012) and some accessions have been crossed with *O. sativa* varieties to develop the popular NERICA (New Rice for Africa) series for upland and lowland ecologies which combines the yield advantage of *O. sativa* and local adaptability of *O. glaberrima* (Jones *et al.*, 1997, Saito *et al.*, 2010).

Some studies identified *O. glaberrima* as a source of tolerant genes for abiotic stresses in rice which can be harnessed in development of climate-resilient varieties (Ndjiondjop *et al.*, 2010; Sikirou *et al.*, 2016; Shaibu *et al.*, 2017). Having evolved from flood-prone ecology, *O. glaberrima* is suggested as a good source of tolerance to flooding stress (Oka, 1974; Watarai and Inouye, 1998; Inouye *et al.*, 1989; Mochizuki *et al.*, 1997; Futakuchi *et al.*, 2001; Sarla and Swamy, 2005; Joho *et al.*, 2008). However, limited research has been carried out to characterise the flooding tolerance of *O. glaberrima*. Futakuchi *et al.* (2009) conducted a physiological study and concluded that *O. glaberrima* has higher resistance to deepwater stress. Sakagami *et al.* (2009) studied physiological responses of 27 accessions of *O. glaberrima* accessions to prolonged submergence and suggested that *O. glaberrima* could be used in breeding for stagnant flooding tolerance. Little is known about tolerance of *O. glaberrima* to short-term flash flooding stress (submergence) although Joho *et al.* (2008) reported that *O. glaberrima* cultivars had the highest shoot elongation ability when submerged for 10 days with 45 cm water depth. In addition, there is no previous report on anaerobic germination (AG) ability in *O. glaberrima*.

Given the importance of *O. glaberrima* as a source of tolerance to flooding, there is a need to assess the genetic variation within this species for flooding-related traits. Diverse *O. glaberrima* accessions are available in the genebank of Africa Rice Center (www.africarice.org) which is accessible for different research purposes. It is important to evaluate available genetic resources to identify materials with novel genes, which can be used to improve released varieties to meet the current climate challenges.

The aim of this study was to screen the entire collection of *O. glaberrima* accessions at Africa Rice Center genebank for tolerance to three types of flooding and identify most tolerant accessions that are suitable asdonors in breeding flood tolerant rice cultivars.

MATERIAL AND METHODS

Experimental site

The experiments were carried out at the Africa Rice Center (AfricaRice) research station at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Latitude 3°54´32" E and Longitude 7°29´15"N). Screening for AG was conducted under screenhouse and field conditions. Screening for submergence was conducted in screenhouse. Stagnant flooding trials including control trials (normal irrigated) were conducted in lowland fields.

Plant material

Two thousand one hundred and six *O. glaberrima* accessions were obtained from the gene bank of AfricaRice and out of these, accessions with poor seed viability and strong dormancy were eliminated (based on earlier experiments) and only 2002 accessions were selected (Table 1). These selected accessions were screened for tolerance to each of the three stresses separately. *Oryza sativa* checks were used for comparison. WITA4 (FARO52) a popular rainfed lowland rice cultivar was used as a local check in all the experiments. In addition, tolerant check for AG (Khao Hlan On; Ismail *et al.*, 2009), submergence (Swarna Sub1; Septiningsih *et al.*, 2009 and stagnant flooding (IRRI 119 and IRRI 154; Vergara *et al.*, 2014; Kato *et al.*, 2014) were used in the respective experiments. The screening involved two stages: in preliminary trials, the entire set of accessions was screened and best performing materials for each stress was selected. The selected set was further screened in advanced trials and about four best accessions for each stress were selected.

Table 1. Geographical origin of selected *O. glaberrima* accessions.

Country of Origin	Selected accessions			
	Total	Anaerobic germination	Submergence	Stagnant flooding
Burkina Faso	50	2	0	0
Cameroun	27	2	1	1
Chad	19	1	0	1
Democratic Republic of the Congo	1	0	0	0
Egypt	1	0	0	0
Gambia	9	0	0	0
Ghana	40	1	0	0
Guinea	165	3	5	1
Guinea-Bissau	19	0	0	0
Guyana	1	0	0	0
India	1	0	0	0
Ivory Coast	54	2	0	4
Japan	1	0	0	0
Liberia	512	4	1	17
Madagascar	2	0	-	0
Mali	272	11	5	8
Nigeria	424	12	2	3
Senegal	83	2	3	2
Sierra Leone	29	1	0	0
Tanzania	2	0	0	0
Togo	10	1	0	0
Zambia	1	0	0	0
Zimbabwe	1	0	0	`0
Unknown	278	1	3	5
Total	2002	43	20	42

Protocols for screening

Anaerobic germination

Dormancy of seeds of the *O. glaberrima* rice accessions was broken by heat treatment in an oven at 50°C for five days, while the same temperature for three days was used for the *O. sativa* checks. Germination tests were carried out after breaking dormancy. Twenty seeds of each accession were placed on moist filter paper in Petri dishes. The petri dishes were kept in an incubator at 30°C and the number of seeds that germinated was counted 5 days after. Only accessions with germination percentage ≥70% were used in all trials. Germination test was conducted prior to every AG experiment and results of germination test were considered as survival under control conditions.

Screenhouse

Screening was done in transparent plastic cups (13 cm length, 500 ml capacity). One cup was used per accession, and each cup was filled with soil up to 2 cm. Soil from lowland rice field on-station was collected, sieved and used to fill the plastic cups. Twenty viable seeds per accession were then placed on the soil. The seeds were covered with 1 cm of soil and thereafter submerged in 10 cm of water (potable quality) and the water level was maintained for 21 days. This protocol is a slightly modified version of IRRI's protocol for AG screening (Ismail *et al.*, 2009; Ella *et al.*, 2011a). In the preliminary trials (in 2012), the plastic cups were directly placed on a table. However, in subsequent trials, for ease of operation, the cups were placed inside transparent plastic trays (55 cm x 35 cm x 23.6 cm) which were filled with water.

Three modifications of this protocol were followed when necessary. One modification was to increase the water depth to 15 cm instead of the usual 10 cm. Second modification was to add soil to the bottom of tray and then place the plastic cup on soil before adding water. Third modification was to use Fe-toxic soil instead of the regular soil. For this, soil from a Fe-toxicity hotspot (Edozhigi, Niger state, Nigeria; 9° 45´N, 6° 17´E) was collected and used. The pH and Fe concentration of the Fe-toxic soil were 5.9 and 500 mg kg^{-1} respectively.

Field

Concrete screening tanks measuring 5 m x 8 m x 0.5 m were constructed in a lowland field in Ibadan. These tanks were at ground level and water depth could be very well controlled. The tanks were filled up to 30 cm with soil. Twenty seeds per accession were sown in seeding trays with 448 cells per tray and the seeds were thereafter covered with a thin layer of soil. The trays were placed on the soil in the screening tank and the tank was flooded with water such that 10 cm water level was maintained over the trays.

Data collection

In the AG trials, data were collected on the number of seedlings that germinated and emerged out of water 21 days after seeding for the computation of survival percentage (Ismail *et. al*., 2009). Survival rate is a measure of the percentage of seedlings emerging above water compared to the total number of seeds sown. Length of the shoot above water was measured and recorded as shoot length. Seedling vigor was scored on a scale of 1 to 9 (SES, 2002). In all trials, daily temperature of water in the cups was measured with a thermometer at 0830 H and 1530 H. Data on ambient temperature were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan Weather Station.

Submergence

The protocol for submergence screening is similar to the one followed at IRRI (Septiningsih *et al.*, 2009). Trial was conducted in a screenhouse. Seeds of *O. glaberrima* accessions were incubated for five days at 50 ^{o}C to break dormancy. Seeds were planted in plastic seeding trays with soil. Twenty seedlings of each entry were allowed to grow for 21 days before submergence. Number of plants before submergence was counted and trays with seedlings were then completely submerged in water in large plastic drums (380 L). Water depth of 40 cm above the seedlings was maintained for 14 days. After 14 days, the trays with seedlings were removed from water and placed on a table to allow recovery. Data on survival was taken after 14 days of recovery.

Stagnant flooding

Seeds of *O. glaberrima* accessions were incubated for five days at 50^{o}C to break dormancy. In all the trials, seeds were first planted in a nursery and 21-day old seedlings were transplanted into a well-levelled puddled field. Plots were either single-row or two-rows and 3 m in length. Spacing of 0.2 m x 0.2 m was followed. Complex fertilizer (NPK, 15-15-15) was applied as basal dose at a rate of 200 kg/ha a day after transplanting. Topdressing was done with urea twice, once at tillering stage (30 kg/ha) and again at panicle initiation (30 kg/ha). Weeds were controlled by application of herbicide in the initial stages and by manual weeding at later stages.

In control trials, standing water of about 2-5 cm was maintained in the field until harvest while in stress trials, water was first maintained at 2–5 cm above the soil surface for 14 days after transplanting. Thereafter, stress was imposed by raising water level to 15 cm on the first day and then gradually increased by about 5 cm on alternate days until the water level reached 50 cm above the soil. This water level was maintained until harvest (about three month duration). Just before harvest, the plots were drained to 2–5 cm water level. The stress screening protocol followed here is similar to the one followed at IRRI (Singh *et al.*, 2011).

Days to flowering was recorded when the panicle was exserted in approximately 50% of the plants in a plot. Plant height was measured at maturity on three randomly selected plants in each plot from the ground to the tip of the panicle and averaged. Number of tillers and panicles were counted from five randomly selected plants from each plot and averaged. Panicles from each plot was harvested, threshed, cleaned, dried to moisture content of about 14% and weighed to obtain grain yield. Under stress conditions, survival was calculated as percentage of number of seedlings in a plot at harvest compared to number of seedlings before imposition of stress.

Preliminary screening

Anaerobic germination

Screening for AG was conducted in a screenhouse in WS 2011. The *O. glaberrima* accessions were screened in an augmented design by dividing them into six batches with number of entries in each batch ranging from 189 to 558. This division was necessary becausenot all the entries could be screened at the same time but checks were included in each batch. Mean water temperature for the duration of the screening in batches ranged between 32.9 °C and 42.6 °C. The mean AG of batches was in the range 13.8 to 87.9% and about 7% of entries with the highest AG were selected in each batch. In all, 147 accessions of *O. glaberrima* were selected. The 147 selected accessions were further screened in dry season (DS) 2012 using a randomized complete block design (RCBD) with two replications. In this trial, the level of water was increased to 15 cm. A total of 79 accessions which had AG tolerance of more than 60% were selected. These 79 accessions were screened again in DS 2012. Fe-toxic soil was used instead of regular soil and a water depth of 10 cm was maintained above the soil. Experimental design was RCBD with two replications. From this experiment, 43 accessions of *O. glaberrima* with AG of up to76% were selected (Table 1).

The selected 43 accessions were screened in WS 2012 in two trials using a RCBD with three replications. In the first trial, regular lowland soil from Ibadan was used while in the second, Fe-toxic soil from Edozhigi was used. In both trials, the plastic cups were placed in large plastic trays to which water was added to a depth of 10 cm above soil in cups. Surviving plants from each entry in first trial were transplanted into a lowland rice field. Single plants from each entry were selected and harvested separately. Seeds from harvested single plants were multiplied in wet season (WS) of 2013. The multiplied seeds were used in all subsequent experiments.

Submergence

The first evaluation was conducted in the wet season (WS) of 2013 in an augmented design. 1732 *O. glaberrima* accessions and two *O. sativa* checks (repeated every 50 plots) were evaluated. In this trial, 20 accessions had higher percentage survival than the best local check (WITA 4) and were selected for further studies. The geographical origin of these accessions is indicated in Table 1.

Stagnant flooding

In DS 2012, 1732 *O. glaberrima* genotypes were evaluated in stagnant flooding conditions in an augmented design. In this trial, accessions with poor survival, strong lodging, or very late flowering (>130 days) were not considered for selection. Of the remaining, 141 entries were selected based on high plant yield, biomass, tiller number and panicle number. The geographical origin of these selected 141 entries is given in Table 1. In WS 2012, the selected accessions were grown in irrigated conditions to purify and multiply them. In DS 2013, the

141 selected *O. glaberrima* accessions and *O. sativa* checks were screened in two separate trials – stress and control. Probably, due to photoperiod sensitivity, some accessions did not flower and such accessions were not considered for selection. Based on grain yield, days to flowering, biomass, height, tiller and panicle number, 42 accessions were selected for further testing.

Advanced screening

Anaerobic germination

The 43 accessions selected from preliminary trials were re-evaluated for AG in the screenhouse and field during WS 2014 (May). In the first trial in the screen-house, the plastic trays had only water and plastic cup with soil and seeds were placed in it. In the second trial, plastic trays were first filled up to 10 cm with regular lowland soil. The plastic cups containing seeds were then placed on the soil in the tray. Experimental design was RCBD with two replications in both trials. In the field trial, a randomized complete block design with two replications was used. Seeding was done in seeding trays with 14 x 32 cells (1.5 cm diameter and 2.5 cm depth) with a hole at the bottom. Twenty seeds were sown per accession using one seed per cell. The seeds were covered with approximately 1 cm soil and placed on soil in the field tank. Water was added to the tank immediately so as to submerge the seeding trays with seeds to a depth of 10 cm. Seeding in trays was necessary to avoid floating of *O. glaberrima* seeds (which were quite light in weight compared to *O. sativa* checks) after submergence and to prevent snail damage.

Submergence

The twenty accessions selected from preliminary studies were screened for submergence tolerance in DS 2014 in two trials conducted one after another. The trials were laid out using Alpha lattice design with three replications.

Stagnant flooding

In WS 2013, 42 selected accessions and *O. sativa* checks were evaluated in two separate trials - stress and control. Trials were laid out as alpha lattice with three replications (in WS). Plots were 3 m long and with two rows. Spacing was 0.2 m x 0.2 m intra- and inter-row. Imposition of stress was carried out as in preliminary trials. Data were collected on days to flowering, plant height, tiller number, panicle number and grain yield.

Data analysis

All data collected were analysed using Breeding View in Breeding Management System (BMS) v 3.0.8 (IBP, 2015). The analysis used mixed models where replications and blocks within replications were treated as random and genotypes were considered as fixed. AG data on survival (%) was arc-sine transformed for analysis. Significant means were separated using Least Significant Difference (LSD) and untransformed means are presented in tables. Broad sense heritability was also obtained from BMS.

RESULTS

Anaerobic germination

Germination (%)

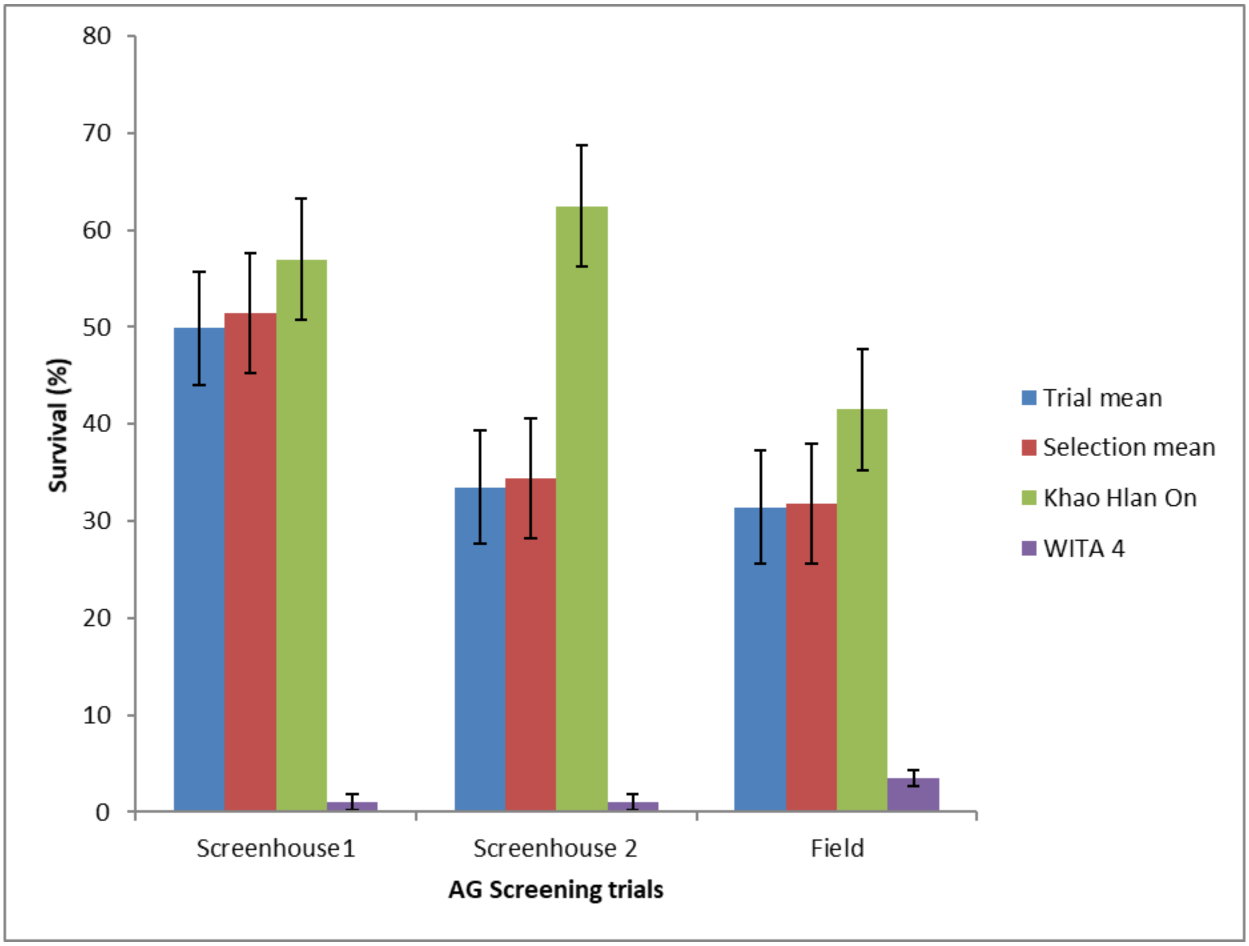
The trial mean for AG in screenhouse trial 1 (outside tray without soil) was higher than that in trial 2 (outside tray with soil) (Table 2). Significant differences were observed among accessions in the two screenhouse trials. In trial 1, AG means of three of the six selected accessions were significantly higher than that of the tolerant check while the AG values of the other three were not significantly different from KHO. In trial 2, all the six entries selected had similar AG values as the tolerant check and they were all significantly higher than WITA 4 (local check). Heritability for anaerobic germination in the screen house trials was 0.8.

Table 2. Anaerobic germination (AG) of selected *O. glaberrima* accessions under screen house and field conditions at AfricaRice, Ibadan, Nigeria during WS 2014.

Genotypes	Anaerobic germination (%)			
	Screen house 1	Screen house 2	Field	Combined
Minimum	0.0	0.00	0.00	0.00
Maximum	90.0	71.3	62.5	65.9
Mean (N = 43)	51.5	34.4	31.9	35.3
Selections				
TOG 5485	72.5	63.2	42.5	57.5
TOG 5505-A	75.0	63.8	32.5	55.9
TOG 5980-A	85.0	68.8	52.5	65.9
TOG 7252-A	80.0	37.5	62.5	51.5
TOG 8347	90.0	56.3	25.0	54.1
TOG 16704	87.5	56.3	32.5	55.3
O. sativa (checks)				
Khao Hlan On	57.0	62.5	41.5	53.1
WITA 4	1.0	1.0	3.5	-1.1
Trial Mean (n=50)	49.9	33.5	31.4	34.3
LSD (0.05)	37.3	28.6	27.9	20.5
P (<0.05)	***	***	***	***
Heritability	0.8	0.7	0.5	0.8

Average ambient temperature was 28 ^{0}C; average water temperature was 31 ^{0}C (screenhouse) and 33.6 ^{0}C (field).

There were significant differences among the 43 entries screened in the field and TOG 7252-A (62.5%) was the best among all the *O. glaberrima* accessions. It was significantly different from three of the selected accessions although not significantly different from Khao Hlan On (KHO). KHO had similar means as other entries in the screening. All the selected entries and the tolerant check were significantly higher than WITA 4. The trial mean in the field was low and heritability for AG was 0.5, which was the lowest in the 2014 trials. In the combined analyses, of all the 2014 WS trials, the six *O. glaberrima* accessions were the best. Heritability for the AG trait was 0.8 (Table 2). The mean performance of the *O. glaberrima* accessions was higher than the trial mean in all trials conducted (Figure 1).



Screenhouse 1: Water only in submergence tank
Screenhoue 2: Paddy soil and water in submergence tank

Figure 1. Anaerobic germination of selected *O. glaberrima* accessions and checks across different selection trials in 2014 WS, Ibadan, Nigeria.

Shoot length

Shoot length above water of the accessions varied significantly in each of the three screening trials (Table 3). Trial mean for shoot length was highest in the field (19.9 cm) and lowest in trial 2 in screenhouse (12.5 cm). The selected accessions had similar shoot lengths with the best check (Khao Hlan On) in all the trials. Heritability for shoot length ranged from 0.7 to 0.9.

Submergence

Swarna-Sub1 outperformed all the *O. glaberrima* accessions in the two trials and this was evident in the combined analysis (Table 4). Three and nine *O. glaberrima* accessions had significantly higher survival than local check (WITA4) in the first and second trials respectively. In the combined analysis, survival of six accessions (TOG 16774, TOG 9365, TOS 16746, TOS 6454, TOS 6455, and YG 146) was significantly higher than WITA 4. Highest survival among *O. glaberrima*

Table 3. Shoot length *O. glaberrima* accessions selected for anaerobic germination under screen house and field conditions at AfricaRice, Ibadan, Nigeria WS 2014.

Genotypes	Shoot Length (cm)			
	Screenhouse1	Screenhouse 2	Field	Combined
Minimum	0.00	0.00	0.00	0.0
Maximum	19.0	18.0	35.4	18.3
Mean (N = 43)	13.3	12.9	20.2	13.7
Selections				
TOG 5485	15.7	16.6	25.2	17.3
TOG 5505-A	15.5	16.0	22.9	16.5
TOG 5980-A	13.7	15.4	19.7	14.8
TOG 7252-A	13.4	15.4	23.1	15.8
TOG 8347	15.9	13.4	22.0	15.0
TOG 16704	14.5	11.7	21.8	13.8
O. sativa (checks)				
Khao Hlan On	14.9	14.5	27.8	16.8
WITA 4	0.1	1.5	4.0	0.7
Trial Mean (n=50)	12.9	12.5	19.9	13.4
LSD (0.05)	6.8	6.6	11.3	5.5
P (<0.05)	***	***	***	***
Heritability	0.8	0.8	0.7	0.9

Average ambient temperature was 28^{0}C; average water temperature was 31^{0}C (screenhouse) and 33.6 ^{0}C (field).

Table 4. Survival of seedlings under submergence in screenhouse condition during DS 2014 at Ibadan, Nigeria.

Genotypes	Survival (%)		
	Trial 1	Trial 2	Combined
O. glaberrima			
TOG 14368	11	4	7
TOG 16769	4	29	17
TOG 16774	49	41	45
TOG 5421	24	25	24
TOG 9365	21	55	38
TOS 12364	9	13	11
TOS 13649	0	5	3
TOS 16673	0	28	14
TOS 16746	25	46	35
TOS 6447	26	21	24
TOS 6454	56	58	57
TOS 6455	41	52	47
UG 28	9	52	30
UI 1085	15	38	27
UI 1145	0	16	8
VIAH	0	0	0
W 1032	0	13	7
W 1573	0	19	10
XLETIETOE EVENTOHI	4	3	3
YG 146	26	36	31
O. sativa			
Tolerant check SWARNA*Sub1*	81	59	73
Local check WITA 4	32	25	29
Trial Mean	20	29	24
LSD (0.05)	3.6	3.8	1.1
Heritability	0.7	0.4	0.6

was recorded in TOS 6454 (57%) and was almost twice that of WITA 4. Heritability ranged between 0.38-0.67.

Stagnant flooding

Grain yield and survival

Highly significant differences among entries were observed for grain yield in both stress and control conditions (Table 5). The mean grain yield of *O. glaberrima* accessions in stress trials was 174 gm^{-2} while under control was 256 gm^{-2}. Thus, relative to control, there was about 32% yield reduction under stress. The tolerant checks (IRRI 119 and IRRI 154) on average showed 52% yield reduction in stress relative to control trial. IRRI 119 out-yielded IRRI 154 both under stress (by ~53%) and control (by ~20%). Interestingly, local check (WITA 4) yielded higher than the tolerant checks under stress, it out-yielded the best tolerant check (IRRI 119) by about 81%, and it also showed very little yield reduction due to stress (~4%). WITA 4 and IRRI 119 had similar yields under control trials. Under stress, four *O. glaberrima* accessions (IG 48, Gervex 2674, IG 133, and TOG 7148) out-yielded the best tolerant check (IRRI 119) and these were selected. In stress trial, the highest yielding *O. glaberrima* accession (IG 48) significantly out-yielded all the *O. sativa* checks. Among the selections, TOG 7148 and IG 48 were the two highest yielders in control and they yielded on par with best *O. sativa* checks (WITA 4 and IRRI 119). Three out of the four selections (IG 48, Gervex 2674, and IG 133) yielded higher under stress than under control (by 22-44%). However, the fourth selection (TOG 7148) yielded lower under stress than in control conditions. All the *O. sativa* checks yielded higher under control than under stress. There were no significant differences in survival (%) between selected accessions of *O. glaberrima* and *O. sativa* checks (Indicating that higher yield is probably because of high yield potential and not just survival).

Days to flowering

Under control conditions, the *O. glaberrima* accessions flowered between 77-101 days while the checks flowered between 95-100 days (Table 6). Under stress, there was an increase in days to flowering both in *O. sativa* and *O. glaberrima*. The *O. sativa* checks increased their flowering duration by about eight days. Among the *O. glaberrima* selections, two accessions (Gervex 2674 and IG 133) increased flowering duration by about 10 days while two others (IG 48 and TOG 7148) increased it by over 20 days.

Plant height

Plant height increased under stress relative to control (by about 26%; Table 6). Plant height was higher under stress than under control. Gervex 2674 was the tallest accession among the selections while IRRI 119 was the tallest among checks. Two out of four selected accessions of *O. glaberrima* were significantly taller than IRRI 119 while the other two were on par with it.

Tiller and panicle number

Tiller number was generally higher in stress than in control (Table 6). Eleven *O. glaberrima* accessions, including IG 48, had higher tiller number in control

Table 5. Grain yield and survival of *O. glaberrima* accessions and checks under stagnant flooding stress and control conditions during WS 2013, Ibadan, Nigeria.

Name	Grain yield (g.m^{-2})		Survival (%)
	Stress	Control	Stress
O. glaberrima			
Minimum	61	141	45
Maximum	435	389	88
Mean (N =42)	174	256	72
Selections			
IG 48	435	357	71
Gervex 2674	418	290	65
IG 133	285	211	82
TOG 7148	269	372	66
O. Sativa			
Tolerant checks			
IRRI 119	220	378	74
IRRI 154	144	316	69
Local check			
WITA 4	385	401	78
Trial Mean	180	264	71
LSD (0.05)	48	49	20
Heritability	0.9	0.9	0.5

than in stress. Under stress, WITA 4 had higher tiller number than the tolerant checks. (Unproductive tiller increased under stress). Panicle number was also generally higher under control than in stress conditions. Nine accessions had higher panicle number in stress than in control (data not shown). IG 48 had the highest panicle number under both stress and control conditions.

Heritability of traits ranged from 0.45 (for survival %) to 0.97 (flowering days under stress) (Tables 5 and 6). High heritability for grain yield was observed both in stress and control trials (0.93 and 0.88, respectively).

DISCUSSION

In this study, we systematically evaluated the entire collection of *O. glaberrima* accessions being conserved at the AfricaRice genebank for tolerance to three types of flooding stresses namely anaerobic germination, submergence, and stagnant flooding. This is the first report of screening *O. glaberrima* for AG.

Anaerobic germination

Significant genetic variation for AG was found among the *O. glaberrima* germplasm. In the preliminary screening, of 2002 accessions, 147 (representing 7.3%) showed AG ability. In contrast, only 19 of the 8114 *O. sativa* (0.2%) rice genotypes screened at IRRI showed high level of survival under AG (Ismail *et al.*, 2009). Thus, the AG trait seems more frequent in *O. glaberrima* gene pool than in *O. sativa*. With experiments, the number of accessions with consistent performance was reduced to 79 and then 43. Finally, six best accessions that could be used in breeding for AG ability were identified.

Given these results, *O. glaberrima* could be considered an important genetic reservoir for the AG trait particularly as *O. glaberrima* is known to have evolved in flood-prone ecology (Agnoun *et al.*, 2012).

Generally, 10 cm water depth is used to screen for AG (Angaji *et al.*, 2010; Septiningsih *et al.*, 2013; Baltazar *et al.*, 2014). However, in this study, we used 10 cm as the standard water depth but we also evaluated for AG at 15 cm water depth. At 15 cm water depth, we found that the selected accessions of *O. glaberrima* significantly outperformed the best check Khao Hlan On (data not shown). Ella *et al.* (2010) observed higher seedling survival when water depth was shallower however; Jiang *et al.* (2004) stated that water depth does not influence anaerobic germination in a soil-free system. Flooding results in decreased oxygen availability as gases diffuse 10,000 times slower in water than in air (Mohanty *et al.*, 2000). Also, Ismail *et al.*, 2009 had previously suggested that oxygen concentration is relatively higher at upper flood water layers and tolerant genotypes rapidly elongate the coleoptile as an escape strategy (Miro and Ismail, 2013). Screening for AG is considered more stringent at 15 cm water depth than at 10 cm water depth as coleoptiles of the tolerant rice genotypes would have to travel a longer distance to access oxygen for transport to under water tissues. Even with this stringent measure, 79 accessions had ≥60% survival rate and the best *O. glaberrima* in this environment had 85% survival. The ability of these accessions to tolerate flooding during germination suggests the innate ability of some *O. glaberrima* for anaerobic germination. There is need for further studies to understand the physiological basis of tolerance of the selected *O. glaberrima* accessions. Direct seeding in un-levelled and poorly-prepared fields, as is widespread in Africa, results in submergence of seeds in water at varying depths when there is excess water and this leads to poor seedling establishment. The ability of some of the *O. glaberrima* accessions to survive at 15 cm water depth is indicative of adaptation to poor management and will be useful particularly in Africa.

Temperature is an important weather component which has effects on AG (Septiningsih *et al.,* 2013). Ismail *et al.* (2012) noted that the range in optimum temperature for germination in flooded soils is narrow. Seedling survival and growth are usually highest at flood water temperatures of 24–26 °C, but significantly less at lower (18–20 °C) or higher (30–32 °C) temperatures (Ismail *et al.*, 2012). In this study, we observed that survival of *O. glaberrima* also reduced with increase in temperature. Generally, the trial means in dry season was lower than in wet season (data not shown). The results also point out that *O. glaberrima* accessions have higher survival rates at higher temperature than the *O. sativa* check. In dry season, *O. glaberrima* accessions had significantly higher AG than the best check (KHO). The ability to tolerate higher temperature during AG was also seen clearly in the preliminary experiments where even with water temperature above 40 °C, some accessions had good AG ability (data not shown). This is a distinct advantage of *O. glaberrima* over *O. sativa* for AG. Ability to tolerate higher temperature during AG may be important in the tropics particularly for irrigated rice in the dry season.

Iron toxicity is recognized as the most widely distributed nutritional disorder in lowland rice production (Dobbermann and Fairhurst, 2000). *O. glaberrima* is known to have good tolerance to Fe–toxicity (Sikirou *et al.*, 2016) but no previous information is available on its reaction to Fe-toxicity during growth under anaerobic conditions. Thus, we considered it important to evaluate AG performance under Fe-toxic conditions. In this study, Fe-toxicity in soil reduced AG of rice cultivars. The *O. sativa* check (KHO) outperformed *O. glaberrima* in AG during the wet season but in the dry season, *O. glaberrima* accessions were better than KHO. As iron toxicity is endemic in Africa and is considered a major abiotic stress in rice production (Lantin and Neue, 1989; Sikirou *et al.*, 2015), ability to tolerate Fe-toxic conditions in soil during AG is important in Africa. Some *O. glaberrima* accessions selected in this study (TOG 8347, TOG 16704) showed tolerance to the two abiotic stresses (AG and Fe-toxicity; data not shown).

Volume of soil used in the screen house trials was quite low compared to a real field situation. In WS 2014 experiments, soil volume was varied between experiments. The first screenhouse trial had similar soil volumes as the preliminary trials but the second screenhouse trial had higher soil volume in the tank and the last trial was under field-like conditions (Table 2). We observed that AG values reduced when the soil volumes increased (Figure 1). *O. sativa* and *O. glaberrima* were affected similarly. We also observed that turbidity of water was higher in field-like situation which could limit interception of solar radiation, increase temperature and negatively affect germination. Hara and Toriyama (1998) have clearly shown that higher ammonium nitrogen content in soil reduces the germination of rice in anaerobic conditions. Higher soil volume could lead to higher ammonium nitrogen content and thus reduce AG. This topic needs further research.

The popular rice variety (WITA 4) grown by farmers was found to be poor in AG compared to the best check (Khao Hlan On) and the best *O. glaberrima* accessions (Figure 1). Similarly, other popular lowland rice varieties in West Africa were found to be inferior in AG performance (AfricaRice, unpublished). Thus, there is an urgent need to improve these varieties with AG trait to make them amenable to direct seeding.

Submergence

Rice plants are known to escape flash floods in two ways: quiescence strategy or by shoot elongation. The quiescence strategy allows plants to conserve carbohydrate to supply energy for regrowth after de-submergence while shoot elongation strategy allows plants to quickly rise above the flood water to gain access to oxygen for maintenance of aerobic metabolism (Joho *et al.*, 2008; Kawano *et al.*, 2008; Septiningsih *et al.*, 2009). It is also known that plants are damaged by flooding due to inability to initiate protection system against oxidative damage when water level recedes (Kawano *et al.*, 2002). In this study, there were significant differences among the *O. glaberrima* genotypes for tolerance to the submergence stress. Majority of the *O. glaberrima* accessions were elongation types. Lodging of seedlings was observed after desubmergence and not all the genotypes were able to recover. We identified one accession (TOS 6454) with appreciable tolerance to submergence at the vegetative stage. Most of the materials identified were better than the local check WITA 4 but none of them was as tolerant as the best check (Swarna-Sub1). TOS 6454 probably contains different tolerance genes than the *O. sativa* and thus may be able to contribute to increase in the level of submergence tolerance of current varieties. Further research is needed to understand the mechanism for tolerance of submergence in TOS 6454.

Stagnant flooding

Out of 1732 accessions tested, four promising accessions (IG 48, Gervex 2674, IG 133, and TOG 7148) that were superior to the tolerant checks were identified. About 20% of entries had a yield reduction of over 50% in the stress trial compared to the control trial and, interestingly, about 20% of the entries showed higher yield under stress than in the control (data not shown). Two of the selections, IG 48 and Gervex 2674, showed strong adaptation to stagnant flooding conditions as these lines yielded higher under stress than in control (Table 4). They yielded higher than the tolerant checks under stress. Higher yield of these lines is probably because of tolerance and not due to better survival (Table 5). In addition, we found that the high-yielding local check, WITA 4, was in fact highly tolerant to stagnant flooding. Thus, WITA 4 could be useful as a variety in stagnant-flood-prone areas and as a donor as well in breeding for stagnant flooding. Importance of using well-characterized donors in breeding that are higher-yielding under stress is well known in rice (Bernier *et al.*, 2007; Venuprasad *et al.*, 2007, 2008). Thus, these high-yielding accessions can similarly be useful in breeding for stagnant flood tolerant rice.

These accessions originated from West Africa and thus these donors are particularly important in Africa as they are well adapted to local stresses (such as Fe-toxicity, RYMV, gall midge) and will be useful when combining tolerance to multiple stresses that are important in rainfed lowlands particularly as rainfed lowlands are said to have the potential to usher in rice 'Green Revolution' in West Africa (Sakurai, 2006). Thus, the selected accessions can serve as new sources of tolerance to breed for a key abiotic stress that constrains rice production in rainfed lowlands.

One main challenge in using *O. glaberrima* in interspecific breeding with *O. sativa* is the problem of sterility (Pham and Bougerol, 1993; Ghesquiere *et al.*, 1997). However, in many cases the sterility barrier can be overcome with backcrossing. Breeding lines can be used to significantly improve fertility while crossing the two species (Deng *et al.*, 2010; Lorieux *et al.*, 2013). Backcrossing approach was used in the development of lowland NERICA varieties (Sie, 2008). Bridge lines can also be used to significantly improve fertility while crossing the two species (Deng *et al.*, 2010; Lorieux *et al.*, 2013).

Recently in Nigeria, new submergence-tolerant varieties have been released using marker-assisted breeding to introgress SUB1 gene into popular rice varieties (AfricaRice, 2017). Identification of QTLs for AG and submergence is being carried out using bi-parental crosses between selected *O. glaberrima* accessions and local sensitive varieties. This could be useful in development of new varieties adapted to direct seeding.

CONCLUSIONS

The results of this study have implications for breeding rice cultivars suited for rainfed conditions particularly in Africa. Six *O. glaberrima* accessions with consistent high level of AG (TOG 5485, TOG 5505-A, TOG 5980-A, TOG 7252-A, TOG 8347 and TOG 16704) were identified. Two accessions, IG 48 and Gervex 2674, with strong adaptation to stagnant flooding were identified. One accession TOS 6454 with moderate submergence tolerance was also identified. These accessions can be used as additional donors in breeding rice varieties better adapted to the increased flooding conditions due to climate change. The new sources of tolerance have the potential of broadening rice adaptation and stabilizing stand establishment and grain yield in rainfed and irrigated lowlands.

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