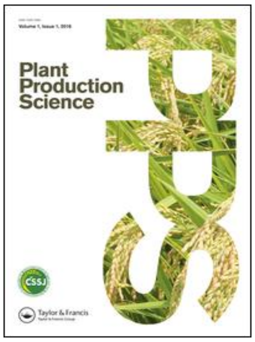

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Soil-based screening for iron toxicity tolerance in rice using pots

Mouritala Sikirou[a,b], Kazuki Saito[b], Khady Nani Dramé[c], Aliou Saidou[a], Ibnou Dieng[b], Adam Ahanchédé[a] and Ramaiah Venuprasad[d]

[a]Laboratoire de Biologie Végétale, Faculté des Sciences Agronomiques, Département de Production Végétale, University of Abomey Calavi, Cotonou, Bénin; [b]Africa Rice Center, Cotonou, Benin; [c]Africa Rice Center (Africa Rice), Dar-es-Salaam, Tanzania; [d]Africa Rice Nigeria Station, Ibadan, Nigeria

ABSTRACT
The objective of this study was to assess the reliability of pot-based screening method for iron (Fe) toxicity tolerance in rice using soils from hot spots. Five lowland rice varieties with known reaction to Fe toxicity were grown in pots in a screen house for three seasons. Fe-toxic soils from two hot spot fields – Edozighi, Nigeria and Niaouli, Benin were used and soil from Africa Rice Center (AfricaRice) experimental farm, Cotonou, Benin was included as control. Leaf bronzing score (LBS) was determined at different stages, and grain yield was determined at maturity. Heritability was estimated using data across the three seasons. High heritability was recorded for LBS and grain yield. Grain yield reduction in stress treatment relative to control varied from 15 to 56% depending on the variety and soil. Bao Thai, Suakoko 8, and WITA 4 had better performance under Fe toxicity in terms of LBS, yield and relative yield reduction, whereas Bouake 189 and IR64 had poorer performance. Grain yield and LBS were significantly correlated but negatively at 60 days after sowing (DAS). Overall, the results found in this experiment were consistent with previous field studies. Therefore, pot screening using soils from hot spots can be used by rice breeding programs to reliably assess Fe toxicity tolerance *ex situ*.

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CLASSIFICATION
Crop Physiology

Fe toxicity is recognized as a widespread nutrient disorder affecting rice production in many inlands and swamps in West Africa (Abifarin, 1989; Chérif et al., 2009). Excessive amounts of Fe^{2+} due to Fe reduction under anaerobic conditions at low pH have been reported to result in significant yield reduction ranging from 12 to 100%, depending on the intensity of Fe toxicity and the tolerance of the rice genotype (Chérif et al., 2009; Sahrawat, 1995). Chérif et al. (2009) reported that up to 55% of the lowland rice areas in several West African countries are affected by this stress, and about 10% of the affected areas were abandoned due to the severity of the stress. A typical symptom of Fe toxicity is a copper coloring of leaves, called 'bronzing' which has been often used as an indicator of Fe toxicity stress level (Bode et al., 1995). The symptom appears initially as tiny brown spots on the lower leaves, starting at the tip and spreading toward the base followed by purplish brown color of the entire leaf (Abifarin, 1989; Becker & Asch, 2005; Fairhurst & Witt, 2002). However, in some cases, growth reduction and significant yield reduction can be observed without significant leaf bronzing (Hua. Xiaoe, & Ancheng, 2001; Onaga, Edema, & Asea, 2012; Sahrawat, 2004; Sikirou, 2009). Fe toxicity also causes delay in phenological development (Audebert & Fofana, 2009; Chérif et al., 2009; Dufey et al., 2009).

To cope with this constraint, development and deployment of varieties with superior tolerance to Fe toxicity are considered as the most affordable and effective approaches for improving rice productivity in the affected areas. However, progress has been slow due to lack of efficient and reliable screening approaches. Most breeding programs rely on screening in Fe-toxic 'hotspots.' However, most hot spots are in rainfed ecologies where screening is limited to one season per year, and year-to-year variations in rainfall cause significant G × E. These factors limit rice selection efficiency (Sikirou et al., 2015). Further, there are no valid managed-stress screening protocols (e.g. hydroponic method, sand based, or soils) which are efficient and can predict rice performance in the target environments according to a detailed literature review by Sikirou et al. (2015). Some researchers have used pot or cement tanks filled with soils for evaluating Fe toxicity tolerance in rice (Abifarin, 1989; Onaga, Edema, & Asea, 2012; Sikirou, 2009). In comparison with field screening at a hot spot, which is usually under rainfed conditions, there are several advantages in use of hot spot soils in pot-based screening. These

CONTACT Ramaiah Venuprasad r.venuprasad@cgiar.org

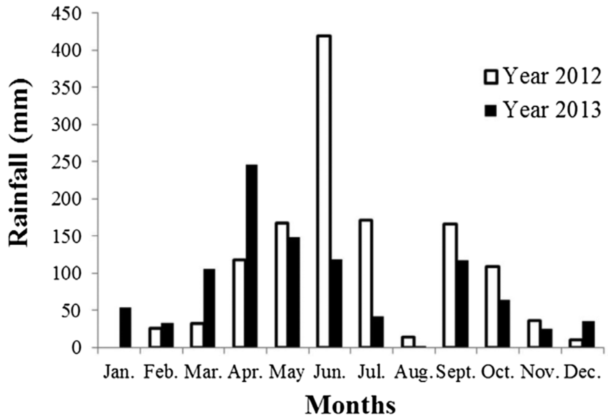

Figure 1. Rainfall in 2012 and 2013 (source IITA Cotonou).

include (i) possibility of two to three screenings within a year, (ii) controlling soil water conditions across years, which is difficult in rainfed fields, and (ii) having an appropriate 'control' along with treatments which is not possible in hot spots. However, the accuracy and reliability of this screening method has not been assessed.

Therefore, the objective of this study was to assess the reliability of pot screening using soil from different Fe toxicity hot spots over three seasons and using five varieties with well-known Fe toxicity tolerance level in the field.

Materials and methods

Experimental site

Experiments were conducted in a screen house at the AfricaRice station, Cotonou, Benin during three seasons: 2012 wet season (WS; March to August), 2013 dry season (DS; November to March), and 2013 WS (May to October). Rainfall during the wet seasons 2012 and 2013 were 923.2 and 673.4 mm, respectively, while 236.7 in the dry season (Figure 1). The station is located in the commune of Abomey-Calavi (6°28′N, 2°21′E) at 12 km of Cotonou west-north in South Benin.

Plant materials

Five varieties with known tolerance to Fe toxicity were evaluated. There were three varieties susceptible to Fe toxicity (Bao Thai, Bouake 189, and IR 64) and two tolerant varieties (Suakoko 8 and WITA 4) (Table 1). Suakoko 8 and WITA 4 are rice varieties developed and cultivated in West Africa, whereas Bao Thai, Bouake 189, and IR64 were developed in Asia. Bao Thai is not cultivated in Africa but IR64 and Bouake 189 are grown in some parts of Africa. Bao Thai was grouped as highly susceptible to Fe toxicity based on hydroponic screening and later confirmed in field in Asia whereas grouping of all other varieties was based on field studies in African conditions (Table 1). Their characteristics in relation to the response to Fe toxicity were based on leaf bronzing score (LBS) and/or yield.

Methodology

Two Fe toxicity hot spots at Edozighi, Nigeria, and Niaouli, Benin were selected for this study. Edozighi and Niaouli are located in the tropical-warm/sub-humid agro-ecological zone (Nwilene et al., 2013). Edozighi soil is acidic (pH 4.2–5.2) and with a clay loam texture (Abah et al., 2012) while Niaouli soil is also acidic (pH 5.2–5.4) and has a hydromorphic silt-clay-sand texture (Hodomihou, 2009). Top soils (0–20 cm) were brought from these sites to AfricaRice station in Cotonou. For the control, top soil (0–15 cm) was collected from AfricaRice experimental farm in Cotonou. This soil is clay sandy and acidic (pH 5.1–5.4) (Saito & Futakuchi, 2009). Even though Cotonou soil was considered as control, after the first season, probably due to continuous saturation, some Fe toxicity symptoms could be observed on the plants.

Plastic pots of 7-liter capacity containing 5 kg of water saturated soil were used, and the pots were watered daily. Water level was constantly maintained at 5 cm above the soil surface in each pot until the end of the trial to create anaerobic conditions favorable for the maintenance of Fe^{2+} form. Paraffin oil (5 ml) was added to each pot to reduce evaporation and aeration; this is to avoid oxidation of Fe^{2+} in the pots (Asch, Mathias, & Kpongor, 2005). The soils were continuously used over three seasons after removing plant debris from the previous crop. From the start of the first experiment in 2012 WS till the end of last experiment in 2013 WS, 5 cm water level above soil in the pots was maintained.

Randomized complete block design with four replications was used in this study. There were 15 pots per replication combining five variety treatments with three soil treatments. The soil treatments included one control (soil from Cotonou) and two stress levels (hot spot soil from Edozighi and from Niaouli). A given variety was

Table 1. Characteristics of rice varieties used in this study.

Variety name	Parentage	Origin	Crop duration (days)	Reaction to Fe toxicity	References
Bao Thai	Land race	Vietnam	130	Sensitive	Elec et al. (2013)
Bouake 189	419C-57/C4-63	Indonesia	125	Sensitive	Sahrawat (2004)
IR 64	5657-33-2-1/IR 2061-465-1–5-5	The Philippines	120	Sensitive	Dufey et al. (2012)
Suakoko 8	Siam 25/*3 Malunja	Liberia	140	Tolerant	Virmani (1977)
WITA 4	11975/IR 13146-45-2-3	Nigeria	125	Tolerant	Gridley et al. (2006)

continuously grown in the same pot in the given replication over the three seasons.

Seeding was done in a nursery and 21-day old seedlings were transplanted at a rate of two plants per pot. Fertilizer dose was calculated based on the pot surface. P and K fertilizers were applied on the soil surface just after transplanting as super triple phosphate and potassium chloride at the dose of 60 and 80 kg/ha, respectively. N fertilizer (100 kg/ha) was applied in the form of urea in three splits: at transplanting (basal, 35 kg/ha), 40 days after sowing (DAS), and at 60 DAS (40 kg/ha).

Data collection

LBS, days to flowering (days from sowing to 50% flowering), plant height, panicle number, shoot dry weight, and grain yield per pot were determined. LBS was recorded based on leaves symptoms and general appearance using a scale from 0 to 9 (0 = normal or nearly normal plant; 9 = nearly dead or dead plant) as described by the Standard Evaluation System for Rice (IRRI, 2002) at 40, 60, and 80 DAS. Plant height at maturity was measured from the top of the soil to the highest panicle. At maturity, panicles from each pot were harvested, dried, and threshed to obtain grain weight. Grain yield expressed as $g.pot^{-1}$ was adjusted to 14% grain moisture content. The remaining shoot was oven-dried at 70 °C until constant weights to obtain shoot dry weight.

Statistical analysis

All the statistical analysis was carried out using Genstat Discovery Edition (Genstat, 2003). For data analysis, we considered soil and variety treatments as fixed factors, and season and replications within season as random factors. For each trait, least square means of varieties by soil treatment over seasons were obtained using the REML option of the MIXED procedure.

Relative yield reduction i.e. percentage reduction of grain yield due to Fe toxicity relative to the control was calculated, using least squares means, according to the formula below.

$$\text{Relative yield reduction} = \left[\left(\text{yield}_{\text{control}} - \text{yield}_{\text{Fe toxic soils}}\right) / \text{yield}_{\text{control}}\right] \times 100$$

Variance components for each trait were obtained using the REML option of the VARCOMP procedure. For this analysis all factors were considered as random. Broad-sense heritability (H) for each trait was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GS}^2}{s} + \frac{\sigma_E^2}{sr}}$$

where σ^2_G, σ^2_{GS}, σ^2_E, s, and r are the varieties, variety x season, error variance, and number of seasons and replications, respectively.

Spearman's rank correlation over seasons was calculated to determine the relationship between yield and LBS for each soil type.

Results

Effect of Fe toxicity on traits

The effect of Fe toxicity on means of different traits is given in Table 2. In general, the Fe toxicity treatments affected all the traits relative to the control. The two different Fe-toxic soil treatments produced similar results and there was no significant difference between them for any of the traits. The Fe treatments significantly reduced grain yield on average, about 36% yield reduction was observed relative to the control. LBS was higher in the Fe-toxic treatment than in the control and the differences were significant at 40 DAS and 60 DAS. In general, there was an increase in LBS with growth stage; between 40 DAS and 80 DAS there was a threefold increase in LBS in the Fe-toxic treatments but in control there was a 36-fold increase. Fe-toxic treatments reduced plant height significantly (by five percent on average) relative to the control. There was no significant difference between control and Fe-toxic treatments for days to flowering, panicle number, and shoot biomass.

Table 2. Trait means over three seasons in three different soils in a screen house study at Cotonou, Benin (2012–2013).

Soil type	Fe-score at 40 DAS†	Fe-score at 60 DAS	Fe-score at 80 DAS	Grain yield (g/pot)	Days to flowering	Plant height (cm)	Panicle number/pot	Shoot dry weight (g/pot)
Control soil								
Cotonou	.1a	1.7a	3.6	17.7a	92.4	100.6a	10.3	27.1
Fe-toxic soil								
Edozighi	1.3 b	2.4b	4.5	10.9b	89.9	94.7b	8.6	20.2
Niaouli	1.4 b	2.8b	4.4	11.7b	88.7	97.2b	9.1	20.9
P	.05	.02	NS	.05	NS	.01	NS	NS

Values in the same column for a trait followed by the same letter are not significantly different ($P < .05$).
NS, Non-significant.
†Days after sowing.

Table 3. Mean grain yield and relative yield reduction of five varieties during three seasons in three different soils in a screen house study at Cotonou, Benin (2012–2013).

	Grain yield (g/pot)			Relative yield reduction (%)		
	Control soil	Fe-toxic soil		Fe-toxic soil		
Variety	Cotonou	Edozighi	Niaouli	Edozighi	Niaouli	Average
Bao Thai	17.7	12.8ab	15.0a	28	15	22
Bouake 189	16.4	8.5b	7.2c	48	56	52
IR 64	19.6	9.7b	11.4bd	51	42	46
Suakoko 8	13.3	8.7b	10.9b	35	18	26
WITA 4	21.6	14.5a	14.4ad	33	33	33
P	NS	.05	.01			
H	.62	.97	.85			

Values in the same column followed by the same letter are not significantly different ($P < .05$).
NS, Non-significant.

Table 4. Mean LBS of five rice varieties during three seasons in three different soils in a screen house study at Cotonou, Benin (2012–2013).

	Leaf bronzing score								
	40 DAS$			60 DAS			80 DAS		
	Control soil	Fe-toxic soil		Control soil	Fe-toxic soil		Control soil	Fe-toxic soil	
Variety	Cotonou	Edozighi	Niaouli	Cotonou	Edozighi	Niaouli	Cotonou	Edozighi	Niaouli
Bao Thai	.1	.7	.6 cd	1.2	2.1	1.8c	1.8b	3.3b	2.5c
Bouake 189	.0	2.3	1.8b	1.2	2.6	3.4ab	3.3a	5.6a	6.0a
IR 64	.1	1.9	3.0a	2.3	3.3	4.1a	4.0a	5.5a	5.8a
Suakoko 8	.2	1.0	1.0bd	2.1	2.5	2.4bc	4.0a	4.2b	3.5bc
WITA 4	.2	.5	.5d	1.7	1.9	2.4bc	3.7a	4.0b	4.2b
P	NS	NS	.01	NS	NS	.01	.05	.03	.001
H	.50	.69	.85	.23	.64	.83	.72	.78	.93

Values in the same column followed by the same letter are not significantly different ($p < 0.05$).
NS, Non-significant.
$days after sowing.

Performance of varieties

Grain yield of the five varieties differed significantly in the two Fe-toxic soils but not in the control (Table 3). All the varieties had higher grain yield in control than in Fe-toxic soils (Table 3). The Fe treatments significantly reduced grain yield. On average, about 36% yield reduction was observed relative to control treatment. In the two Fe-toxic soils, Bao Thai and WITA 4 performed better than the other varieties. Even though Bao Thai was considered as susceptible based on earlier studies in Asia, it yielded higher than the other varieties in this study. In Edozighi soil, WITA 4 was the highest yielder (14.5 g/pot) followed by Bao Thai and the other three varieties yielded similarly (<9.7 g/pot). In Niaouli soil, Bao Thai yielded higher (15 g/pot) than the rest while Bouake 189 was the lowest yielder (7.2 g/pot). The average relative yield reduction varied between varieties – Bao Thai and Suakoko 8 showed the lowest yield reduction (22 and 26%, respectively) while Bouake 189 and IR 64 showed the highest reduction (52 and 46%, respectively).

In Edozighi soil, no significant differences were observed at 40 and 60 DAS but at 80 DAS varieties Bao Thai, Suakoko 8 and WITA 4 had significantly lower LBS than Bouake and IR 64 (Table 4). However, in Niaouli soil, significant differences between varieties were observed at all the three stages. At 40 DAS, IR 64 had the highest LBS (3.0) while WITA 4 had the lowest (.5). At 60 and 80 DAS, IR 64 had the highest LBS whereas Bao Thai had the lowest. Leaf bronzing was also observed in Cotonou soil (control) and the differences between varieties were significant at 80 DAS. In most cases, the performance of susceptible varieties (Bouake 189 and IR 64) was similar and differences between them were not significant. A similar trend was observed for tolerant varieties (Suakoko 8 and WITA 4).

Significant differences between the varieties tested were observed also for others traits including days to flowering, plant height, number of panicles, and shoot dry weight (Tables 5 and 6). For all these traits, higher values were found in the control treatment compared to the Fe-toxic treatments (Tables 5 and 6). Suakoko 8 had the highest number of days to flowering, plant height, and shoot dry weight irrespective of the treatments. WITA 4 and Bouake 189 were intermediate while Bao Thai and IR 64 flowered the earliest. WITA 4 and Bao Thai had intermediate plant height while IR 64 and Bouake 189 were the shortest varieties.

In general, for all the traits, the H values were higher than .50 (Tables 3–6). H for grain yield was .62 in control,

Table 5. Mean days to flowering and plant height of five varieties during three seasons in three different soils in a screen house study at Cotonou, Benin (2012–2013).

	Days to flowering			Plant height (cm)		
	Control soil	Fe-toxic soil		Control soil	Fe-toxic soil	
Variety	Cotonou	Edozighi	Niaouli	Cotonou	Edozighi	Niaouli
Bao Thai	84c	77c	76b	105.5b	102.4a	103.0b
Bouake 189	93bc	90b	89b	84.9c	79.6b	82.3c
IR 64	80c	83bc	81b	82.1c	78.9b	78.5c
Suakoko 8	118a	117a	113a	127.3a	118.3a	124.3a
WITA 4	100b	90b	90b	102.5b	95.2a	97.8b
P	.001	.001	.001	.001	.001	.001
H	.93	.92	.89	.98	.96	.98

Values in the same column followed by the same letter are not significantly different ($P < .05$).

Table 6. Mean panicle number and shoot dry weight of five varieties during three seasons in three different soils in screen house study at Cotonou, Benin (2012–2013).

	Panicle number			Shoot dry weight (g/pot)		
	Control soil	Fe-toxic soil		Control soil	Fe-toxic soil	
Variety	Cotonou	Edozighi	Niaouli	Cotonou	Edozighi	Niaouli
Bao Thai	12ab	10a	11a	33.9ab	17.0b	17.5b
Bouake 189	10ab	9ab	8ab	16.7b	14.5b	14.6b
IR 64	13a	8ab	11a	18.1b	14.0b	14.3b
Suakoko 8	7b	7b	7b	40.6a	32.3a	38.5a
WITA 4	9ab	9ab	8ab	26.6ab	19.7b	19.8b
P	.05	.05	.05	.01	.001	.001
H	.58	.53	.78	.57	.86	.92

Values in the same column followed by the same letter are not significantly different ($P < .05$).

Table 7. Correlation of average leaf bronzing score at different days after sowing (DAS) with grain yield and relative yield reduction in a screen house study at Cotonou, Benin during 2012–2013.

	Leaf bronzing score								
	Control soil			Fe-toxic soil					
	Cotonou			Edozighi			Niaouli		
Traits	40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS	40 DAS	60 DAS	80 DAS
Grain yield	$.16^{NS}$	$.15^{NS}$	$-.05^{NS}$	$-.58^{NS}$	−.87*	$-.58^{NS}$	$-.45^{NS}$	−.87*	$-.74^{NS}$
Relative yield reduction	–	–	–	.93*	$.85^{NS}$	.99**	$.61^{NS}$	$.79^{NS}$	.95*

*$p < .05$; **$p < .01$.
^{NS}Non-significant.

.97 in Edozighi soil, and .85 in Niaouli soil. LBS had high heritability in both Fe-toxic soils and at all the three stages, and it ranged between .64 and .93.

Relationship between traits

The rank correlation between LBS, and grain yield and relative yield reduction is shown in Table 7. Significant negative correlation between grain yield and LBS at 60 DAS was found in Fe treatments. In the other cases correlation was not significant. The correlation between LBS and relative yield reduction was positive and significant at 40 DAS for Edozighi and 80 DAS for both Fe-toxic soils. There was no strong relationship between grain yield and other traits (days to flowering, plant height, panicle no. and shoot dry weight) determined in this study (data not shown). Correlation analysis showed that varietal ranking for LBS was similar between the two Fe-toxic soils at all the three sampling dates (data not shown).

Discussion

The efficacy of Fe toxicity tolerance screening using hot spot soil in a pot experiment was studied. Five rice varieties with known reaction to Fe toxicity and including both tolerant and susceptible varieties were used. Overall, performance of tolerant and susceptible varieties was consistent with what was observed in the field in different countries of West Africa. Besides, similar trend was observed for the two hot spot soils (Edozighi and Niaouli; Table 2). The susceptible varieties IR64 and Bouake 189 showed high LBS with high yield reduction (Tables 3 and 4) while the tolerant varieties Suakoko 8 and WITA 4 showed low LBS with low yield reduction. However, performance of Bao Thai was contrary to what was previously reported by Elec et al. (2013). In our screening conditions, Bao Thai showed tolerance to Fe toxicity. Interestingly, Bao Thai's tolerance to Fe toxicity was also clearly observed in natural field conditions at Edozighi, Nigeria (AfricaRice, unpublished results), confirming our results. Elec et al. (2013) have shown that Bao Thai is highly susceptible in hydroponic screening and later found similar results in field condition (in Philippines). The reasons for the observed differences in performance of Bao Thai in the two field studies are not clear but it could be due to differences in soil type. While Elec et al. (2013) observed good correlation between hydroponic screening and field testing others have not found similar results (Becker & Asch, 2005; Nozoe et al., 2008).

Yield reduction in our pot study ranged between 20 and 50% depending on the variety, and is in accordance with field reports (Audebert & Fofana, 2009; Chérif et al., 2009). All traits recorded in this study had high heritability, indicating that this method of screening can generate repeatable results (Tables 3–6). In field trials conducted during 2012 and 2013 WS at Edozhigi, Nigeria heritability for grain yield ranged between .52 and .55 while for LBS it ranged from .12 to .34 (AfricaRice, unpublished results). Therefore, pot screening using soil from a hot spot can be considered as a reliable method to evaluate reaction of rice varieties to Fe toxicity.

In rice plant, the most prominent symptom of Fe toxicity is leaf bronzing. It has also been used as the secondary trait of choice in breeding for Fe toxicity tolerance (Abifarin, 1989; Gridley et al., 2006). Indeed LBS has all the requirements of a good secondary trait. Firstly, LBS is relatively easy and inexpensive to measure compared to grain yield. This is particularly important when the hot spot field is distant from the research station. Rapidly assessing Fe toxicity tolerance using LBS could considerably reduce travel and labor costs. Secondly, there is a strong correlation between LBS and grain yield as seen in this study at 60 DAS and several others (Audebert & Fofana, 2009; Audebert & Sahrawat, 2000; Elec et al., 2013; Onaga,Edema, & Asea, 2012). However, in some other studies no significant correlation between LBS and grain yield was observed (Nozoe et al., 2008; Sahrawat et al., 1996) as seen at 40 and 80 DAS. Level of correlation between the two traits is likely to depend on stress intensity, testing condition, and type of varieties used. When the stress level is moderate, there is no strong relationship between LBS and yield (Audebert & Fofana, 2009). In our experiment, stress level was rather high with regards to the LBS of the susceptible variety (IR64) which reached the score of 6. On average, in Fe-toxic soils one unit increase in LBS was related to a yield decrease of about 20%. In field situations, it is reported that increase in LBS score by one unit reduced yield by 390 kg ha^{-1} (Audebert & Fofana, 2009). The correlation between LBS and relative yield reduction was not consistent; this could be because of the fact that the control also showed considerable yield reduction in later seasons. For example, under control, the average grain yield reduced from 24 g/pot in 2012 WS to 14 g/pot in 2013 WS while at the same time the LBS score (80 DAS) increased from 3 to 4 (data not shown). Lastly, the H values for both LBS and grain yield is quite high and comparable (Tables 3 and 4). H values for grain yield and LBS have been rarely reported in literature. Our results indicate that both these traits could be measured at good precision and thus LBS could be used in rice breeding for Fe toxicity tolerance.

In field studies, 80 DAS was considered as the best stage to record LBS (Aboa & Dogbe, 2006; Audebert & Fofana, 2009; Chérif et al., 2009; Gridley et al., 2006). Similarly, in our pot study we found that varieties could be better differentiated with LBS score at 80 DAS in both Fe-toxic soils (Table 4). However, grain yield was correlated with LBS measured at 60 DAS. Thus, it may be better to measure LBS at both 60 and 80 DAS, but measuring LBS earlier at 40 DAS has little value. In other experimental setups such as hydroponics, LBS can be assessed earlier and a higher throughput can be achieved compared to our pot experiment. However, in some cases hydroponic results could not be repeated in the field (Becker & Asch, 2005; Nozoe et al., 2008, AfricaRice, unpublished results), probably because of the complexity of soil factors that influence the occurrence of Fe toxicity.

Even though pot screening method is fairly simple and reliable it is not without limitations. For this screening, large volume of soil has to be transported from far off places to research stations. Besides, moving soil from one country to another may be subjected to quarantine restrictions. Unless, the throughput is increased using concrete beds or large tanks as described earlier (Abifarin, 1989; Yamauchi, 1989), this method could only be used to screen a limited number of varieties and may not be adapted for screening of large breeding populations. Most of the soils in West Africa have low pH (Takow, Doumbia, & Hossner, 1991) and are prone to Fe toxicity upon saturation, thus, care should be taken in choosing a proper control soil. In our pot experiment, Cotonou soil initially used as control developed symptoms of Fe toxicity in later seasons. Thus, yield reduction calculated on the basis of Cotonou soil could have been underestimated. Further, care also needs to be taken while screening in pots as, unlike in field, nutritional imbalances can easy occur and impact plant performance (Hua, Xiaoe, & Ancheng, 2001; Onaga, Edema, & Asea, 2012). To increase the precision of Fe toxicity screening, it would be good to accurately determine the level of Fe the plants were subjected to throughout the growth duration.

In conclusion, pot screening using Fe-toxic soils is relatively simple to put in place and reproduces field tolerance. Besides, it is reproducible over seasons and thus can be reliably used to characterize rice varieties for tolerance to Fe toxicity. Further research is needed to scale up this method for developing high-throughput phenotyping protocol for Fe toxicity tolerance.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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