SUBMERGENCE TOLERANCE IN SOME RICE (*ORYZA SPECIES*) GENOTYPES

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Abstract

To elucidate tolerance of rice genotypes to submergence stress and validate the efficiency of submergence protocol in rice, twenty nine genotypes obtained from the Africa Rice, Ibadan station Nigera were evaluated. The evaluations were conducted at the Botanical garden, Ahmadu Bello University, Zaria. All genotypes showed escape (6.89%) and quiescent (93.11%) strategies to mitigate submergence stress. Submergence stress significantly ($p<0.05$) reduced plant survival (0 – 99%). Six genotypes (20.69%) showed growth recovery as water receded in the first week of desubmergence. Growth recovery was lost at seven and fourteen days after desubmergence. The efficiency of submergence evaluation protocol was best at 7DAS as against 14DAS. FARO 57, NERICA-L-52, IR64 SUB1, W 8-45 and FKR 54 varieties showed significant ($p<0.05$) recovery plasticity in morphological traits ranging from 0-11.5% within 7 to 14 days of de- submergence. Three cultivars (FARO 36, GAMBIAKA and NERICA-L-52) showed high tolerance to submergence stress and exhibited both escape and quiescent strategies during submergence and after de-submergence. Thus, FARO 36 and NERICA-L-52 can be cultivated in flood prone regions to mitigate flooding. They could be further utilized in breeding programs for the enhancement of rice landraces.

Keywords: Oryza species, Shoot elongation, Quiescence, Submergence tolerance.

Introduction

Climate change has become a major concern worldwide. In Africa, major symptoms of such change include disruption of normal climate patterns over large areas, increasing incidence of drought and extreme temperatures, heavy flooding and increasing levels of salt stress both in inland and coastal areas. These problems have progressively became more severe in frequency and severity (Desanker and Magadza, 2001; Hulme *et al.,* 2001). Rice is cultivated on 144 million farms worldwide, more than for any other crop with productions spreading across at least 114 countries (FAO, 2013). It is grown in widely diverse production environments topographically and strongly affected by soil type, water regime and climatic factors (Khush, 1984). There are five types of growth environments for rice based mainly on the water regime, namely; irrigated, rainfed lowland, tidal wetland, deepwater, and upland or dryland areas (Khush, 1984). In Africa, rice is grown in four ecosystems: upland (38% of planted area), rainfed wetland (33%), deepwater and mangrove swamps (9%) and irrigated wetland (20%) (Balasubramanian *et al*., 2007). More than 50% of the world's rice crop area is irrigated and it contributes about 73% to total rice production. This is an indication of the importance of water regime in the development and yield of rice plant. Complete or partial submergence is an important abiotic stress that affects 10–15 million ha of rice fields in South and Southeast Asia, causing yield losses estimated at US$1 billion every year (Dey and Upadhyaya, 1996). This number is anticipated to increase considerably in the future given the increase in sea-water level, as well as an increase in frequencies and intensities of flooding caused by extreme weather events (Bates *et al.,* 2008; Akinwale *et al*., 2012). Flash floods are highly unpredictable and can occur at any growth stage of the rice crop, resulting in yield loss of 10% to 100% depending on water depth, duration of submergence, temperature, turbidity of water, light intensity and age of the crop (Aliyu *et al*., 2015; Akinwale *et al*., 2012; Setter *et al*., 1995). In Nigeria, approximately 70% of rainfed lowland rice farms are prone to seasonal flooding which is a major constraint to rice production in some major rice producing states. Yield loss can range from 10% to total destruction. Among the most frequently affected states in Nigeria are Kebbi, Niger, Kogi and Taraba states which account for 80% of rice ecology in Nigeria (Erenstein *et al*., 2003). As a semiaquatic plant, rice is generally intolerant of complete submergence and plants die within a few days when completely submerged (Mohanty *et al.,* 2013). Since deep water rice and lowland rice comprise nearly 33% socio-economic vulnerability to climate change of the world's rice-growing area (Bailey-Serres *et al.,* 2010), flood is a major source of production losses in these important rice ecosystems. Often, transient flash flood occurs and is followed by longstanding stagnant floods. This type of flood reduces the survival chance of rice plants and causes severe damage to rice yields. For example, rice production losses in Bangladesh and India due to flash floods were estimated to be around 4 million tons per year, and this lost in production (had there been no floods) could have fed 30 million people (IRRI, 2010). Africa is particularly vulnerable to climate change because of the over-dependence on rainfed agriculture and the high incidence of poverty. Considering that changes in the global climate will result in more extreme events, such as floods, substantial economic benefits can be achieved from the development of improved rice varieties that are more resilient to climate change. This would allow rice producers to adapt to worsening global climate and allow them to mitigate the adverse effects of climate change in the future. Systematic screening of rice germplasm in Asia has shown that there are excellent flood-tolerant rice genotypes locally available. Among these are FR13A and FR43B of India, Kurkaruppan of Sri Lanka and GodaHeenati of Indonesia (IRRI, 2010). It is from these local genotypes within Nigeria, we intend to establish flood-tolerant rice varieties and validate the submergence protocol.

Materials and Methods

Plant Materials, Sterilization and Pre-germination

Preliminary phenotypic screening and germination test was conducted at the Botanical garden of the Department of Biological sciences, Ahmadu Bello University. A total of twenty nine (29) genotypes were obtained from the Africa Rice center, Ibadan station, pre-screened for viability and subjected to complete submergence stress.

Rice seeds were cleaned and placed in an oven at 50°C for five days to break seed dormancy. The seeds were surface sterilized with 0.1% Mercury Chloride ($HgCl_2$), and rinsed with distilled water. Sterilized seeds were soaked in water in a Petri-dish lined with Whatman's filter paper and placed on laboratory bench until germination. Benlate solution was added to prevent fungal infection. Pre-germinated seeds were sown, two seeds per genotype, in rows of three in 30x30cm plastic trays which served as nursery. Each genotype was labeled appropriately and watered regularly (200ml/day).

Preparation of seed pots and transplanting

Sterilized loamy soil, mixed with NPK (15:15:15) fertilizer at the rate of 30 kg/ha was placed in black polythene bags (15cm diameter). Each polythene bag was filled with about 3kg of soil, and watered until the soil was fully saturated. Seeds were pre-germinated for 21 days on nursery bed before they were transplanted to the polythene bags. Each genotype was labeled appropriately using plastic tags. Each polythene bag was placed in a 1m x 1m plastic tank in rows of five in a randomized block design (RBD) with three replications (Aliyu *et al.*, 2015).

Submergence evaluation

Submergence treatment was administered 30 days after transplanting by gradually increasing water level at 5cm per day until a maximum of 100cm water level was attained. Plants were submerged for a period of 14days. Control set up was left un-submerged. Water was raised to 2cm above the soil surface (Akinwale *et al.*, 2012).

Data collection

Survival count was taken immediately after de submergence (0DAS), seven days after desubmergence (7DAS) and 14 days after desubmergence (14DAS) (Aliyu *et al.*, 2015; Akinwale *et al.,* 2012; Sarkar and Bhattacharjee, 2011; Septiningsih *et al.,* 2009; Xu *et al.,* 2006). Other phenotypic parameters measured after desubmergence were; Plant Height (cm): measured from soil level to the tip of the flag leaf, Tiller Number: the number of tillers per genotype was counted at 0DAS, 7DAS and 14DAS, number of leaves per plant, leave diameter (cm) values were taken. Data obtained were subjected to analysis of variance (ANOVA). Where significant ($p<0.05$), Duncan's Multiple Range Test was used to separate the means. The degrees of association of the morphological parameters were determined using the Pearson's correlation index.

RESULTS

Performance of 29 Rice Genotypes Screened for Submergence Tolerance

Response to submergence tolerance was largely genotype dependent. Percentage survival after desubmergence (immediately after desubmergence (0DAS), 7 days after desubmergence (7DAS) and 14 days after desubmergence (14DAS) ranged from 0-100%. Tiller number, number of leaves and leaf diameter were not significantly ($P<0.05$) affected by submergence stress in genotypes evaluated. Immediately after de-submergence (0DAS), percentage survival varied between 57.67% and 100%. Four genotypes (CG14, FKR 19, NERICA-L- 43 and TOG 7148) recorded 100% survival upon de-submergence. TOG6632 was most susceptible with a percentage survival of 57.67%. The tolerant check (IR64 SUB1) showed an 89.00% survival percentage (Table 1). At 7DAS, percentage survival ranged between 40% and 100%. IR64, NERICA-L-43 and TOS6455 were most tolerant with a survival percentage of 100. This was significantly ($p<.01$) higher than survival scores for all other genotypes at this growth stage. NERICA-L-43, IG 133 and IR 64 SUB1 sustained the survival percentages from 0DAS to 7DAS. FARO44, NERICA-L-53, TOG6632 and W8-45 showed significant recovery within this period with increasing survival scores. TOG7218 was most susceptible to submergence stress at 7DAS.

Survival percentages at 14DAS ranged from 17.67% to 73.33%. IR 64 SUB-1 was the most tolerant genotype with a survival percentage of 89.00%. NERICA-L-52 showed consistent tolerance to submergence stress with a 73.33% tolerance between 7DAS and 14DAS. This consistency was similar to that of IR 64 SUB-1, though with differences in tolerance level. FKR54 showed slight recovery of 40% to 44.67% from 7DAS to 14DAS while TOG 7412 was most susceptible to submergence stress as it was completely lost at 14DAS (Table 1).

Table 1: Performance of 29 Rice Genotypes Screened for Submergence Tolerance

GENOTYPES	Survival (%) 0DAS	Survival (%) 7DAS	Survival (%) 14DAS	Plant Height (cm) at 0DAS	Tiller Number at 0DAS	Number of Leaves at 0DAS	Leave Diameter cm 0DAS
CG 14	100.00^{a}	78.00^{ab}	51.00^{b}	19.33^{c}	2.33^{a}	3.67^{b}	0.23^{a}
FARO 36	78.00^{b}	73.33^{ab}	51.00^{b}	26.00^{b}	2.33^{a}	3.00^{b}	0.20^{a}
FARO 44	73.33^{b}	89.00^{a}	55.67^{b}	31.00^{ab}	2.67^{a}	5.33^{a}	0.33^{a}
FARO 57	73.33^{b}	62.33^{b}	62.33^{ab}	31.33^{ab}	2.67^{a}	3.67^{b}	0.23^{a}
FKR 54	78.00^{b}	40.00^{c}	44.67^{c}	24.00^{b}	2.33^{a}	2.67^{bc}	1.33^{a}
FKR 19	100.00^{a}	73.33^{ab}	55.67^{b}	21.67^{b}	2.67^{a}	3.67^{b}	0.27^{a}
GAMBIAKA	89.00^{ab}	78.00^{ab}	51.00^{b}	24.33^{b}	2.33^{a}	3.33^{b}	0.23^{a}
IG 133	78.00^{a}	78.00^{ab}	40.00^{c}	31.67^{ab}	2.00^{a}	2.33^{bc}	0.23^{a}
IR 64	89.00^{ab}	89.00^{ab}	66.67^{ab}	32.67^{ab}	3.00^{a}	4.33^{ab}	0.40^{a}
IR 64 SUB1	89.00^{a}	89.00^{ab}	89.00^{a}	23.67^{b}	2.67^{a}	3.33^{b}	0.10^{a}
NERICA L-43	100.00^{a}	100.00^{a}	40.00^{c}	25.67^{b}	2.00^{a}	2.33^{bc}	0.20^{a}
NERICA L-52	78.00^{b}	73.33^{ab}	73.33^{a}	42.33^{a}	3.00^{a}	6.33^{a}	0.30^{a}
NERICA L-53	73.33^{b}	84.33^{ab}	53.00^{b}	22.33^{b}	2.00^{a}	1.33^{c}	0.20^{a}
RAM 133	66.67^{bc}	62.33^{ab}	33.33^{e}	11.67^{c}	2.00^{a}	4.00^{ab}	0.13^{a}

TOG 7218	89.00^{a}	40.00^{c}	35.33^{e}	9.00^{d}	1.67^{a}	2.00^{bc}	0.10^{a}
TOG 7250-A	89.00^{ab}	73.33^{ab}	55.67^{b}	24.67^{b}	2.67^{a}	4.67^{ab}	0.17^{a}
TOG 7412	84.33^{a}	89.00^{ab}	0.00^{f}	0.00^{f}	0.00^{a}	0.00^{b}	0.00^{a}
TOG 7439-B	89.00^{ab}	68.67^{b}	22.33^{f}	16.00^{c}	1.67^{a}	2.00^{bc}	0.13^{a}
TOG 8347	89.00^{ab}	44.67^{bc}	40.00^{c}	17.33^{c}	2.00^{a}	1.33^{c}	0.13^{a}
TOG 6632	57.67^{c}	73.33^{ab}	40.00^{c}	19.67^{c}	2.00^{a}	2.33^{bc}	0.17^{a}
TOG 6640-B	84.33^{ab}	73.33^{ab}	50.33^{f}	17.67^{c}	1.67^{a}	2.33^{bc}	0.30^{a}
TOG 7148	100.00^{a}	73.33^{ab}	51.00^{b}	27.33^{ab}	2.33^{a}	2.67^{bc}	0.15^{a}
TOS 6455	89.00^{ab}	86.00^{ab}	17.67^{f}	12.00^{c}	1.33^{a}	0.67^{d}	0.10^{a}
VANDANA	89.00^{ab}	89.00^{ab}	55.67^{b}	29.33^{b}	2.67^{a}	4.67^{ab}	0.40^{a}
W 8 45	62.33^{bc}	89.00^{ab}	66.67^{ab}	31.67^{ab}	2.00^{a}	6.33^{a}	0.40^{a}
WITA 9	73.33^{b}	66.67^{b}	40.00^{c}	27.67^{b}	2.00^{a}	2.33^{bc}	0.23^{a}
WITA 12	68.67^{bc}	73.33^{ab}	57.67^{b}	18.67^{c}	2.33^{a}	3.67^{b}	0.33^{a}
WITA 4	73.33^{b}	68.67^{b}	57.67^{b}	35.33^{ab}	2.33^{a}	3.33^{b}	0.40^{a}
POKALLI	73.33^{b}	55.67^{b}	51.00^{b}	26.67^{b}	2.33^{a}	2.00^{bc}	0.27^{a}
CV	26.14	30.94	83.52	87.35	98.28	88.06	70.13
R2	0.34	0.12	0.33	0.3	0.29	0.33	0.34
P VALUE	0.55	0.01	0.05	0.05	0.8	0.61	0.58

Means with the same superscript along columns do not differ significantly ($p < .05$) from one another using the Duncan's multiple range tests. DAS= days after de submergence.

Survival percentages for non-submerged (control) genotypes at 14DAS indicated that all genotypes except IR 64 SUB-1, TOG7218 and TOG6640-B showed 100% survival. This was significantly higher than the survival percentages observed in submerged genotypes. Submergence caused total loss of some plants at this evaluation stage. The positive control checks showed greater tolerance and survival compared to the un-submerged control. In response to submergence stress, NERICA-L-52 and IR64 exhibited greater shoot elongation of 24.5% and 21% over the un-submerged control treatment. No genotype showed greater tillering ability or increase in leaves numbers upon comparison with the control treatment. FKR 54 showed wider leaf diameter under submergence stress than in the control treatment. Few genotypes were lost as water receded at 14DAS; a 100% loss in plant developmental traits was inputted for these genotypes (Table 2).

Table 2: Percentage Effect of Submergence Stress on 29 Rice Genotypes at 14DAS

GENOTYPE	SURVIVAL AT 14DAS (%)	PH(%)	TN (%)	NL (%)	LD (%)
CG 14	49.00	50.40	22.30	75.50	71.30
FARO 36	49.00	38.80	22.30	84.20	77.80
FARO 44	44.30	33.30	11.00	76.30	61.20
FARO 57	37.70	30.40	11.00	73.80	75.80
FKR 54	55.30	53.80	22.30	84.70	-26.70
FKR 19	44.30	36.30	11.00	73.80	68.20
GAMBIAKA	49.00	39.20	22.30	79.20	64.60
IG 133	60.00	54.40	33.30	88.10	80.80
IR 64	33.30	-21.00	0.00	71.10	42.90
IR 64 SUB1	-6.60	45.60	11.00	73.40	85.70
NERICA L-43	60.00	37.40	33.30	85.00	71.40

NERICA L-52	26.70	-24.50	0.00	63.80	64.70
NERICA L-53	47.00	34.30	33.30	91.40	69.20
RAM 133	66.70	81.80	33.30	74.20	84.70
TOG 7218	57.70	85.10	16.50	84.60	92.00
TOG 7250-A	44.3	57.50	11.00	76.1	83.00
TOG 7412	100.00	100.00	100.00	100.00	100.00
TOG 7439-B	77.70	72.40	44.30	87.90	87.0
TOG 8347	60.00	71.10	33.30	91.40	86.30
TOG 6632	60.00	34.40	33.30	85.90	81.10
TOG 6640-B	73.30	71.00	44.30	84.50	71.40
TOG 7148	49.00	55.60	22.30	83.80	85.00
TOS 6455	82.30	70.40	55.70	94.40	88.90
VANDANA	44.30	41.30	11.00	69.50	55.60
W 8 45	33.30	28.80	33.30	72.50	42.90
WITA 9	60.00	27.20	33.30	89.40	69.30
WITA 12	42.30	48.10	22.30	75.50	49.20
WITA 4	42.30	4.50	22.30	80.40	33.30
POKALLI	49.00	54.40	22.30	84.60	66.30

14DAS= survival at 14 days after de submergence. PH=Plant height, TN= Tiller number, NL= Number of leaves, LD= leave diameter.

There was a strong positive correlation between survival at 14DAS with and plant height (r^2=0.94), tiller number (r^2=0.94), number of leaves (r^2=0.89) and leave diameter (r^2=0.85) (Table 3). All other morphological parameters showed some degree of positive correlation with one another (Figure 1).

Table 3: Correlation Coefficient for Traits Measured in 29 Rice Genotypes Subjected to Submergence Stress.

	14DAS	PH	TN	NL	LD
14DAS	1				
PH	0.94*	1			
TN	0.94*	0.82*	1		
NL	0.89*	0.81*	0.87*	1	
LD	0.85*	0.88*	0.79*	0.83*	1

*= significant at p<0.01

DISCUSSION

Submergence stress affects the physiological and metabolic processes (Sarkar *et al.,* 2006; Bailey-Serres and Voesenek, 2010) of plants. Expectedly, the decline in percentage survival of the genotypes due to submergence stress indicates that submergence stress strongly influences survival of rice plants (Aliyu *et al.*, 2015). Differential response observed amongst genotypes in tolerance or susceptibility to submergence stress was largely due to their escape or quiescence strategies to mitigate the prevailing stress conditions. Plants with escape mechanisms responds to submergence stress by exhibiting enhanced shoot elongation. In contrast, minimum shoot elongation which promotes growth recovery upon de-submergences was characteristic of the quiescence mechanism (Fukao and Bailey-serres, 2008). Genotypes that exhibited escape strategies showed some degree of lodging after de-submergence which ultimately affected their survival as the water level recedes. Growth recoveries observed in some of the genotypes indicates the ability of those genotypes to survive under short periods of submergence. Ability to recover after de- submergence is a desirable trait in genotypes as it ensures production of sufficient biomass for plant productivity. This was corroborated by Sarkar and Bhattacharjee. (2011). Pandey and Bhandari. (2007) reported that plants ability to quickly re-generate after submergence is key to its survival. This in return is hinged on plants ability to maintain a high carbohydrate content. This will lead to quick development of new leaves and accumulation of greater biomass (Sarkar and Bharttacharjee, 2011). Ram *et al*. (2002) reported that stem elongation competes for energy with maintenance process and could reduce the chances of survival of rice during submergence. It is hence imperative for submergence tolerant genotypes to exhibit limited stem elongation. Akinwale *et al.* (2012) suggested that shoot elongation is associated with cost, as energy and carbohydrate are expended in cell division and elongation. Singh *et al.* (2010) also mentioned that limited stem elongation is associated with variety's ability to survive submergence as energy required for maintenance process are made readily available, not used for stem elongation. Luo *et al.* (2011); Sarkar and Bharttacharjee (2011) reported that rice adapts to submergence either by elongation or quiescence (reduced activity) depending on the nature of the flood. This was also supported by Xu *et al.* (2006); Sarkar and Panda (2009) and Hattori *et al.* (2009). Plant height was strongly correlated with survival at 14DAS. This could be attributed to the fact that most of the genotypes exhibited the escape strategy rather than the quiescence mechanisms. This was contrary to the findings of Akinwale *et al.* (2012) and Singh *et al*. (2010) who found stem elongation (plant height) to have a negative relationship with percentage survival. Their report was based on the evaluations of progenies from tolerant parents. Sarkar and Bhattacharjee (2011) stated that great elongation benefits plants during submergence by restoring contact with the air above the flood water. This improves internal aeration for aerobic respiration and photosynthesis. Setter *et al*. (1995) reported that one of the causes of intolerance to complete submergence in some rice cultivars was the desiccation of leaves after de submergence. The sharp decline in number of leaves between control and stressed genotypes could be attributed to desiccation of leaves after de submergence. This could also be explained that plants might have reduced their utilization of resources during submergence stress thus affecting other growth traits. Furthermore, loss of genotypes after 14DAS could be due to inability of the plant to continuously sustain itself in the stress environment owing to energy depletion. Tolerance to submergence stress as exhibited by Nerica L-52 is indicative of the fact that there exists some degree of tolerance to abiotic stresses within local germplasms of plant genotypes. The ability of IR64 sub-1 to maintain high survival percentages could be because of the presence of the SUB-1 gene which has been introgressed into it. Fukao and Bailey-Serres (2008) stated that submergence tolerance conferred by SUB1A gene is related to suppression of elongation thereby enhancing survival by reducing carbohydrate consumption.

This result clearly indicates that NERICA-L-52 showed great potentials for submergence tolerance. It hence implies that this genotype can be considered as a candidate in breeding for submergence. This will also increase the gene pool of submergence tolerant genotypes.

In conclusion, rice genotypes showed escape and quiescent strategies to mitigate submergence stress. Submergence stress affected plant survival (0 – 100%) and phenotypic growth parameters evaluated. FARO 36, GAMBIAKA and NERICA-L-52 showed high tolerance to submergence stress by exhibiting moderate plant height and reduced number of leaves during submergence and after de-submergence. Six genotypes (20.69%) showed growth recovery as water recedes in the first week. This was however lost at 14DAS thus validating the efficiency of the submergence evaluation protocol. FARO 57, NERICA-L-52, IR64 SUB1, W 8-45 and FKR 54 showed recovery plasticity in morphological traits ranging from 0-11.5% within 7 to 14 days of de- submergence. Hence, FARO 36 and NERICA-L-52 which are released rice varieties can be cultivated in flood prone regions and for future breeding programs for the enhancement of other local rice genotypes.

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