

*Full Length Research Paper*

## **Identification of rice genotypes tolerant to submergence at seedling stage in Uganda**

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Received 2 June, 2019; Accepted 18 July, 2019

Floods have destroyed over 2000 acres of rice in Uganda which affected yield and caused losses to farmers. This problem is more pronounced when fields are not well leveled, and the mode of irrigation is by surface flooding. Majority of lowland rice fields in East African region are of this nature and are thus prone to yield losses. There are no submergence tolerance varieties identified in Uganda, so far. To address this problem, breeding for submergence tolerance is the most ideal and promising strategy in rice. As a first step, genotypes tolerant to submergence need to be identified which is the objective of this study. 29 rice genotypes were morphological characterized in screen house and field conditions while 34 rice genotypes were molecularly characterized. Results suggested significant differences in the performance of genotypes both in the screen house and under field conditions in which varieties Swarna, IRRI SUPA 3 and KOMBOKA showed approximately 80% and above survival rate with Swarna variety ranking first. Molecular characterization of rice genotypes revealed that, out of 34 genotypes, 30 genotypes scored presence for *Sub 1A-2* allele while, four genotypes were neither *Sub1A-1* nor *Sub 1A-2* alleles. None of the tested genotypes were carrying *Sub 1A-1* allele.

**Key words:** Flash floods, submergence, tolerance, sub1, swarna

### **INTRODUCTION**

Globally, total area under rice cultivation is estimated to be 150 million hectares with annual production averaging 500 million metric tons (Tsuboi, 2004). This represents 29% of the total grain crop output worldwide (Xu and Shen, 2003). Rice is a dominant staple food crop in developing countries, particularly in the humid tropics with almost 90% of rice being produced and consumed in Asia and in developing countries in the tropics and

subtropics (Conteh et al., 2012; Hossain, 2004; Yoshida, 1981). In 2000, Food and Agriculture Organization (FAO) classified rice as the most important food crop depended on by over 50% of the world population. Due to increase in consumption rate of 7.2% per year, rice demand is expected to rise (Africa Rice, 2012). Given the higher rate of population growth (4% per annum) and change in customer preference in urban areas, rice has become the

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most rapidly growing source of food and income in sub-Saharan Africa (Sohl, 2005). Rice demand in urban areas has grown faster than elsewhere in the world (Balasubramanian et al., 2007; WARDA, 2005). In African countries, population size of urban dwellers is expected to increase from 38 to 48% by 2030, which will make rice consumption increase even more (Africa Rice Center, 2011). Consumption of rice in Africa averages 16 million metric tons while production is at 14 million metric tons, creating a deficit of 2 million metric tons (UNRDS, 2009).

In East Africa, rice is a vital crop, primarily grown by smallholder farmers as a lowland rainfed crop excluding Kenya, where the majority of rice is irrigated (Adhikari et al., 2015). Unlike other East African countries, Uganda grows both upland and low land rice. According to the cost benefit analysis study done by National Agriculture Research Organization (NARO), both rice types are profitable; although lowland rice has slightly higher margins than upland rice (MoFPED, 2015). With the promotion of upland rice in Uganda, production is now carried out in all regions including Western (upland genotypes), Eastern (lowland genotypes), Northern (upland/lowland) and Central regions (upland). It has been estimated that 59% of area under rice is rain-fed lowland conditions, 36% is upland rain-fed rice and 5% irrigated rice in Uganda (MoFPED, 2015). In Uganda, rice production trends indicated that demand for rice has grown at an average rate of about 9.5% per year since 2000 (MoFPED, 2015). It is the second most important grain commodity after maize, making it one of the three major grain commodities (maize, rice and sorghum) that are grown for commercial purposes in Uganda (MoFPED, 2015) with a significant contribution to Agricultural Gross Domestic Product (UBOS, 2015). The current demand for rice in Uganda is 223,000 metric tons while production is at 214,000 metric tons (MoFPED, 2015).

With a per capita consumption of 8 kg, population growth rate of 3.2% and a production growth rate of 3%, the demand still outweighs production and will continue to grow (MoFPED, 2015).

Rice productivity in Uganda is considered low and has stagnated between 1.3 to 2.4 tons per hectare over the last 15 years (Akongo et al., 2016). High rainfall variability, which is not optimal is one of the major underlying factors for low rice production in Uganda (Akongo et al., 2016; EPRC, 2016; Republic of Uganda, 2010; USAID, 2013). Drastic changes in rainfall patterns can introduce unfavorable growing conditions due to flooding or drought into cropping calendars and by modifying growing seasons, which can subsequently reduce crop productivity. IFPRI (2007), forecasts rice yield losses between 10 and 15% by 2050 as a result of flooding and drought associated with climate change. Variation in climate can also affect rice productivity in rice production systems in other ways. In some areas like Otuke district in Uganda, an new invasive rice weed was reported to be linked to excessive rainfall in rice fields and can cause up to 100% yield loss due to flooding

(Akongo et al., 2016).

Although rice is a crop that requires flooded and irrigated condition for cultivation, most of the rice genotypes are susceptible to flooding if the water stagnates and keeps plants submerged for more than seven days (Adkins et al., 1990). This causes leaf or stem elongation, leaf rotting, loss of dry mass and lodging after the flood water recedes (Jackson and Ram, 2003). Excess water triggers serious damage to the growth and survival rate of rice plants (HilleRisLambers and Vergara, 1982). Flooding is expected to increase as a result of erratic weather patterns, which includes frequent lengthy and storms associated with climate change that could severely affect food production if mitigation measures are not sought, and will further reduce crop yields especially in the tropics and subtropics (Onaga and Wydra, 2016). In Uganda, rain fed lowland ecologies especially in Eastern Central region (Soroti) and Northern parts (Katakwi and Amaria) receive above normal rains, which can reach flood level. Varieties grown in these regions suffer yield penalty due to high rainfall (Odogola, 2006). According to New Vision of 5<sup>th</sup> December 2011, floods have destroyed over 2000 acres of rice in Uganda (Butaleja district) which affected yield and caused losses to farmers. Frequency and intensity of flash floods has increased due to changes in global weather patterns (Singh et al., 2011); therefore, sustainable and permanent solutions are needed to overcome this problem. One of the most promising solutions is to develop high yielding varieties that are tolerant to submergence. This work, therefore aims at identifying rice genotypes that are tolerant to submergence at seedling stage that can adapt well in floods condition in order to reduce yield losses especially in flood prone areas. This was done based on hypothesis that, rice genotypes that are tolerant to seedling submergence are available among selected rice genotypes in Uganda.

## MATERIALS AND METHODS

A total of 29 rice genotypes were used in this study. Of these, six genotypes namely, *O. barthii* interspecific lines which were obtained from a cross of *O. barthii* and *O. sativa*, where *O. glaberrima* is a monocarpic annual derived from *O. barthii* (Sakagami et al., 1999), which is well adapted to lowland condition and is known to be tolerant to diseases and other abiotic stress such as flooding conditions (Sakangami, 2012). Other eight genotypes (NamChe-5, NamChe-2, ARS 126-3-B-1-2, ARU1189, ARU1190, ARU1191, E20 and E22) are potential candidate varieties for rain-fed lowland condition. 11 genotypes (AGRA 65, SUPA 1052, IRRISUPA3, TXD306, ART84 SANDE, KOMBOKA, SUPA 5, NamChe-3, NamChe-4, NamChe-6 and AGRA 60) have been adapted to rain fed lowland condition. Genotypes IR 64, Mahsuri and Swarna from IRRI were included as submergence susceptible checks and CG 14 as tolerant check.

### Evaluation of rice genotypes tolerant to submergence at seedling stage

An experiment was conducted at National Crop Resources

**Table 1.** Submergence scoring/survival (IRRI, 1996).

Submergence scale	Description (%)	Category
1	100% submergence check	Tolerant
3	95-99% submergence	Tolerant
5	75-94% submergence	Moderately tolerant
7	50-74% submergence	Moderately susceptible
9	0-49% poor submergence	Highly susceptible

Research Institute (NaCRR) - Namulonge-Uganda. Rice genotypes were evaluated under controlled flood condition in both screen house and under field condition according to Joho et al. (2008). Nursery bed was prepared, where seeds of 29 cultivars were sown separately in soil (3 seeds per hole). Seventeen days after planting, seedlings were transplanted in small plastic basins (15 cm depth, 40 cm length and 30 cm width) in the screen house and on the field condition. Di-ammonium phosphate (DAP) fertilizer was applied in both experiments one day after transplanting. 13 days after transplanting; seedlings were completely flooded in big containers (water tanks) at 45 cm water depth above the soil level in the basins for 10 days in the screen house and 45 cm water depth from the soil level under field condition. Water depths were maintained by adding water regularly in the water tanks. 10 days after, flooded plants were removed from water tanks where identification of submergence tolerant genotypes was done 19 days after submergence stress based on rate of seedling survival. Screen house experiment was laid in alpha lattice design while field experiment was laid in randomized complete block design. Both experiments were replicated twice. A total of 12 rice genotypes were selected from the first experiment based on their tolerance level and tested under submergence stress following IRRI standard protocol as described by Seiji (2002). Rice seedlings were submerged to a water depth of 100-cm for 14 days in the screen house. The experiment was laid in randomized complete block design with two replications.

Molecular analysis of submergence tolerance was done on 34 rice cultivars where genomic DNA of 34 rice cultivars (NERICA-L19 Sub 1, WITA-4 Sub 1, ARS 37, ARS 38 and Wita 9 in addition to 29 genotypes) were isolated according to a pre-standardized protocol of Borges et al. (2012) in the molecular laboratory at Kabanyolo. The quantity of the extracted DNA was checked by a Nano Drop spectrophotometer machine (ND-1000). Gel electrophoresis was performed using 1.5% agarose gel to check the quality of the DNA. Genetic relationship among the 34 studied rice genotypes was assessed for five reported tightly linked SSR markers (RM 219, RM 316, RM464A, RM 444 and RM 285) mapped on rice at the Sub1 region on chromosome 9 as reported by Goswami et al. (2015). The study of the allelic diversity in Sub1 loci among the studied cultivars was done using gene specific primers for three components of Sub1 loci which are, Sub1A, Sub1B and Sub1C.

## Data collection

### Measurement of plant, leaf and root properties

Seedling height and leaf length were measured using a meter ruler one day before submergence, one day after submergence and eleven days after submergence and recorded in centimeters (cm). Seedling height was measured from the base of the shoot to the tip of the tallest leaf blade. Shoot elongation during submergence was computed as a percentage of the pre-submergence value. Leaf area index (LAI) was measured one day before submergence and eleven days after submergence using leaf area meter (Ceptometer

AccuPAR LP-80). Leaf senescence (LS) was characterized by dramatic yellowing resulting from chloroplast degradation; and it was assessed immediately after submergence on a plot basis using a SPAD-502 chlorophyll meter (Monilta Camera Co.Ltd, Japan). Whole root systems of five plants were washed carefully and root length of each plant was measured (cm) with a meter ruler after submergence. Number of tillers was counted manually in the five plants before and after submergence.

### Assessment of lodging

The degree of lodging was determined five days after the water level receded and expressed in percentage as shown in Equation 1:

$$\% \text{ lodging} = \frac{\text{Number of plant lodged after submergence} \times 100}{\text{Total number of plants after submergence}} \quad (1)$$

### Assessment of dry matter content

Five plants from each experimental plot were harvested and separated into leaves, shoot, and root parts. The different plant parts were then dried in an oven for 48 h at 80°C, weighed to determine the dry weights of separate organs after submergence and presented as total dry matter.

### Assessment of seedlings survival

Seedling survival was rated 19 days after submergence by counting the number of plants able to produce at least one new leaf and expressed as the percentage of the initial number of plants before submergence. Submergence tolerant genotypes were identified in terms of percentage survival (Equation 2):

$$\% \text{ Survival} = \frac{\lambda_2}{\lambda_1} \times 100 \quad (2)$$

Where:  $\lambda_1$  = Number of plants before submergence;  $\lambda_2$  = Number of survived plants after recovery effect.

Scores were categorized into 4 groups: tolerant (1-3), moderately tolerant (4-5), moderately susceptible (6-7) and highly susceptible (8-9), based on the standard evaluation system of IRRI (1996) as shown in Table 1.

Effect of submergence was measured as the height reduction or height gain after submergence compared to height of pre-submergence period as shown in equation 3:

$$\Delta = \mu_2 - \mu_1 \quad (3)$$

Where,  $\Delta$  = Height reduction or height gain during submergence;  $\mu_2$  = Average height of plants just after the submergence stress;  $\mu_1$  = Average height of the plants just before the submergence stress. Polymorphism testing was done between identified tolerant and susceptible genotypes and was scored based on presence (1) or

absence (0) of a particular band. Allelic diversity study data were collected based on differences in molecular weights for gene specific primers. Individual alleles in form of differences in molecular weight of the amplified product for individual loci were scored based on the presence (1) or absence (0) of a particular band.

### Statistical analysis

Collected data were subjected to analysis of variance (ANOVA) using Genstat software (18<sup>th</sup> edition) at  $P < 0.05$  to obtain the mean squares and differences in different parameters. For screen house experiment, the Restricted Maximum Likelihood (REML) analysis was used to generate analysis of variance (ANOVA). The linear model for alpha lattice design used in this study was  $Y_{ijk} = \bar{Y} + G_i + R_j + B/R_{jk} + e_{ijk}$  where  $\bar{Y}$  Grand mean,  $G_i$  is genotype mean effect,  $R_j$  replications effect,  $B/R_{jk}$  block within replication effect, and  $e_{ijk}$  is the experimental error. However, when lattice incomplete block was not effective the data were analyzed as Randomized Complete block design to generate ANOVA. For the screening at 100-cm water depth experiment, Randomized Complete block design was used to generate ANOVA. The linear model used was  $Y_{ijk} = \bar{Y} + G_i + R_j + B/R_{jk} + e_{ijk}$  where  $\bar{Y}$  Grand mean,  $G_i$  is genotype mean effect,  $R_j$  replications effect, and  $e_{ijk}$  is the experimental error.

Means were separated using Fisher's protected least significant difference (LSD) test at 5% probability level if the F value was significant. The genotypes that recorded to have a recovery score of 1-3 were grouped as tolerant, 4-5 moderately tolerant while a score of 8-9 were highly susceptible. In addition, Pearson correlations of main traits were determined to find the association among traits. Polymorphism information was obtained by using five tightly reported simple sequence repeat (SSR) markers between identified tolerant and susceptible genotypes. Chi-square test of independence was done to determine association between marker and submergence tolerance scores.

## RESULTS

Analysis of variance for parameters evaluated at seedling stage in the screen house immediately after submergence stress showed significant difference ( $P \leq 0.05$ ) for seedling height, suggesting the variation among genotypes in seedling height soon after submergence stress. Moreover, there were no significant differences in computed shoot elongation among genotypes suggesting that, there is not enough evidence that variation of genotypes under submergence were different in terms of elongation rate. All genotypes were observed for high rate of lodging, though variation on lodging rate was not significant among genotypes. There were significant differences in chlorophyll content ( $P \leq 0.05$ ) assessed immediately after submergence, suggesting differences in rate of photosynthesis among genotypes. Moreover, significant differences for the computed leaf senescence (differences in chlorophyll content before and after submergence) ( $P \leq 0.01$ ) suggest variation among genotypes in chlorophyll content before and after submergence stress (Table 2)

Seedling height measured immediately after submergence was the highest compared to seedling

height measured one day before submergence and seedling height measured eleven days after submergence (Figure 1), indicating that there was an increase in seedling height under submergence stress which suggest low oxygen escape syndrome as response of genotypes under flooded water. Variety MET 41 recorded maximum mean seedling height of 72.86 cm higher compared to CG 14 (check genotype) (61.7cm) while the lowest seedling height was recorded for variety ARS 126-3-B-1-2 (25.22 cm) (Figure 1). Variety CG14 (check) recorded maximum shoot elongation rate of 196.1% while MET 59 recorded lowest shoot elongation of 77%. Unlike seedling height, a high rate of leaf chlorosis was observed among genotypes soon after submergence (Figure 2).

Analysis of variance for parameters evaluated at seedling stage in the screen house at 11 and 19 days after submergence showed significant differences in seedling height and leaf area index (LAI) among rice genotypes at ( $P < 0.05$ ). Significant differences in LAI suggest differences in amount of light intercepted among genotypes. Total dry matter content did not differ among genotypes 19 days after submergence, suggesting that during recovery time both tolerant and susceptible genotypes were capable of conserving equal dry matter contents. Leaf chlorophyll content assessed 11 day after submergence showed significant differences among genotypes ( $P \leq 0.01$ ), suggesting a variation in leaf chlorophyll content, which reflects photosynthesis rate among genotypes during recovery. Survival rate recorded at 19 days after submergence showed significant difference ( $P < 0.01$ ) among the genotypes suggesting differences on the performance of genotypes under submergence stress, which indicates diversity among genotypes (Table 3).

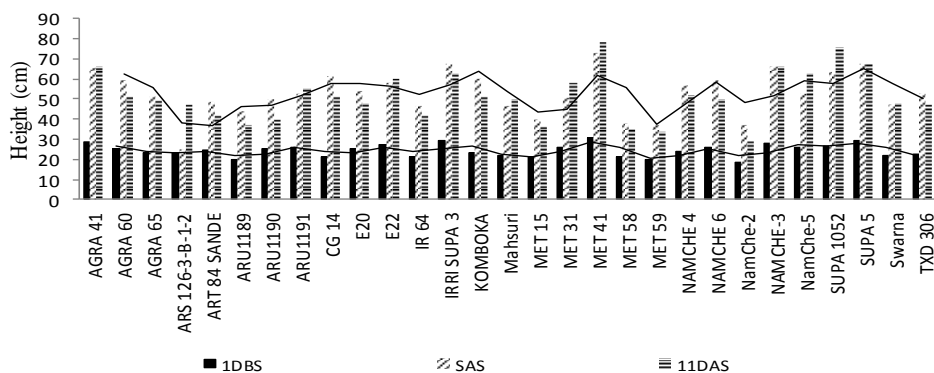
Differences in survival rate were observed across the two experiments, where the performances of genotypes was somewhat higher in the screen house than in the field (Figure 3a) with tolerant genotypes being observed in screen house and moderately tolerant genotypes in the field conditions (Figure 3b).

A Fisher protected multiple range test, results suggested that Swarna, IRRI SUPA 3, Mahsuri, SUPA5 and KOMBOKA showed stable performance in terms of survival rate under submergence stress with percentage survival of 93, 89, 88, 86 and 84, respectively; while MET 59, (AGRA 60, Namche-2) and MET 58 showed poor performance (30, 32 and 35) respectively across the two experiments (Figure 4). Evaluation of submergence tolerant rice genotypes following the IRRI standard protocol revealed a significant difference in seedling height assessed immediately after submergence stress ( $P \leq 0.01$ ). Lack of significant differences in percentage shoot elongation suggested that there was no evidence that the elongation rate among genotypes were different. There was no variation in leaf chlorophyll content assessed immediately after submergence stress, but leaf

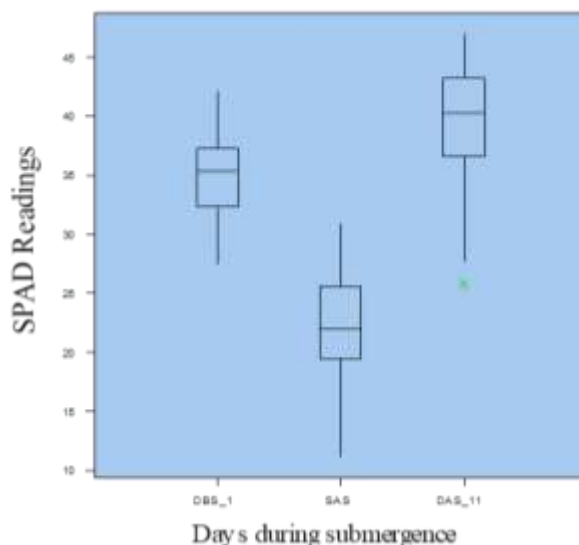
**Table 2.** ANOVA table of 29 genotypes immediately after submergence in screen house condition.

SOV	df	Seedling height (cm)	Shoot elongation (%)	Lodging (%)	Chlorophyll (SPAD readings)	Leaf senescence (SPAD readings)
Rep	1	1328.6*	25619.5**	9791*	314.1*	236.69*
Rep/Block	10	180.2ns	1739.8ns	NA	48.6**	28.04ns
Genotypes	28	285.0*	1561.5ns	1267ns	29.3*	52.30**
Residual	18 or 26	101.7	912.9	1303	10.9	14.3
LEE	17	122.3	1156.7	NA	12.3	15.8
<b>SD</b>		<b>11.1</b>	<b>34.01</b>	<b>36.1</b>	<b>3.5</b>	<b>4</b>
<b>%CV</b>		<b>21.72</b>	<b>28.92</b>	<b>52.79</b>	<b>19.09</b>	<b>28.35</b>

NA=Not applicable, Lattice method was not effective. Ns = non-significant; \*\*, \*\*\* = significant at 0.01 and 0.001 probability level, respectively; SOV=Source of variation, df=degree of freedom, LEE=Lattice effective error, SD=Standard deviation, CV=Coefficient of variation, %=Percentage.



**Figure 1.** Genotypes response to seedling height before and after submergence stress. 1DBS=One day before submergence, SAS=Soon after submergence stress, DAS=Days after submergence

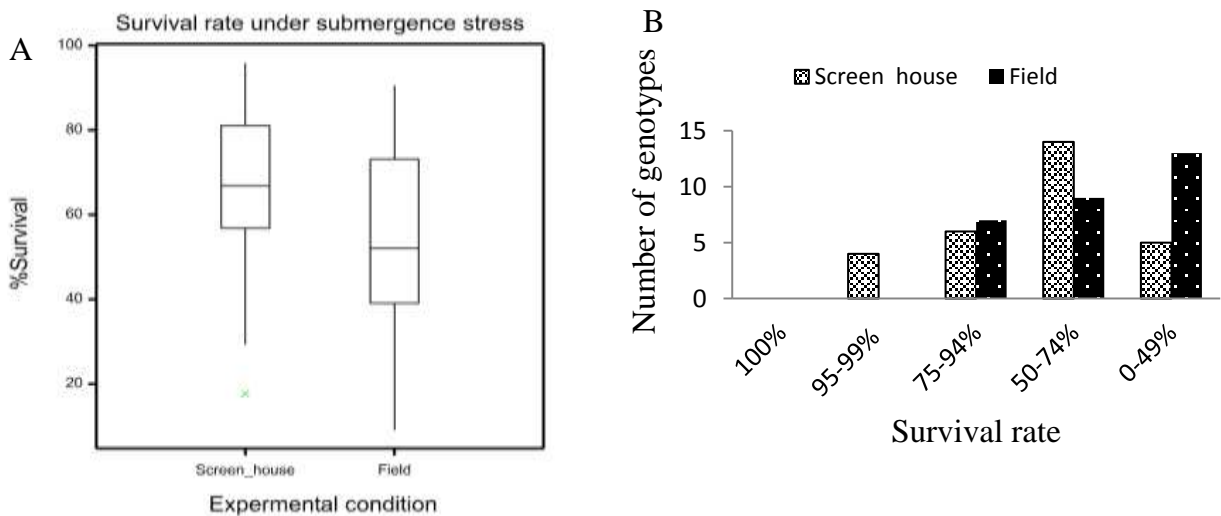


**Figure 2.** Effects of submergence on chlorophyll content. DBS-1=One day before submergence, SAS=Soon after submergence stress, DAS-11=Eleven days after submergence.

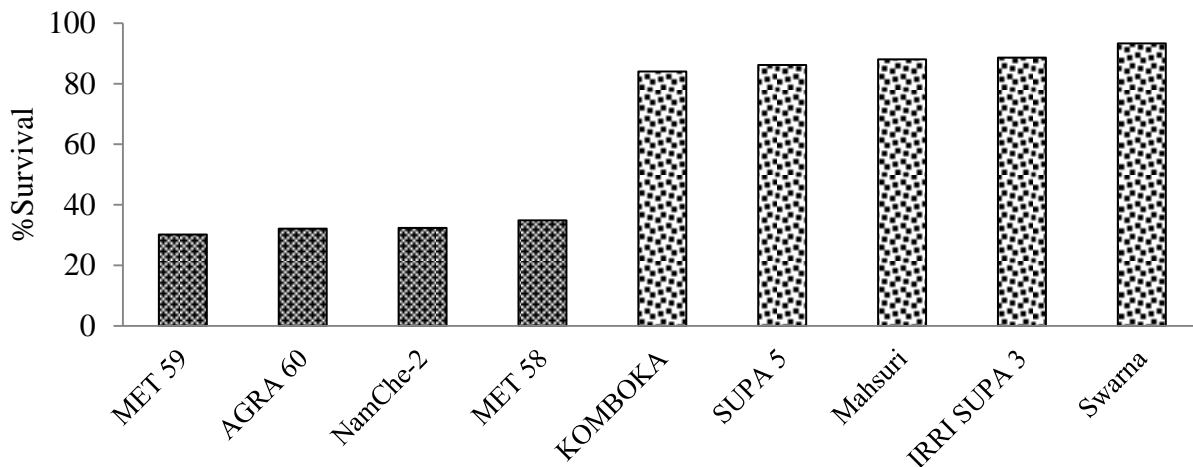
**Table 3.** ANOVA table of 29 genotypes 11DAS and 19 DAS in the screen house.

SOV	df	11DAS			19DAS	
		Seedling height (cm)	Leaf area index ( $\mu\text{mols/m}^2\text{s}$ )	Chlorophyll (SPAD readings)	Total dry matter (g)	Survival (%)
Rep	1	1.6 <sup>ns</sup>	0.002 <sup>ns</sup>	39.5 <sup>ns</sup>	6.21 <sup>ns</sup>	1159.4 <sup>ns</sup>
Rep/Block	10	NA	NA	16.84 <sup>ns</sup>	NA	378.5 <sup>ns</sup>
Genotype	28	307.3*	0.0254*	44.0**	41.44 <sup>ns</sup>	879.2**
Residual	18	153	0.013	11.02	33.6	248.2
LEE	17	NA	NA	13.29	NA	280.9
<b>SD</b>		<b>12.37</b>	<b>0.114</b>	<b>3.65</b>	<b>5.8</b>	<b>16.8</b>
<b>%CV</b>		<b>23.87</b>	<b>50.15</b>	<b>9.69</b>	<b>66.25</b>	<b>28.5</b>

NA=Not Applicable lattice method was not effective. Ns = non-significant, \*\*, \*\*\* = significant at 0.01 and 0.001 probability level, respectively; SOV=Source of variation, df=degree of freedom, Rep=Replication, LEE=Lattice effective error, SD=Standard deviation, CV=Coefficient of variation, %=Percentage, DAS=Days after submergence.



**Figure 3.** Survival rates of genotypes between field and screen house.



**Figure 4.** Survival rate and selection of rice genotypes tolerant to submergence.

**Table 4.** ANOVA table for genotypes under 14 day's submergence period at 100 cm water depth.

After submergence							
SOV	df	SH (cm)	SE (%)	CHL (SPAD readings)	PL (%)	LS (SPAD readings)	PS (%)
Rep	1	2.64 <sup>ns</sup>	32 <sup>ns</sup>	1.399 <sup>ns</sup>	37.5 <sup>ns</sup>	94.43 <sup>ns</sup>	2016.7*
Genotypes	11	91.53**	1172.2 <sup>ns</sup>	22.085 <sup>ns</sup>	1458.71***	265.23*	2384.8***
Residual	11	20.49	613.5	8.925	64.77	76.93	225.8
<b>SD</b>		<b>4.53</b>	<b>24.77</b>	<b>2.99</b>	<b>8.05</b>	<b>8.77</b>	<b>15.03</b>
<b>%CV</b>		<b>11.89</b>	<b>16.33</b>	<b>10.9</b>	<b>12.3</b>	<b>-91.94</b>	<b>26.52</b>

Ns = non-significant, \*\*, \*\*\* = significant at 0.01 and 0.001 probability level, respectively; SOV=Source of variation, df=degree of freedom, PS=Percentage survival, SH=Seedling height, CHL=Leaf chlorophyll content, SE=Shoot elongation, LS=Leaf senescence, PL=Percentage lodging.

senescence among genotypes was significantly different ( $P \leq 0.05$ ), suggesting differences in chlorophyll among genotypes which reflect differences in photosynthesis rate. Significant differences in survival ( $P \leq 0.001$ ), suggests genotypes responded differently under submergence stress (Table 4).

Evaluation of submergence at 100-cm water depth for 14 days showed that, the survival rate of check genotype (CG14) was low ( $\leq 40\%$ ) compared to varieties Swarna, KOMBOKA, Mahsuri and SUPA 5 ( $\geq 80\%$ ); where variety Swarna ranked first in terms of seedlings survival rate of 100%. Varieties Swarna, SUPA 5, IRRI SUPA 3, KOMBOKA Mahsuri and IR 64 showed stable survival rate at both water depths with  $\geq 75\%$  survival. On the contrary, the survival rate of check genotype (CG14) and SUPA 1052 showed a decrease in survival rate at 100-cm water depth. Survival rates of CG14 and SUPA 1052 decreased from 75 and 67% at 45-cm water depth to 35 and 35%, respectively, at 100-cm water depth. Varieties MET 58 and MET 59 were also found to be highly susceptible to submergence at both 45 and 100-cm water depths (Figure 5).

#### Pearson correlation analysis of parameters taken before and after submergence stress in screen house

Associations among parameters were studied before and after submergence stress. In relation to survival rate, before submergence stress seedling height and leaf area index were positively correlated with %survival at ( $r=0.40$ ,  $P \leq 0.05$ ) and ( $r=0.41$ ,  $P \leq 0.05$ ), respectively. Soon after submergence stress seedling height, chlorophyll content and %shoot elongation were positively correlated with survival rate ( $r=0.47$ ,  $P \leq 0.01$ ), ( $r=0.49$ ,  $P \leq 0.01$ ) and ( $r=0.43$ ,  $P \leq 0.05$ ) respectively. At 11 days after submergence, %survival was positively correlated with seedling height ( $r=0.59$ ,  $P \leq 0.001$ ) and Leaf area index ( $r=0.62$ ,  $P \leq 0.001$ ). At 19 days after submergence, a significant positive correlation was observed between %survival and total dry matter content at ( $r=0.74$ ,  $P \leq 0.001$ ), and also between %survival and root length ( $r=0.63$ ,  $P \leq 0.001$ ). Association among parameters are

presented in Table 5

Polymorphism screening results, based on five reported tightly linked simple sequence repeat (SSR) markers, revealed that markers RM 464A, RM 285, RM 219 and RM 316 were not polymorphic to submergence tolerance in this study. On the other hand, marker RM 444 was seen to be polymorphic to submergence tolerance scores between variety Swarna (tolerant) and MET 58 (susceptible). Based on an hypothesis that, there is no association between marker and variation in submergence tolerance score, a chi square test showed a statistically significant deviation from the hypothesis ( $\chi^2_{cal} = 857.2$ ,  $\chi^2_{crit} = 3.84$ ,  $\alpha = 0.05$ ). Results from the allelic diversity study on Sub 1 region based on gene specific primers (Sub 1A, Sub 1B and Sub1C) revealed that, all genotypes except NERICA L19-Sub 1 and WITA-4 Sub 1 scored presence in Sub 1B and Sub 1C at  $\sim 0.45$  and  $\sim 0.40$  Kb, respectively (Figure 6). Analysis of Sub1-A regions showed that, all genotypes scored bands at *Sub 1A-2* at  $\sim 0.700$  Kb while none of the genotypes in this study scored bands at *Sub 1A -1* allele at  $\sim 0.956$ Kb, suggesting that all genotypes survived submergence stress following low oxygen escape strategy which is conferred by *Sub 1A-2* allele; and none of the tested genotypes followed quiescent strategy which is conferred by *Sub-1A-1* allele. Genotypes WITA-4-Sub1, IRRISUPA 3, TXD 306 and strong-S did not score a band at Sub 1A locus.

#### DISCUSSION

In this study, two genotypes groups, based on seedling height differences, were able to survive. The first group belongs to genotypes that are tall and had ability to elongate (SUPA 5, SUPA 1052 and IRRI SUPA3) and the second group includes short varieties and had very little elongation ability (Swarna, IR64 and Mahsuri). Most of the tall varieties in this study exhibited good survival rate under submergence and also during recovery after submergence periods. In this study, varieties SUPA 5, SUPA1052 and IRRI SUPA 3 are tall varieties compared to IR64, Mahsuri and Swarna; however, the general

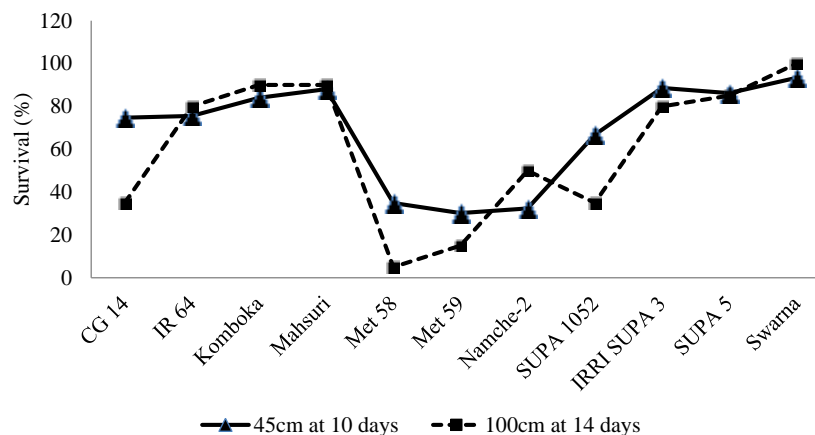


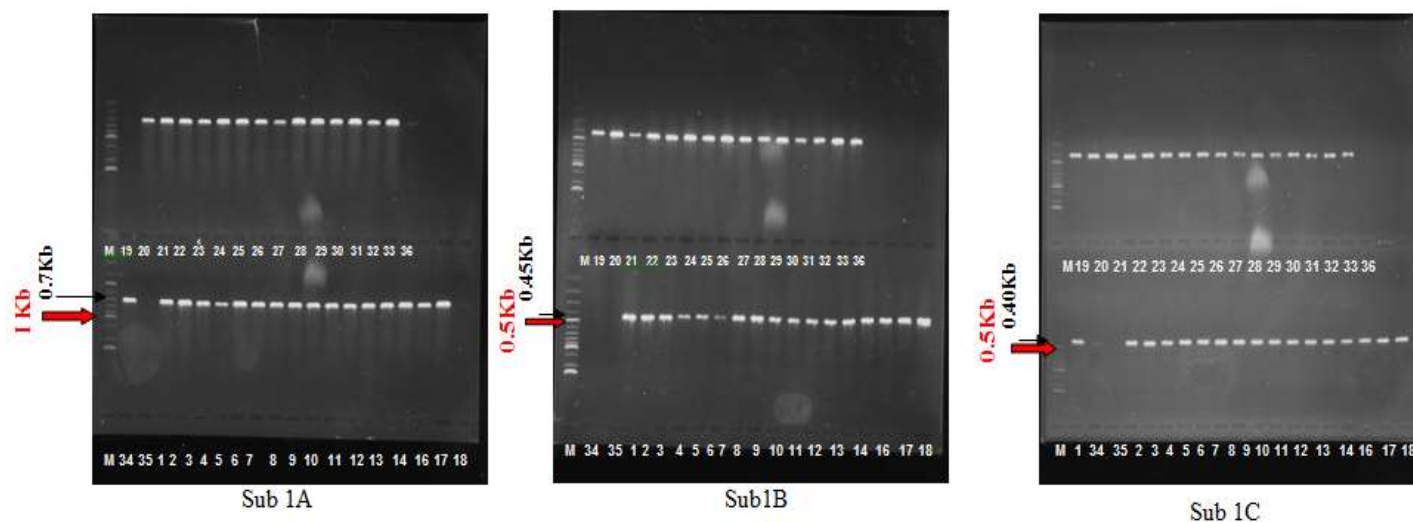
Figure 5. Percentage survivals of genotypes at 100 cm for 14 days submergence period.

Table 5. Pearson correlation analysis between parameters in the glasshouse before, soon after, 11DAS and 19DAS submergence stress.

Correlation parameters	PS	SE	SVS	SH 1DBS	TN 1DBS	CHL 1DBS	LAI 1DBS	SH SAS	CHL SAS	%SE	SH 11DAS	LAI 11DAS	CHL 11DAS	TN 19DAS	RL 19DAS
PS															
SE	-0.23														
SVS	-0.20	<b>-0.63***</b>													
SH 1DBS	<b>0.41*</b>	-0.12	0.06												
TN 1DBS	0.32	0.08	-0.15	-0.27											
CHL 1DBS	0.18	-0.08	-0.02	-0.07	<b>0.44*</b>										
LAI 1DBS	<b>0.40*</b>	0.17	-0.30	0.02	0.15	0.13									
SH SAS	<b>0.47**</b>	-0.10	-0.04	<b>0.78***</b>	-0.27	-0.34	0.09								
CHL SAS	<b>0.49**</b>	-0.19	-0.05	0.15	0.12	-0.09	0.29	<b>0.38*</b>							
%SE	<b>0.43*</b>	-0.01	-0.24	0.21	0.13	-0.32	0.17	<b>0.66***</b>	<b>0.35*</b>						
SH 11DAS	<b>0.59***</b>	-0.28	0.06	<b>0.87***</b>	-0.13	-0.05	0.17	<b>0.80***</b>	0.31	<b>0.40*</b>					
LAI 11DAS	<b>0.62***</b>	-0.29	0.04	0.22	<b>0.39*</b>	0.28	0.01	0.18	0.24	0.21	<b>0.43*</b>				
CHL11DAS	<b>0.46*</b>	-0.22	-0.09	<b>0.53**</b>	0.08	<b>0.42*</b>	0.15	<b>0.41**</b>	0.16	0.22	<b>0.54**</b>	0.25			
TN 19DAS	0.23	0.14	-0.20	-0.06	<b>0.70***</b>	0.23	0.23	0.00	0.21	0.10	-0.01	<b>0.38*</b>	0.06		
RL 19DAS	<b>0.63***</b>	-0.08	-0.17	<b>0.62***</b>	0.11	0.26	<b>0.39*</b>	<b>0.54**</b>	0.16	0.32	<b>0.66***</b>	<b>0.38*</b>	<b>0.68***</b>	0.34	
TDM 19DAS	<b>0.74***</b>	-0.16	-0.13	<b>0.43*</b>	0.31	0.21	<b>0.47**</b>	<b>0.47**</b>	<b>0.40*</b>	0.27	<b>0.57***</b>	<b>0.59***</b>	<b>0.43*</b>	<b>0.58***</b>	<b>0.76***</b>

Ns = non-significant, (\*, \*\* \*\*\*) = significant at (0.05, 0.01, 0.001) probability level respectively, DBS=Days before submergence, SAS=soon after submergence, DAS=Days after submergence PS=Percentage survival, SE=Seedling emergence, SVS=Seedling vigor scores, SH=Seedling height, TN=Tiller number, CHL=Leaf chlorophyll content, LAI=Leaf area index, SE=Shoot elongation, RL=Root length, TDM=Total dry matter, %=Percentage.





**Figure 6.** Allelic diversity of the Sub 1 region among the tested rice genotypes. Labeled numbers 1-36 are the different rice genotypes as indicated on Table 6. Genotype number 15 was not evaluated in this study. M = 2Kb DNA ladder used for reference made by Bioneer Corporation in Korea.

performance of tall varieties under submergence did not exceed that of Swarna variety. Short varieties (Swarna and Mahsuri) were found to be more tolerant than tall varieties; while tall varieties were moderately tolerance to submergence. Palada and Vergara (1975) also observed taller varieties to be more tolerant than the susceptible varieties; which suggested that, longer leaf blades made it possible for some leaf tips to be above the water.

Following post-submergence period, two strategies in this study were observed by the genotypes during recovery. The first strategy was no drying or shedding off the leaves. This strategy was mostly observed by taller varieties (SUPA5, IRRI SUPA 3 and SUPA1052) in which most parts of their plant parts were out of the water surface for 10 days during submergence. The plants that follow this strategy continued with the normal photosynthesis and after submergence periods they lodged, but there was no shedding or drying of the leaves that were out of the water during submergence. These genotypes were able to maintain and increase the seedling height 11DAS. The second strategy was by drying and shedding off the submerged parts; and new leaves were produced. Under this strategy, some genotypes (Swarna and Mahsuri) were able to produce new leaves with little shedding off; these genotypes were able to survive at the end of submergence period unlike other genotypes (MET 58 and MET 59) that showed high rate of leaf dying and shedding off, with no production of new leaves. These genotypes (MET 58 and MET 59) showed high rate of leaf and plant mortality at the end of submergence period. The genotypes that followed the strategy of drying and shedding off, showed decrease in seedling height 11 DAS. This has also been reported by Srivastava et al. (2007), who observed a progressive

drying and subsequent death of plants several days after de-submergence. According to Srivastava et al.(2007), plants exposed to air after a period of anoxia suffer due to production of highly reactive species of free radicals of oxygen (ROS ) indicating the occurrence of a series of events following post-submergence period. However, in this study, varieties Swarna and Mahsuri followed the second strategy, which was observed for quick recovery. This support the findings of Srivastava et al. (2007) which suggested that apart from nature of seedling height, post-flooding responses could be associated with submergence tolerance. This includes prevention of leaf dehydration (Setter et al., 2010) and post-submergence up-regulation of scavengers of reactive oxygen species (Ella et al., 2003).

In this study, none of the cultivars tested survived submergence stress by using a quiescence strategy, but most cultivars showed elongation; which also depends on submergence periods, water depth and nature of seedling height. These findings disagree with the prior research results (Ito et al., 1999; Ram et al., 2002; Jackson and Ram, 2003; Das et al. 2005; Fukao et al. 2006) which reported that a quiescence strategy can help rice plants to maintain high levels of stored carbohydrates coupled with minimum shoot elongation which has been considered as a strategy for tolerance against submergence stress. However, the results agreed with the findings of Ranawake et al. (2014) who observed 100% submergence survival by the elongation mechanism. This was also supported by Redona and Mackill (1996) and Ismail et al. (2009), who pointed out that submergence stress significantly promotes shoot elongation in young rice seedlings; but shoot elongation ability relates to the submergence period and growth

**Table 6.** Genotype names as labelled in Figure 6.

No.	Genotype name
1	NamChe-5
2	NamChe-2
3	ARS 126-3-B-1-2
4	ARU1189
5	ARU1190
6	ARU1191
7	E22
8	E20
9	MET 15
10	MET 31
11	MET 41
12	MET 58
13	MET 59
14	AGRA 41
16	AGRA 65
17	SUPA 1052
18	IRRI SUPA 3
19	TXD 306
20	ART 84 SANDE
21	KOMBOKA
22	SUPA 5
23	NAMCHE-3
24	NAMCHE 4
25	NAMCHE 6
26	AGRA 60
27	CG 14
28	IR 64
29	Mahsuri
30	Swarna
31	ARS37
32	ARS 38
33	Wita 9
34	Nerica L 19 Sub 1
35	Wita 4 Sub1
36	Strong-S

stage of rice (Joho et al., 2008). Internodes elongation was observed in the CG 14 variety, which measured 5 to 8 cm; moreover, CG 14 is an early maturity variety and it has been reported by Datta (1980) that, early-maturing varieties show relatively vigorous elongation capacity in the early vegetative growth phases. In addition to internodes elongation, root length was significantly positively correlated with plant survival after submergence ( $r=0.64$ ,  $P<0.001$ ) in the screen house condition. Similar results have been reported by Singh et al. (2014) who observed a greater root elongation in tolerant genotypes than sensitive ones.

Earlier studies indicated that submergence tolerant genotypes accumulate higher dry matter before

submergence and maintained enough dry matter after de-submergence to sustain recovery growth (Singh et al., 2001; Chaturvedi et al., 1995). In this study post-submergence total dry weight was positively correlated with survival rate ( $r=0.74$ ,  $P<0.001$ ) in the screen house following 10 days of submergence. Moreover, seedling height, leaf area index, chlorophyll content, tillers number and root length were positively correlated with total dry matter ( $r=0.57$ ,  $P<0.01$ ), ( $r=0.59$ ,  $P<0.001$ ), ( $r=0.43$ ,  $P<0.05$ ), ( $r=0.58$ ,  $P<0.01$ ) and ( $r=0.74$ ,  $P<0.001$ ), respectively, in the screen house after submergence. Chlorophyll content was at a peak in all genotypes 1 DBS and 11 DAS but a high decline was observed immediately after submergence in the screen house. In this study, there was significant variation in leaf chlorophyll one day before submergence stress, soon after submergence and 19 DAS in the screen house, suggesting differences in photosynthetic rate among genotypes before and after submergence stress. A significant positive correlation was observed between chlorophyll 11 DAS and survival rate ( $r=0.46$ ,  $P<0.05$ ) which suggests tolerant genotypes had more chlorophyll than sensitive ones. This has been supported by Singh et al. (2014) who pointed out that, when genotypes are submerged, tolerant genotypes maintained more chlorophyll than non-tolerant genotypes. In addition, a significant positive correlation between chlorophyll content (assessed immediately after submergence) and percentage of seedlings survival ( $r=0.49$ ,  $P<0.01$ ) was observed. This implies that, genotypes that showed some level of tolerance, showed little leaf senescence under submergence stress.

Despite increase in water depth, variety Swarna showed 100% survival while KOMBOKA, Mahsuri, SUPA 5, IRRI SUPA 3 and IR 64 showed moderate tolerance to submergence with above 80% seedling survival. Varieties MET 59, MET 58 and Namche-2 were susceptible at 100-cm water depth for 14 days. Variety SUPA 1052, which was moderately tolerant across 45-cm water depth, was highly susceptible at 100-cm for 14 days with 35% survival, suggesting that, probably the tolerant nature of variety SUPA1052 was favored by the nature of seedling height and water depth used. It has been reported by Bailey-Serres and Voesenek (2008), that tolerance to shallow, deep submergence is characterized by the low oxygen escape strategy. This clearly indicates the effect of water depth on selection of submergence tolerance genotypes as reported by Adkins et al (1990) that submergence tolerance depends on many factors apart from water depth.

Association was determined between a polymorphic marker RM 444 based on hypothesis that there is no association between markers and variation in submergence scores, test showed significant deviation from null hypothesis ( $\chi^2_{cal} = 857.2$ ,  $\chi^2_{crit} = 3.84$ ,  $\text{Alpha}=0.05$ ) suggesting that, marker RM 444 is associated with submergence tolerance and could be

used in marker assisted selection in breeding program. In this study molecular data suggested that, all genotypes except NERICA L19-Sub 1 and WITA-4 Sub 1 were scored for the presence of Sub 1B and Sub 1C at 450 and 400 bp, respectively, suggesting probably NERICA L9-Sub1 and WITA 4Sub-1 are not indica or japonica species while other genotypes could be from either indica or japonica species. Analysis of Sub1-A regions suggested 30 genotypes to have *Sub1A-2* allele where four genotypes score absence in Sub1A region. These findings showed that Sub1A is completely absent (Sub1A0) in varieties WITA-4-Sub1, IRRISUPA 3, TXD 306 and Strong-S; however, the rest of genotypes scored presence for *Sub 1A-2* at ~ 0.700 kb where none of the genotypes screened were scored for *Sub 1A -1* allele. Based on phenotypic data, some of the genotypes, showed to be moderately tolerant to submergence stress, with little elongation, while others were susceptible to submergence. However, molecular data suggested all genotypes to be susceptible to submergence as explained by others (Xu et al., 2006; Singh et al., 2010) that, submergence tolerance is strongly correlated with the presence and pronounced expression of *Sub1A-1* allele; whereas, susceptible to submergence is associated with the *Sub1A-2* allele or with the complete absent of the Sub1A(Sub1A0). Most of genetic variation studies on submergence tolerance have revealed that slow shoot elongation during submergence is always related to the high flash flood tolerance and the expression of Sub1A gene (Xu et al., 2006). In contrast, few reports have described that the slow shoot elongation during submergence, is not always linked with high flash flood tolerance (Jackson and Ram, 2003; Perata and Voesenek, 2007). In this study there were 4 identified genotypes that showed moderately tolerance to tolerance for submergence; however, are not carrying the *Sub 1A-1* allele, suggesting that, probably the tolerant level was not influenced by *Sub1A-1* allele and could be due to other factors including presence of other novel genes or differences in gene expression level. These findings corresponded with findings of Masuduzzaman et al. (2017) in their study of haplotype diversity in the Sub1 region, where they observed that most tolerant varieties are in A1C1 haplotype, which showed slow elongation, having tolerant specific Sub1A1 and Sub1C1 alleles. But they also observed moderate tolerant level for varieties Madabaru and Kottamali (A2C2) tolerance, without a Sub1A1 allele, and they were suspected to carry different novel tolerant genes at other loci.

## Conclusion

The study revealed four rice genotypes which are Swarna, IRRISUPA 3, KOMBOKA and SUPA 5 to be tolerant to submergence at 45 cm water depth for 10 days ( $\geq 85\%$  survival) that could be utilized in the Ugandan rice breeding programme for introgression

submergence tolerance into susceptible preferred rice varieties. This indicates that the hypothesis tested in this study is accepted since rice genotypes that are tolerant to seedling submergence are available among selected rice genotypes. It has also been observed that, the performance of genotypes under submergence stress depend on many factors in addition to depth and duration. From evidence of marker-based screening among susceptible genotype (MET 58) and genotypes selected to be most potent in this study (Swarna) revealed that marker RM 444 can be used as a polymorphic marker for marker assisted selection (MAS) involving a submergence tolerance breeding programme. For allelic diversity study, data suggested the presence of *Sub 1A-2* allele in both tolerant and susceptible genotypes; which suggests probably the tolerance nature of the identified genotypes was not due to *Sub1A-1* allele, which is known to confer tolerance to submergence using a quiescent strategy.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The authors deeply acknowledge AGRA for the scholarship and research funds for this study, NaCRRI for hosting the study and Makerere University / MaRCCI for the knowledge, skills and guidance.

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