VALIDATION OF SUBMERGENCE TOLERANCE IN CANDIDATE *ORYZA SPECIES* WITH ART5 AND SC3 MARKERS

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BY

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FEBRUARY, 2017

DECLARATION

I hereby declare that the work in this thesis titled "VALIDATION OF SUBMERGENCE TOLERANCE IN CANDIDATE *ORYZA SPECIES* WITH ART5 AND SC3 MARKERS" was performed by me in the Department of Biology, Ahmadu Bello University, Zaria, Nigeria under the supervision of Dr. (Mrs.) R.E Aliyu and Dr. D. B. Dangora.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at this or any institution.

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Name of student	Signature	Date

CERTIFICATION

This thesis titled "VALIDATION OF SUBMERGENCE TOLERANCE IN CANDIDATE *ORYZA SPECIES* WITH ART5 AND SC3 MARKERS" by Wasiu A. AZEEZ meets the regulation governing the award of the degree of Masters of Biology in the Department of Biology, Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

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ABSTRACT

This research was conducted to validate the efficiency of ART5 and SC3 markers in identifying submergence tolerant genotypes and novel sources of tolerance to submergence between and within Oryza species.Twenty Nine (29) candidate genotypes which consisted of seventeen (17) *Oryza sativa*, three (3) lowland NERICAs and Nine (9) *Oryza glabberima*were obtained from Africa Rice Center (Africa Rice), International Institute of Tropical Agriculture (IITA), Ibadan station, Nigeria. These genotypes included widely cultivated and released rice varieties, lines under trials and lines that served as donor parents to some abiotic stresses. They were phenotypically evaluated and genotypically validated following standard field and laboratory procedures. Rice seeds of each genotype were sown by adopting a completely randomized block design with three replications. Submergence treatment was administered 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. Non-submerged treatment served as control check. Genotypic validation with ART5 and SC3 markers was adopted. The amplified products were subjected to 2% agarose gel electrophoresis and 10% poly acrylamide gel electrophoresis (PAGE) for separation. The genetic associations among varieties were evaluated by comparing the amplicon of the genotypes against IR64 Sub1. Dendrograms were constructed to determine association with DARwin 6.0.01 software. Submergence tolerant rice genotypes (IR64 Sub1, FARO36, GAMBIAKA, and NERICA-L-52) showed high percentage survival (89%, 51%, 51% and 73.33% respectively) by exhibiting moderate plant height (23.67cm, 26.00cm, 24.33cm and 42.33cm respectively) and reduced number of leaves (3.33cm, 3.00cm, 3.33 and 6.33cm respectively). Six genotypes showed recovery at 7DAS (days after de-submergence). These recovery was lost at 14DAS thus validating the desubmergence evaluation protocol. FARO57, NERICA-L-52, IR64 Sub1, W 8 45 and FKR54 showed recovery ranging from 0-11.5% from 7 to 14DAS. Submergence significantly ($P>0.01$) induced plant mortality (57.67 – 100%). Upon genetic validation, IR64 Sub1 (tolerant check) clustered with moderately tolerant *Oryza sativa* genotypes but failed to cluster with NERICA-L-52 which displayed potentials for submergence tolerance. Conclusively, ART5 and SC3 submergence tolerance markers were species specific and not efficient in accurate validation of submergence tolerance between rice

species. This indicates the need for QTL analyses aimed at tagging submergence tolerant genes with large effect on other rice species.

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LIST OF ABBREVIATIONS

SDS:	Sodium dodecyl sulphate
TEMED:	N,N,N,N: Tetramethylethylenediamine
FAO:	The Food and Agriculture Organization of the United Nations
%:	Percentage
Ha or ha:	Hectare
t:	tonnes
t/ha:	Tonnes per hectare
OFN:	Operation Feed the Nation
RBDA:	*River Basin Development Authority*
IRRI:	International Rice Research Institute
NAFPP:	National Accelerated Food Production Project
US:	United States
USA:	United States of America
USD:	United States Dollar
&:	and
BLP:	Back to Land Programme
DFRRI:	Directorate of Food, Roads and Rural Infrastructures
NALDA:	National Land Development Authority
NERICA:	New Rice for Africa
NERICA:	New Rice for Africa Lowland
LPDR:	Lao People's Democratic Republic
g:	gramme
Kg:	Kilogram
ILRI:	*International Livestock Research Institute*

SSA:	Sub Saharan Africa
CGIAR:	Consortium of International Agricultural Research Centers
Sub1A:	genes Submergence Tolerance Regulator A
Sub1C:	genes Submergence Tolerance Regulator C
ART5:	ADP-Ribosyltransferase 5
SC3:	Single-cell Consensus Clustering
WARDA:	*West Africa Rice Development Association*
IFPRI:	*International Food Policy Research Institute*
IPCC:	*Intergovernmental Panel on Climate Change*
IFAD:	International Fund for Agricultural Development
FR13A:	a traditional Indian flood-resistant rice
QTL:	quantitative trait locus
LOD:	logarithm of the odds
ERF:	ethylene:response:factor
cM:	centimorgans
SK1:	genes snorkel 1
SK2:	genes snorkel 2
MAB:	Marker-assisted backcrossing
IITA:	*International Institute of Tropical Agriculture*
$HgCl_{2:}$	Mercury Chloride
cm:	centimeter
mm:	millimeter
RBD:	randomized block design
DNA:	Deoxyribonucleic acid
PCR:	Polymerase chain reaction
CTAB:	Cetyl trimethylammonium bromide
EDTA:	Ethylenediaminetetraacetic acid

µl:	microliter
C: IAA; 24:1:	Chloroform:Isoamyl alcohol 24:1
rpm:	*Revolutions per minute*
SSRs:	Simple sequence repeats markers
TBE Buffer:	*Tris/Borate/EDTA*
PAGE:	Poly Acrylamide Gel electrophoresis
Pb:	petabyte
bp:	bases pair (10^{15} byte)
mA:	milliampere
UV:	Ultraviolet
ANOVA:	Analysis of variance
PIC:	Polymorphism information content
$I_{b:}$	Band Informativeness
Rp:	Resolving power
NTSYS-pc:	Numerical Taxonomy and. Multivariate Analysis System
SAHN:	sequential, agglomerative, hierarchical, and nested clustering methods
SIMQUAL:	association coefficients for qualitative data
UPGMA:	unweighted pair group method with arithmetic average
RBD:	Randomised Block Design
GxE:	Genotype by environment interaction
BC:	Before Christ
kb:	Kilo base
F_2:	Second filial generation
^oC:	degree Celsius
hr:	Hours
NPK:	Nitrogen Phosphorus and Potasium
ng:	Nano gramme

ml:	milliliter
CV:	coefficient of variance
pH:	Potential of Hydrogen
TE:	Tris EDTA
DNTP:	deoxynucleotide triphosphate
DAS:	days after de submergence
NY:	New York
p-value:	probability of obtaining a value equal to or greater than null value
R^2:	coefficient of determination
L:	Carolus Linnaeus
S:	Ernst Gottlieb von Steudel

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Rice is the most important staple food for about half of the world population (IRRI, 2006) Production and consumption is concentrated in Asia, china and India account for approximately 50% of world rice consumption (FAO, 2013). World rice production is spread across at least 114 countries (FAO 2013) and rice is grown on 144 million farms worldwide more than for any other crop. Rice is grown in widely diverse production environments topographically and strongly affected by soil type, water regime and climatic factors. There are five types of growth environments for rice based mainly on the water regime, namely; irrigated, rainfed lowland, tidal wetland, deep water, and upland or dry land areas (Khush, 1984).

In Africa, rice is grown in four ecosystems: upland (38% of planted area), rain fed wetland (33%), deep water and mangrove swamps (9%) and irrigated wetland (20%) (Balasubramanian *et al*., 2007).

World demand for rice is expected to increase by 2.5% per year over the next decade as a result of growth in population and increase in per capita income (World food Council, 1991; Johnson, 1999). However, large numbers of the world remain in poverty and can hence lack the income to translate its needs into wants. This is expected to drive down demand for agricultural products to 1.5 percent over the next 30 years (FAO, 2002). Although this projection is slightly lower than historical rates, increases in rice production required to feed the growing population in the 1990s and beyond will have to depend almost entirely on higher productivity per unit production area as against

expansion of production area. The need to increase rice production through technological development is even more pressing for the coming decades than it was in the 1960s (Tilman *et al.,* 2002). The research strategy to increase and sustain future rice productivity will have to do with rising yield potential of irrigated rice, incorporation of durable resistance to disease and insects and tolerance to abiotic stresses and finally increase of productivity in less favourable production environments (David, 1991).

Nigeria, along with many countries across the world, has ecologies that are suitable for different rice varieties and that can be harnessed to boost rice production to meet domestic demands, and even to produce a surplus for export (Anonymous, 1997a; Imolehin and Wada, 2000). The country has a potential land area for rice production of between 4.6 million and 4.9 million ha. However, only 1.7 million ha, or 35 percent of Nigeria's total cultivable land mass, is cropped for rice. The cultivable land to rice is spread over five major ecologies - upland, inland or shallow swamp, irrigated rice, deep water or floating rice, and tidal mangrove or swamp. The latter is not fully developed because there is lack of appropriate technology (Singh *et al.*, 1997). In spite of the presence of these suitable environments, Nigeria is not among the leading world rice producers.

1.2. Global rice production

World rice production has increased markedly by almost 140% since the start of the green revolution. The world's largest rice producers are China and India (Dawe *et al.*, 2010). This is followed by Indonesia, Bangladesh, Vietnam, Myanmar, and Thailand. These seven countries all had average production of more than 30 million tones between 2008 and 2010. Collectively, the top seven countries account for more than 80% of world production. Although rice is grown worldwide, world rice production is

dominated by Asia which accounted for almost 91% of world rice production, on average, in 2008-10 (Dawe *et al.*, 2010). In Africa, production has grown rapidly with West Africa been the main producing sub region, accounting for more than 45% of African production between 2008-2010. In terms of individual countries, the leading producers of rice (2008-10) are Egypt (5.7 million tonnes), Nigeria (3.6 million t), and Madagascar (4.4 million t) (Dawe *et al.*,2010).

1.2.1 Rice production in Nigeria

Rice production started in Nigeria in 1500 BC with the low-yielding indigenous red grain species *O. glaberrima* Steud, then widely grown in the Niger delta area (Hardcastle, 1959). The high-yielding white grain *O. sativa* L. was introduced in about 1890, and by 1960 it accounted for more than 60 percent of the rice grown in Nigeria (WARDA, 1996). Since the yield level of *O. glaberrima* is regarded as generally low compared to that of *O. sativa* due to grain shattering and poor resistance to lodging, its growing area has been decreasing (Linares, 2002).The main areas of rice cultivation in Nigeria include the middle belt and Northern states of Benue, Kaduna, Niger and Taraba, as well as the South Eastern states of Enugu, Cross River and Ebonyi. Kaduna is the main producing state, followed by Niger, Benue, Ebonyi, Taraba, Kano and Borno. The latter seven states account for over 67 percent of total rice production in the country (Ezedinma, 2008). With expansion of the cultivated land area to rice, there has been a steady increase in rice production and consumption in Nigeria. The production increase has, however, not been enough to meet the consumption demand of the rapidly growing urban population, who has a great preference for parboiled rice (Singh *et al.*, 1997).

In spite of these programmes, local rice production has not kept up with the domestic consumption demands of the Nigerian populace and, consequently, rice is still imported (Singh *et al.,* 1997). Inconsistent government policy on rice imports has seriously affected local production. Nigerian farmers reacted to the ban on rice imports in 1985 by starting to prepare and use their fields for rice cultivation, but imported rice was soon back on the market because of another government policy that liberalized rice imports in 1997. This led to another drop in local rice production. Though, rice is grown on approximately 3.7 million hectares of land in Nigeria, covering 10.6 percent of the 35 million hectares of land under cultivation, out of a total arable land area of 70 million hectares, 77 percent of the farmed area of rice is rain-fed, of which 47 percent is lowland and 30 percent upland. The range of grown varieties is diverse and includes both local (such as Dias, Santana, Ashawa, Yarsawaba, and Yarkuwa) and enhanced varieties of traditional African rice such as NERICA (Bayou, 2009).

Paddy rice production has been increasing between 2001 and 2006, followed by a decline in 2007 and a positive peak in 2008. From 2008 to 2010 production statistics show a decreasing trend in production, associated with a decline in area harvested between 2006 and 2010. This trend resulted into higher yields between 2008 and 2010, despite declining production. Increasing production between 2002 and 2006 can be explained as result of the implementation of the Presidential Initiative on increased Rice Production, although decreasing production between 2008 and 2010 is not in line with policies aimed at the development of the rice sector during those years, such as the National Rice Development Strategy and the Federal Market Stabilization Programme (Erenstein, 2003).

1.2.2 Global rice consumption

China and India alone account for over 50% of the world's rice consumption, Highest consumers per capita are found in much of South and Southeast Asia, West Africa, Madagascar, and Guyana (Nelson, 2011). Several of these countries have per capita consumption rates surpassing 100 kg per year. In Africa, rice has been the main staple food for at least 50 years in parts of West Africa (Guinea, Guinea-Bissau, Liberia and Sierra Leone). In Nigeria, the most populous country on the continent, rice consumption increased from 1% to 8% (Nelson, 2011). Seck*et al.,* (2012) project global rice consumption to rise from 439 million tons (milled rice) in 2010 to 496 million tons in 2020 and further increase to 555 million tons in 2035. Current rice area is at an all-time high and increasing rice production through area expansion in the future is highly unlikely in most parts of the world because of water scarcity and competition for land from non-agricultural uses such as industrialization and urbanization. Thus, it is prudent to assume that additional production will have to come entirely from yield growth. In that case, annual yield growth of 1.2–1.5% will be needed compared with current yield growth of less than 1% to keep rice affordable to millions of poor people in the world (Mohanty*et al.,* 2013).

1.2.3 Floods

Floods are a significant problem for rice farming, especially in the lowlands of South and Southeast Asia, and with no alternatives, subsistence farmers in these areas depend on rice, which—in contrast to other crops—thrives under shallow flooding (Dey and Upadhyaya, 1996).Floods are also becoming more frequent in Africa despite the generally reduced mean rainfall over most of the continent. The east Africa floods of 1998, the Mozambique floods of 2000 and the more recent floods in West Africa

(Ghana, Benin, Togo, Burkina Faso and Nigeria) in 2007 were devastating and led to the loss of much farmland, damage to transport networks, disease outbreaks and loss of human life. The continent already experiences a major deficit in food production in many areas, and potential further declines in soil moisture or inundation of crop lands will be added burdens. The extent of loss of production due to climate change differs among the five rice production systems recognized in Sub Saharan Africa – rainfed upland, rainfed lowland, irrigated lowland, mangrove swamp and Deepwater. Among these, the greatest threats to rice production due to climate change are anticipated in the rainfed rice production systems due to their often total dependence on rainfall as a source of water. However, mangrove rice production systems also face increased risks of flooding, coastal erosion, saline intrusion and storm surges (Field, 1995; Ball *et al.*, 1997).

Historical climate records shows worrying trends in climate variables for most of Africa: warming of approximately 0.7ºC over most of the continent during the 20th century at the rate of about 0.05°C per decade with slightly greater warming in the June–November seasons than in December–May; a decrease in rainfall over large portions of the Sahel (the semi-arid region south of the Sahara); and an increase in rainfall in east central Africa (Desanker and Magadza, 2001; Hulme *et al*., 2001). Increase in submergence stress caused by climate change affects rice production in rice growing areas worldwide (Dey and Upadhyaya, 1996). More than 16 million ha of rice lands of the world in lowland and deep-water rice areas are unfavorably affected by flooding due to complete submergence (Dey and Upadhyaya, 1996). The estimated annual economic loss of this is more than US$ 600 million. Two types of flooding that cause damages to rice are: (i) flash flooding which results in rapid ascending of water levels from 5 to about 50cm with submergence for 1-2 weeks. This type of flooding

could also occur after sowing in areas where direct seeding is practiced and could result in substantial reduction in stand establishment; (ii) deepwater flooding where water depth exceeds 100 cm and remains at these depths for short periods or for several months if flooding is severe (Septiningsih *et al.,* 2009, Singh *et al.,* 2011).

1.3. Statements of Research Problem

In Nigeria approximately 70% of rainfed lowland rice farms are prone to seasonal flooding. This constitutes a major constraint to rice production in major rice producing states with yield loss ranging from 10% to total destruction (Akinwale *et al.,* 2012). 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; Odeh, 2014). According to Anugwara and Emakpe (2013), the flooding which occurred in 2012 damaged over 1.9 million hectares of land and reduced food production along the flood plains. Rice production in the submerged areas was reduced by 22.4% and was the most affected of all crops.

1.4. Justifications for the study

Considering that changes in the global climate will result in more extreme events, such as floods, droughts, and cyclones; 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 (Mohanty *et al.,* 2013). Sarkar and Bhattarcharjee, 2011, stated that germplasm survey could be conducted for allele-specific markers targeting Sub1A and Sub1C, two of the three transcription-factor

genes within the Sub1 locus along with some physiological traits to identify novel sources of tolerance. SSR markers have merits which include quickness, simplicity; rich polymorphism and stability and are thus widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhou *et al*., 2003; Zhang *et al.,* 2007, Ma *et al.,* 2011). ART5 and SC3 markers are capable of providing diagnostic evidence of submergence tolerance (Septiningsih *et al.,* 2009, Iftekharuddaula *et al.,* 2011). Study on submergence screening in Africa especially Nigeria is quite limited (Akinwale *et al.,* 2012). This research work seeks to bridge this gap by providing information on the submergence tolerance of some cultivated rice varieties.

1.5. Aims and Objectives

This research was aimed at identifying sources of tolerance to submergence among *Oryza species* and to validate the efficiency of tagged submergence tolerant markers.

The objectives of the study were to:

1. evaluate phenotypic response of *Oryza species* to submergence stress
2. evaluate growth recovery upon de-submergence
3. validate the presence of Sub1 QTL in tolerant genotypes under survey

1.6. Hypotheses

The research hypothesizes that:

1. *Oryza species* under survey do not show significant phenotypic response to submergence
2. *Oryza species* do not exhibit significant growth recovery upon de-submergence
3. *Oryza species* under survey do not possess the Sub1 QTL

CHAPTER TWO

2. LITERATURE REVIEW

One of the most serious long-term challenges to achieve sustainable growth in rice production is climate change (Adams *et al.,* 1998; Vaghefi *et al.,* 2001, Wassmann and Dobermann, 2007;IFPRI 2010). By 2100, the mean surface temperature of the Earth is expected to rise by 1.4 to 5.8 ºC and extreme events, such as floods, droughts, and cyclones, are likely to become more frequent (IPCC, 2007). In delta/coastal regions, climate change is expected to raise sea levels, and this will increase the risk of flooding and salinity problems in major rice-growing areas (Wassmann *et al.,* 2004, Mackill *et al.,* 2010b). Droughts and floods already cause widespread rice yield losses across the globe (Pandey and Bhandari, 2007, IFAD 2009, IRRI 2010). The research strategy to increase and sustain future rice productivity will have to raise yield potential of irrigated rice, incorporate durable resistance to disease and insects, tolerance to abiotic stress and increased productivity in less favourable production environments (David, 1991).

Flooding is a serious constraint to rice plant growth and survival in rainfed lowland and deepwater areas (Toojinda *et al.,* 2003). Recently, the extent of submergence stress has increased due to extreme weather events including unpredicted heavy rains that have flooded wider areas across many states of the country. Among the most frequently and severely affected states in Nigeria are Kebbi, Niger, Kogi and Taraba states which together account for over 80% of lowland rice ecology in Nigeria (Erenstein *et al.,* 2003).

2.1 Physiology of Submergence Tolerance

Submergence of plants inhibits aerobic respiration and photosynthesis, and stimulates a variety of responses that can enhance survival, such as a switch from aerobic to

anaerobic respiration (Fukao and Bailey-Serres, 2004). Plant submergence restricts the diffusion of oxygen by 104-folds and this has a dramatic impact on biochemical activities such as aerobic respiration and photosynthesis (Armstrong and Drew, 2002). Environmental characterization of flood water causing complete submergence in South and Southeast Asia shows that this is a complex environment which may need different strategies for optimizing different plant types. One very important factor in all locations is gas diffusion. In all locations where complete submergence occurs, gas diffusion is 10000-folds less in the water than in air (Armstrong, 1979). In most environments, flood water oxygen concentrations during flash floods are usually below saturation. In some, oxygen is completely absent (anoxia) particularly during the night, but may even become supersaturated during the day (Heckman, 1979; Setter *et al*., 1987a, 1995a; Whitton *et al*., 1988). Anoxia for 24hours can result in death of many plants including intolerant rice cultivars like IR8 (Crowford, 1989), probably because the absence of oxygen stops respiration and reduces energy production for survival or elongation growth processes.

Deep-water rice avoids submergence stress by growing above the water surface thereby restoring gas exchange. Submergence-tolerant rice however can survive 10-14 days of complete submergence and renew growth when water level subsides (Catling, 1992). The duration of survival however depends on the character of flood water such as water turbidity, temperature, O_2 diffusion, CO_2 concentration and light penetration (Ram *et al.*, 1999, 2002; Jackson *et al.*, 1987). Limited CO_2 supply is a common characteristic of flood-prone environment. During turbulent flash floods the flood water tends to be at equilibrium with the CO_2 partial pressure in air due to rapid mixing. However, once the water level stabilizes, the flood water may become stagnant resulting in low CO_2 supply due to large boundary layer effects (Smith and Walker, 1980; Setter *et al*., 1989a). Rice

has adapted to flood-prone environments by either submergence tolerance or elongation ability (Mohanty and Chaudhary, 1986, Luo *et al.*, 2011). These two responses are usually associated with different water regimes. Submergence tolerance is required in rainfed areas where flash flooding results in rapid rise in water level and submergence occurs for up to about 14days. Elongation under such condition is a disadvantage because it results to lodging once water level recedes. In contrast leaf and internode elongation is appropriate for deepwater and floating rice areas (>100cm water) where water remains at these depths for several months.

Submergence tolerance is aimed at reducing yield lose in rain fed lowland and deepwater rice areas but this has enjoyed limited success in Africa (Kwanano *et al.*, 2009; Mackill, 1986). Systematic screening of rice germplasm is 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. It is from these local genotypes that flood-tolerant rice types were developed through marker-assisted backcrossing. Some of the newly developed lines include Swarna-Sub1, Swarna Mahsuri-Sub1, TDK1-Sub1 and BR11-Sub1 (Singh *et al.*, 2009).

2.2 Genetics of submergence tolerance

Submergence tolerance is controlled by a single major quantitative trait locus (QTL) on chromosome 9, along with a number of minor QTLs (Xu and Mackill, 1996; Nandi *et al.*, 1997; Toojinda *et al.*, 2003). Most studies used the landrace FR13A, which is one of the most submergence-tolerant donor varieties. The major QTL, named *Sub1*, with a LOD score of 36 and an R^2 value of 69% (Xu and Mackill, 1996), provide tolerance to complete submergence for up to 2 weeks. The Sub1 locus was mapped to an interval of 0.06 centimorgans on chromosome 9 using a mapping population of 4022 plants developed from the hybridization of a tolerant indica derivative of FR13A and an

intolerant japonica cultivar M-202. The region physically spans over 182 kilobases (kb) and encodes three genes containing ethylene-response-factor (ERF) and is designated Sub1a, Sub1B and Sub1C, ten non- ERF genes, including four transcribed and six hypothetical protein-coding genes, and >50% retrotransposon related sequences (Xu *et al.,* 2006). The fine-mapping of *Sub1* employing 2950 F_2 segregating individuals indicated that it occupies a genomic region of approximately 0.06 centimorgans (cM) (Xu *et al.,* 2000). Sequencing the Sub1 region in an FR13A-derived line revealed the presence of three genes encoding putative ethylene responsive factors (ERF); *Sub1A, Sub1B* and *Sub1C* were subsequently identified (Xu *et al.,* 2006). It was also observed that *Sub1C* alleles were associated with tolerance; however, it was not known if the tolerant *Sub1C* allele had any effect on the level of tolerance. The ethylene response factors genes *snorkel 1* (*SK1*) and *snorkel 2* (*SK2*) allow rice to adapt to deep water whereas *submergence 1A-1* (*sub1A-1*) allows rice to acclimatize under flash flooding (Xu *et al.,* 2006; Hattori *et al.,* 2009; Nagai *et al.,* 2010).

Both SKs genes and *Sub1A-1* are connected with gibberellins biosynthesis or signal transduction, yet deepwater and submergence-tolerant rice seem to have opposite flooding response; namely, escape by elongation or remain stunted under water until flood recedes (Xu *et al.,* 2006; Hattori *et al.,* 2009; Sarkar and Panda 2009; Bailey-Serres *et al.,* 2010; Bailey-Serres and Voesenek, 2010). Genetics research has demonstrated that for 15 to 50 days-old seedlings there is one major dominant gene involved in inheritance of submergence tolerance from FR13A and Kurkaruppan, this was shown by analysis of segregating populations and backcrossing populations produced by crossing submergence tolerant with intolerant cultivars were tested along with the parents for submergence tolerance in greenhouse concrete tanks and field ponds. The results obtained was consistent with the hypothesis that submergence

tolerance is governed by one dominant gene which has a high probability of 3:1 in segregating F_2, 1:1 in backcross to intolerant parent and 1:0 in backcross to tolerant parent (Mazaredo and Vergara, 1982; Mohanty and Chaudhary, 1986).

Previous studies have reported the development of submergence tolerant varieties by introducing the *Sub1* locus using marker-aided selection (Siangliw *et al.,* 2003; Toojinda*et al.,* 2005). More recently this gene has been successfully introgressed through marker-assisted backcrossing (MAB) into a popular high-yielding variety from India, Swarna, within a 2-years' time frame (Neeraja *et al.,* 2007). Swarna-Sub1 is currently being evaluated in submergence-prone areas of India and Bangladesh. Under non-submerged control conditions, no significant differences in agronomic performance, grain yield and grain quality between Swarna and Swarna-Sub1 were observed indicating complete restoration of the Swarna background in Swarna-Sub1 (Sarkar *et al.,* 2006; Neeraja *et al.,* 2007), but Swarna-Sub1 showed a 2-fold or higher yield advantage over Swarna after submergence for 10 days or more during the vegetative stage (Singh *et al.,* 2009). These results highlighted the opportunity to develop additional high-yielding varieties that are adapted to other regions or environments. In addition, hybrid rice has been viewed as a promising approach that can be used as a vehicle to boost rice production. In recent years, there has been increasing interest to grow hybrid rice in several countries, and the potential use of Sub1 in hybrid rice has great promise for flood-prone areas.

Introgression of *Sub1* QTL into 'Swarna' greatly enhanced its survival under submergence, and plant productivity under flash flood conditions (Neeraja *et al.,* 2007; Sarkar *et al.,* 2009b). A wide-ranging on farm and on station trials showed that under normal conditions both cultivars have similar grain yield potential whereas under complete submergence (submergence period varied between 3 and 14 days in different

locations), a yield advantage of 1.65 t / ha (an average of 0.81 t / ha over five locations) were obtained from Swarna-Sub1 compared to Swarna (Sarkar *et al*., 2009b). Subsequently several submergence tolerant mega varieties namely IR64-Sub1, SambaMahsuri-Sub1, Thadokkam1-Sub1 and BR11 Sub1 were developed (Singh *et al*., 2009; Iftekharuddaula *et al*., 2011). Swarna-Sub1 was released in India, Indonesia and Bangladesh; BR11-Sub1 was released in Bangladesh; and IR64-Sub1 was released in the Philippines and Indonesia. Breeders are now using the Sub1 locus to develop tolerant rice varieties for submergence-prone areas in Asia and Africa (Singh *et al.,* 2010).

2.3 Molecular markers and submergence tolerance

A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily monitored. It could be a piece of DNA or a protein. They can either be hybridization based markers or polymerase chain reaction (PCR) based markers. PCR based markers involve in vitro amplification of specific DNA sequence by using specially designed or arbitrarily chosen random primers. Among the commonly used types of PCR based markers are randomly amplified DNAs (RAPDs), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSR) etc (Kottearachchi, 2013). SSR markers are the most abundant and are also the easiest to use. In rice, many DNA markers linked to genes have been mapped and are been used to detect the presence of gene without waiting for phenotypic observation (Kottearachchi, 2013).Many functional markers have not been developed due to the fact that many mapped genes have not been cloned, researchers use markers such as SSR, Single Nucleotide Polymorphism (SNP) etc that are tightly linked with genes in Marker Assisted breeding (MAB) of rice.

QTLs (Quantitative Trait Loci) consist of one or more genes that quantitatively affect a phenotype of interest and have physical boundaries defined by linked molecular markers (Kottearachchi, 2013). Molecular markers are usually applied in mapping of QTLs by observing crossover between linked markers in bipolar population. Once linkage map is constructed, QTLs can be identified by dissecting the association between marker and the variation of phenotype through statistical analyses. Many major QTLs were identified by flanking markers that are tightly linked with the QTL of many traits such as drought, submergence tolerance, resistance to pest etc in rice. Best performing varieties are formed by introgression of several agronomically important QTLs into one line (Kottearachchi, 2013).

Study of QTL mapping has shown that a major QTL, named *Sub1*provides tolerance to complete submergence of rice for up to 2 weeks (Xu and Mackill, 1996). Marker, RM464A, was shown to be tightly linked to the gene and is flanked by markers RM219 and RM316. RM219 was found to be highly polymorphic among diverse cultivars and hence have the potential for usage in *Sub1* QTL studies.Cuc *et al.,* 2012 used eight closely linked markers around the *Sub1*region of chromosome 9 in the development of elite variety AS996-*Sub1*. In this study, preliminary selection was confirmed by the use of ART5 (6.3 Mb) and SC3 (6.6Mb) markers. Xu *et al.,* 2000 also indicated that ART5 and RM25181 are closely linked markers to the *Sub1*region of chromosome 9 in rice genome. Khanh *et al.,* 2013 reported that SC3 and ART5 are two markers distally flanking the *Sub1* locus and can be used in foreground selection of submergence tolerant genotypes.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted in two phases:

Phenotypic Screening- Preliminary phenotypic screening and germination test were conducted at the Botanical Garden of the Department of Biological sciences, Ahmadu Bello University Zaria located on Latitude 11^0 13' North, Longitude 7^0 12' East and on Altitude 630 meters above sea Level, between January and March, 2015.

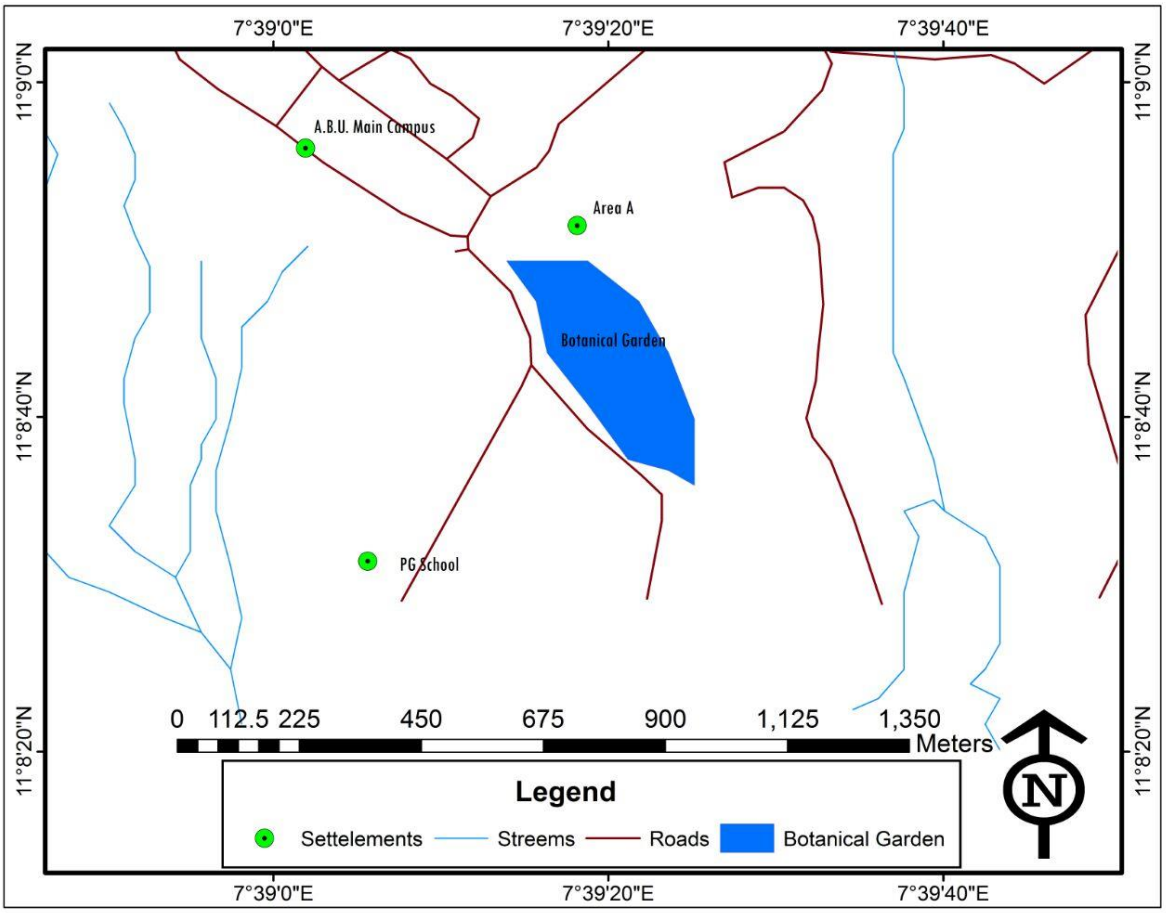


Figure 3.1: Map showing the study area.

Molecular characterization- Genotypic validation was conducted at the Central Biotechnology laboratory of IITA, Ibadan, Nigeria.

3.2. Plant Materials

A total of One hundred and Twenty-one (121) genotypes were obtained from the Africa rice center, Ibadan station and pre-screened for viability. Twenty Nine (29) viable genotypes which consisted of seventeen (17) *Oryza sativa L*, three (3) lowland NERICAs and Nine (9) *Oryza glabberima S* were subjected to submergence stress.

3.3. Phenotypic Screening

3.3.1. Plant Materials, Sterilization and Pre-germination

Rice seeds were cleaned and placed in an oven at 50^{o}C for five days to break seed dormancy. The seeds were then surface sterilized with 0.1% mercury chloride ($HgCl_2$), and rinsed with distilled water. The sterilized seeds were then soaked in water in a Petri-dish lined with No 1Whatman'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 a 30x30x30cm plastic buckets which served as nursery. Each genotype was labeled accordingly using a board marker. The seeds were then watered regularly for a period of two weeks.

At the end of this period, twenty nine (29) genotypes that survived were subjected to submergence stress. They consisted of seventeen (17) *Oryza sativa* genotypes, three (3) lowland NERICA genotypes and Nine (9) *Oryza glabberima* S genotypes (Table 3.1).

Table 3.1: List of genotypes and sources

S/N	GENOTYPE	SOURCE
1	CG 14	AfricaRice, IITA Ibadan
2	FARO 36 (ITA222)	AfricaRice, IITA Ibadan
3	FARO 44	AfricaRice, IITA Ibadan
4	FARO 57	AfricaRice, IITA Ibadan
5	FKR 54	AfricaRice, IITA Ibadan
6	FKR 19	AfricaRice, IITA Ibadan
7	GAMBIAKA	AfricaRice, IITA Ibadan
8	IG 133	AfricaRice, IITA Ibadan
9	IR 64	AfricaRice, IITA Ibadan
10	IR64 Sub1	AfricaRice, IITA Ibadan
11	NERICA L-43	AfricaRice, IITA Ibadan
12	NERICA L-52	AfricaRice, IITA Ibadan
13	NERICA L-53	AfricaRice, IITA Ibadan
14	RAM 133	AfricaRice, IITA Ibadan
15	TOG 7218	AfricaRice, IITA Ibadan
16	TOG 7250-A	AfricaRice, IITA Ibadan
17	TOG 7412	AfricaRice, IITA Ibadan
18	TOG 7439-B	AfricaRice, IITA Ibadan
19	TOG 8347	AfricaRice, IITA Ibadan
20	TOG 6632	AfricaRice, IITA Ibadan
21	TOG 6640-B	AfricaRice, IITA Ibadan
22	TOG 7148	AfricaRice, IITA Ibadan

23	TOS 6455	AfricaRice, IITA Ibadan
24	VANDANA	AfricaRice, IITA Ibadan
25	W 8 45	AfricaRice, IITA Ibadan
26	WITA 9	AfricaRice, IITA Ibadan
27	WITA 12	AfricaRice, IITA Ibadan
28	WITA 4	AfricaRice, IITA Ibadan
29	POKALLI	AfricaRice, IITA Ibadan

3.3.2. Preparation of Seed bed

Seed bed was prepared in a screen house with dimension 1mx1mx5cm. The soil was properly drained by allowing excess water flow away. Pre germinated seeds were then transferred from petri dishes to the seed bed at day 21.

3.3.3. Preparation of seed pots, transplanting and Experimental design

Sterilized soil, mixed with NPK (15:15:15) fertilizer at the rate of 30 kg/ha was placed in black polythene bags (15cm diameter and 30cm depth). Each polythene bag was filled with 1kg of soil and soaked with 2 liters of water. Each genotype was labeled appropriately using plastic tags and board markers. Each polythene bag was then placed in the 1mx1mx1m plastic tank in rows of five in a randomized complete block design (RCBD) with three replications.

3.3.4. Submergence evaluation

Submergence treatment was administered at day 30 after transplanting by gradually increasing water level at $5cm^3$ per day until a volume of $100cm^3$was attained. Plants were submerged for a period of 14days. Control set up was left un-submerged. Water was barely a few inches above the soil surface.

3.3.5. Data collection

Survival count was taken immediately after de-submergence (0DAS), seven days after de submergence (7DAS) and 14 days after de-submergence (14DAS) (Xu *et al.,* 2006; Septiningsih *et al.,* 2009; Sarkar and Bhattacharjee, 2011; Akinwale *et al.,* 2012; Aliyu *et al*., 2015). Other phenotypic parameters measured after de-submergence 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, and average taken; number of leaf per plant: the number of leaf on each genotype was counted at

0DAS, 7DAS and 14DAS, and average taken. Leaf diameter (cm): the diameter of three leafs per plant was measured at the widest part, and the average taken (Wazed, 2014).

3.4. Molecular characterization with ART5 and SC3 markers

3.4.1. Collection of leave samples

To optimize DNA quality and concentration, young leaves were collected from 21 days old seedlings (before they were subjected to stress) of each rice genotype. Leaves were harvested with sterilized scissors. This was then washed in distilled water and ethanol to rid it of all forms of contamination. The leaves were placed in white polythene bags, labeled and placed in a plastic container and sealed. This was then stored at -20^{o}C in the freezer in the laboratory (Wazed, 2014).

3.4.2. Isolation and quantification of DNA for PCR:

Genomic DNA for all the 29 rice genotypes was isolated separately. DNA was extracted using the modified CTAB protocol (Fulton *et al*., 1995). Twenty (20) milligram fresh leaf tissue was collected from each sample and inserted into labeled 1.5µl eppendorf tubes (Thermo fisher scientific) and covered. The tubes were then kept in ice on the laboratory bench for onward DNA extraction. The leaves were grinded using a Geno Grinder 2000 (SP2100-115) in the deepwell tube. 500µl extraction buffer (standard buffer-Tris, EDTA, SDS) was added and mixed by vortexing. It was placed in a water bath at 65^{o}C for 20 minutes, 500µl chloroform; isoamylalchohol (C:IAA; 24:1) was added and mixed. The mixture was centrifuged for 10 minutes at 13000 rpm (revolutions per minute) in a micro centrifuge (5415D, Thermo fisher scientific). Thereafter, the upper aqueous layer was transferred into another set of labeled 1.5µl eppendorf tubes. 500µl ice-cold isopropanol was then added and mixed by inversion.

The tubes were placed in -20^{0}C freezer for twenty minutes and centrifuged for 12 minutes at 13000 rpm. The supernatant was discarded taking care not to lose the DNA pellet. This was then washed in 800µl ice-cold 70% ethanol. The DNA pellet was air dried for 1 hour and re-suspended in 200µl low salt TE buffer (1M Tris, pH 8.0 and 0.5M EDTA pH 8.0) and stored at -20^{0}C.The concentration of the DNA was measured (ng/µl) using a Nano-Drop Spectrophotometer (ND-1000, Nano Drop inc.).

3.4.3. Genotype validation with molecular markers

Two polymorphic simple sequence repeats (SSRs) markers (ART5 and SC3) were chosen on the basis of their ability to align with major genes linked to submergence tolerance (Xu *et al.,* 2006; Fukao *et al.,* 2009; Singh *et al.,* 2010; Iftekharuddaulah *et al.,* 2011;Sakar and Bhattachargee, 2011; *Cuc et al.,* 2012). The original source, repeat motifs, primer sequences, expected length, chromosomal localizations and repeat types of the markers can be found in the gramene data base (http://www.gramene.org) (Table 3.2).

Table 3.2: Details of SSR markers used for the study

Name of markers	Mb	Primer sequence	PCR band size (bp)	Annealing temperature (^{O}C)
ART5	2.6	F: CAGGGAAAGAGATGGTGGA	124	55
		R:TTGGCCCTAGGTTGTTTCAG		
SC3	6.8	F:GCTAGTGCAGGGTTGACACA	217	55
		R: CTCTGGCCGTTTCATGGTAT		

bp=bases pair, F=forward, R: reverse

3.4.4. Polymerase Chain Reaction (PCR)

DNA samples from each genotype were subjected to PCR amplification with the SSR primers. PCR was conducted in 10µl reaction mixture containing 1µl PCR buffer, 1µl of 25mM $MgCl_2$, and 0.2µl of 10mM dNTPs', 0.25µl of 20 pm primer pair, 0.5µl of Taq polymerase, 2.15µl nuclease free water and 5µl of 10ng/µl DNA using Perkin-Elmer thermo cycler (Gregorio *et al.*,1997). Pre heating was at 94^0C for 2 minutes, followed by 34 cycles of denaturation (94^0C), 30 seconds annealing at 55^0C, then 30 seconds initial primer extension at 72^0C and final extension for 2 minutes at 72^0C. Amplified products were then stored at -20^0C until further use.

3.4.5. Agarose Gel Electrophoresis

The amplification products were subjected to electrophoresis in a 2% agarose gel in 1×TBE buffer at 70-80 volts for 2-3 hrs for separation of amplified products (Mehede *et al.,* 2014, IRRI 1997). Two grammes (2g) of agarose was poured into a 100ml 0.5 TBE buffer in a conical flask. The mixture was heated in a microwave until agarose completely dissolved. The conical flask was placed on a stirrer, with a magnetic stirrer placed in it, and allowed to cool for 5 minutes. The warm gel was poured in a gel clamped casting stand at equilibrium with gel combs placed in it. This was allowed to cast, then the combs and clamps were removed. The gel was placed in a gel tank and 1×TBE buffer poured to cover it. The gel was loaded with 2µl of each PCR product mixed with 2X loading dye, $1KB^+$ ladder was used for size determination. Finally the tank was covered and the electrodes connected to power. The DNA samples were allowed to run for 2-3 hours at 70-80 volts for good separation. The gel was removed from the electrophoresis system and placed carefully in an ethidium bromide staining solution (5ml ethidium and 1L of distilled water). The gel was stained for 5 minutes and viewed under an ultraviolet transilluminator (Fisher scientific). Where the staining was

too deep, the gel was distained for 2 minutes and viewed using gel documentation system.

3.4.6. Polyacrylamide Gel Electrophoresis (PAGE)

The amplified products with SC3 marker were further subjected to Poly Acrylamide Gel electrophoresis (PAGE) using pre cast gel (Bio-Rad). 10% polyacrylamide gels concentration (1mm thick) was used in order to achieve good separation of the PCR products. Along with the PCR products, a 100pb DNA ladder was also loaded in the gel. Electrophoresis was carried out in a BioRad cell in a non-dissociating and discontinuous buffer system (stacking gel buffer Tris-HCl 1M pH 6.8 and separating gel buffer Tris-HCl 3M pH 8.7). Electrophoresis was performed in running buffer pH 8.9 (0.3% Tris, 1.44% Glycine, 0.1% SDS) during 2hr at 60mA (Barril and Nates, 2012). After electrophoresis, polyacrylamide gels were stained with ethidium bromide for 5 mins and destained in two 20 minutes washes in distilled water. The gel was viewed under UV Transilluminator (Fisher scientific) and documented.

3.4.7. Gel scoring and statistical analysis

For each marker, allele bands were scored based on a 1 (present) and 0 (absent) binary codes. Only prominent and unambiguous bands were scored for data reliability.

Phenotypic data collected were subjected to analysis of variance (ANOVA) using SAS analysis package. Where significant, means were separated using the Duncan's multiple range test (DMRT).

SSR allelic composition was determined for each genotype. Cluster analysis was used to determine the genotypic association of the genotypes based on the markers. The allelic frequencies, allele per locus, Polymorphic information content (PIC) value and band informativeness were determined as follows:

3.4.8. Polymorphic Information Content (PIC): The value of a marker for detecting polymorphism between genotypes was calculated for each primer combination depending on the number of detectable alleles and the distribution of their frequency using the formula proposed by Anderson *et al.,* (1993).

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where PIC=polymorphism information content; i= marker I; $P_{ij}$is the frequency of the J^{th}allele for marker 'i' and summation extends over n alleles. The number of alleles per locus was based on an evaluation of 29 rice genotypes based on 2 SSRs.

3.4.9. Band Informativeness (I_b):The ability of the primer to distinguish between accessions was assessed by calculating their resolving power (Rp) as $Rp=\sum I_b$ where $I_b$is band informativeness, $I_b= 1-[2\times(0.5-P_i)\ ]$ and P_i is the proportion of accession containing band I (Prevost and Wilkinson, 1999).

3.4.10. Cluster Analysis*:* The genetic association among varieties was evaluated by comparing the size of the PCR products with that of IR64 *Sub1*. The estimated repeats amplified fragment was treated as a unit character scored as a binary code. Genetic similarities were evaluated using Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by the primers. Similarity matrices were generated using 'SIMQUAL' module of NTSYS-pc software. Similarity coefficients were used for cluster analysis of genotypes using 'SAHN' module of NTSYS-pc software and dendrograms were obtained using un-weighted pair-group method with arithmetic average (UPGMA) module of NTSYS-pc software version 2.0 from Exeter software, NY, USA (Rohlf, 2000). Genotypes that showed

similar banding pattern and clustered with IR64 *sub1* were considered as tolerant, whereas those with different banding pattern based on the tagged markers were considered as susceptible.

CHAPTER FOUR

4. RESULTS

PHENOTYPIC SCREENING

4.1. Survival and growth performance of 29 rice genotypes screened for submergence tolerance

Percentage survival was assessed immediately after de- submergence (0DAS), 7DAS and 14DAS. Plant height (cm), tiller number, number of leaves and leave diameter (cm) where recorded at 14DAS (Table 4.1a). Percentage survival varied between 57.67% and 100% immediately after de submergence (0DAS). Four genotypes (CG14, FKR 19, NERICA-L- 43 and TOG 7148) recorded 100% survival. TOG6632 had the least percentage survival with 57.67% while the tolerant check (IR64 Sub1) recorded 89.00% survival (Table 4.1a).

At 7DAS, percentage survival ranged between 40% and 100%. IR64, NERICA-L-43 and TOS 6455 recorded a 100% survival. This was significantly higher than survival scores for all other genotypes at this stage. NERICA-L-43, IG 133 and IR 64 Sub1 sustained their survival percentages recorded immediately after de-submergence (0DAS). FARO 44, NERICA-L-53, TOG 6632 and W 8 45 showed significant ($p>0.01$) recovery (15.67%, 11%, 15.66% and 26.67% respectively) within a period of one (1) week as their survival scores increased appreciably. TOG 7218 recorded the least survival percentage and the steepest decline (122.5%) in submergence tolerance survival within 7DAS.

Survival at 14DAS ranged from 0.00% to 73.33%. IR64 Sub1 was the most tolerant genotype and recorded the highest survival percentage of 89.00%. NERICA-L-52 showed consistent submergence tolerance of 73.33% between 7 and 14DAS. This

consistent submergence tolerance is similar to that of IR64 Sub1 with a survival percentage upon de-submergence of 89% across the test period. FKR 54 showed slight recovery from 40% to 44.67% at 14DAS while TOG 7412 was most susceptible as it was completely lost at 14DAS (Table 4.1a).

Plant morphological characteristics were significantly ($P<0.05$) affected by submergence stress. Plant height ranged from 0.00cm to 42.30cm (Table 4.1a). NERICA-L-52 recorded the highest plant height of 42.33cm which is significantly higher than that recorded for all other genotypes. Submergence stress had no significant ($P<0.05$) effect on tiller numbers and leaf diameter of the genotypes. IR 64 and NERICA-L-52 recorded the highest mean number of tillers while TOS 6455 had the least mean number of tillers (1.33). W 8 45 and NERICA-L-52 recorded the highest number of leaves. This was significantly ($p<0.05$) higher than for all other genotypes. Highest leaf diameter (0.4cm) was recorded in IR 64, VANDANA, W 8 45 and WITA 4 (Table 4.1a).

Table 4.1a: Survival and growth performance of 29 rice genotypes screened for submergence tolerance.

GENOTYPES	Survival (%) 0DAS	Survival (%) 7DAS	Survival (%) 14DAS	Plant Height(cm)	Tiller Number	Number of Leaf	Leaf Diameter (cm)
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 (ITA222)	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^{ab}	55.67^{b}	31.00^{ab}	2.67^{a}	5.33^{a}	0.33^{a}
FARO 57	73.33^{b}	62.33^{ab}	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}	100.00^{a}	66.67^{ab}	32.67^{ab}	3.00^{a}	4.33^{ab}	0.40^{a}
IR64 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^{ab}	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}	100.00^{a}	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^{ab}	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^{ab}	57.67^{b}	35.33^{ab}	2.33^{a}	3.33^{b}	0.40^{a}
POKALLI	73.33^{b}	55.67^{ab}	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	108.06	100.13
R^2	0.34	0.12	0.33	0.3	0.29	0.33	0.34
P VALUE	0.55	0.01	0.05	0.72	0.8	0.61	0.58

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

Survival percentages for un-submerged (control) genotypes at 14DAS indicated that all genotypes except IR64 Sub1, TOG7218 and TOG6640-B recorded 100% survival (Table 4.1b, fig 4.1). These were significantly higher than the survival scores in submerged genotypes. In the un-submerged treatment, plant height was significantly higher than in submerged genotypes (fig 4.2). Plant heights ranged from 69.50cm in IG 133 to 27cm in IR 64. NERICA-L-52 had a height of 34.00cm. Tiller number averaged 2.98 in un-submerged genotypes as against 1.17 obtained in submerged genotypes (Table 4.1b, fig 4.3).Number of leafs ranged between 12.0 and 23.0 in un-submerged genotypes (table 4.1b, fig 4.4). This is significantly higher than values recorded in submerged genotypes. Variation in leaf diameter among un-submerged genotypes is as indicated in table 4.1b and figure 4.5.

Table 4.1b: Survival and growth performance of 29 rice genotypes, treatments and control, screened for submergence tolerance.

GENOTYPE	Survival (%) 14DAS	Survival (%) Control	Plant Height(cm)	Plant height Control (cm)	Tiller Number	Tiller Number Control	Number of Leaf	Number of Leaf Control	Leaf Diameter(cm)	Leaf Diameter Control (cm)
CG 14	51.00^{a}	100.00^{a}	19.33^{ab}	39.00^{a}	1.33^{a}	3.00^{a}	3.67^{a}	15.00^{a}	0.23^{a}	0.80^{a}
FARO 36 (ITA222)	51.00^{a}	100.00^{a}	26.00^{ab}	42.50^{a}	1.33^{a}	3.00^{a}	3.00^{a}	19.00^{a}	0.20^{a}	0.90^{a}
FARO 44	55.67^{a}	100.00^{a}	31.00^{ab}	46.50^{a}	1.67^{a}	3.00^{a}	5.33^{a}	22.50^{a}	0.33^{a}	0.85^{a}
FARO 57	62.33^{a}	100.00^{a}	31.33^{ab}	45.00^{a}	1.67^{a}	3.00^{a}	3.67^{a}	14.00^{a}	0.23^{a}	0.95^{a}
FKR 54	44.67^{a}	100.00^{a}	24.00^{ab}	52.00^{a}	1.33^{a}	3.00^{a}	2.67^{a}	17.50^{a}	1.33^{a}	1.05^{a}
FKR 19	55.67^{a}	100.00^{a}	21.67^{ab}	34.00^{a}	1.67^{a}	3.00^{a}	3.67^{a}	14.00^{a}	0.27^{a}	0.85^{a}
GAMBIAKA	51.00^{a}	100.00^{a}	24.33^{ab}	40.00^{a}	1.33^{a}	3.00^{a}	3.33^{a}	16.00^{a}	0.23^{a}	0.65^{a}
IG 133	40.00^{a}	100.00^{a}	31.67^{ab}	69.50^{a}	1.00^{a}	3.00^{a}	2.33^{a}	19.50^{a}	0.23^{a}	1.20^{a}
IR 64	66.67^{a}	100.00^{a}	32.67^{ab}	27.00^{a}	2.00^{a}	3.00^{a}	4.33^{a}	15.00^{a}	0.40^{a}	0.70^{a}
IR 64 SUB1	89.00^{a}	83.50^{a}	9.67^{ab}	43.50^{a}	0.67^{a}	3.00^{a}	1.33^{a}	12.50^{a}	0.10^{a}	0.70^{a}
NERICA L-43	40.00^{a}	100.00^{a}	25.67^{ab}	41.00^{a}	1.00^{a}	3.00^{a}	2.33^{a}	15.50^{a}	0.20^{a}	0.70^{a}
NERICA L-52	73.33^{a}	100.00^{a}	42.33^{a}	34.00^{a}	2.00^{a}	3.00^{a}	6.33^{a}	17.50^{a}	0.30^{a}	0.85^{a}
NERICA L-53	53.00^{a}	100.00^{a}	22.33^{ab}	34.00^{a}	1.00^{a}	3.00^{a}	1.33^{a}	15.50^{a}	0.20^{a}	0.65^{a}
RAM 133	33.33^{a}	100.00^{a}	11.67^{ab}	64.00^{a}	1.00^{a}	3.00^{a}	4.00^{a}	15.50^{a}	0.13^{a}	0.85^{a}
TOG 7218	35.33^{a}	83.50^{a}	9.00^{ab}	60.50^{a}	0.67^{a}	2.00^{a}	2.00^{a}	13.00^{a}	0.10^{a}	1.25^{a}
TOG 7250-A	55.67^{a}	100.00^{a}	24.67^{ab}	58.00^{a}	1.67^{a}	3.00^{a}	4.67^{a}	19.50^{a}	0.17^{a}	1.00^{a}
TOG 7412	0.00^{a}	100.00^{a}	0.00^{b}	43.00^{a}	0.00^{a}	3.00^{a}	0.00^{a}	17.00^{a}	0.00^{a}	1.10^{a}
TOG 7439-B	22.33^{a}	100.00^{a}	16.00^{ab}	58.00^{a}	0.67^{a}	3.00^{a}	2.00^{a}	16.50^{a}	0.13^{a}	1.00^{a}
TOG 8347	40.00^{a}	100.00^{a}	17.33^{ab}	60.00^{a}	1.00^{a}	3.00^{a}	1.33^{a}	15.50^{a}	0.13^{a}	0.95^{a}
TOG 6632	40.00^{a}	100.00^{a}	19.67^{ab}	30.00^{a}	1.00^{a}	3.00^{a}	2.33^{a}	16.50^{a}	0.17^{a}	0.90^{a}
TOG 6640-B	22.33^{a}	83.50^{a}	17.67^{ab}	61.00^{a}	0.67^{a}	3.00^{a}	2.33^{a}	15.00^{a}	0.30^{a}	1.05^{a}
TOG 7148	51.00^{a}	100.00^{a}	27.33^{ab}	61.50^{a}	1.33^{a}	3.00^{a}	2.67^{a}	16.50^{a}	0.15^{a}	1.00^{a}
TOS 6455	17.67^{a}	100.00^{a}	12.00^{ab}	40.50^{a}	0.33^{a}	3.00^{a}	0.67^{a}	12.00^{a}	0.10^{a}	0.90^{a}
VANDANA	55.67^{a}	100.00^{a}	29.33^{ab}	50.00^{a}	1.67^{a}	3.00^{a}	4.67^{a}	15.30^{a}	0.40^{a}	0.90^{a}
W 8 45	66.67^{a}	100.00^{a}	31.67^{ab}	44.50^{a}	1.00^{a}	3.00^{a}	6.33^{a}	23.00^{a}	0.40^{a}	0.70^{a}
WITA 9	40.00^{a}	100.00^{a}	27.67^{ab}	38.00^{a}	1.00^{a}	3.00^{a}	2.33^{a}	22.00^{a}	0.23^{a}	0.75^{a}
WITA 12	57.67^{a}	100.00^{a}	18.67^{ab}	36.00^{a}	1.33^{a}	3.00^{a}	3.67^{a}	15.00^{a}	0.33^{a}	0.65^{a}
WITA 4	57.67^{a}	100.00^{a}	35.33^{ab}	37.00^{a}	1.33^{a}	3.00^{a}	3.33^{a}	17.00^{a}	0.40^{a}	0.60^{a}
POKALLI	51.00^{a}	100.00^{a}	26.67^{ab}	58.50^{a}	1.33^{a}	3.00^{a}	2.00^{a}	13.00^{a}	0.27^{a}	0.80^{a}
CV	83.52	73.65	87.35	96.63	98.28	11.56	108.06	22.83	100.13	62.84
R^2	0.33	0.35	0.3	0.28	0.29	0.32	0.33	0.30	0.34	0.30
P VALUE	0.05	0.05	0.72	0.05	0.8	0.05	0.61	0.05	0.58	0.05

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range tests. 14DAS= percentage survival at 14 days after de submergence. CTRL= Control, PH=Plant height (cm), TN= Tiller number, NL= Number of leaves, LD= leave diameter (cm).

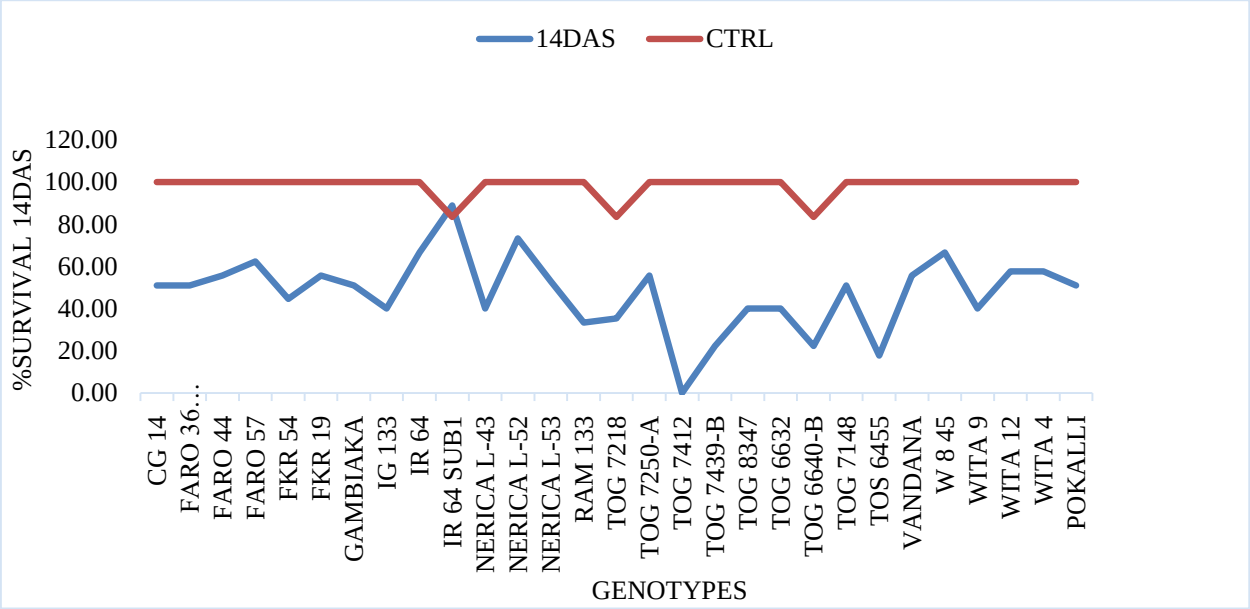


Figure 4.1: Variation in percentage survival among 29 rice genotypes, treatment and control, for submergence stress at 14 DAS.

CTRL= control, DAS= days de-submergence, %= percentage

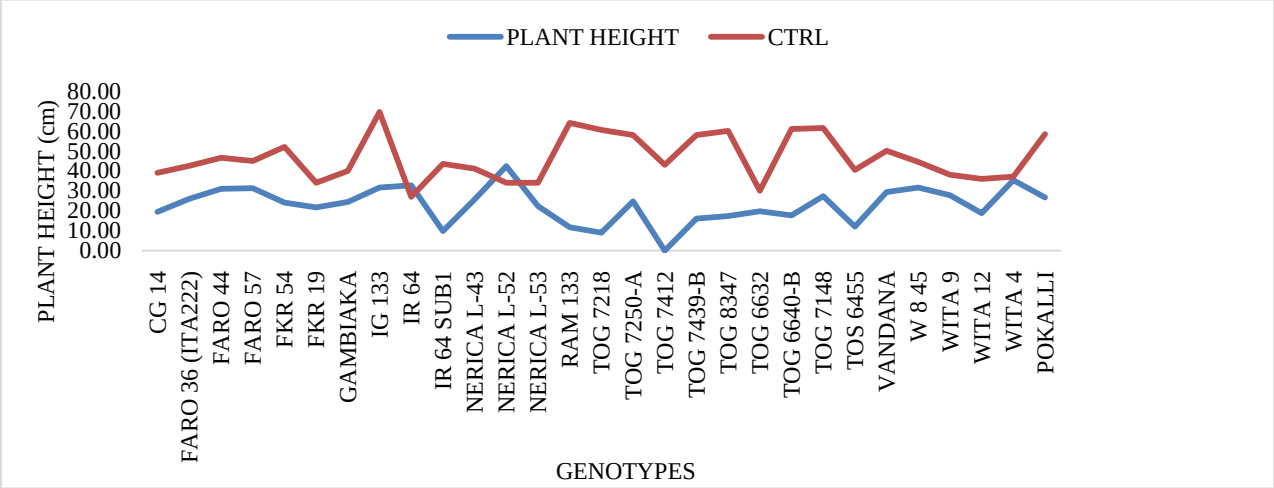


Figure 4.2: Variation in plant height among 29 rice genotypes, treatment and control, for submergence stress at 14DAS.

CTRL= control, DAS= days after de-submergence.

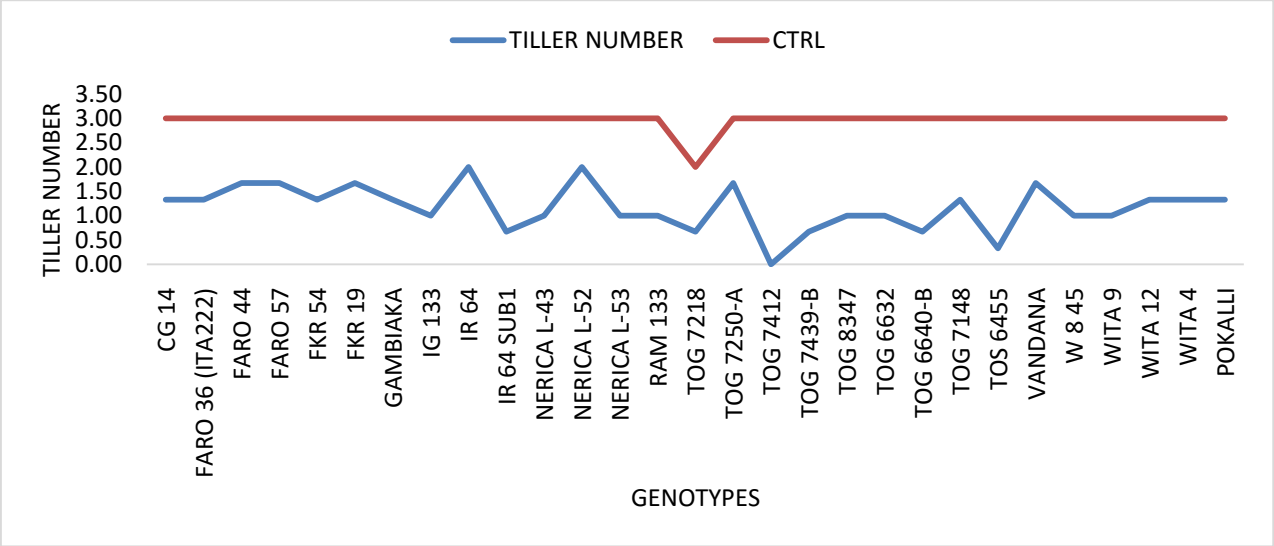


Figure 4.3: Variation in number of tillers among 29 rice genotypes, treatment and control, for submergence stress at 14DAS.

CTRL= control, DAS= days after de-submergence.

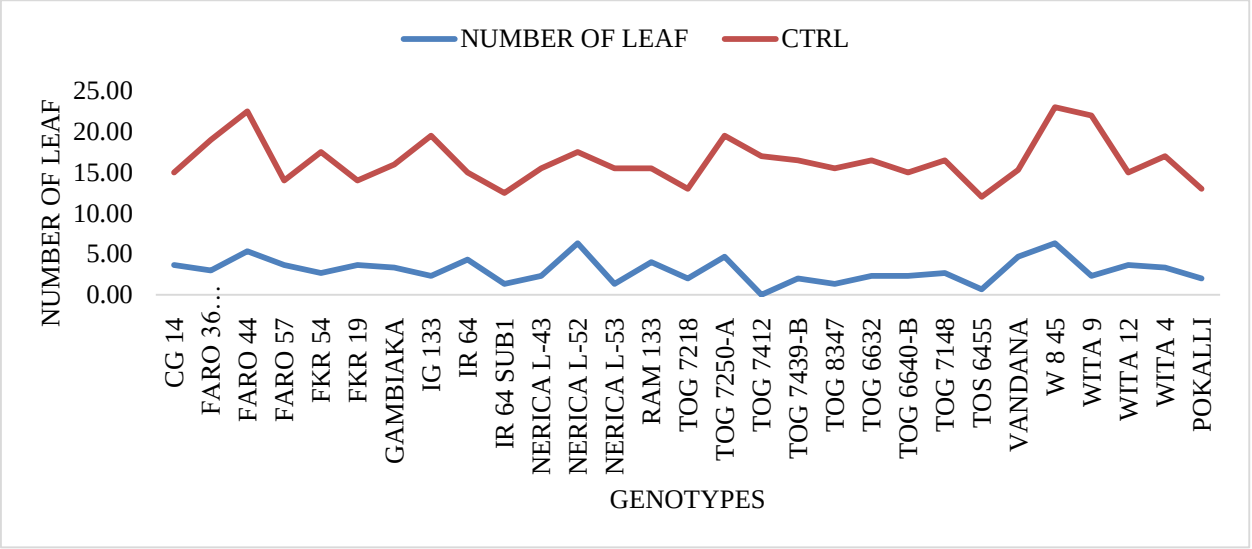


Figure 4.4: Variation in Number of leaf among 29 rice genotypes, treatment and control, for submergence stress at 14 DAS.

CTRL= control, DAS= days after de-submergence.

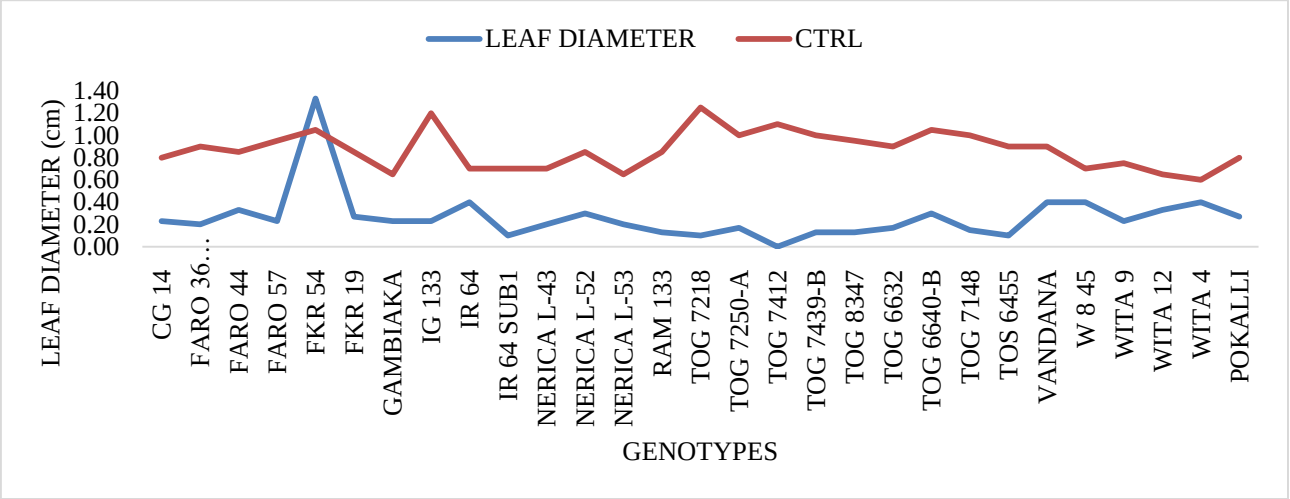


Figure 4.5: Variation in leaf diameter among 29 rice genotypes, treatment and control, for submergence stress at 14DAS.

CTRL= control, DAS= days after de-submergence.

Response to submergence stress indicated that most genotypes were strongly affected by the stress (Table 4.1c). TOG 7412 was most affected by submergence stress at 14DAS (100%) as it was totally lost at 14DAS (Table 4.1b). This was followed by TOS 6455 (82.3%), TOG 7439-B (77.7%), RAM 133 (66.7%), IG 133 (60.0%), TOG 8347 (60.0%), TOG 6632 (60.0%) and WITA 9 (60%). Only IR64 Sub1 showed a negative percentage effect (-6.6%) indicating that percentage survival of the genotype at 14DAS was higher than that recorded in control genotype (Table 4.1c).

Submergence stress significantly ($p<0.05$) affected all phenotypic parameters. In plant highest, TOG 7412 showed the least tolerance since the plant was totally lost at 14DAS. Other genotypes (RAM 133, TOG 7218, TOG 7439-B, TOG 8347, TOG 6640-B and TOS 6455) showed more than 70% decline in plant height compared to control genotypes. Only IR 64 and NERICA-L-52 showed recorded greater plant height relative to the control genotypes (Table 4.1b). Most genotypes recorded decrease in tiller number compared to control genotypes. IR 64 and NERICA-L-52 recorded 3 tillers each both under submergence stress and in control set up. All genotypes showed decrease in number of leaves compared to control at 14DAS (Table 4.1b). FKR 54 showed wider leave diameter than under stress condition than in control (-26.7%). All other genotypes showed decrease in this parameter (Table 4.1c).

Table 4.1c: Percent mortality or survival and change in growth due to submergence stress on 29 rice genotype at 14DAS

GENOTYPE	Mortality/ Survival 14DAS (%)	Plant Height (%)	Tiller Number (%)	Number of Leaf (%)	Leaf Diameter (%)
CG 14	49.0	50.4	22.3	75.5	71.3
FARO 36 (ITA222)	49.0	38.8	22.3	84.2	77.8
FARO 44	44.3	33.3	11.0	76.3	61.2
FARO 57	37.7	30.4	11.0	73.8	75.8
FKR 54	55.3	53.8	22.3	84.7	-26.7
FKR 19	44.3	36.3	11.0	73.8	68.2
GAMBIAKA	49.0	39.2	22.3	79.2	64.6
IG 133	60.0	54.4	33.3	88.1	80.8
IR 64	33.3	-21.0	0.0	71.1	42.9
IR64 Sub1	-6.6	45.6	11.0	73.4	85.7
NERICA L-43	60.0	37.4	33.3	85.0	71.4
NERICA L-52	26.7	-24.5	0.0	63.8	64.7
NERICA L-53	47.0	34.3	33.3	91.4	69.2
RAM 133	66.7	81.8	33.3	74.2	84.7
TOG 7218	57.7	85.1	16.5	84.6	92.0
TOG 7250-A	44.3	57.5	11.0	76.1	83.0
TOG 7412	100.0	100.0	100.0	100.0	100.0
TOG 7439-B	77.7	72.4	44.3	87.9	87.0
TOG 8347	60.0	71.1	33.3	91.4	86.3
TOG 6632	60.0	34.4	33.3	85.9	81.1
TOG 6640-B	73.3	71.0	44.3	84.5	71.4
TOG 7148	49.0	55.6	22.3	83.8	85.0
TOS 6455	82.3	70.4	55.7	94.4	88.9
VANDANA	44.3	41.3	11.0	69.5	55.6
W 8 45	33.3	28.8	33.3	72.5	42.9
WITA 9	60.0	27.2	33.3	89.4	69.3
WITA 12	42.3	48.1	22.3	75.5	49.2
WITA 4	42.3	4.5	22.3	80.4	33.3
POKALLI	49.0	54.4	22.3	84.6	66.3

14DAS= percentage mortality 14 days after de submergence. PH=Plant height, TN= Tiller number, NL= Number of leaf, LD= leaf diameter.

There was a strong positive correlation between survival at 14DAS and plant height (0.94), tiller number (0.94), number of leaves (0.89) and leave diameter (0.85) (Table 4.1d).All other morphological parameters showed some degree of correlation with one another (Table 4.1d).

Table 4.1d: Correlation coefficient for traits measured in 29 rice genotypes subjected to submergence stress

	Survival(%) 0DAS	Survival(%) 7DAS	Survival(%) 14DAS	Plant Height(cm)	Tiller Number	Number of leaf	Leaf Diameter(cm)
Survival(%) 0DAS	1.00						
Survival(%) 7DAS	0.20	1.00					
Survival(%) 14DAS	0.05	0.18	1.00				
Plant Height(cm)	-0.02	0.17	0.94*	1.00			
Tiller Number	0.06	0.17	0.94*	0.82*	1.00		
Number of leaf	0.03	0.20	0.89*	0.81*	0.87*	1.00	
Leaf Diameter(cm)	-0.07	0.21	0.85*	0.88*	0.79*	0.83*	1.00

*= significant at $p<0.01$

4.2. Survival and growth performance of *Oryza sativa L* genotypes screened for submergence tolerance

Percentage survival at 0DAS was significantly ($p<0.05$) different among sativa genotypes. CG14 and FRK 19 recorded 100% survival at this period. The most susceptible genotype was W 8 45 (62.33%) followed by RAM 133 (66.67%) (Table 4.2a). Four genotypes (GABMIAKA, IR 64, IR64 Sub1 and VANDANA had a survival percentage of 89.00% at 0DAS (Table 4.2a). IR64 showed a significant ($p<0.05$) recovery after de-submergence (89% to 100%) while FARO 44 recovered from 73.33% to 89%, W8 45 (62.33% to 89%) and WITA 12 (68.67% to 73.33%). Other *Oryza sativa* genotypes decreased in survival. The sharpest decline was observed in FKR 54 (95% decline) between 0DAS and 7DAS (Table 4.2a). IG 133, IR64 Sub1 and VANDANA recorded consistent survival percentages between 0DAS and 7DAS.

Survival percentages at 14DAS did not significantly increase amongst *Oryza sativa* genotypes. However, the highest survival percentage at this period was recorded in IR64 Sub1 (89%) this was followed by IR64 and W8 45 (66.67%) (Table 4.2a).

Table 4.2a: Survival and growth performance of 17 *Oryza sativa L* genotypes screened for submergence tolerance

GENOTYPE	Survival (%) 0DAS	Survival (%) 7DAS	Survival (%) 14DAS	Plant Height(cm)	Tiller Number	Number of Leaf	Leaf Diameter (cm)
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^{ab}	55.67^{b}	31.00^{ab}	2.67^{a}	5.33^{a}	0.33^{a}
FARO 57	73.33^{b}	62.33^{ab}	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}	100.00^{a}	66.67^{ab}	32.67^{ab}	3.00^{a}	4.33^{ab}	0.40^{a}
IR64 Sub1	89.00^{a}	89.00^{ab}	89.00^{a}	23.67^{b}	2.67^{a}	3.33^{b}	0.10^{a}
RAM 133	66.67^{bc}	62.33^{ab}	33.33^{e}	11.67^{c}	2.00^{a}	4.00^{ab}	0.13^{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^{ab}	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^{ab}	57.67^{b}	35.33^{ab}	2.33^{a}	3.33^{b}	0.40^{a}
POKALLI	73.33^{b}	55.67^{ab}	51.00^{b}	26.67^{b}	2.33^{a}	2.00^{bc}	0.27^{a}
CV	29.82	31.44	72.98	77.31	88.84	95.64	86.85
R^2	0.28	0.5	0.4	0.32	0.33	0.36	0.36
P VALUE	1.79	1.08	1.31	1.66	1.6	1.49	1.49

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range test. DAS=Days after de submergence.

Plant height ranged between 35.33cm in WITA 4 and 11.67cm in RAM 133 amongst *Oryza sativa* genotypes (Table 4.2a). IR64 Sub1 with the best percentage survival at 14DAS (89%) recorded a plant height of 23.67cm. Submergence stress did not significantly affect tiller numbers at 14DAS. Number of tillers ranged between 3 and 2 (Table 4.2a). *Oryza sativa* genotypes with the highest and lowest number of leaves were W 8 45 (6.33) and POKKALI (2.0) respectively. Leaf diameter ranged between 0.1cm in IR64 Sub1 to 0.4cm in IR 64, VANDANA, W 8 45 and WITA 4 (Table 4.2a).

Oryza sativa genotypes showed strong positive correlation between percentage survival at 14DAS and plant height (0.89), tiller number (0.92), number of leaf (0.89) and leaf diameter (0.90). The morphological parameters also showed positive correlation with one another. Genotype survival at 0DAS and 7DAS showed no significant ($p<0.01$) association the traits measured (Table 4.2b). All the phenotypic parameters showed highly significant correlation with one another (Table 4.2b).

Table 4.2b: Correlation coefficient for traits measured in *Oryza sativa L*.

	Survival(%) 0DAS	Survival (%) 7DAS	Survival(%) 14DAS	Plant Height (cm)	Tiller Number	Number of leaf	Leaf Diameter (cm)
Survival(%) 0DAS	1.00						
Survival(%) 7DAS	0.28	1.00					
Survival(%) 14DAS	0.09	0.34	1.00				
Plant Height (cm)	0.05	0.31	0.89*	1.00			
Tiller Number	0.10	0.26	0.92*	0.80*	1.00		
Number of leaf	0.09	0.32	0.89*	0.79*	0.84*	1.00	
Leaf Diameter(cm)	0.05	0.38	0.90*	0.88*	0.81*	0.86*	1.00

*= significant at $p<0.01$

In control set up, all *Oryza sativa* genotypes (except IR64 Sub1; 83.5%) recorded 100% survival. This is significantly higher than the values recorded in the stressed (submerged) genotypes (Table 4.2c). In IR64 Sub1 percentage survival of the stressed genotype was higher than that of control by 5.5% (Table 4.2c). All other genotypes were greatly affected by submergence stress at 14DAS with IG 133, RAM 133 and WITA 9 recording 40%, 33.33% and 40% respectively while exhibiting 100% survival in control (Table 4.2c).

Submergence stress also affected plant height. IR 64 showed a decline in plant height of 5.67cm compared to the control. All other genotypes recorded greater heights in control (Table 4.2c).All *Oryza sativa* genotypes recorded 3 tillers each under control (un-submerged) set up (table 4.2c). Among submerged genotypes, only IR 64 had two tillers.

Number of leaves is the most visibly affected of all phenotypic parameters. The number of leaf in submerged genotypes was less than in control for all genotypes (Table 4.2c).

Leaf diameter was also affected by submergence stress. All genotypes in control set up recorded higher leaf diameter except FKR 54 (Table 4.2c).

Table 4.2c: Survival and growth performance of *Oryza sativa* genotypes, treatment and control, screened for submergence tolerance.

GENOTYPE	Survival 14DAS (%)	Survival 14DAS (%) Control	Plant Height(cm)	Plant height Control (cm)	Tiller Number	Tiller Number Control	Number of Leaf	Number of Leaf Control	Leaf Diameter(cm)	Leaf Diameter Control (cm)
CG 14	51.00^{a}	100.00^{a}	19.33^{ab}	39.00^{a}	1.33^{a}	3.00^{a}	3.67^{a}	15.00^{a}	0.23^{a}	0.80^{a}
FARO 36 (ITA222)	51.00^{a}	100.00^{a}	26.00^{ab}	42.50^{a}	1.33^{a}	3.00^{a}	3.00^{a}	19.00^{a}	0.20^{a}	0.90^{a}
FARO 44	55.67^{a}	100.00^{a}	31.00^{ab}	46.50^{a}	1.67^{a}	3.00^{a}	5.33^{a}	22.50^{a}	0.33^{a}	0.85^{a}
FARO 57	62.33^{a}	100.00^{a}	31.33^{ab}	45.00^{a}	1.67^{a}	3.00^{a}	3.67^{a}	14.00^{a}	0.23^{a}	0.95^{a}
FKR 54	44.67^{a}	100.00^{a}	24.00^{ab}	52.00^{a}	1.33^{a}	3.00^{a}	2.67^{a}	17.50^{a}	1.33^{a}	1.05^{a}
FKR 19	55.67^{a}	100.00^{a}	21.67^{ab}	34.00^{a}	1.67^{a}	3.00^{a}	3.67^{a}	14.00^{a}	0.27^{a}	0.85^{a}
GAMBIAKA	51.00^{a}	100.00^{a}	24.33^{ab}	40.00^{a}	1.33^{a}	3.00^{a}	3.33^{a}	16.00^{a}	0.23^{a}	0.65^{a}
IG 133	40.00^{a}	100.00^{a}	31.67^{ab}	69.50^{a}	1.00^{a}	3.00^{a}	2.33^{a}	19.50^{a}	0.23^{a}	1.20^{a}
IR 64	66.67^{a}	100.00^{a}	32.67^{ab}	27.00^{a}	2.00^{a}	3.00^{a}	4.33^{a}	15.00^{a}	0.40^{a}	0.70^{a}
IR 64 SUB1	89.00^{a}	83.50^{a}	9.67^{ab}	43.50^{a}	0.67^{a}	3.00^{a}	1.33^{a}	12.50^{a}	0.10^{a}	0.70^{a}
RAM 133	33.33^{a}	100.00^{a}	11.67^{ab}	64.00^{a}	1.00^{a}	3.00^{a}	4.00^{a}	15.50^{a}	0.13^{a}	0.85^{a}
VANDANA	55.67^{a}	100.00^{a}	29.33^{ab}	50.00^{a}	1.67^{a}	3.00^{a}	4.67^{a}	15.30^{a}	0.40^{a}	0.90^{a}
W 8 45	66.67^{a}	100.00^{a}	31.67^{ab}	44.50^{a}	1.00^{a}	3.00^{a}	6.33^{a}	23.00^{a}	0.40^{a}	0.70^{a}
WITA 9	40.00^{a}	100.00^{a}	27.67^{ab}	38.00^{a}	1.00^{a}	3.00^{a}	2.33^{a}	22.00^{a}	0.23^{a}	0.75^{a}
WITA 12	57.67^{a}	100.00^{a}	18.67^{ab}	36.00^{a}	1.33^{a}	3.00^{a}	3.67^{a}	15.00^{a}	0.33^{a}	0.65^{a}
WITA 4	57.67^{a}	100.00^{a}	35.33^{ab}	37.00^{a}	1.33^{a}	3.00^{a}	3.33^{a}	17.00^{a}	0.40^{a}	0.60^{a}
POKALLI	51.00^{a}	100.00^{a}	26.67^{ab}	58.50^{a}	1.33^{a}	3.00^{a}	2.00^{a}	13.00^{a}	0.27^{a}	0.80^{a}
CV	83.52	73.65	87.35	96.63	98.28	11.56	108.06	22.83	100.13	62.84
R^2	0.33	0.35	0.3	0.28	0.29	0.32	0.33	0.3	0.34	0.3
P VALUE	0.05	0.05	0.72	0.05	0.8	0.05	0.61	0.05	0.58	0.05

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range test. DAS=Days after de submergence.

4.3. Survival and growth performance of NERICA genotypes screened for submergence tolerance

NERICA genotypes had the highest survival percentages (Table 4.3a). At 0DAS, NERICA-L-43 recorded the highest survival score of 100%. This was followed by NERICA-L-52 with a survival score of 78%. Average survival score at 0DAS for NERICA genotypes was 83.78% (Table 4.3a). At 7DAS, NERICA-L- 43 sustained its survival percentage of 100%. NERICA-L-52 showed a decline of 4.67% between 0DAS and 7DAS; however NERICA-L-53 showed an appreciable recovery of 11 %.(Table 4.3a). At 14 DAS, only NERICA-L-52 could withstand the prolonged submergence stress regime. Survival percentage for other genotypes declined. NERICA-L-43 showed the sharpest decline of 60%. `

Table 4.3a: Survival and growth performance of 3 NERICA genotypes screened for submergence tolerance.

GENOTYPE	Survival (%) 0DAS	Survival (%) 7DAS	Survival (%) 14DAS	Plant Height(cm)	Tiller Number	Number of Leafs	Leaf Diameter(cm)
NERICA L-43	100.00[a]	100.00[a]	40.00c	25.67[a]	2.00[a]	2.33[ab]	0.20[a]
NERICA L-52	78.00[a]	73.33[a]	73.33[a]	42.33[a]	3.00[a]	6.33[a]	0.30[a]
NERICA L-53	73.33[a]	84.33[a]	53.00[b]	22.33[a]	2.00[a]	1.33[b]	0.20[a]
CV	24.66	29.65	34.63	58.66	43.3	54.77	78.25
R^2	0.45	0.3	0.72	0.51	0.78	0.85	0.26
P VALUE	0.57	0.78	0.05	0.49	1.13	0.06	0.83

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range tests.

NERICA genotypes also recorded the highest plant heights. NERICA-L-52 recorded the highest plant height of 42.33cm (Table 4.3a) which was not significantly ($p<0.05$) higher than the other genotypes. Tiller number ranged between 2 and 3 among NERICA genotypes. Number of leafs was highest in NERICA-L-52, 6.33cm. Survival at 14DAS was greatly affected by submergence stress in NERICA genotypes as all submerged genotypes recorded less than 100% survival. NERICA-L-52 recorded higher plant height (42.33cm) than in control (34.00cm). All other phenotypic parameters recorded lower plant height than in control (Table 4.3b).

Correlation coefficients indicated that survival at 14DAS was strongly dependent on plant height (0.69), tiller number (0.94), number of leaf (0.82) and leaf diameter (0.51) (Table 4.3c). All phenotypic parameters were also positively correlated with one another in NERICA genotypes (Table 4.3c).

Table 4.3b: Survival and growth performance of NERICA genotypes, treatment and control, screened for submergence tolerance.

GENOTYPE	Survival 14DAS (%)	Survival 14DAS (%) Control	Plant Height(cm)	Plant height Control (cm)	Tiller Number	Tiller Number Control	Number of Leaf	Number of Leaf Control	Leaf Diameter (cm)	Leaf Diameter Control (cm)
NERICA L-43	40.00[a]	100.00[a]	25.67[ab]	41.00[a]	1.00[a]	3.00[a]	2.33[a]	15.50[a]	0.20[a]	0.70[a]
NERICA L-52	73.33[a]	100.00[a]	42.33[a]	34.00[a]	2.00[a]	3.00[a]	6.33[a]	17.50[a]	0.30[a]	0.85[a]
NERICA L-53	53.00[a]	100.00[a]	22.33[ab]	34.00[a]	1.00[a]	3.00[a]	1.33[a]	15.50[a]	0.20[a]	0.65[a]
CV	83.52	73.65	87.35	96.63	98.28	11.56	108.06	22.83	100.13	62.84
R^2	0.33	0.35	0.3	0.28	0.29	0.32	0.33	0.3	0.34	0.3
P VALUE	0.05	0.05	0.72	0.05	0.8	0.05	0.61	0.05	0.58	0.05

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range tests. DAS=Days after de-submergence

Table 4.3c: Correlation coefficient for traits measured in NERICA

	Survival(%) 0DAS	Survival(%) 7DAS	Survival(%) 14DAS	Plant Height(cm)	Tiller Number	Number of leaf	Leaf Diameter(cm)
Survival(%) 0DAS	1.00						
Survival(%) 7DAS	0.82	1.00					
Survival(%) 14DAS	-0.03	-0.03	1.00				
Plant Height(cm)	-0.09	-0.45	0.69*	1.00			
Tiller Number	0.11	0.06	0.94*	0.66*	1.00		
Number of leafs	0.15	-0.04	0.82*	0.69*	0.90*	1.00	
Leaf Diameter(cm)	-0.19	-0.51	0.51*	0.88*	0.38	0.52*	1.00

*= significant at $p<0.01$

4.4. Survival and growth performance of *Oryza glaberrima* S genotypes screened for submergence tolerance

Percentage survival amongst *Oryza glaberrima* genotypes indicated that TOG 7148 was the best surviving (100%) immediately after de-submergence. However, at 7DAS, the genotype declined in survival to 73.33%. TOS 6455and TOG 7412 showed 11% and 5% recovery. At 14DAS, there was a sharp decline in survival. TOG 7412 was completely susceptible (0% survival) while TOS 6455 declined by 82.33%. TOG7250-A was most tolerant at 14DAS (Table 4.4a). Two *Oryza glaberrima S* genotypes (TOG 7218 and TOG 6640-B) had survival percentage of 83.5% each in the un-submerged treatment (Table 4.4b).

Plant height ranged from 9.00cm to 27.33cm among submerged genotypes. The tallest plant at 14DAS was TOG 7148 with a height of 27.33cm; it was also among the most tolerant to submergence stressed. This was closely followed by TOG 7250-A with a height of 24.67cm (Table 4.4a). Plant height in un-submerged genotypes was significantly ($p<0.05$) higher than was obtained under submergence. It ranged from 40.5cm to 61.5cm (Table 4.4b).

Table 4.4a: Survival and growth performance of 9 *Oryza glaberrima S* genotypes screened for submergence tolerance

GENOTYPE	Survival (%) 0DAS	Survival (%) 7DAS	Survival (%) 14DAS	Plant Height(cm)	Tiller Number	Number of Leaf	Leaf Diameter(cm)
TOG 7218	89.00^{ab}	40.00^{b}	35.33^{b}	9.00^{c}	1.67^{a}	2.00^{a}	0.10^{a}
TOG 7250-A	89.00^{ab}	73.33^{ab}	55.67^{a}	24.67^{ab}	2.67^{a}	4.67^{a}	0.17^{a}
TOG 7412	84.33^{ab}	89.00^{a}	0.00^{d}	0.00^{d}	1.00^{a}	0.00^{a}	0.00^{a}
TOG 7439-B	89.00^{ab}	68.67^{ab}	22.33^{c}	16.00^{b}	1.67^{a}	2.00^{a}	0.13^{a}
TOG 8347	89.00^{ab}	44.67^{b}	40.00^{b}	17.33^{b}	2.00^{a}	1.33^{a}	0.13^{a}
TOG 6632	57.67^{b}	73.33^{ab}	40.00^{b}	19.67^{b}	2.00^{a}	2.33^{a}	0.17^{a}
TOG 6640-B	84.33^{ab}	73.33^{ab}	22.33^{c}	17.67^{b}	1.67^{a}	2.33^{a}	0.30^{a}
TOG 7148	100.00^{a}	73.33^{ab}	51.00^{a}	27.33^{a}	2.33^{a}	2.67^{a}	0.15^{a}
TOS 6455	89.00^{ab}	100.00^{a}	17.67^{c}	12.00^{bc}	1.33^{a}	0.67^{a}	0.10^{a}
CV	20.8	31.46	117	134.5	127.47	144.58	163.08
R^2	0.48	0.62	0.32	0.24	0.34	0.3	0.27
P VALUE	0.23	0.04	0.05	0.87	1.62	0.72	0.83

Means with the same superscript along columns do not differ significantly from one another using the Duncan's multiple range tests.

Among un-submerged *Oryza glaberrima* genotypes, average tiller number of 2.89 was obtained (Table 4.4b). The highest number of leaves was recorded in TOG 7250-A (4.67). This was not significantly different from figures obtained for other *Oryza glaberrima* genotypes (Table 4.4b). This genotype was the most tolerant to submergence stress at 14DAS. The number of leaves observed in TOG 7250-A was almost twice that recorded in any other *Oryza glabberima* genotype. Number of leaves under un-submerged condition was significantly higher in genotypes under control set up. The widest leaf diameter was recorded in TOG 7250-A and TOG 6632, 0.17cm each. Leaf diameter was considerably wider in un-submerged genotypes (Table 4.4b).

Table 4.4b: Survival and growth performance of *Oryza glaberrima* genotypes, treatment and control screened for submergence tolerance.

GENOTYPE	Survival 14DAS (%)	Survival 14DAS (%) Control	Plant Height(cm)	Plant height Control (cm)	Tiller Number	Tiller Number Control	Number of Leaf	Number of Leaf Control	Leaf Diameter (cm)	Leaf Diameter Control (cm)
TOG 7218	35.33^{a}	83.50^{a}	9.00^{ab}	60.50^{a}	0.67^{a}	2.00^{a}	2.00^{a}	13.00^{a}	0.10^{a}	1.25^{a}
TOG 7250-A	55.67^{a}	100.00^{a}	24.67^{ab}	58.00^{a}	1.67^{a}	3.00^{a}	4.67^{a}	19.50^{a}	0.17^{a}	1.00^{a}
TOG 7412	0.00^{a}	100.00^{a}	0.00^{b}	43.00^{a}	0.00^{a}	3.00^{a}	0.00^{a}	17.00^{a}	0.00^{a}	1.10^{a}
TOG 7439-B	22.33^{a}	100.00^{a}	16.00^{ab}	58.00^{a}	0.67^{a}	3.00^{a}	2.00^{a}	16.50^{a}	0.13^{a}	1.00^{a}
TOG 8347	40.00^{a}	100.00^{a}	17.33^{ab}	60.00^{a}	1.00^{a}	3.00^{a}	1.33^{a}	15.50^{a}	0.13^{a}	0.95^{a}
TOG 6632	40.00^{a}	100.00^{a}	19.67^{ab}	30.00^{a}	1.00^{a}	3.00^{a}	2.33^{a}	16.50^{a}	0.17^{a}	0.90^{a}
TOG 6640-B	22.33^{a}	83.50^{a}	17.67^{ab}	61.00^{a}	0.67^{a}	3.00^{a}	2.33^{a}	15.00^{a}	0.30^{a}	1.05^{a}
TOG 7148	51.00^{a}	100.00^{a}	27.33^{ab}	61.50^{a}	1.33^{a}	3.00^{a}	2.67^{a}	16.50^{a}	0.15^{a}	1.00^{a}
TOS 6455	17.67^{a}	100.00^{a}	12.00^{ab}	40.50^{a}	0.33^{a}	3.00^{a}	0.67^{a}	12.00^{a}	0.10^{a}	0.90^{a}
CV	83.52	73.65	87.35	96.63	98.28	11.56	108.06	22.83	100.13	62.84
R^2	0.33	0.35	0.3	0.28	0.29	0.32	0.33	0.3	0.34	0.3
P VALUE	0.05	0.05	0.72	0.05	0.8	0.05	0.61	0.05	0.58	0.05

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

There was no significant ($p<0.01$) correlation between survival at 0DAS and 7DAS with the phenotypic parameters measured (Table 4.4c). However a strong positive correlation was obtained between survival at 14DAS and plant height (0.92), tiller number (0.97), number of leaf (0.89) and leaf diameter (0.76) (Table 4.4c). All phenotypic parameters also showed strong positive correlation with one another (Table 4.4c).

Table 4.4c: Correlation Coefficient for Traits Measured in *Oryza glaberrima S*

	Survival(%) 0DAS	Survival(%) 7DAS	Survival(%) 14DAS	Plant Height(cm)	Tiller Number	Number of leafs	Leaf Diameter(
Survival(%) 0DAS	1.00						
Survival(%) 7DAS	-0.08	1.00					
Survival(%) 14DAS	0.07	-0.15	1.00				
Plant Height(cm)	-0.07	-0.02	0.92*	1.00			
Tiller Number	0.03	-0.05	0.97*	0.91*	1.00		
Number of leafs	-0.06	-0.03	0.89*	0.90*	0.93*	1.00	
Leaf Diameter(cm)	-0.26	-0.03	0.76*	0.89*	0.76*	0.80*	1.00

*= significant at p<0.01. DAS=days after de-submergence

4.5. Validation of Rice genotypes with ART5 and SC3 Markers

4.5.1 Allelic and Loci Variation within Genotypes

PCR based molecular validation was conducted using the standard procedures elucidated earlier (Fulton *et al.*, 1995, Gregorio *et al.*, 1997). Allelic bands obtained with ART5 and SC3 are presented in plates 1, 2 and 3 below. The primer pair produced polymorphic bands among the rice genotypes. Allelic bands for 4 genotypes (CG14, IR 64, RAM 133 and VANDANA) were missing in the SSR profile on Agarose using ART5 marker. Plate 3 indicates the SSR profile of the genotypes with SC3 on PAGE. There-in, three genotypes (FARO36, GAMBIAKA and TOG 6640-B) showed band patterns similar to that obtained for IR64 Sub1.

4.5.2 Number of Alleles per Locus

A total of 7 alleles were detected with an average of 2 alleles per marker. Botha markers recorded 2 alleles each on agarose while SC3 on PAGE recorded 3 alleles. ART5 has an allelic frequency of 93% while SC3 recorded 100% both on agarose as well as on PAGE with an average allelic frequency of 97.7% (Table 4.7).

4.5.3 Polymorphism Information Content (PIC) Value

Polymorphism Information Content values were calculated to access how informative the markers were. The PIC values and resolving power of the marker was best and highest with SC3 on PAGE (Table 4.7). ART5 recorded a PIC value of 0.40 on agaraose while SC3 recorded 0.45 on the same medium. SC3 on PAGE recorded a PIC score of 0.48. Average PIC value for all markers was 0.44. SC3 was also more informative on PAGE with a resolving power of 2.04 relative to 2.00 on agarose and 1.86 recorded for ART5 on agarose. The average resolving power for all the markers is 1.97 (Table 4.7).

Table 4.7: Data of summary statistics of 29 rice genotypes for 2 microsatellites (SSR) markers

Primers	Allele	Allele Frequency (%)	PIC	Pb (Rp)
ART5	2	93	0.40	1.86
SC3	2	100	0.45	2.00
SC3 (PAGE)	3	100	0.48	2.04
Avg.	2	97.7	0.44	1.97

PIC=Polymorphism information content, Pb (Rp)= resolving power.

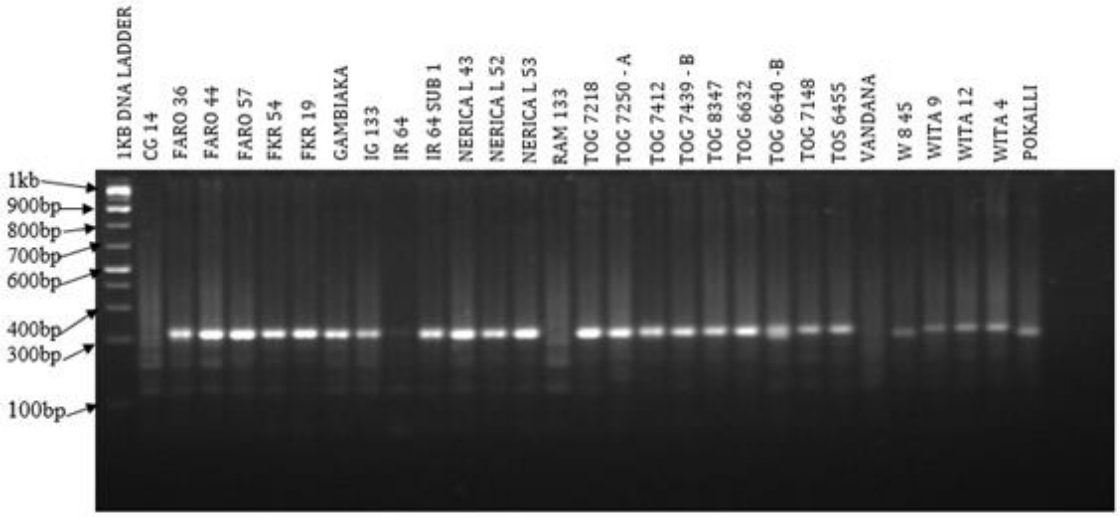


Plate 4.1: SSR profile of 29 rice genotype using primer ART 5

NB: Genotype 1,9,14, are missing

Size standard used is 1kb ladder.

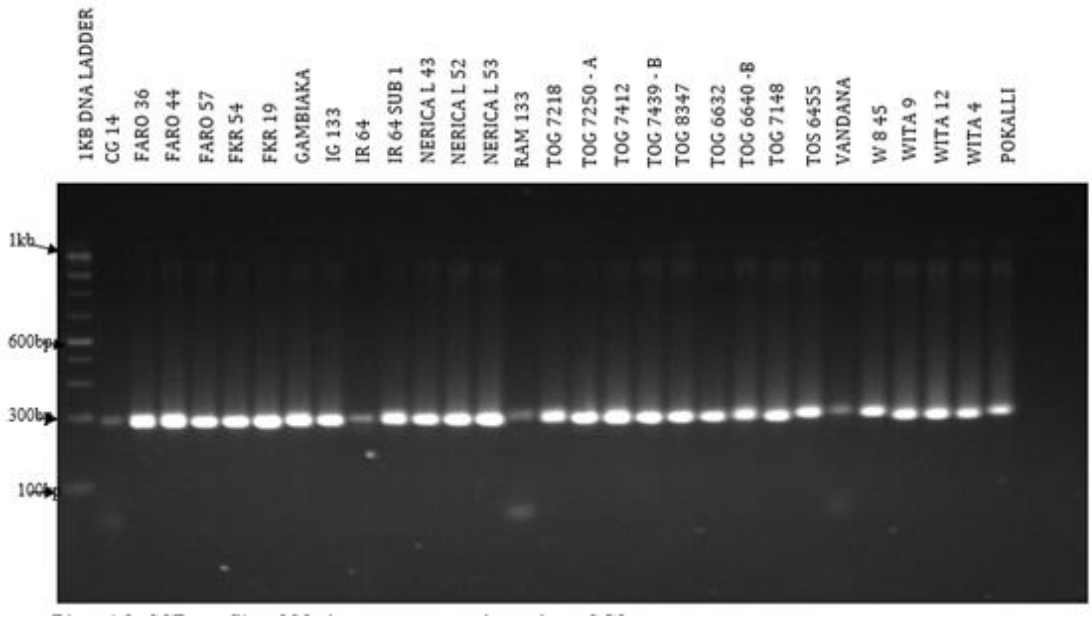


Plate 4.2 SSR profile of 29 rice genotypes using primer SC3

NB: Genotype 1,9,14 and 24 are missing

Size standard used is 1kb ladder.

Gel pictures on 2% SFR agarose (Superfine Resolution Agaarose)

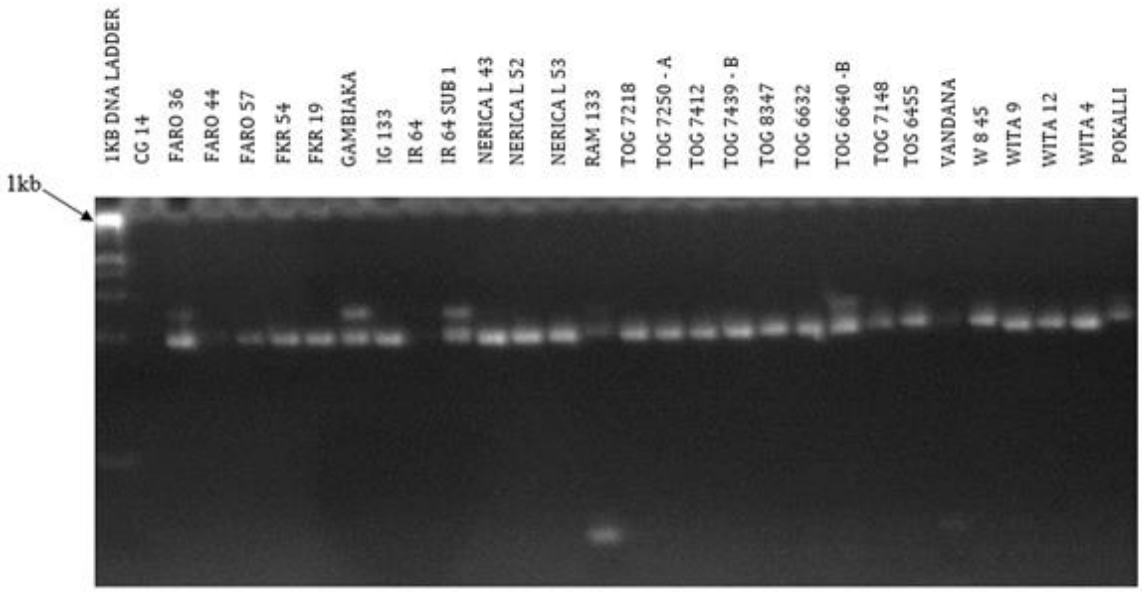


Plate 4.3: SSR profile of 29 rice genotypes using primer SC3 on PAGE(Polyacrylamide Gel Electrophoresis)

4.5.4 Genetic Similarities of the Genotypes based on ART5 and SC3 Markers

Cluster Analysis

Cluster analyses revealed three (3) major clusters (Figure 4.6) using DARwin 6.0.01. Cluster I had just one genotype (NERICA-L-52). Cluster II consisted of 9 genotypes, 1-NERICA (NERICA-L-43), 4-*Oryza glaberrima* (TOG 7412, TOG-7250-A, TOG7439-B and TOG 8347) and 4-*Oryza sativa* genotypes (IG 133, FKR 19, FKR 54 and FARO 57) while Cluster III was composed of 19 genotypes in 3 major sub clusters. Sub cluster A has only NERICA-L-53 in it. Sub cluster B consists of IR64 sub1, FARO 36, TOG 6640-B and GAMBIAKA. Sub cluster C consists of the 14 genotypes of sativa and glaberrima extraction. All NERICA genotypes did not cluster with any other genotype. NERICA-L-52 maintained a lone cluster position. IR64 sub1 clustered in the same group with other submergence tolerant genotypes viz FARO 36, TOG 6640-B and GAMBIAKA with a 70% similarity score.

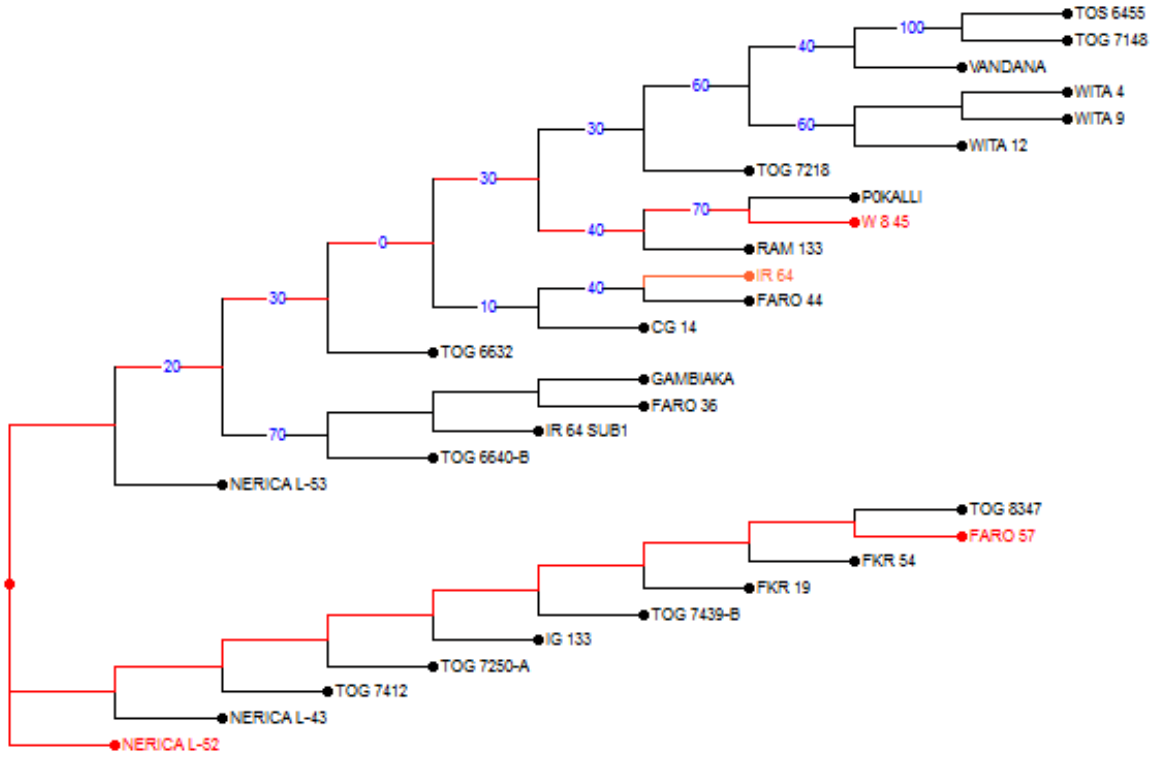


Figure 4.6 Cluster dendrograms showing genetic relationship among 29 Oryza species based on 2 SSR markers using DARwin 6.0.01

The cluster patterns obtained with SC3 on PAGE was consistent with the corresponding DNA band pattern. Three genotypes which showed the same band pattern with IR64 Sub1 (Plate 4.3) clustered with it (Figure 4.7).Three major clusters were obtained. Cluster I consisted of 4 *Oryza glaberrima* genotypes (TOG 7439-B, TOG 7412, TOG 7250-A and TOG 7218). Cluster II consisted of 13 genotypes in two sub clusters; sub cluster A consists of GAMBIAKA, FARO 36, IR64 sub1 and TOG 6640-B with 50% similarity score; sub cluster B consists of 9 genotypes (TOS 6455, TOG 7148, POKALLI, VANDANA, W 8 45, FARO 44, CG 14, IR 64 and RAM 133) with 60% similarity score. Cluster III consisted of 12 genotypes, including all the 3 NERICAs (NERICA-L-43, NERICA-L-52 and NERICA-L-53), 2 glaberrima (TOG 6632 and TOG 8347) and 7 sativa genotypes (WITA 4, FARO 57, FKR 54, FKR 19, WITA 9, IG 33 and WITA 12).

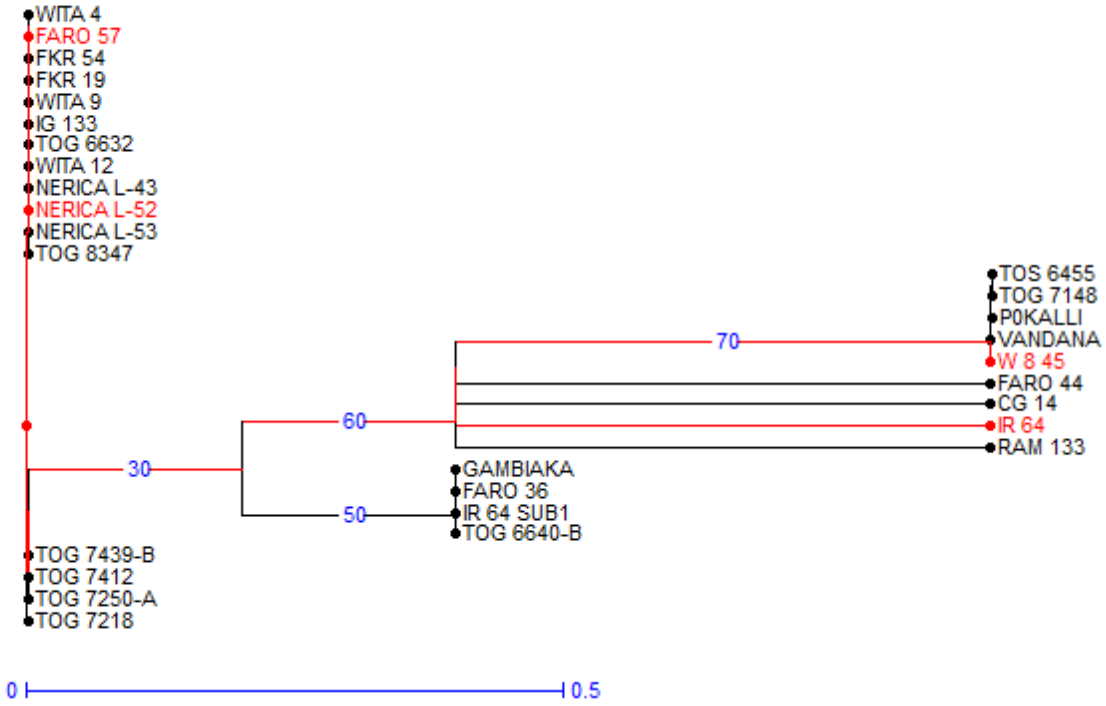


Figure 4.7: Dendrogram showing genetic relationship among 29 Oryza species based on SC3 marker on PAGE using DARwin 6.0.01

CHAPTER FIVE

5. DISCUSSION

Submergence affects both physiological and metabolic processes (Sarkar *et al.,* 2006; Ella *et al.,* 2003; Bailey-Serres and Voesenek, 2008) in plants. Percentage survival of all genotypes in this study declined due to submergence stress. This indicates that submergence gravely affects survival of rice plants and this will ultimately affect grain yield. Singh *et al.,* 2009 and Sarkar *et al.,* 2009a reported that grain yield in submergence tolerant genotypes was significantly higher than in susceptible genotypes. Akinwale *et al.,* 2012 concluded that increased grain yield could be attributed to higher survival rate, higher number of tillers and higher number of panicles. Erenstein *et al.* (2003) stated that yield loss due to submergence may range between 10% to total destruction. In the present study, TOG 7412 was completely lost as 14DAS indicating that such genotype could be totally wiped out of farmer's field under 2 weeks of flooding. Voesenek *et al.,* 2004 and Pierik *et al.,* 2009 stated that shoot elongation is associated with cost, as energy and carbohydrate are expended in cell division and elongation. This implies that genotypes that continuously elongate during flooding will not be idea for long duration flood regime. Singh *et al.,* 2001 and Das *et al.,* 2005 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. It then means that an idea genotype will device a means of conserving energy for use in growth recovery after de-submergence. This pattern was observed in IR64 sub1 in this current study. It recorded a plant height of 9.67cm under stress relative to 43.5cm recorded when it was un-submerged. Even though NERICA-l-52 showed promising survival scores and general growth pattern, it displayed worrisome elongation score with a height of 42.33cm under submergence compared to

34.0cm when it is un-submerged. This will increase its tendency for lodging when flood water recedes. This is similar to the position of Bailey-Serres, 2010. The survival score obtained in the tolerant check, IR64sub1 at 14DAS (89%) was similar to that obtained by Septiningsih *et al.,* 2009. Bailey-Serres *et al.,* 2010 obtained a survival score of 98% with a submergence tolerant genotype, M202 sub1 after 16 days of submergence stress. In the current study, the genotypes suspected of submergence tolerance had survival scores ranging from 51.0% in FARO 36 to 98% in IR64 sub1.

Plant height was seen to be strongly correlated with survival at 14DAS. This could be due to the fact that the first and most common response of plants to submergence stress is the tendency to escape from it through extended growth. Luo *et al.,* 2011 and Sarkar and Bharttacharjee, 2011 reported that rice adapts to submergence either by elongation or quiescence (reduced activity) depending on the nature of the flood water, particularly its temperature and turbidity. It is established that gas diffuses 10^4 less in water than in air. This ultimately reduces exchange of carbon dioxide and oxygen available for photosynthesis and respiration (Das *et al.,* 2009). This was also reported by Xu *et al.,* 2006; Sarkar and Panda, 2009 and Hattori *et al.,* 2009. 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. Pederson *et al.,* 2009 stated that to mitigate submergence to so extent, plants retain a thin film of gas on the surface of submerged leaves which provides a microenvironment for exchange of carbon dioxide and oxygen. This could explain the gas bubbles observed on the tip of submerged leaves. Bailey-Serres *et al.,* 2010 in supporting this position mentioned that rainfed lowland rice genotypes respond to transient or partial flooding by moderate elongation of leaves and

the portion of stems that are underwater. This is however contrary to the findings of Akinwale *et al.,* 2012 and Singh *et al*., 2001 who found stem elongation to have a negative relationship (-0.80) with percentage survival in intolerant genotypes. Jackson and Ram, 2003 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.

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 in order to reduce their utilization of resources during submergence stress, plants could adopt a survival strategy which involves reducing its less critical body parts. Setter *et al.,* (2010) reported that one of the causes of intolerance to complete submergence in some rice cultivars was the desiccation of leaves after de submergence. Ella *et al.,* 2003 reported that chlorosis and leaf senescence in submerged plants is due to reduction in light energy that get to underwater leaves especially in turbid water. 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. This could be as a result of submergence stress. Ella *et al.,* (2003); Sarkar *et al. (*2006); Bailey-Serres and Voesenek, (2008). 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. Sarkar *et al.*, (2009); Singh *et al.,* (2009) and Akinwale *et al.,* (2012) reported that submergence tolerance is associated with higher survival rate, higher number of tillers and higher number of panicles which will ultimately lead to increase in grain yield. 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.

The strong correlation of plant height with 14DAS could be as a result of adaptation mechanisms upon de-submergence as observed by Singh *et al,* (2001) and Akinwale *et al.,* (2012) who reported that plant height was positively correlated with percentage survival.

Growth recoveries observed in some of the genotypes indicates the ability of those genotypes to survive under short flash flood condition. This trait is desirable since it ensures production of sufficient biomass for plant productivity (Sarkar and Bhattacharjee, 2011). Panda *et al.,* (2008) reported that plants ability to quickly re-generate after submergence is important 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).Growth recovery of 0 to 11.5% was observed in this current study. It is believed that recovery came from plants ability to conserve energy during submergence and use it when the flood receeds. This can also be deduced from the findings of Bailey-Serres *et al.,* 2010.

The hardiness and inherent tolerance of NERICA to abiotic stress (Jones *et al.,* 1997) must have accounted for their high tolerance to submergence stress. It could also be due to their pedigree history. NERICA genotypes are interspecific hybrids derived from crosses between *Oryza sativa* and *Oryza glaberrima*. Further checks indicated that NERICA-L-52, which is the best performing NERICA genotype has sativa genotype IR64 in its pedigree line (IR64/TOG5681//4*IR64). Additionally, high susceptibility observed in *Oryza glaberrima* genotypes was due to the inability of the species to withstand the stress factor. This could be attributed to the low domestication and

adaptation of these genotypes to soil and environmental conditions. This feat is however ironic given that glaberrima genotypes are indigenous to African soil and environment. It hence implies that more research effort need to be invested in developing mega varieties from glaberrima as it is known that it a reservoir of gene pool with features peculiar to Africa. This further indicates that survival during submergence is not hinged on plant elongation alone. Das *et al*., (2005) and Singh *et al*., (2009) stated that stem elongation exhausts energy reserve and can cause lodging when water level recedes.

The number of alleles obtained (2 to 3 alleles) is lower than that obtained in previous works using SSR markers. This could be due to the use of limited number of SSR markers. Ni *et al.,* 2002 obtained 1 to 17 alleles using 111 SSR markers in *Oryza sativa* and its close relatives with an average PIC value of 0.62. Sajib *et al.,* 2012, using nine SSR markers obtained between 2 and 6 alleles with PIC values ranging from 0.14 to 0.71 with an average of 0.48. Aliyu *et al.,* 2013, using 17 SSR markers, obtained 1 to 4 alleles per marker with PIC values of 0.19 to 0.83in *Oryza sativa,* 0.13 to 0.71 in NERICA and 0 to 0.63 in *Oryza glaberrima*. These suggest that the markers were more informative in sativa genotypes and least informative in glaberrima. These differences amongst species must have accounted for the disparity in clustering. PIC values of between 0.4 and 0.48 obtained in the current study could be due to limitation in the number of markers used.

NERICA-L-52 which showed phenotypic traits geared towards submergence tolerance but did not cluster in the same group with the tolerant genotype (IR64 Sub1). This could indicate that the markers are species specific, hence could not detect genetic similarity between the tolerant *Oryza sativa* genotypes and the interspecific hybrids. This could

also account for the clustering of tolerant genotype (*O. sativa*) with few *Oryza glaberrima* genotypes. The clustering of the averagely tolerant FARO 36, GAMBIAKA and TOG 6640-B with the tolerant genotype (IR64 Sub1) at a 70% genetic similarity was in consonance with their survival percentages and other morphologies. This could be attributed to the growth pattern exhibited by these genotypes in the bid to survive in the stressed environment. This was achieved by probably reducing the photosynthesizing ability and conserving energy needed for growth and expending them on survival. Similar findings were reported by Aliyu *et al*, 2015 on their work on the effects of submergence tolerance within *Oryza glaberrima* genotypes. Aliyu *et al.*, 2013also observed *Oryza sativa* genotypes clustering with interspecific hybrids as obtained in this current study. This could be due to pedigree history, since the recurring parent in the pedigree history is of sativa origin.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATONS

6.1 SUMMARY

This study was conducted to assess the effects of submergence stress on some rice genotypes and species. Twenty nine (29) genotypes including 17 *Oryza sativa*, 9 *Oryza glaberrima* and 3 NERICA genotypes were used for the study. For molecular screening, DNA was extracted using modified CTAB protocol. Two microsatellite (SSR) markers, ART5 and SC3 were used.

Results obtained 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.

6.2 CONCLUSIONS

1. Submergence affected plant survival (57.67 – 100%) and phenotypic growth parameters. Submergence tolerant rice genotypes (IR64 Sub1, FARO 36, GAMBIAKA, NERICA-L-52) showed higher (insert values in % here) percentage survival by exhibiting moderate plant height and reduced number of leaves immediately after de-submergence.

2. Six genotypes (20.69%) exhibited recovery at 7DAS, this was however lost at 14DAS thus validating the de-submergence evaluation protocol. However, FARO 57, NERICA-L-52, IR64 Sub1 8 45 and FKR 54 showed recovery plasticity ranging from 0-11.5% within 7 to 14 days of de- submergence.

3. IR64 Sub1 (tolerant check) did not cluster with NERICA-L-52 which displayed potentials for submergence tolerance. This indicates that submergence tolerant markers were species specific. However its clustering with other moderately tolerant genotypes validates its potential for tagging submergence tolerance in *Oryza sativa* genotypes.

6.3 RECOMMENDATIONS

Based on the outcome of this research, it is recommended that:

1. The four promising genotypes (IR64 Sub1, FARO 36, GAMBIAKA, NERICA-L-52) obtained can be utilized for possible use in future breeding programs for the development of submergence tolerance in local genotypes
2. Species specific submergence markers can be developed to allow for effective diagnostic detection of submergence tolerance across Oryza species.
3. Further evaluations on quantitative trait loci for submergence tolerance is recommended for validating submergence tolerant genotypes with a higher degree of association.

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