RESPONSE OF RICE (*Oryza sativa* L.) GENOTYPES UNDER VARYING SUBMERGENCE DURATIONS

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DECEMBER, 2015

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REGISTRATION NO. 11-04688

A Thesis
Submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka,
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

IN

AGRICULTURAL BOTANY

SEMESTER: July-December, 2015

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CERTIFICATE

*This is to certify that the thesis entitled, "**RESPONSE OF RICE (Oryza sativa L.) GENOTYPES UNDER VARYING SUBMERGENCE DURATIONS**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL BOTANY**, embodies the results of a piece of bona fide research work carried out by **Nasima Akhter** Registration No. **11-04688** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation, has duly been acknowledged.

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*Place: **Dhaka, Bangladesh***

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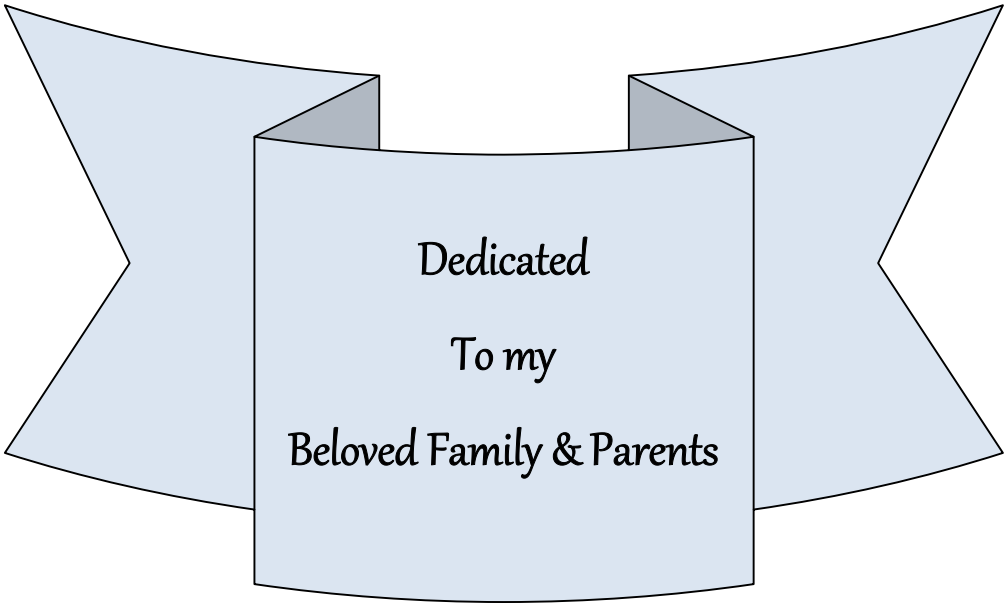
Dedicated
To my
Beloved Family & Parents

ACKNOWLEDGEMENTS

All praises go to “Almighty Allah”, the supreme ruler of the universe, who has given the author the ability to complete her dissertation in time.

It is the privilege to express her deepest gratitude, sincere appreciation and deep sense of respect to her Supervisor and Chairman of her Ph.D. advisory committee, Dr. Kamal Uddin Ahamed, Professor, Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, for his scholastic guidance, valuable suggestions, continuous encouragement and all kinds of support and help throughout the research period and for the preparation of this manuscript.

The author expresses her heartfelt gratitude to the respectable members of her Ph.D. advisory committee, A.M.M. Shamsuzzaman, Professor, Department of Agricultural Botany; Dr. Parimal Kanti Biswas, Professor, Department of Agronomy; and Dr. Jiban Krishna Biswas, Director General, Bangladesh Rice Research Institute, for their kind co-operation, valuable suggestions and constructive criticisms during the period of the present investigation and in preparation of the manuscript.

The author appreciates the support and co-operation of other teachers, staffs, and students of the Department of Agricultural Botany. The author is also thankful to the scientific officers of the physiology division of Bangladesh Rice Research Institute for their assistance during this study.

The author would like to express her deepest honor and respect to her parents, brothers and sisters who always inspire her to pursue higher education. She expresses her special gratefulness to her father-in-law, mother-in-law and all her relatives whose blessing and constant inspiration supported her during this study.

The author also expresses her gratefulness to her beloved husband for his patience, inspiration and sacrifice so that she might complete the degree. The author also expresses her most sincere gratitude to her beloved daughter for her co-operation and sacrifice during this study.

The author would like to acknowledge the co-operation of Sher-e-Bangla Agricultural University authority for granting her leave on deputation for this study. The author is also grateful to her colleagues and friends for their enthusiastic support during this study.

Finally, the author acknowledges the co-operation of HEQEP (Higher Education Quality Enhancement Project) authority for their financial support to complete her research work.

The Author

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ABSTRACT

Three pot culture experiments were carried out in the field at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during *aman* seasons of 2012 - 2014 to study the response of different rice genotypes under varying submergence durations and to identify the morphological, physiological and anatomical characters those contribute to submergence tolerance and to study the variations among the selected rice genotypes. The experiments were conducted with three tolerant (BRRI dhan52, IR64Sub1, FR13A) and one susceptible genotype (BR5) with four submergence treatments (0, 7, 14, and 21 days of submergence). All experiments were laid out in two factors RCBD with four replications. Different morphological and physiological processes were hampered due to submergence and there existed genotypic variations also. The lower stem elongation and less affected root dry matter help plants to survive under submergence condition. After desubmergence, tiller number, stomatal conductance, net assimilation rate, relative growth rate and absolute grain growth rate were less affected which helped BRRI dhan52 and FR13A to perform better. Different yield components such as number of panicles per plant, number of filled grain per plant, filled grain weight per plant and 100-grain weight were also less affected in BRRI dhan52 and FR13A compared to other two genotypes (IR64Sub1 and BR5). The results indicated that 7DS treatment in BRRI dhan52 produced more or less similar (99%) yield as compared to control which was followed by FR13A(81% of the control). Decreased grain yield per plant under submergence treatment was observed due to reduction in tiller number rather than grain weight per panicle. Larger aerenchyma formation, stronger arrangement of parenchyma, lower membrane leakage, higher proline content, higher stem carbohydrate and lower chlorophyll depletion in BRRI dhan52 and FR13A provided better adaptation capabilities of these genotypes under submergence condition. But in BR5, though the proline content was higher just after desubmergence, the injury label was much higher in this genotype and cells lost their normal physiological functions. Lower chlorophyll a/b ratio in BRRI dhan52 and FR13A indicated that chlorophyll a content was more affected and chlorophyll b content was less affected due to submergence. The genotype BRRI dhan52 showed more submergence tolerance compared to the other genotypes.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABBREVIATION	FULL WORD
AEZ	Agro-Ecological Zone
ANOVA	Analysis of Variance
AGR	Absolute Grain Growth Rate
@	At the rate of
ATP	Adenosine Tri Phosphate
BRRI	Bangladesh Rice Research Institute
BY	Biological Yield
cm	Centimeter
cv.	Cultivar(s)
CV	Coefficient of Variation
CGR	Crop Growth Rate
DAA	Days after anthesis
DMRT	Duncan's Multiple Range Test
DS	Days of Submergence
e.g.	*exempi gratia* (for example)
et al.	*et alibi* (and others)
EY	Economic Yield
etc.	*et cetra* (and so on)

ABBREVIATION	FULL WORD
g	Gram
HI	Harvest index
H_2O_2	Hydrogen per oxide
i.e.	*id est* (that is)
IRRI	International Rice Research Institute
kg	Kilogram
LA	Leaf area
LSD	Least significant difference
LWR	Leaf weight ratio
mg	miligram
MP	Muriate of potash
NAR	Net assimilation rate
pH	Hydrogen ion concentration
RCBD	Randomized complete block design
RGR	Relative Growth Rate
ROS	Reactive oxygen species
SAU	Sher-e-Bangla Agricultural University
SRDI	Soil Resources and Development Institute
SPAD	Soil Plant Analysis Development

ABBREVIATION	FULL WORD
SLW	Specific leaf weight
Sub	Submergence
TDM	Total dry matter
TSP	Triple super phosphate
t ha^{-1}	Ton per hectare
Viz.	*Videlicet* (namely)

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population. It is the major crop in most flood prone areas of South and South-East Asia. There is a tremendous pressure for increasing rice production in order to keep pace with population growth. By the year 2035, 26% increase in rice production will be necessary to feed the rising population (Seck *et al*., 2012).

Submergence is one of the major constraints for rice production in Bangladesh along with many other rice growing countries in the world. In many monsoon areas of the world where the intensity of rainfall is extremely high, the rice plant is completely flooded for a period ranging from a day to a week. This kind of inundation is generally known as "submergence". A submerged plant is defined as "a plant standing in water with at least part of the terminal above the water or completely covered with water" (Nishiuchi *et al*., 2012). Of the lowland rainfed rice farms worldwide, over 22 million hectares are vulnerable to flash flooding, representing 18% of the global supply of rice (Khush, 1984). More than half of the total rice grown area in Bangladesh is considered to be submergence-prone during the *kharif* season. Flash flood is a common phenomenon in the northern, north eastern, eastern and coastal region of Bangladesh. Ismail *et al*. (2010) reported that more than one million hectare of rice fields in the coastal areas of Bangladesh suffer from prolonged flooding during the wet season.

The soil and overlying water of flooded habitats are characterized by hypoxia and anoxia, the partial and complete depletion of oxygen. The negative impacts of submergence on rice plant are mainly related to low light intensity, slow gas diffusion (10,000 times slower in water than air) and changes in root environment including the accumulation of phytotoxic compounds (Gambrell *et al*., 1991; Bailey-serres and Voesenek, 2008). The submergence effect on rice plant also depends on water depth, duration of submergence, turbidity of water, light intensity, O_2 concentration of water, pH of water, water temperature and age of the plant (Sarkar *et al*., 2006). As a result, when submerged, rice plants usually face reduced ATP production by rapid alcoholic fermentation, limited photosynthesis, carbohydrate starvation, degradation of chlorophyll and mechanical damage (Ella *et al*., 2003a). After desubmergence, on the other hand, plants face

aerobic shock induced photoinhibition, production of reactive oxygen species and accumulation of acetaldehyde (Luo *et al*., 2009).

The ability of rice to endure waterlogging varies with its growth stage. Zhang *et al*. (2015) have reported that the reduction in yield is maximum when complete submergence is imposed at flowering and this is followed by seedling establishment and maximum tillering stages. The decrease in grain yield on submergence at flowering occurs due to impaired anthesis causing high sterility and unfilled spikelets. Improper grain filling under submergence is reported to be due to reduction in carbohydrate content and less translocation of the same to sink (Palada and Vargara, 1972; Yoshida, 1972).

The influence of flooding on rice tillering has been examined through numerous studies (Huo *et al*., 1997; Sasidharan and Voesenek, 2013; Wang *et al*., 2014). The plants submerged at seedling establishment stage increase in height rapidly. As a result tillering is affected adversely. It is reported that the decrease in number of total tillers, air bearing tillers and grain yield is higher due to submergence at early rather than at late vegetative stage (Reddy and Mittra, 1985). The tolerance of plant to submergence increase with age (Pervin *et al*., 2010) and has a positive relation with carbohydrate content. The reduction division stage of the pollen mother cells is most susceptible to submergence. Most of the stored starch from source is translocated to sink during the period from 10 to 20 days after heading. So submergence at late flowering stage causes the reduction in grain number as well as grain weight and thereby the yield (Reddy and Mittra, 1985).

Tolerant to flash flood submergence may be defined as "the ability of a rice plant to survive from 10 to14 days of complete submergence and renew its growth when the water subsides" (Catling, 1992). Rice varieties differ in their tolerance to submergence. Under flash flooding, few characters have been identified as playing a key role in submergence tolerance in rice; the most critical are: maintenance of high carbohydrate concentration in shoot, optimum rates of alcoholic fermentation and energy conservation by maintaining low shoot elongation growth rates during submergence, lower degradation of chlorophyll, antioxidant defense system against reactive oxygen species (ROS) and larger aerenchyma formation. According to Sarkar *et al.* (2006), "submergence tolerance is a metabolic adaptation in response to anaerobiosis that enables cells

to maintain their integrity so that the plant survives hypoxia without major damages". Submergence tolerance includes a number of anatomical (formation of higher aerenchyma tissue in nodal region), physiological (less shoot elongation) and biochemical (inhibition of chlorophyll degradation, less utilization of storage carbohydrates and increased activity of antioxidative enzymes) adaptations (Hattori *et al*., 2011; Banerjee *et al*., 2015a). For flash-flood affected areas, a major breakthrough was the recent discovery and cloning of the *Sub1A* gene and its subsequent deployment through popular high yielding varieties (Xu *et al*., 2006; Neeraja *et al*., 2007; Septiningsih *et al*., 2009). The effectiveness of this gene under field conditions has been validated in many countries in South and Southeast Asia, with a consistent yield advantage of 1 to 3.5 tons per hectare. *Sub1A* promotes the expression of genes related to the detoxification of reactive oxygen species (ROS) and reduces the accumulation of ROS (Nishiuchi *et al*., 2012).

Subsequently, several submergence tolerant mega varieties namely IR64-Sub1, SambaMahsuri-Sub1, Thadokkam1-Sub1 and BR11Sub1 have been developed (Singh *et al*., 2009; Iftekharuddaula *et al*., 2011). Swarna-Sub1 has been released in India, Indonesia and Bangladesh, BR11Sub1 has been released in Bangladesh and IR64-Sub1 in the Philippines and Indonesia. New rice varieties tested in India and Bangladesh can survive up to two weeks of complete submergence (Iftekharuddaula *et al*., 2011). The flood-resistant gene, when transferred into popular rice varieties, allows them to retain their characteristics. The Bangladesh Rice Research Institute (BRRI) scientists have developed some submergence tolerant lines using conventional breeding methods as well as biotechnological and physiological tools. Out of these lines, Swarna Sub1 and BR11 Sub1 lines have been released as BRRI dhan51 and BRRI dhan52, respectively. Recently Bangladesh Institute of Nuclear Agriculture (BINA) has also released submergence tolerant varieties, Binadhan-11 and Binadhan-12. Iftekharuddaula *et al.* (2015) have done the yield trial and the adaptability test of those genotypes. Pervin *et al*. (2010) state the different factors that affect submergence tolerance. Those submergence tolerant rice varieties are reported to performing well in flood-prone areas. Now it deems necessary to identify the characters that help plants to survive under varying submergence conditions and to give better yield after desubmergence in comparison with submergence susceptible ones. After identifying the tolerant characters, further improvement of the existing submergence tolerant rice genotypes and incorporation of those tolerant genes to other high yielding rice cultivars will be possible.

Through this process we shall be able to reduce submergence effect as well as to get better a*man* yield. However, a little research works have been done on these aspects in Bangladesh.

Therefore, the ultimate goal of the present study is to find out the characters those help in submergence tolerance. This knowledge should facilitate designing suitable screening criteria for breeding programs. For achieving this goal, three pot-culture experiments have been done in consecutive three *aman* seasons.

Objectives of the dissertation work

1. To study the response of different rice genotypes under varying durations of submerged condition, and
2. To identify the morphological, physiological and anatomical characters of rice plants those contribute in submergence tolerance.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Rice under submergence stress

Submergence or waterlogging imposes a complex abiotic stress on rice plant. It affects various physiological and metabolic processes. It was observed that water can inflict serious injuries on plants (Jackson and Ram, 2003). Submergence is a situation when floodwaters rise to levels that shoots are completely under water. It was reported that three contrasting submergence regimes, based on temporal and depth criteria, have been studied in relation to natural and agricultural systems: short duration floods; long duration, but shallow floods; and long duration, but deep floods (Piedade *et al.,* 1991; Jackson and Ram, 2003; Voesenek *et al.,* 2004).

Colmer and Voesenek (2009) reported that short duration submergence occurs during flash-flooding, with variable time and depth in most low-lying land areas of the world. Long-term submergence can either be shallow or deep. In river systems, the deep floods typically occur on areas that are in direct contact with the river. Long-term shallow floods occur in more distal areas of the plains when water flows over embankments. In many cases, the flooding durations are further prolonged due to poor drainage of soils in those areas.

It was defined that a submerged plant is a plant standing in water with at least part of the terminal above the water or completely covered with water (Nishiuchi *et al*., 2012). On the other hand, flooding or submergence was also classified into “flash flooding” and “deepwater flooding” according to the duration of flooding and the water depth (Catling, 1992; Jackson and Ram, 2003).

It was observed that the negative impacts of submergence on terrestrial plants are mainly related to low light intensity and slow gas diffusion, which severely affects carbohydrate and energy availability for plants underwater (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Plant survival in submergence is greatly affected not only by depth of floodwater but also by physico-chemical characteristics (O_2 and CO_2 concentration, pH, turbidity) of floodwater. The adverse effects on growth and metabolism are likely due to limited gas diffusion (Setter *et al*., 1989) and light penetration (Raha, 2009).

The life cycle of rice can be roughly divided into seedling, tillering, booting, heading, flowering, milk, and maturation stages. The ability of rice plant to endure waterlogging varies with growth stages. It was reported that waterlogging in the booting and heading-flowering stages causes more serious yield loss than in the other stages (Huo *et al*., 1997). It was also reported that rice plants in the tillering stage have higher submergence tolerance than at other stages, and submergence at this stage has little influence on rice yield because rice plants in the tillering stage possess sufficient carbohydrates for growth (Sasidharan and Voesenek, 2013; Wang *et al*., 2014). It was observed that rice submergence can result in yellow leaf discoloration, a decrease in the number of green leaves and white roots, a decrease in root absorption, impaired growth and development, and a decrease in lodging tolerance, which can result in underproduction or even a complete lack of yield (Crawford, 2003).

2.1.1. Mechanical damage

It was observed that plants can suffer physically from strong water flow or from abrasion by suspended particles during flooding (Jackson and Ram, 2003). Growth and developmental processes in plants can be strongly disturbed and restricted under submergence. It was also reported that seeds of most terrestrial plants lose viability under submergence stress (Hook, 1984) and seedlings, germinated in water, have high mortality in flood-prone areas (Parolin, 2002; Das *et al.*, 2009). It was also stated that above and belowground growth and biomass accumulation become severely impaired, or even completely ceased, during submergence in many terrestrial plants (Mauchamp *et al.*, 2001; Macek *et al.*, 2006; Sarkar *et al.*, 2006; Parolin, 2009).

2.1.2. Poor light transmission

Light is an important factor to consider during submergence. It was showed that photosynthetically active radiation (PAR) declined sharply with increasing water depth to 50 and 100 cm during submergence. About 60-70% of the incident light was received at the water surface (2.5 cm), but it decreased to 40-50% at 50-cm depth and further to 20-30% at 100 cm (Singh *et al*., 2014).

When floodwater is turbid, solar radiation under water becomes very low and limits the capacity of plants to photosynthesis. Palada and Vergara (1972) observed a decrease in survival of rice seedlings after complete submergence in turbid water because of lower light transmission (40% of that in air). It was also observed that there was a reduction in solar radiation to <1% that in air at only 40 cm depth in one flood-affected location in eastern India (Setter *et al*., 1995). Flood turbidity reduces light transmission and deposits silt on the submerged plant. Low irradiance in surface water is occurring to surface algal colony and turbidity. Submergence in turbid water was confirmed to be more detrimental than in clear water for several cultivars, however, the submergence period is often too long and the differences are insignificant to confirm this in intolerant cultivars. It was also reported that irradiance under water is a major factor affecting the O_2 and CO_2 concentrations, hence photosynthesis and respiration that influence the balance between gain and loss of CO_2 (Ram *et al.*, 2002).

It was observed by Whittier *et al*. (1988) that poor light transmission through floodwater is an important limiting factor for plants in deep water, in highly turbid floodwater or under thick algal growth. Under submergence conditions, light reaching the submerged plants/leaves is attenuated by water, dissolved organic matter, silt, and/or phytoplankton suspended in the water (Das *et al*., 2009). Due to the attenuation, little amount of solar radiation reaches the plant canopy level, and thus limiting the capacity for underwater photosynthetic carbon fixation (Setter *et al*., 1995).

Light limitation during submergence has been shown to cause severe injuries and accelerate plant mortality (Adkins *et al*., 1990; Jackson and Ram, 2003). It was also observed that light availability under water may be relatively low, due to surface reflection and absorption by water, suspended particles, zooplankton, and algae (Holmes and Klein, 1987).

2.1.3. Slow rate of gas exchange

Setter *et al*. (1995) stated that limited gas diffusion is the most important factor during flooding. Reduced movement of gases to the submerged plant alters the concentration of O_2, CO_2 and ethylene inside the plants. The depletion of O_2 creates a condition of low O_2 (hypoxia) or no O_2 at all (anoxia) around the plant tissues such as seeds or root apices and stele.

Armstrong (1979) described that the solubility and diffusion coefficient of O_2 decrease 33 and 8.8×10^3 times, respectively, in fully aerated water at 25°C compared with the situation in air. Compared with O_2, the ratio of dissolved CO_2 relative to gas-phase CO_2 in water is approx. 0.76 at 25°C, depending on the pH values of the water (Sisler and Wood, 1988). Setter *et al*. (1989) reported that photosynthesis of submerged leaves is severely CO_2 limited because of slow gas diffusion in water and the inevitable boundary layer formed around leaves.

2.1.4. Hypoxia and anoxia

Roots of the plants are usually in contact with oxygen at a partial pressure which is equivalent to the gaseous atmosphere. It was described that hypoxia, reduction of oxygen below optimum level, is the most common form of stress which occurs during partial submergence of plant due to short-term flooding where the root goes under water and shoots remain in the atmosphere. Anoxia, another kind of water stress, where plant goes under water completely, hence complete absence of oxygen as a result of long term flooding (Ahmed *et al*., 2013). The embyo and coleoptiles of rice and rice grass can survive weeks of anoxia. It was observed that during germination of paddy rice and rice grass, the coleoptiles breaks the water surface and becomes a diffusion pathway for O_2 to the rest of the plant. Even though rice is a wetland species, its roots are as intolerant of anoxia (Taiz and Zeiger, 1998).

2.1.5. Changes in root environment

It was found that as water saturates the soil, air spaces are filled, leading to the modification of several soil physical and chemical characteristics (Krik *et al*., 2003; Dat *et al*., 2004). It was reported that soil redox potential (Eh) is often considered the most appropriate indicator of the chemical changes taking place during soil flooding (Pezeshki and Delaune, 1998). Eh generally declines during soil waterlogging (Pezeshki and Delaune, 1998; Pezeshki, 2001; Lu *et al*., 2004). It was also reported that soil reduction induces the release of cations and phosphorus through adsorbtion of ferrous ion dissolution of oxides (Boivin *et al*., 2002). According to them, soil reducing conditions also favour the production of ethanol, lactic acid, acetaldehyde and acetic and formic acid.

2.1.6. Toxicities of reduced soil components

In water-saturated conditions, electrochemical properties of soils change because of the activities of microorganisms that use oxidized chemicals as electron acceptors (Ponnamperuma, 1984; Laanbroek, 1990). It was found that the concentrations of certain potentially toxic compounds, such as Mn^{2+}, Fe^{2+} and S^{2-} often increase in submergence condition that may lead to their accumulation in root tissues (Jackson and Drew, 1984). It was also reported that when soil is completely depleted of molecular O_2, anaerobic soil microorganism take their energy from the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) or to nitrous oxide (N_2O) and molecular nitrogen (N_2). Under submergence condition these gases (N_2O + N_2) are lost to the atmosphere in a process called denitrification. It was also stated that under more reducing condition Fe^{3+} is reduce to Fe^{2+} and because of its greater solubility and Fe^{2+} toxicity increases when soils are anaerobic for many weeks. Other anaerobes may reduce sulfate (SO_4^-) to hydrogen sulphide (H_2S), which is a respiratory poison (Taiz and Zeiger, 1998). It was also demonstrated that volatile lower organic acids (e.g. propionic and butyric acids) can also accumulate in flooded soils and damage roots (Armstrong and Armstrong, 1999). These organic acids, together with high CO_2, can impose "acid loads" on root cells in submerged plants (Greenway *et al*., 2006).

2.1.7. Carbohydrate starvation

It was reported that soluble sugars and starch in plants are important sources of energy for cellular metabolism and maintenance during submergence as well as subsequent regeneration of new tissues after de-submergence (Das *et al*., 2005; Panda *et al*., 2008b). It was also reported that some flood-tolerant varieties have the ability to retain carbohydrate pools during submergence, provided that they are capable of underwater photosynthesis and/or carbohydrate consumption can be reduced during submergence (Das *et al*., 2005). It was found that in susceptible cultivars, submergence causes degradation of chlorophyll, decrease of Rubisco activity and damage to photosynthetic apparatus, resulting in strong reduction of photosynthesis (Ella *et al*., 2003a; Panda *et al*., 2008b). It was observed that anaerobic metabolic pathways induced under hypoxic or anoxic conditions (hypoxia, < 20.9% and > 0% O_2 at 20^oC; anoxia, 0% at 20^oC) are far less efficient in energy conversion than aerobic respiration, and thus they can deplete carbohydrate reserves more rapidly (Laan and Blom, 1990; Guglielminetti *et al.*, 1997).

Bailey-Serres and Voesenek (2008) stated that if the reserves of soluble sugars and starch are not replenished, exhaustion of carbohydrate will ultimately lead to cell and organ death.

2.1.8. Energy crisis

It was observed that submergence causes carbohydrate shortage in plants, diminishing important substrates to sustain glycolysis and ATP generation. The most of ATPs for cellular metabolism in plants are generated by oxidative phosphorylation (i.e. through respiration) under aerobic conditions. It was also observed that submergence often causes hypoxia within shoot tissues and can cause anoxia in non-photosynthesizing tissues such as roots (Armstrong *et al*., 1994; Mommer *et al*., 2004). Due to hypoxia or anoxia, plants alter metabolism to generate ATP via glycolysis followed by fermentation. Gibbs and Greenway (2003) stated that energy (ATP) crisis ensues because glycolysis is inefficient, yielding 2 to 3 mol ATP per mol hexose, as compared with 24 to 36 mol ATP generated by oxidative phosphorylation. Although studies in different plant species have demonstrated that glycolysis and fermentation are necessary for cell survival under O_2 deprivation, enhancement of these processes is not necessarily correlated with prolonged submergence tolerance in plants (Gibbs and Greenway, 2003). They also stated that energy shortage due to hypoxia/anoxia may lead to nutrient imbalance and pronounced root injury during submergence. Whether the end products of fermentation (mainly ethanol) are toxic to plants and responsible for cell injuries during anoxia.

2.1.9. Water temperature

Temperature is a factor affecting the survival of plants during submergence. It was reported that high temperature (30ºC) accelerates plant mortality, where as low temperature (20ºC) improves survival. High temperature decreases O_2 and CO_2 solubility in submergence water and accelerates anaerobic respiration leading to faster starvation and faster death of the plant (Ram *et al*., 2002). Das *et al*. (2009) hypothesize that warmer water increases seedling mortality, possibly through increased carbohydrate depletion during submergence and turbid water enhance plant mortality by effects similar to those caused by natural shading, the common consequence of cloudiness during the wet season. This could be due to reduction in light penetration, the subsequent chlorophyll degradation and reduced under-water photosynthesis. Singh *et al*. (2014)

found that Water temperature was slightly cooler in the morning and warmer in the afternoon. In general, water temperature ranged from 27 to 32 ºC.

2.1.10. Water pH

Singh *et al*. (2014) found that in the morning, floodwater pH was a little lower (7.4-7.8) than in the afternoon (7.9-8.1) and was lower at the water surface than at lower depths. The pH also increased from 7.4 at the beginning to 8.1 at the end of the submergence period.

2.2. Rice after desubmergence

It was revealed that after de-submergence, tissue injuries which developed underwater can be intensified as the floodwater recedes and shoots become re-exposed to the atmosphere (Sarkar *et al*., 2006). The major forms of stress plants must encounter upon de-submergence are explained in the following sections.

2.2.1. Photoinhibition

The photosynthetic carbon fixation and accumulation of carbohydrate are essential for biomass accumulation during submergence and after desubmergence. However, it was also reported that sudden increase in light intensity upon de-submergence threatens leaves accustomed to low-light underwater environments, causing photoinhibition to the photosynthetic apparatus (Osmond, 1994; Ella *et al.*, 2003a). For example, submergence increased minimal fluorescence in leaves of rice cultivars, which signifies photoinhibition (Panda *et al*., 2006). So, the first challenges for de-submerged plants are photoprotection and recovery from photoinhibition.

2.2.2. Production of reactive oxygen species (ROS)

It was observed that the main cellular components susceptible to damage by free radicals are membrane lipids (peroxidation of unsaturated fatty acids), proteins (denaturation), carbohydrate and nucleic acids (Blokhina *et al*., 2003). ROS can be generated under hypoxic conditions during submergence; severe lipid peroxidation by ROS can have a fatal consequence in submerged plants (Santosa *et al*., 2007). Hence, maintenance of membrane integrity is one of the key factors for plant survival underwater (Blokhina *et al*., 2003). It was observed that anoxia-tolerant plant species, such as *Acorus calamus* and *Schoenoplectus lacustris*, are able to preserve lipids during

submergence and after de-submergence, while significant lipid peroxidation happens in anoxia-sensitive plants, such as *Iris germanica*, upon re-oxygenation (Henzi and Braendle, 1993). It was reported that the production of ROS is far more intensified upon de-submergence, which is associated with an abrupt and concomitant increase in light intensity and O_2 concentration. Excessive formation of ROS is commonly found in plants re-exposed to the ambient conditions following submergence (Blokhina *et al.*, 2003; Bailey-Serres and Chang, 2005; Santosa *et al.*, 2007). On the other hand, some ROS and oxidation products can serve as important signaling molecules in plant cells (Bailey-Serres and Chang, 2005; Moller *et al*., 2007). They also indicated that the ROS species, superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical (OH^-) can be produced in a number of cellular reactions.

2.2.3. Accumulation of acetaldehyde

It was demonstrated that excess accumulation of acetaldehyde is harmful for organisms because of its tendency to form acetaldehyde-protein and acetaldehyde-DNA adducts. Acetaldehyde can be produced enzymatically from pyruvate or ethanol under both anoxic and hypoxic conditions with the levels produced during hypoxia being higher than anoxia (Boamfa *et al*., 2005). It was indicated that re-aeration can induce a transient burst of acetaldehyde emission (Zuckermann *et al.*, 1997; Tsuji *et al.*, 2003) as a result of rapid ethanol oxidation without coordinated oxidation of acetaldehyde (Boamfa *et al*., 2005).

2.2.4. Drought

It was observed that when the floodwater recedes, low hydraulic conductivity of submerged roots cannot provide enough water to meet aboveground transpirational demand, causing wilting of shoot (Luo *et al*., 2009).

2.3. Submergence effect on rice morphology and physiology

2.3.1. Shoot elongation

Most rice cultivars show shoot elongation in response to submergence. It was stated that shoot elongation during submergence is an ‘escape strategy’, which enables rice plants to resume aerobic metabolism and photosynthetic fixation of CO_2 by raising their shoots above the water surface (Ram *et al*., 2002; Jackson and Ram, 2003).

It was also stated that the shoot elongation of genotypes grown under turbid water condition was significantly reduced as compared with those grown under clear water and control condition (Cisse *et al*., 2010). The difference between turbid and clear water could be the clear indication that turbidity is a limiting factor to the plant shoot elongation. Elanchezhian *et al.* (2013) found that short term submergence enhanced the shoot elongation by about 56.9 %.

Singh *et al*. (2014) stated that shoot elongation was similar in different genotypes under controlled conditions, but it increased progressively with the duration of submergence. Rice genotypes that elongate only slowly underwater (*Sub1* types) are suitable for cultivation in flash-flood-prone areas, whereas genotypes that elongate faster during flooding are more useful for semi-deep and deepwater areas where floodwater stagnates in the field for durations of several weeks to months (Luo *et al*., 2011, Sarkar and Bhattacharjee, 2011; Vergara *et al*., 2014). Recently, it has been stablished that *Sub1* rice cultivars survive flooding by minimizing ethylene-promoted elongation underwater. The tolerant checks and *Sub1* introgression lines elongated at significantly slower rates compared with the sensitive cultivars. FR13A, the most tolerant genotype, had the slowest underwater elongation in their experiment.

2.3.2. Survival percentage

The *Sub1* gene was introgressed in Swarna (which is a popular rice cultivar in south and southeast Asia) through breeding and is currently being tested in farmers fields in India and Bangladesh. Introgression of *Sub1* into Swarna, greately enhanced its survival under submergence (Sarkar *et al*., 2006). Setter and Laureles (1996) found that survival of five rice cultivars was negatively correlated with elongation growth during complete submergence, with deepwater rice cultivars tending to have the lowest survival and greatest elongation relative to lowland cultivars. Elanchezhian *et al.* (2013) stated that submergence up to 14 days caused significant decrease in the survival of different rice genotypes, and the average survival was 59.71 %. However, the survival percentage was higher (*90 %) in landraces viz. FR 13A, Kusuma, Atiranga, Kalaputia, Gangasuili, and HYVs viz. Swarna *Sub1*, IR 64 *Sub1*, Sambha Mahsuri *Sub1* and Savitri *Sub1* than in the other genotypes. Setter and Laureles (1996) using five rice cultivars, showed a negative correlation (r = -0.81) between survival and shoot elongation growth.

Singh *et al*. (2014) found that survival decreased substantially due to submergence. When submerged for 12 and 17 days, Swarna *Sub1* showed, respectively, twofold and fourfold greater survival than Swarna. Among the tolerant genotypes, FR13A showed the highest survival, followed by IR49830 and Swarna *Sub1*. A similar response was observed for IR64 *Sub1* and Samba Mahsuri *Sub1* introgression lines. They also found that Submergence-induced shoot elongation correlated negatively with plant survival.

2.3.3. Leaf area, specific leaf weight and relative water content after desubmergence

It was recorded that the range of LAI was 3.70-5.21 in control condition, whereas it ranged from 1.35 to 4.69 under submergence condition (Elanchezhian *et al.,* 2013). Fukao *et al*. (2011) examined two rice genotypes, to evaluate the contribution of *Sub1A* to leaf water management during the early recovery period, RWC was monitored in the upper leaves of M202 and M202 (*Sub1*) plants after reoxygenation. Upon desubmergence, the RWC decreased rapidly in both genotypes, but in M202 (*Sub1*) RWC was moderated. Thus, *Sub1A* variety maintains higher leaf water content following reoxygenation under drought. It was observed that waterlogging can cause wilting of shoot organs in many plant species. This response is mediated by a decrease in the hydraulic conductivity of roots (Holbrook and Zwieniecki, 2003). Sakagami *et al*. (2009) found that the increase in shoot biomass and leaf area during submergence was strongly influenced by depth of submergence water. Mazaredo and Vergara (1982) showed that submergence-tolerant genotypes tended to have higher leaf area with greater levels of carbohydrate during submergence compared with submergence-susceptible genotypes. It was also revealed that submergence-tolerant genotypes had a larger leaf area and dry weight in the leaf blade and sheath when submerged for 10 d in water 45 cm deep (Joho *et al*., 2008).

2.3.4. Plant height and tiller number

Elanchezhian *et al*. (2013) found that short term flooding significantly affected the growth of different rice genotypes. It reduced the mean tiller number, plant biomass, and harvest index by 42.20, 15.15 and 52.63 %, respectively. Significant differences in plant height were observed between different varieties under control and stress treatment. The substantial reduction in tiller number was responsible for the decrease in grain yield. Haque *et al*. (2015) found that the number of effective tillers per hill was not significantly influenced by different water levels. The

number of effective tillers hill^{-1} obtained with submerged condition was 12.92 and with continuous saturated condition was 12.28. Chowdhury (1988) also revealed that the highest number of effective tillers m^{-2} with continuous flooding was significantly different from that obtained with continuous saturation. Zhang *et al*. (2015) found that compared with the control treatment, the panicle number increased by 4.2% after 1 day of submergence, with significant increases observed after 3 and 5 days of submergence (17.5% and 18.2%, respectively); short term submergence increased panicle number.

2.3.5. Duration of growth and development

Elanchezhian *et al*. (2013) found with different rice genotypes that short term flooding delayed days to 50 % flowering, however, the responses varied among genotypes. Sarkar *et al*. (2009) have also reported that partial flooding delayed flowering in Swarna *Sub1*, IR 42 and Varshadhan by 12, 6 and 9 days, respectively. Their study suggested that these responses are not related to *Sub1* but rather they are dependent on the genotypic background.

2.3.6. Dry matter accumulation and distribution to different organs after desubmergence

Singh *et al*. (2014) found that the *Sub1* lines and the tolerant checks (FR13A and IR49830) showed a significant increase in stem dry weight, the sensitive variety showed decrease in stem dry weight under submergence. They also found that the decreasing trends in leaf dry weight were similar to those of stem dry weight, but the magnitude of reduction in dry weights measured immediately after submergence was greater for leaves than for stems. Elanchezhian *et al*. (2013) observed significant difference in leaf and stem dry weight between submerged and control conditions. Above ground biomass decreased considerably under submergence stress as compared to control condition.

Significant genotypic differences were observed in above ground total dry matter accumulation both under normal and submerged conditions by Sarkar and Bhattacharjee (2011). They also found that the total above ground dry matter contents were greater under control condition followed by 14 and 20 days of submergence. They also recorded that the reduction in dry matter content was more than 90% in susceptible genotypes after 14 days of submergence.

Zhang *et al*. (2015) found that as the submergence deepened and the duration increased, different treatment plants showed decreases in the aboveground dry weight and root dry weight, but short-term submergence increased aboveground dry weight and root dry weight.

2.3.7. Soil Plant Analysis Development (SPAD) value, stomatal conductance and transpiration rate from anthesis to maturity

It was stated that the plants showing high SPAD values indicate non-senescent leaves with high content of chlorophyll (Siangliw *et al*., 2003). Debabrata and Kumar (2011) also stated that stomatal conductance found to be significantly decreased in both Swarna and Swarna *Sub1* during the progression of submergence as compared to control plant. They also found that after 7days of submergence stomatal conductance significantly more in Swarna *Sub1* compared to Swarna. It was observed that at physiological level, flooding modifies water relations and plants carbon fixation. Closing of stomata, with or without leaf dehydration, reduction of transpiration and inhibition of photosynthesis, are responses that can occur in hours or days, depending on the tolerance to flooding of each plant species (Bradford & Hsiao, 1982; Else *et al*., 1996; Insausti *et al*., 2001; Striker *et al*., 2005; Mollard *et al*., 2008; Mollard *et al*., 2010). Toojinda *et al*. (2003) measured leaf greenness with SPAD meter and found that submergence-tolerant plants are able to retain green leaves for longer than intolerant lines.

2.3.8. Intercellular CO_2 concentration and net assimilation rate

Sakagami *et al*. (2009) found that complete submergence adversely affected leaf area, indicating that contact with the air and the associated photosynthesis and a fully aerobic state is required. Emerged leaves of partially and completely submerged plants displayed an increase in photosynthetic rate. The photosynthetic rate at 37 Days after submergence in partial and complete submergence was closely related to the net assimilation rate (NAR) during submergence in the pot experiment.

2.3. 9. Relative growth rate (RGR), absolute grain growth rate (AGR) and stem reserve translocation (SRT)

The RGR represents the rate of increase in mass per unit of mass already present over a time interval. Yu *et al*. (2011) found that submergence significantly reduced relative growth rates (RGRs) in different grass plants. Sarkar and Bhattacharjee (2011) reported that the absolute growth rate (AGR, mg/plant) during the period of desubmergence was greater in control plants compared to the 14 days and 20 days of submerged plants. AGR during re-emergence was greater in 14 days submerged plants compared to the 20 days submerged plants. Improper grain filling under 10 days of submergence was reported to be due to reduction in carbohydrate content and less translocation of the same to sink (Palada and Vergara, 1972; Yoshida, 1972). When the current photosynthetic source is inhibited, grain filling becomes more dependent on mobilized stem reserves (Blum, 1988).

2.4. Submergence effect on rice physiology

2.4.1. Proline content after desubmergence

We know that plants incorporate 22 amino acids into their proteins. However, many plants also contain unusual amino acids, called non protein amino acids that are not incorporated into protein but are present instead in free form and act as protective substances. They are the organic compounds that do not interfere with enzyme functions and are known as compatible solutes. It was reported that proline is a non protein amino acid and compatible solute commonly accumulates in many plants exposed to various stress conditions such as water stress (Barnett and Neylor, 1966), salinity (Steward and Lee, 1974), air pollution (Godzic and Linskens, 1974) and unfavourable temperature (Chu *et al*.,1974; Chu *et al*.,1978). In plants proline is synthesized from glutamic acid through a pathway catalyzed by pyrroline-5-carboxylate synthetase and pyrroline-5-carboxylate reductase. It is suggested that it acts as an osmolyte/compatible as well as a source of nitrogen during recovery from stress. Proline accumulates in many plant species in response to environmental stress. Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism.

Proline accumulation has been reported during conditions of drought, high salinity, high light and UV irradiation, heavy metals, oxidative stress and in response to biotic stresses. Proline accumulation is important for the tolerance of certain adverse environmental conditions. It was also reported that compartmentalization of proline metabolism implies that extensive intracellular proline transport must occur between the cytosol, chloroplasts and mitochondria (Szabados and Savoure, 2009). Physiological data suggest that proline uptake into mitochondria is an active process. Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes. It was revealed that proline treatment can diminish ROS (reactive oxygen species) levels in fungi and yeast, thus preventing programmed cell death (Chen and Dickman, 2005), can protect human cells against carcinogenic oxidative stress (Krishnan, 2008), and can reduce lipid peroxidation in alga cells exposed to heavy metals (Mehta and Gaur, 1999). Proline treatment also alleviated Hg^{2+} toxicity in rice (*Oryza sativa*) through scavenging ROS, such as H_2O_2 (Wang *et al*., 2009). He also mentioned that the damaging effects of oxygen and hydroxyl radicals on Photosystem II (PSII) can be reduced by proline in isolated thylakoid membranes (PSII). Proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways. It was also reported that during stress conditions, the rate of the Calvin cycle is diminished, which prevents oxidation of NADPH and restoration of NADP+. Stabilization of proteins and protein complexes in the chloroplast and cytosol, protection of the photosynthetic apparatus and enzymes involved in detoxification of ROS are an important. Proline has also been found to serve as a substrate for respiration and as a source of nitrogen and other metabolites (Steward and Boggess, 1978). It was also reported that the relationship between proline accumulation and environmental stress suggests that proline could have a protective function. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress, 1997). But the over accumulation of active oxygen species and their contribution to cell damage induced by environmental stress is well known. In order to deal this effect, plants have evolved a number of protective scavenging or antioxidant defense mechanisms. It was also reported that apart from the enzymatic defense system (Bowler *et al*., 1992), the accumulation of free proline may also contribute to the scavenging of these active oxygen species by enhancing photochemical electron transport activities (Alia *et al*., 1991).

Banerjee *et al*. (2015b) found that proline content was significantly induced by submergence and there were variably in the varieties. Thus, the maximum accumulation of this particular stress responsive amino acid was observed in the variety Swarna with 1.88 fold and minimum in that of Swarna *Sub1A* by 1.168 fold. FR13A had the intermediatory value by 1.182 fold. On account of proline consideration, Swarna *Sub1A* had the minimum increase of proline content and thereby assumed to be more sensitive to water deficit stress so developed under submergence.

2.4.2. Relative injury of leaf tissue after desubmergence

It was stated that the reoxygenation injury commonly occurs primarily due to the overproduction of ROS upon sudden reexposure to atmospheric oxygen (Bailey-Serres and Voesenek, 2008; Blokhina and Fagerstedt, 2010). Upadhyay *et al*. (2009) revealed that submergence stress increased the membrane damage and was evident from increased value of electrical conductivity and lipid peroxidation. It was also reported that when ATP formation is reduced, the oxidation-reduction state between cell membranes becomes unbalanced and membrane permeability is increased. Hence, the solute leakage and electrical conductivity are increased, deteriorating cell membrane (Kawano *et al*., 2009).

It was revealed that the imposition of submergence may increase generation of reactive oxygen species (ROS) within the cell, particularly within the chloroplast of flood stressed plants, leading to lipid peroxidation, protein degradation, enzyme inactivation and affect nucleic acid and almost every component of cell leading to cell death (Blokhina *et al*., 2003; Sakagami *et al*., 2009).

2.4.3. Chlorophyll content after desubmergence

Singh *et al*. (2014) found that chlorophyll concentration in leaves decreased under submergence and with increasing submergence duration from 12 to 17 days. The sensitive and tolerant genotypes had similarly high leaf chlorophyll concentrations before submergence but, when submerged, the tolerant genotypes maintained more chlorophyll than the intolerant genotypes.

Submergence resulted in significant reduction of chlorophyll content both in Swarna and Swarna *Sub1* cv. Debabrata and Kumar (2011) found that after 7days of submergence the reduction in chlorophyll content was greater in Swarna (76%) than Swarna *Sub1* (56%) compared to the respective control plant.

Sarkar and Bhattacharjee (2011) stated that submergence caused a greater reduction of chlorophyll content. There were great genotypic differences in retention of chlorophyll level among the tolerant rice genotypes. Ella and Ismail (2006) stated that the relative concentration of both chlorophyll a and b, measured 3 days after submergence, decreased significantly, with a greater decrease in chlorophyll a than in chlorophyll b in both cultivars.

2.4.4. Soluble sugar and starch content after desubmeregence

Singh *et al*. (2014) found that the reduction in total soluble sugar and starch under submergence was about 60-70% in tolerant genotypes and about 80-90% in the sensitive genotypes. But there was no specific trend in differences between tolerant and sensitive genotypes in their stem sugar and starch concentrations before submergence.

In an experiment Pervin *et al*. (2010) determined the soluble sugar content in four varieties of rice separately at different ages of seedling. The sugar level of plant tissue was generally higher before submergence which decreased markedly due to submergence. Irrespective of variety, with the increase of seedling age soluble sugar increased. The positive relationship between amount of carbohydrate in plant parts and submergence tolerance was reported by many researchers (Panda *et al.,* 2008b, Chaturvedi *et al*., 1993). Sarkar and Bhattacharjee (2011) found that in general NSC concentrations before submergence were higher in tolerant genotypes compared to the susceptible genotypes with one exception.

It was reported that non-structural carbohydrate (total soluble sugar and starch) concentrations in shoot have long been associated with genotypic differences in ability to tolerate submergence in rice (Das *et al*., 2009). Elanchezhian *et al*. (2013) found that total soluble sugars (TSS) and starch concentration were higher in control than submerged condition. Das *et al*. (2009) also reported similar findings. Elanchezhian *et al*. (2013) confirmed that tolerant *sub1* containing genotypes and land races consistently maintained higher concentration of starch and total soluble sugars under control and submergence stress treatments.

Banerjee *et al*. (2015a) found that the variation of total carbohydrate depletion in the plants under submergence as compared to control was recorded maximum in case of shoot than root with 44.36 % and 40.56 % respectively. On varietal performance, the depletion in carbohydrate

content was maximum in Swarna (63.6 %) followed by Swarna *Sub1A* (38.3 %) and FR13A (19.8 %) in case of root. For shoot, the trend of decline in carbohydrate content was in order of Swarna *Sub1A* (65.8 %), FR13A (34.7 %) and Swarna (32.6 %). Therefore, Swarna *Sub1A* was evident to exercise more carbohydrate utilization from its shoot. Starch accumulation recorded a significant down regulation in case of Swarna under submergence and that was 26.5 % less compared to control. Similarly, the other two varieties namely Swarna *Sub1A* and FR13A observed the similar trend in depletion of starch content by 53.1 % and 44 % respectively.

2.5. Submergence effect on rice anatomy

Formation of aerenchyma is essential to the survival and for the functioning of plants subjected to waterlogging. It was reported that the aerenchyma contributes to O_2 supply from shoots to roots and to the ventilation of toxic gases (e.g. CO_2 and methane) from roots to shoots (Colmer 2003; Evans 2003). The ventilation of gases in aerenchyma is mainly caused by gas diffusion in rice, but in some wetland species with 'through-flow pathways,' gas flows can also occur by humidity- and Venturi-induced pressure flows (Armstrong *et al*., 1996). It was also reported that the aerenchyma may provide a photosynthetic benefit by concentrating CO_2 from root respiration and transporting it to the leaf intercellular spaces in some wetland plant species (Constable and Longstreth, 1994). It was decribed that the aerenchyma can be classified into two types: (i) schizogenous aerenchyma, which develops by cell separation and differential cell expansion that creates spaces between cells, in e.g. Rumex palustris; and (ii) lysigenous aerenchyma, formed by the death and subsequent lysis of some cells, e.g., in rice (Jackson, 1985), maize (Drew *et al*., 1981) and wheat (Trought and Drew, 1980). In the roots, lysigenous aerenchyma forms in the cortex, whereas in the stems it can form in the cortex and pith cavity (Armstrong, 1979).

In an experiment it was found that under flooded condition there was no clear change in leaf and stem tissues, while in flooded roots aerenchyma had been developed in comparison with control (Pourabdal *et al*., 2008). Aerenchyma formation creates an internal gas exchange channel from the aerobic shoot to the hypoxic roots. Air enters through stomata of leaves or lenticels on the stem and passes through the network of aerenchyma channels to the submerged roots. In an experiment with two rice cultivars, Arborio Precoce (AP) and 'FR13A' under complete submergence Parlanti *et al*. (2011) found that 'AP' leaf sheaths showed more constitutive

aerenchyma than 'FR13A' that increased following submergence in both 'AP' and 'FR13A'. In rice, aerenchyma develops through lysigeny (Hoshikawa, 1989; Matsukura *et al*., 2000) resulting from the selective death of root and shoot cortex cells (Kawai *et al*., 1998).

Aerenchyma formation in rice stems was found to be dependent on both ethylene and ROS accumulation (Steffens *et al*., 2011). Moreover, it was found that in arabidopsis hypocotyls, both ethylene and ROS are involved in aerenchyma formation under hypoxic conditions (Mühlenbock *et al*., 2007). A clear increase in H_2O_2 concentrations was found in submerged 'FR13A' plants (Parlanti *et al*., 2011)

2.6. Submergence effect on rice yield

2.6.1. Grain number and sterility percentage

Zhang *et al.* (2015) found an increasing panicle number and decreasing grain number per panicle and seed-setting rate as the submergence duration and depth increased. Haque *et al*. (2015) found that total grains per panicle were significantly influenced by the interaction effect of water levels and seedling number hill^{-1}. Continuous submergence showed higher grains panicle^{-1} irrespective of their seedling number hill^{-1} but in case of in case of saturated condition higher seedling number hill^{-1} resulted the lowest grains panicle^{-1}. The filled grains panicle did not differ significantly for water levels. They also found that the maximum number of filled grains (145.35) was found in submerged condition and the minimal number (137.20) of grains panicle^{-1} at saturated condition. The percent filled grain was 89.50 and 90.05 of total grains under saturated and submerged condition respectively. Analysis of variance showed that number of unfilled grain panicle^{-1} was not statistically differed due to different water levels. The maximum (16.07) and minimum (15.96) number of unfilled grains panicle^{-1}was recorded under continuous submergence and saturated condition respectively.

Zhang *et al*. (2015) observed that short term (1-5 days) submergence exhibited a significantly increased seed-setting rate (2.5%). They also observed an increased panicle number and decreased grain number per panicle and seed-setting rate as the submergence duration and depth increased. Taiz and Zeiger, (1998) stated that crop yield can be severely reduced and garden pea yields can be halved by only 24 hours of flooding.

2.6.2. 1000 grains weight and grain yield

Elanchezhian *et al*. (2013) found that the range of grain yield in control condition was 2.65-6.14 t ha^{-1}, whereas it ranges from 0.13 to 3.18 t ha^{-1} under submergence stress. Highest grain yield under submergence stress was observed in Swarna *Sub1*. The reduction in grain yield under short term stagnant flooding condition in rainfed lowlands is attributed to the alternation of growth and yield attributes, such as plant height, tiller number, dry matter accumulation, number and weight of panicles and harvest index (Sarkar, 1998; Sarkar and Das, 2003). Elanchezhian *et al*. (2013) found a highly significant positive correlation (r = 0.91) between seedling survival and grain yield. It was suggested that increase in grain yield was a consequence of number of seedlings survived after submergence. Zhang *et al*. (2015) found that the yields in all submergence treatment plants were significantly decreased compared with that of the control plant with an exceptional plants (1 day complete submergence), where submergence treatment increased grain yield. They also stated that short-term (1 day) complete submergence significantly increased rice yields. Wang *et al*., (2014) investigated the influence of slight submergence (2 and 4 days) on mid-season rice at the final phase of the tilling stage and found that the yields of the experimental groups were close to those of the control group.

Haque *et al*. (2015) found that the weight of 1000 grains was statistically unaffected by the variation of water levels. Comparatively heavier (19.86 g) 1000 grain weight was found under saturated condition which was similar with that of submergence condition. Result was similar with the findings of Patel (2000) who observed similar 1000 grain weight under saturated and submerged condition. Short term submergence significant increase in the seed-setting rate as well as increases in panicle number, grain number per panicle, and 1000-seed weight compared with the control plant after 1 day of submergence, which was the primary reason for the significant yield increase in the 1day treatment.

2.6.3. Total dry matter content and harvest index

According to the literature, rice submergence can result in leaf discoloration into yellow, a decrease in the number of green leaves and white roots, a decrease in root absorption, impaired growth and development, and a decrease in lodging tolerance, which can result in

underproduction or even a complete lack of yield (Vriezen *et al*., 2003; Kawano *et al*., 2009; Colmer and Voesenek, 2009; Crawford, 2003)

Haque *et al*., (2015) demonstrated that the biological yield was not significantly varied for the water levels. The maximum biological yield (14.47 t ha^{-1}) was obtained from continuous submerged condition which was statistically similar (13.86 t ha^{-1}) with the saturated condition. Statistically similar biological yield might be due to the similar grain yield and straw yield under different water levels. They also found that the harvest index was not statistically influenced by the water levels. However, the maximum (45.03 %) harvest index was found at submerged condition and the minimum harvest index (44.65) was at saturated condition.

Kawano *et al*. (2009) found that submerged rice plants did not show significant increases in dry matter weight during submergence while gaining significant shoot length. The question arises how the plants accomplish shoot elongation during submergence under energy-limited conditions. Shoot elongation in different rice cultivars was achieved by means of extension growth by developing leaf sheaths during submergence. Some cultivars did not show significant correlations between whole-plant dry matter weight and shoot elongation during submergence.

2.7. Strategies for submergence tolerance

Some cultivars use two distinct strategies of growth controls to survive under submerged conditions. One of the strategies is a quiescence strategy [i.e., the low-oxygen quiescence syndrome] (Colmer and Voesenek, 2009), in which shoot elongation is suppressed to preserve carbohydrates for a long period (10-14 days) under flash-flood conditions, which could positively affect the survival rate and the generation of new tissues after desubmergence (Panda *et al*., 2008b). Submergence-tolerant cultivars can restart their growth during desubmergence by using preserved carbohydrates. Another strategy is an escape strategy [i.e., the low-oxygen escape syndrome] (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009), which involves fast elongation of internodes to rise above the water level and is used by deepwater rice cultivars. The costly escape strategy is adventitious in shallow flood water. But their elongation growth can exhaust energy reserves and cause death when the flooding depth is deep and the flooding period is long (Bailey-Serres *et al*., 2010; Jackson and Ram, 2003); both strategies depend on ethylene-responsive transcription factors (Hattori *et al*., 2009; Xu *et al*., 2006).

2.8. Plants adaptation traits under submergence stress

2.8.1. Morphological and anatomical adaptation

a) Shoot elongation

Most rice cultivars elongate their shoot during submergence. It was suggested that in small seedlings, this response is restricted to emerging leaves. This is one of the escape strategies for adaptation to submergence that promotes a return of part of the foliage to the air (Kende *et al*., 1998). The mechanisms of plant adaptation to excessive flooding depend on the water regime. They also reported that in deepwater areas with >100 cm water depth for 2-3 months, cultivars with sufficient capacity for internode elongation maintain their foliage above the water surfaces to sustain leaf photosynthesis and oxygen transport, leading to better survival.

It was observed that in contrast to deep water rice, rainfed low land rice is subjected to flash flood (>50 cm water depth), ranging from 1 to 2 weeks, where elongation growth is not sufficient for leaves to regain contact with the air (Ram *et al*., 2002). Reduced elongation of plants occurs under flash flood conditions which is necessary for survival because elongating plants would tend to lodge as soon as the water level recedes (Jackson and Ram, 2003). It was affirmed that the negative relationship between flash-flood tolerance and shoot elongation during submergence was confirmed using 903 cultivars from the International Rice Research Institute (IRRI) gene bank collection (Setter and Laureles, 1996).

b) Aerenchyma formation

It was reported that the presence of gas filled spaces, known as aerenchyma, in roots of numerous plant species is considered to be an important anatomical adaptation for survival under flooded conditions (Justin and Armstrong, 1987). It was also reported that the aerenchyma may provide a photosyntheticbenefit by concentrating CO_2 from root respiration and transporting it to the leaf intercellular spaces in some wetland plant species (Constable and Longstreth, 1994). Formation of aerenchyma may be initiated by ethylene. It was also suggested that the formation of aerenchyma in rice roots has little or no requirement for ethylene, and rice roots normally form aerenchyma in well aerated conditions (Jackson, 1985). Although O_2 transport through aerenchyma is most significant when the shoot is above water, this pathway may be used to

transport some of the O_2 produced in the underwater photosynthesis when sufficient light penetrates into the above canopy through water. It was found that the formation of aerenchyma during flooding occurs not only in the roots but also in the leaves (Vartapetian and Jackson, 1997). It was also reported that the aerenchyma allows rapid gas movement from shoots to roots and promote root growth and survival in O_2 deficient conditions (Ap Rees and Wilson, 1984; Armstrong, 1979). Such gas-filled channels would supply O_2 for root respiration and release O_2 into the rhizosphere for oxygenation. Recently, aerenchyma formation in rice stems was found to be dependent on both ethylene and ROS accumulation (Steffens *et al*., 2011). *Sub1A* gene represses submergence-induced ethylene formation (Bailey-Serres and Voesenek, 2010), and may hamper aerenchyma formation in *Sub1A* varieties. *Sub1A* varieties do not grow in response to submergence. It was suggested that the important of aerenchyma in deep flooding not only to have access to O_2 above the water level, but rather to rely on O_2 produced and stored during photosynthesis in the underwater organs, or retained by the leaf surface gas film (Colmer and Pedersen, 2008b; Pedersen *et al*., 2009). The aerenchyma formation was examined (Parlanti *et al*., 2011) in 'FR13A', a *Sub1A* variety, and in 'Arborio Precoce' ('AP'), a non-*Sub1A* variety displaying fast shoot elongation when submerged. The results showed the presence of constitutive aerenchyma in both 'AP' and 'FR13A' varieties, which further increased under submergence. Submergence-induced ethylene synthesis was observed in 'AP' only, 'FR13A' did not show any increase in ethylene production. The results suggest that aerenchyma formation in 'FR13A' is independent of ethylene signalling, and ROS appear to be important to regulate aerenchyma formation in this *Sub1A* variety.

c) Formation of adventitious root

Ahmed *et al*. (2013) found that the portion of the plant stem in flooded condition produces adventitious roots grows horizontally (diageotropism). This may be the new roots which replace old root systems (Jackson and Drew, 1984). Because of the position of the new roots is close to water surface and they are connected to the stem, close to the formatted aerenchyma, they have more access to oxygen than the old root system. Large air space between these roots enables diffusion of gas between roots and shoots. It was stated that the adventitious root primordia of deep water rice initiates as a normal plant development but the formation is initiated by the death of nodal epidermal cell covering the tip of primordia which occurred as a result of flood induced

ethylene development (Mergemann and Sauter, 2000). It was also reported that the adventitious roots are those that emerge from stem tissue under conditions of partial to complete submergence. The formation of these specialized roots takes place when the original root system becomes incapable of supplying the shoot with the required water and minerals (Mergemann and Sauter, 2000). These can replace compromised roots and provide efficient aerenchymatous connections between aerial shoot tissues and submerged organs. Adventitious roots can form via de novo meristem initiation or the emergence of preexisting root primordia. It was indicated that in case of adventitious root emergence at lower stem internodes of flooded rice, the process involves signal transduction within the growing root and the overlying epidermal cells (Steffens and Sauter, 2009, Steffens *et al*., 2011). Ethylene and ROS-dependent signaling creates mechanical force to the overlying epidermal cells. The force exerted on the tightly attached epidermal cells directly above the primordia triggered localized cell death (Bailey-Serres *et al*., 2012).

d) Formation of gas film layer

Pedersen *et al*. (2009) stated that under completely submerged condition, the leaves of rice species retain a surface gas film micro layer. Moreover, leaf gas films, which are a micro-layer of air trapped between submerged leaves and the surrounding water, contribute to the internal aeration during submergence, thereby increasing submergence tolerance in rice (Colmer and Pedersen, 2008b; Pedersen *et al*., 2009; Raskin and Kende, 1984). Gas film layer on submerged leaves enlarge the water gas interface. Gas films can facilitate O_2 and CO_2 exchange of completely submerged leaves, with benefits for leaf respiration and net photosynthesis (Colmer and Pedersen, 2008a). It was reported that the gas films on leaves of rice can enhance underwater photosynthesis by supplying CO_2 during the day to provide additional sugars and O_2 which can increase tolerance of plants to complete submergence (Mommer and Visser, 2005), as it provides O_2 for internal aeration (Waters *et al*., 1989; Colmer and Pedersen, 2008b) and photosynthates (Setter *et al*., 1989) for respiration/fermentation and growth at night. Even in darkness, O_2 entry from floodwaters was improved, and O_2 that entered shoots moved via the aerenchyma to the roots.

e) Formation of a barrier to radial O_2 loss (ROL)

It was observed that suberization and/or lignification of the cell walls in the root layers exterior to the aerenchyma is implicated in the development of a tight barrier to ROL (Armstrong *et al*., 2000; Garthwaite *et al*., 2008; Kotula *et al*., 2009; Soukup *et al*., 2007). It was also observed that the levels of suberin, as well as lignin, released from the outer root sleeves are several times greater in plants grown in oxygen-deprived media compared with plants grown in aerated solution (Kotula *et al*., 2009; Ranathunge *et al*., 2011). The extra suberin and lignin deposited in the roots in stagnant solutions may effectively clog the wall pores, making a barrier sufficient to block the passage of oxygen and ions, but not water, which is mainly bulk and viscous in nature (Kotula *et al*., 2009; Ranathunge *et al*., 2011). It appears that rice roots living in anaerobic media can retain oxygen in the aerenchyma while taking up sufficient water (Kotula *et al*., 2009; Ranathunge *et al*., 2011). Such a barrier on the periphery of the cortex may not only reduce the loss of O_2 to the rhizosphere but could also protect the plant from phytotoxin produced by microorganisms in the environment surrounding the roots (Soukup *et al*., 2007).

It was reported that the oxygen molecules diffusing longitudinally through aerenchyma toward the root tips may be either consumed by respiration or diffused radially to the rhizosphere (Nishiuchi *et al*., 2012). ROL, the flux of O_2 from the aerenchyma to the soil, is determined by the concentration gradient, the physical resistance to O_2 diffusion in a radial direction, and consumption of O_2 by cells along this radial diffusion path (Colmer, 2003). It was also reported that the roots of many wetland species, including rice, have the ability to prevent ROL to the rhizosphere by forming a barrier in the root peripheral cell layers exterior to the aerenchyma (McDonald *et al*., 2002; Visser *et al*., 2000). This adaptive trait enhances longitudinal O_2 diffusion through the aerenchyma towards the root apex by diminishing losses of O_2 to the rhizosphere, thereby enabling the roots to elongate into anaerobic substrates (Armstrong, 1979).

2.8.2 Physiological adaptation

a) Sustained sugar supply and energy metabolism

Singh *et al*. (2001) stated that submergence tolerance is related to high carbohydrate supply during submergence. Carbohydrate metabolism during submergence seems to be an important

factor in flash-flood tolerance and this strategy is characterized by slow expansion growth that is presumed to conserve energy.

It was reported that carbohydrate concentration before and during submergence has long been recognized as an important factor in submergence tolerance in rice. For example a strong positive correlation between carbohydrate concentration prior to submergence and tolerance to submergence is commonly observed (IRRI, 2006). The carbohydrates remaining after submergence is more important than that before submergence. Studies using techniques that alter the concentration of carbohydrates before or during submergence support the significant role carbohydrates plays during submergence.

b) Alcohol fermentation

It was revealed that the anaerobic response of plant tissues is the adaptive metabolic mechanism of increasing rate of alcoholic fermentation (AF) which involves alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) as the two key enzymes. Submergence can shift aerobic respiration to the less efficient anaerobic fermentation pathway as the main source of energy production. It was also revealed that the acetaldehyde is one of the intermediate of alcoholic fermentation, which can be oxidized by aldehyde dehydrogenase (ALDH) and found to be low in plants having higher activities ALDH with concomitant increase in submergence tolerance (Sarkar *et al*., 2006). Alcoholic fermentation is the key catalytic pathway for recycling NAD (nicotinamide adenine dinucleotide) to maintain glycolysis and substrate level phosphorylation in the absence of oxygen (Ap Rees *et al*., 1987)

c) Ethylene production

C_2H_4 (ethylene) is a plant hormone and it is related to the stress response. It plays a vital role in modifying the plant's morphology, physiology, biochemistry, and gene expression to allow the plant to withstand adverse conditions (Zhang *et al*., 2015). It was reported that the accumulation of ethylene in waterlogged soil and plants occurs at concentration of 10 cm^3 dm^{-3} (Musgrave *et al*., 1972). This accumulation of ethylene occurs in mainly two ways, firstly ethylene diffusion rate from root to water is 10 times slower than in air (Stünzi and Kende, 1989) and, ethylene synthesis is increased in the hypoxic root system and in the aerobic shoot (Jackson, 1985). It was

also reported that at first ethylene may be released to the internal channels of aerenchyma and then diffused to the root area. In roots high amount of ACC (1-amino cyclopropane 1-carboxylic acid) synthesis is occurred, which is a precursor of ethylene (Bradford and Yang, 1980). ACC to ethylene conversion cannot happen without oxygen. Highest amount of ACC accumulation occurs in the lowest portions of the stem and ethylene is released in the presence of oxygen. They also found that in partial submergence rice undergoes through a special pathway initiated by the entrapment of ethylene in the plant cell. As a result ethylene encourages degradation of abscisic acid (ABA) and increases the content of gibberellic acid (GA) and their downstream effects. Remarkably, the rates of stem elongation in deepwater rice varieties may be up to 25 cm/day.

d) Hormonal regulation

It was stated that the interplay of ethylene, abscisic acid (ABA) and gibberellin (GA) regulates shoot elongation in submerged plants. Ethylene accumulation reduces ABA levels by inhibiting ABA biosynthesis (Benschop *et al*., 2005) and at the same time increasing ABA degradation (Saika *et al*., 2007). Benschop *et al*. (2006) mentioned that this decline of ABA, in turn, releases repression of GA biosynthesis and thus increases the GA concentration in cells, leading to shoot elongation. On the other hand, Hattori *et al*. (2009) stated that flooding-induced elongation growth and carbohydrate catabolism are regulated by ethylene-responsive transcription factors in rice; *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*) genes stimulate elongation growth in leaves and stems of deepwater cultivars, whereas *SUBMERGENCE1A-1* (*Sub1A-1*) gene suppresses elongation growth and carbohydrate catabolism in rice cultivars (Fukao *et al*., 2006; Xu *et al*., 2006)

e) Anaerobic proteins (ANPs)

It was stated that the plant reaction to oxygen deprivation is expressed as the repression of most aerobic protein synthesis and induction of a number of so called ANPs. Submergence and/or anoxia-tolerant and intolerant species may differ in the number and the level of production of these ANPs. Most studies on the expression of ANPs under hypoxia or anoxia emphasizes the importance of energy metabolism, since the majorities are enzymes of glycolysis and fermentation. It was also reported that these proteins fall mainly in three functional group: (i)

enzymes mobilizing sucrose (sucrose synthase) or starch hydrolysis (α-amylase), (ii) main glycolytic enzymes (glucose phosphate isomerase, fructose1,6-bisphosphate aldolase, glyceraldehydes-3-phosphate dehydrogenase) and (iii) enzymes of alcoholic fermentation (PDC and ADH). Lactate dehydrogenase is another enzyme expressed at higher levels under anoxia indicating the importance of lactate production in submergence and anoxia tolerance in plants (Ram *et al*., 2002).

f) Antioxident defense systems

Luo *et al*. (2009) stated that chloroplasts and peroxisomes are major organelles of reactive oxygen species (ROS) generation in green tissues while it is mitochondria in non-green tissues. To prevent or minimize damage caused by ROS, plant cells are equipped with antioxidant defense systems, consisting of antioxidants such as ascorbate, α-tocopherol, glutathione and carotenoids as well as antioxidant enzymes. He also indicated that that the major pathways of ROS scavenging occur in chloroplasts, peroxisomes and mitochondria of plant cells. Peroxidation reactions triggered by ROS can be terminated by the action of superoxide dismutase (SOD), the major scavenger of superoxide anion (O^{2-}) in almost all cellular compartments. H_2O_2 is detoxified by ascorbate peroxidase (APX) in the chloroplasts, cytosol, mitochondria, apoplast or in peroxisomes.

CHAPTER 3

MATERIALS AND METHODS

This research work comprises of three pot-culture experiments in the field. The materials and methods of the experiments are as follows-

3.1. Description of the experimental site

3.1.1. Location and duration

The experiment was conducted at the research farm (Plot no. 42) and Plant Physiology Laboratory, Dept. of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The research area was located under the Agro-ecological zone of Madhupur Tract (AEZ-28) which was in 23º77ˈN latitude and 90º33ˈE longitude at an altitude of 9m above the sea level (BCA, 2004). The duration of the experiment was July to November (Aman season) of 2012. The morphological characters of the experimental field are presented in Appendix 2.

3.1.2. Climate and soil

The meteorological data during the crop growth period are presented in Appendix 3. The climate of this area is sub-tropical. The soil used in this experiment was deep red brown terrace soil under Tejgaon series. The textural class of the soil was silty loam. The soil analysis was done at the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka. The physical and chemical properties of soil are furnished in Appendix 4.

3.2. Experimental design and treatment

Design : The experiment was conducted in two factors randomized complete block design with four replications.

Treatments: The experiment consisted of two factors.

(I) Factor A- Submergence durations:

i) 0 day under water or control (no submergence)

ii) 7 days under water

iii) 14 days under water

iv) 21 days under water

(II) Factor B- Rice genotypes:

i) BRRI dhan52

ii) IR 64 sub1

iii) FR 13A (tolerant check)

iv) BR5 (susceptible check)

There were altogether 16 treatment combinations in case of data collection after recovery period (15 days after desubmergence) to harvest. But there were 8 treatment combinations in case of data collection just after desubmergence.

3.3. Plant materials collection

Seeds of the different rice (*Oryza sativa* L) genotypes, such as BRRI dhan52, IR 64 sub1, FR 13A and BR5, were collected from the gene bank of Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

BRRI dhan52

This variety was developed by Bangladesh Rice Research Institute. The variety became suitable for flashflood area where submergence occurs for 12 to 14 days and it gives more yield in this situation compared to BR11. It gives more or less same yield as BR11 does under normal situation. This variety was released in 2010 (Anon. 2010).

Characters:

1. It is tolerant to flash flood submergence.
2. Its average plant height is 116 cm.
3. Strong stem prevents lodging.
4. Sightly photoperiod sensitive.

5. Lifecycle is completed in 140-145 days under normal situation and 150-160 days under 14 days of submergence.
6. Yield ranges from 4.5 to 5.0 t/ha under normal condition and 4.0 to 4.5 t/ha when submergence in effect.

IR64Sub1

The flood tolerant IR64Sub 1 variety is a paddy of short duration and has created new hope among farmers for its early harvest to combat monga. It also gives excellent yields even after its 15 days submergence under floodwaters. IR64Sub1 flood tolerant variety can sustain submergence for 15 days and gives average yield of about 4 t/ha. The farmers harvest the paddy within 115 to 120 days after sowing seeds using dry or wet Direct Seeded Rice methods and in 90 days after transplantation of 25 to 30 day old seedlings everywhere including the flood-prone areas. Farming of IR64Sub1 paddy is more beneficial as it survives submergence for 15 days when the other short duration paddies like BRRI dhan33, Binadhan-7 and BU dhan1 cannot survive if submerged under floodwaters for a long. It gives excellent slender grain rice. The farmers have been cultivating the anti- monga and short duration flood tolerant IR64Sub 1 paddy during the past couple of years and getting excellent harvests during the peak monga hour in Aswin-Kartik (Anon. 2010).

FR 13A

FR13A (FR stands for 'flood resistant') has long been a traditional Indian rice cultivar that could withstand up to 14 days of flooding. It is only one submergence tolerant land race (Anon. 2010).

Characters:

1. Its lifecycle is around 140 days
2. Its days to flowering occur within 114 days
3. Its average plant height is 76.9 cm
4. Number of tiller ranges from 12 to13.
5. Number of panicles per plant ranges from 7 to 8.
6. 1000 grain weight approx. 23.4 g.

BR5

BR5 or Dulavog is a rice of aman season. BR5 rice was released by Bangladesh Rice Research Institute in 1976 from local Badshavog through pure line selection method. BR5 is a photo-sensitive variety and very suitable for late cultivation. Its yield is almost double compare to other local variety. Its market prize is good, so the farmer would be by cultivating this variety.

Characters:

1. This is an aromatic rice and is suitable for making fried rice or polao.
2. Its average height is 120 cm.
3. Kernel is small and round in shape.
4. Its lifecycle is completed around 150 days.
5. Average yield 3 t ha^{-1}.

Justification of selecting these genotypes for the present study

The number of submergence tolerant rice variety is not enough in Bangladesh; moreover, the present exiting submergence tolerant rice varieties need to be improved. In this situation, it is important to figure out the nature of morpho-physiological traits under submergence condition for further improvement of those tolerant varieties.

3.4. Seed treatment and germination

The seeds were treated with Bavistin solution for 20 minutes. The solution was prepared by dissolving 3 gm of Bavistin in 1 liter of water. When the treatment was completed then the seeds were washed with clean water and placed for sprouting in a traditional practice using straw and cloth. After two days all the seeds were well sprouted.

3.5. Seed bed preparation, sowing and seedlings raising

The seed beds were prepared in a plastic rack. Separate rack was used for each genotype. A plastic sheet was placed at the bottom of the soil. Fertilizer was applied as per recommendation in the seed beds. After pudling of the soil the sprouted seeds were sown in the separate seed beds as per 60-80 g seeds per square meter. Then, the seed beds were covered with mosquito net to

prevent from bird damage and watered regularly. The seedlings were raised for 30 days in the seed beds.

3.6. Pot preparation

Sufficient earthen pot was taken as per treatment, replication and data collection. The pots were 25cm in height and 28cm in upper portion diameter. Each pot contained 10kg of pot soil. Each pot was tagged with enamel paint.

3.7. Fertilizer application

The pots were fertilized with cow dung 40g/pot, urea 1.72g/pot, TSP 1.44 g/pot, MP 0.8g/pot corresponding to 15 ton/ha cow-dung, 215 kg urea/ha, 180 kg TSP/ha and 100kg MP/ha. All TSP, MP and 1/3 of the total Urea were applied as basal dose. The remaining 2/3 of the Urea was applied in two equal splits in each pot at 30 and 50 days after transplanting (DAT).

3.8. Transplanting

Thirty-days-old seedlings were transplanted to the pots. Each pot contained one healthy seedling only. The seedlings were transplanted immediately after uprooting from the seed bed.

3.9. Intercultural operations

Different intercultural operations were done to ensure the growth and development of the crops. The pots were watered regularly. Weeding was done as and when necessary. Plant protection measures, such as mechanical and chemical methods were used to keep the free from insect attack. Top dressing of urea was done as per schedule.

3.10. Making a submergence tank

A concrete submergence tank was prepared at the middle of the experimental field in front of the administrative building of Sher-e-Bangla Agricultural University. Sufficient water supply was ensured by making pipe line and tap. There was an outlet at the bottom of the submergence tank for drainage of water. The water level was maintained around 125cm from the bottom of the tank. Water level was checked daily.

3.11. Submergence induction

After 10 days of transplanting when the seedlings were well established in the pot soil then all the pots except control treatments (no submergence) were placed inside the tank and the water level was gradually increased up to 125cm. The pot containing plants were submerged for different days (7 days, 14 days and 21 day) as per treatments. The water was made turbid daily by mixing mud. Different data was collected just after desubmergence, 5 days after desubmergence, 15 days after desubmergence, during anthesis, during grain filling and finally after harvest.

3.12. Characterization of experimental environment

Submerged water O_2 concentration (measured by Dissolved oxygen meter, model-HI9142, Hanna instruments, USA), pH (measured by G5-pH meter, Model: sensION+PH3), temperature and incident irradiance (measured by G10-Light meter, Model- LX, Taiwan) of underwater canopy area of the submerged plants and in the air were measured during submergence as discussed by Singh *et al*. (2009).

Table 1. Environmental characters of the experimental site

Water levels	Light intensity (μ mol photons m^{-2} s^{-1})	pH range	Dissolve O_2 range (mg/L)	Water temperature (ºC)
Upper level	1250-1400 (in air)			
Mid level of submerged plant canopy	285-350	7.5-8.5	8.2-9.7	29-31
Lower level submerged plant canopy	11-52			

3.13. Experiment 1:

Morpho-physiological responses of selected rice genotypes as influenced by different submergence durations

The present study was undertaken with the following objectives-

►to study the responses of different rice genotypes under various submergence durations considering their morphological and physiological characters

►identifying the important morphological and physiological characters that help plant to survive under submergence.

3.13.1. Data collection:

The data were recorded on the following parameters

3.13.1.1. Percent elongation of shoot

The shoot length (the height of the seedlings from the base to the tip of the leaf) of the seedlings of all the rice genotypes was taken before submergence and just after de-submergence and percent elongation was computed using following formula according to Hossain *et al*. (1983).

$$\text{Percent elongation} = \frac{\text{Final plant height} - \text{Initial plant height}}{\text{Final plant height}} \times 100$$

3.13.1.2. Percent survival

The seedling survival was observed after 15 days of desubmergence (of 7, 14 and 21 DS treatments) and the percent survival was calculated as described by Supapoj *et al*. (1979). The percent survival of rice genotype for each submergence treatment was calculated as follows-

$$\text{Percent survival} = \frac{\text{Number of live seedlings after 15 days of desubmergence}}{\text{Number of live seedlings before submergence}} \times 100$$

3.13.1.3. Submergence tolerance score:

Submergence tolerance score was recorded after 5 days of desubmergence from each treatment (7, 14 and 21DS) as Hossain *et al*. (1983). Scoring for submergence tolerance, were made based on the phenotypic appearance as follows-

Table 2. Plant scoring after desubmergence.

Plant characters	**Score**
Erect, green, no elongation or slight elongation	1
Erect, green, elongated considerably	3
Elongated and bent at the middle, pale in color	5
Much elongated and lodged (flat)	7
Very much elongated and apparently dead	9

3.13.1.4. Days to flowering

Days to flowering was calculated from the date of germination to the date of 50% of the spikelet of a panicle were open for anthesis.

3.13.1.5. Days to maturity

The maturity of grain was determined when the grain weight was found maximum and the color of the grain turned yellowish.

3.13.1.6. Plant height at maturity

The plant height was measured at maturity from soil level to tip of the panicle. It was measured in cm unit.

3.13.1.7. Tiller number at maturity

The tiller number of the plant was counted at maturity.

3.13.1.8. Relative growth rate (RGR)

RGR is a measure to quantify the speed of plant growth. It expresses the rate of increase in dry matter per unit amount of dry matter produced. RGR was calculated growth rate per unit plant weight according to Tanaka *et al*. (1996).

$$\text{RGR } (g\ g^{-1}day^{-1}) = \frac{W2 - W1}{W1(T2 - T1)}$$

Where,

W2 = Total dry weight (g) at the time T2

W1 = Total dry weight (g) at the time T1

T2 – T1 = Time interval between first and second sample collection

3.13.1.9. SPAD value from anthesis to maturity (from the flag leaf of main stem)

The greenness of the flag leaf of main stem was observed by SPAD meter (model- SPAD-502 Plus, Japan) starting from the day of anthesis to maturity at five days intervals.

3.13.1.10. Leaf conductance / stomatal conductance

The stomatal conductance of main stem flag leaf was measured by leaf porometer (G9-Leaf Porometer, model: SC-1, USA) from the day of anthesis to maturity at five days interval.

3.13.1.11. Photosynthesis gas exchange system

1. **Net assimilation rate of CO_2 from anthesis to maturity**

The net assimilation rate of main stem flag leaf was measured by 'LCpro+ photosynthesis gas exchange system' (model: LI-6400XT, USA) from the day of anthesis to maturity at five days interval and the average value was calculated for analysis. At the same time, the following parameters were also measured by this equipment and the average value was calculated for analysis.

2. **Intercellular CO_2 concentration**
3. **Transpiration rate**

3.13.1.12. Stem reserve translocation

To determine the pre-anthesis photosynthetic stem reserve translocation towards the final kernel weight the method described by Gallagher *et al*. (1976) has been used. This is based on the net loss in weight of stem between anthesis and maturity with the difference between yield and net assimilation. It was calculated as follows-

$$\text{Stem reserve translocation (\%)} = \frac{S1 - S2}{G2 - G1} \times 100$$

Where,

S_1 = Stem dry weight at anthesis

S_2 = Stem dry weight at maturity

G_1 = Grain dry weight at anthesis

G_2 = Grain dry weight at maturity

The main stems were used to calculate stem reserve translocation. The main stems were marked by color thread for easy identification during subsequent sampling.

3.13.1.13. Estimation of leaf area (LA), specific leaf area (SLA), specific leaf weight (SLW) and leaf weight ratio (LWR)

LA, SLA and SLW were calculated after desubmergence. LWR was calculated after harvest as Hossain *et al*. (2011). Plant samples were uprooted carefully and separated into leaf, stem, panicle and root. For leaf area, randomly three upper fully expanded leaves from each treatment were collected and their length and breadth were measured. The area of each leaf blade was computed as follows-

$$\text{Leaf area} = k \times l \times w$$

Where,

k = adjustment factor

l = length of leaf blade

w = breadth of leaf blade

The value of k varied with the genotypes, growth stage and slope of the leaf. To determine the dry weight of leave and stem, the samples were at first air dried for 6 to 8 hours. The leaf and stem were then packed in separate brown paper packet and were oven dried for 72 hours at 75 ºC. Dry weight of leaf and stem were recorded separately with an electronic balance. Dry weight of leaf, stem and panicle were altogether regarded as total above ground dry matter. For the calculation of LWR, the panicle dry matter was also taken. Then the SLW, SLA and LWR were calculated with the following formulae-

$$\text{SLW} = \frac{\text{Dry weight of leaf}}{\text{Leaf area}}\ \text{g/cm}^2$$

$$\text{LWR} = \frac{\text{Leaf dry weight}}{\text{Above ground dry weight}}$$

3.13.1.14. Dry matter content of leaf blade, stem and root at harvest

The maturity of the plants was determined by SPAD value of flag leaf (below 20) and grain observation. Plants were carefully uprooted at harvest. All the plant parts such as panicle, leaf blade, stem with leafsheath and roots of each plant were separated. The panicles were packed in separate brown paper packet for each treatment. To determine the dry weight of leaves, stems and roots the samples were at first air dried for 6 to 8 hours. Leaves, stems and roots were then packed in separate brown paper packet and were oven dried (by G8-Drying Oven, Model: FN500, Turkey) for 72 hours at 70ºC temperature. Dry weight of leaves, stems and roots were recorded separately with an electronic balance. Dry weight of leaves, stems and roots were altogether regarded as total vegetative dry matter. Dry weight of leaves, stems, panicle and roots were altogether regarded as total dry matter.

3.13.1.15. Dry matter content of main stem and leaf at harvest

The panicle, leaf blade and stem with leaf sheath of main stem were packed separately and were oven dried and weighted as the same procedure described above.

3.13.1.16. Relative water content of leaf

The fully developed upper three leaves of each plant were carefully sampled after two hours of desubmergence from each treatment. Their fresh weight was measured immediately after sampling. Thereafter, the leaves were immersed in distilled water for 24 hrs at room temperature in the dark. Then the leaves were weighed after removing excess water by gently wiping with paper towel to determine their turgid weight. The leaves were then dried in oven for 72 hrs at 70ºC temperature to determine their dry weight. The fresh, turgid and dry weight of the leaves were used to calculate the relative water content of leaves according to Ghannoum *et al*. (2002) as follows-

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

3.13.1.17. Absolute grain growth rate

During anthesis, different main panicles were tagged; some were sampled and packed in separate brown paper packet as per treatment. Then the packets were kept in oven at 70ºC for 72 hrs. The tagged panicles were collected after 10 days interval from anthesis to maturity. Then the panicles were packed and oven dried at the same procedure. After drying 20 grains were randomly collected from each panicles and the weight of one grain was calculated. The absolute grain growth rate (AGR) was calculated using the following formula according to Hasan (2009). The average values were taken from each treatment for analysis.

$$\text{AGR (mg/grain/day)} = \frac{W2 - W1}{T2 - T1}$$

Where,

W_1 = Grain dry weight at initial time

W_2 = Grain dry weight at final time

T_1 = Initial time

T_2 = Final time

3.13.1.18. Statistical analysis

The data were analyzed in two factor randomized complete block design and the means were separated by LSD at 5% level of significance using the statistical computer package programme MSTAT-C (Russell, 1986). Correlation analysis was also done.

3.14. Experiment-II:

The yield and yield parameters responses of rice genotypes under different submergence durations

The experiment was undertaken with the following objectives-

» to study the effect of submergence stress on yield components of different rice genotypes

» to assess the relatively submergence tolerant rice genotypes considering their yield attributes.

In the experiment-II, the location of the experimental field, experimental design and treatment, plant materials collection, seed treatment and germination, seed bed preparation, sowing and seedlings raising, pot preparation, fertilizer application, transplanting, intercultural operations, submergence induction etc were same as experiment I except the duration of the experiment. The duration of the experiment-II was July to November of 2013.

3.14.1. Data collection

Different yield data were collected from the following parameters-

3.14.1.1. Tiller number per plant

Total number of tiller was counted during anthesis and maturity from each treatment plant.

3.14.1.2. Effective tiller number per plant

The number of effective or panicle bearing tiller was counted during anthesis and maturity from each treatment.

3.14.1.3. Panicle length of main stem

The panicle length (cm) was measured from the fast node of the rachis to the tip of each main stem panicle.

3.14.1.4. Filled grain number of main stem panicle

After harvesting of the whole plant, the main stem was separated and the filled grain number of each main stem panicle was counted.

3.14.1.5. Unfilled grain number of main stem panicle

The unfilled grain number of main stem panicle was also counted at the same time.

3.14.1.6. Grain weight of main stem panicle and the whole plant grain weight

The samples were collected from each treatment pot by cutting the plant at ground level. Then the panicles were counted and collected in brown paper packet. The samples were dried in sun, threshed and cleaned manually and fresh weight of grain was taken. The empty panicle and representative samples of grain were dried in an oven at 70ºC for 72 hrs to obtained dry weight. The grain weight of main stem panicle and the whole plant was recorded separately for each treatment.

3.14.1.7. 100-grains weight

From each treatment, thousand grains were taken randomly from dried sample and weighed. From this weight average individual seed size was calculated.

3.14.1.8. Biological yield

Grain yield and straw yield were altogether regarded as biological yield.

3.14.1.9. Grain yield/plant

The grain weight of each treatment plant was recorded in grams (g/plant) which was adjusted to 12% moisture.

3.14.1.10. Harvest index

It is the ratio of economic yield (grain yield) to biological yield. The harvest index (HI) was computed as

$$\text{HI}\ (\%\ ) = \frac{\text{Grain yield}}{\text{Biological yield}} \times\ 100$$

3.14.1.11. Spikelet sterility percentage:

The spikelet sterility percentage was recorded from the main stem panicle. The filled and unfilled grain were separated and counted manually.

The spikelet sterility percentage was calculated using the following formula-

$$\text{Spikelet sterility}\ (\%) = \frac{\text{Sterile spikelets per panicle}}{\text{Total spikelets per panicle}} \times 100$$

3.14.1.12. Statistical analysis

The data were analyzed as same as experiment I

3.15. Experiment-III:

The anatomical and physiological changes of rice genotypes under different submergence durations

The present study was undertaken with the following objectives-

» to assess the anatomical and physiological response of different rice genotypes due to submergence treatments.

» identification of the anatomical features and physiological parameters that play an important role in submergence tolerance.

In the experiment-III, the location of the experimental field, experimental design and treatment, plant materials collection, seed treatment and germination, seed bed preparation, sowing and seedlings raising, pot preparation, fertilizer application, transplanting, intercultural operations,

submergence induction etc were same as experiment I except the duration of the experiment. The duration of the experiment-III was July to November of 2014.

3.15.1. Data collection

Different biochemical data were collected from the following parameters-

3.15.1.1. Membrane leakage

Cell membrane leakage of electrolytes is termed as an indicator of crop plant stress tolerance. There is a strong relationship between membrane leakage and yield under stress. A cell membrane system that remains functional during stress finally contributes to adaptation of plants to this unfavorable condition. This is very important during grain filling period.

Measurement procedure:

Hossain *et. al*. (1995) developed an improved method of determination of membrane thermostability for screening heat tolerance and sensitive varieties in Brassica. In case of rice, fully expanded leaf samples were collected from five randomly selected plants. Ten leaf discs (10 mm in diameter) were collected from a flag leaf using a leaf puncher. The leaf discs were washed three times with deionized water (collected from G14 Deionizer water set, model: TKA Micromed, Germany) to remove electrolytes adhering to leaf tissue as well as electrolytes released from cut cells on the periphery of the leaf discs. The test tubes (25mm x 150mm) were also rinsed with deionised water. Then the leaf discs were placed in a test tube and a piece of cotton was put on the leaf discs inside the test tube to prevent any injury of the discs by the electrode bar during conductance measurement. Thereafter 20 ml of deionized water was added in each tube.

The initial conductivity reading (I) was taken with an electric conductivity (EC) meter (model: CD-4303). The test tubes were covered by aluminium foil and placed in a thermostatistically controlled water bath (model:DSB-1000E, Taiwan) incubator maintaining a constant temperature of 55^0 C. Electrical conductivity reading (E) was also taken at 30 minutes interval up to 4.5 h, subsequently the samples were autoclaved (model: DAC-45, Korea) at 121^0 C for 15 minutes to kill the leaf tissues completely. After autoclaving the samples were again placed in water bath to adjust the elevated temperature of 55^0 C. Final conductance (F) was measured after 30 minutes

of incubation. The percentage of membrane leakage induced by the elevated temperature during the time course (30 minutes) was calculated as follows:

$$\text{Cell membrane leakage } (\%) = \frac{\text{E} - \text{I}}{\text{F} - \text{I}} \times 100$$

Where,

I = Initial conductance

E = Elevated temperature conductance

F = Final conductance after autoclaving

The relative injury (RI) was measured as follows-

$$\text{RI} = \frac{\text{Injury level after 4.5 hours treatment}}{\text{Injury level after autoclaving}}$$

3.15.1.2. Estimation of soluble sugar and starch

The soluble sugar and starch content of upper fully expanded leaves were determined as Yoshida *et al.* (1976) from control and submerged plant just before submergence and after desubmergence.

Reagents used in sugar and starch analysis

80% Ethanol: 420 ml of 95% ethanol (Merk, Germany) was mixed with 80ml of distilled water. The solution was stored at room temparature.

9.2 *N* Perchloric acid ($HCLO_4$): 793 ml of 70% perchloric acid (Merk, Germany) was mixed with 207 ml of distilled water. The solution was stored at room temperature.

4.6 *N* Perchloric acid ($HCLO_4$): 397 ml of 70% perchloric acid (Merk, Germany) was mixed with 603 ml of distilled water. The solution was stored at room temperature.

Anthrone: 1 g of anthrone (BDH che. Ltd.) was dissolved in 500 ml of conc. sulfuric acid. The solution was prepared in ice bath and stored at 4^0C. A fresh solution was prepared every day.

Glucose stock solution: 0.1 g of anhydrous glucose (AR Grade) was put into a 1000 ml volumetric flask. The volume was made up with distilled water. This solution contained 100 ppm glucose. The stock solution was prepared daily and kept cool.

0.46 *N* perchloric acid ($HClO_4$): 10 ml of the 4.6 *N* $HClO_4$ solution was diluted to 100 ml with distilled water.

Extraction procedure

The plant materials were dried in an oven at 72^0C for 3 days. These plant materials were then grinded finely by grinding machine. The grinded plant materials were stored in desiccators. These grinded plant materials were used for the extraction as follows following the procedure of Yoshida *et al.* (1976) –

Flow chart for sugar extraction

100 mg of dried powder was taken in 15 ml centrifuge tubes

↓

10 ml of 80% ethanol was added

↓

The tubes were then covered with aluminum foil

↓

The tubes were kept in a water bath (measured by Water Bath, Model: DSB-1000E,Taiwan) at 80-85^0C for 30 minutes

↓

The extracts was centrifuged at 5000 rpm(by G-13 Centrifuge Machine, Model: NF200, Turkey) for 10 minutes at room temperature

↓

The supernatant was collected in a 50 ml beaker

↓

The procedure was repeated 3 more times

↓

The beaker was placed on a water bath at 80-85^0C to evaporate the alcohol until the volume was reduced to about 3 ml

↓

After cool down the extract was transferred by 3-4 times wash to a 25 ml volumetric flask and made the volume with distilled water

↓

This was the sugar extract

Preparation of standard curve for sugar analysis

The standard curve for the estimated of sugar was produced as follows following the procedure of Yoshida *et al.* (1976)-

0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mg/50ml standard glucose solution was taken in separate 50 ml Pyrex test tube

↓

The volume was made up to 5 ml with distilled water

↓

The tubes were kept in ice bath

↓

10 ml of anthrone reagent was slowly added, the reagent being allowed to run down the side of the test tube and slowly stirring

↓

The tubes were put into a boiling water bath for exactly 7.5 minutes

↓

The tubes were immediately cooled in ice (made by Ice Maker, Model: EC-S020A, China)

↓

When cooled, the absorbance (G14 Spectrophotometer, Model:SB-10, Australia) were measured at 630 nm

↓

A calibration curve was plotted taking absorbance as ordinate and respective glucose conc. abscissa

This curve was used as the standard curve for the estimation of sugar content of the sample.

Estimation of Sugar from the extracts **(**following the procedure of Yoshida *et al.*, 1976)

5 ml of sugar extract was taken in a 100 ml volumetric flask and made the volume with distilled water

↓

5 ml of diluted extracts were taken in separate 50 ml Pyrex test tubes

↓

The tubes were kept in ice bath

↓

10 ml of anthrone reagent was slowly added, the reagent being allowed to run down the side of the test tubes and slowly stirring and cooled in ice

↓

When cooled, the absorbance were measured at 630 nm

Finally the sugar content as mg/g dry weight was estimated using the standard curve.

Flow chart for starch extraction **(**following the procedure of Yoshida *et al.*, 1976)

The residue left over from the extraction of sugar was dried in an oven at 70^0C for 2 hrs

↓

2 ml of distilled water was added

↓

The tubes were put in a boiling water bath for 15 minutes and stirred occasionally

↓

The tubes were then cooled

↓

2 ml of 9.2 N $HClO_4$ was added and stirred occasionally for 15 minutes

↓

The volume was made up to 10 ml with distilled water

↓

The extract was centrifuged as in the sugar extraction

↓

The supernatant was collected in a 50 ml conical flask

↓

2 ml of 4.6 N $HClO_4$ was added to the residue and stiring occasionally for 15 minutes

↓

The volume was made up to 10 ml with distilled water

↓

The extract was centrifuged as above and the supernatant was collected in the conical flask where previous supernatant was present

↓

The volume was made up to 50 ml with distilled water

↓

This was the starch extract

Preparation of standard curve for starch analysis

The standard curve for the estimated of starch was produced as follows following the procedure of Yoshida *et al.* (1976)-

0, 1.0, 2.0, 3.0 , 4.0, 5.0 and 6.0 mg/50ml standard glucose solution was taken in separate 50 ml Pyrex test tube

↓

0.6 ml 0.46 N $HClO_4$ was added to each test tube

↓

The volume was made up to 5 ml with distilled water

↓

The tubes were kept in ice bath

↓

10 ml of anthrone reagent was slowly added, the reagent being allowed to run down the side of the test tube and slowly stirring

↓

The tubes were put into a boiling water bath for exactly 7.5 minutes

↓

The tubes were immediately cooled in ice

↓

When cooled, the absorbance were measured at 630 nm

↓

A calibration curve was plotted taking absorbance as ordinate and respective glucose conc. abscissa

This curve was used as the standard curve for the estimation of starch content of the sample.

Estimation of Starch from the extracts **(**following the procedure of Yoshida *et al*., 1976)

5 ml of starch extract was taken in a 50 ml volumetric flask and made the volume with distilled water

↓

5 ml of diluted extracts were taken in separate 50 ml Pyrex test tubes

↓

The tubes were kept in ice bath

↓

10 ml of anthrone reagent was slowly added, the reagent being allowed to run down the side of the test tubes and slowly stirring and cooled in ice

↓

When cooled, the absorbance were measured at 630 nm

Finally the starch content as mg/g dry weight was estimated using the standard curve.

3.15.1.3. Percent depletion of non structural carbohydrate (soluble sugar and starch)

In the present experiment, the soluble sugar and starch content was considered as non structural carbohydrate. The percent depletion of total non structural carbohydrate (NSC) contents due to

submergence was determined by the formula, described by Sarkar and Bhattacharjee (2011), as follows-

Reduction %= {(NSC before submergence – NSC at the end of submergence) / (NSC before submergence)×100}.

3.15.1.4. Chlorophyll content

Chlorophyll content of rice leaf was determined just after desubmergence both from control and 7, 14 and 21 days of submergence. Chlorophyll content of rice leaf was also determined at grain filling stage on fresh weight basis using the method proposed by Witham *et al*. (1986). Hundred milligram of rice leaf sample was broken into small pieces and dipped into 80% acetone in a twenty five-milliliter vial. The vial was made up to the volume with 80% acetone. Then the sample was kept over forty eight hours in a dark place. Finally the absorbance of the filtrate was taken by spectrophotometer (G14 Spectrophotometer, Model:SB-10, Australia) at 663 nm and 645 nm, respectively.

Amount of chlorophyll were calculated using the following equations / formula

Chlorophyll a (mg/g) = $[12.7 (OD_{663}) - 2.69 (OD_{645})] V/1000W$

Chlorophyll b (mg/g) = $[22.9 (OD_{645}) - 4.68 (OD_{663})] V/1000W$

Total Chlorophyll (mg/g) = $[20.2 (OD_{645}) + 8.02 (OD_{663})] V/1000W$

Where,

OD= Optical density regarding of the chlorophyll extract at the specific indicated wavelength (645 and 663 nm).

V= Final volume of the 80% acetone chlorophyll extract (ml).

W= Fresh weight in gram of the tissue extracted.

3.15.1.5. Proline content

Free proline content in the leaves was determined following the method of Bates *et al.,* 1973. The protocol was based on the formation of red colored formazone by proline with ninhydrin in acidic medium, which is soluble in organic solvents like toluene.

Instruments and glassware

Test tubes, test tube stand, micro-pipettes (20-200 µl, 100-1000 µl and 5 ml), Whatman No. 1 filter papers, visible range spectrophotometer (G14 Spectrophotometer, Model:SB-10, Australia).

Reagents

1. Glacial acetic acid (Analytical grade)
2. Sulphosalycylic acid (3%): Three gram of sulphosalycylic acid was dissolved in 100 ml of distilled water.
3. Orthophosphoric acid (6 N): Required volume of orthophosphoric acid (38.1 ml) was taken and volume was made to 100 ml, using distilled water to get 6 N orthophosphoric acid.
4. Acid ninhydrin: Ninhydrin (1.25 g) was dissolved in a blend of 30 ml of glacial acetic acid and 20 ml of 6 N orthophosphoric acid.

Procedure

1. Plant tissue (0.5 g) was taken and homogenized in 5ml of 3% sulphosalycylic acid using pre-washed mortar and pastle.
2. Then the homogenate was filtered through Whatman No.1 filter paper and the collected filtrate was used for the estimation of proline content.
3. Exactly 2 ml of extract was taken in a test tube and add 2ml of glacial acetic acid and 2ml of ninhydrin reagent was added.
4. Then the reaction mixture was heated in a boiling water bath (by Stirrer hot water bath, Model: DSB-1000E, Taiwan) at 100ºC for 1 hour. Brick red colour was developed.
5. After cooling the reaction mixtures, 4ml of toluene was added and each tube shaken vigorously for 15-20 second in an electric shaker (Model: KS260 basic, Germany) then transferred to a separating funnel.
6. After thorough mixing, the chromospheres containing toluene was separated and its absorbance was read at 520 nm in spectrophotometer (G14 Spectrophotometer, Model: SB-10, Australia) against toluene blank.

7. The proline standard curve was prepared by taking 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg ml^{-1} concentration.

The proline content was determined from a standard curve and calculated on a fresh weight basis as follows-

µmoles proline / g of fresh plant material = {(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample/5)

3.15.1.6. Anatomy of rice plant

The thickness of leaf blade, thickness of leaf sheath and root diameter was taken from control and submerged plant during just after desubmergence. The thickness of leaf blade, thickness of leaf sheath was taken from the 6^{th} leaf which was developed during submergence. The root diameter was taken from 2 cm behind the root tip. The section was taken with free hand sectioning technique and the photograph was taken with photomicroscope (Olympus microscope with camera G7, Model- Primo Star, Germany).

3.15.1.7. Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

3.15.1.8. Statistical analysis

The data were analyzed as same as experiment I.

CHAPTER 4

RESULTS AND DISCUSSION

In this research work, the behavior of four wetland *aman* rice genotypes was studied providing complete submergence at seedling stage conducting three pot-culture experiments in the field. In an attempt to infer the strategies of submergence tolerance in these plants, the morphological and physiological responses of these genotypes were studied under submergence in the first experiment. In the second one, different yield contributing characters of the genotypes under different submergence durations were studied. In this experiment, attention had been paid to evaluate the yield losses due to submergence. In the third experiment, the results of the above experiments were evaluated further considering a few anatomical and physiological parameters.

4.1. Experiment I: Morpho-physiological responses of selected rice genotypes as influenced by different submergence durations

The results of different parameters have been discussed under the following headings:

4.1.1. Percent elongation of shoot

Percent elongation of rice seedlings was measured after desubmergence from 0, 7, 14 and 21 days of submergence (Figure 1). The elongation was higher in submergence treated plants than the control in all the genotypes due to 7 DS treatment. But the differences between control and submerged treatments were significant in all the genotypes except FR13A. Considering all the genotypes and 7 DS treatment, the highest (57.17%) elongation recorded was in BR5 under submergence and the lowest (32.60%) elongation recorded was in the same genotype in control.

Due to 14 DS treatment, all the genotypes (except FR13A) showed higher elongation in treated plants than the control and the difference between control and submerged treatment was significant only in IR64Sub1. After 14 DS treatment it recorded was that elongation due to submergence was little lower than the control in FR13A. Among the genotypes, the highest (63.51%) elongation recorded was in BR5 at 14 DS treatment and the lowest (44.94%) elongation recorded was in IR64Sub1 at control treatment. Due to 21 DS treatment, the genotype IR64Sub1 and BR5 showed higher elongation at submergence treatment compared to control

treatment. But the difference between control and submerged treatment was nonsignificant. In FR13A, control plants showed significantly higher (58.74%) elongation than submerged (21 DS) plants. In BRRI dhan52, the elongation of control plants and submerged (21 DS) plants were almost similar. In this (21 DS) treatment, considering all the genotypes, the highest (66.70%) elongation recorded was in BR5 at submerged plant and the lowest (48.07%) elongation recorded was in FR13A at submerged plant. It was recognized that under submergence condition, the genotype in which less shoot elongation occurred, might be considered as submergence tolerant, as shown by BRRI dhan52 and FR13A in the present experiment. The rapid shoot elongation is disadvantageous in flash flood conditions since lodging usually occurs when flood water recedes and sometimes dead. Zhang *et al*. (2015) reported that waterlogging promotes the production of "stress ethylene", which interacts with growth hormones to promote cell elongation; however, such growth is not classified as normal growth because it is not caused by an increase in cell number.

In the present experiment, excess shoot elongation occurred in BR5 and IR64Sub1 in all the submergence treatments. But the difference between control and submerged treatment was significant only at 7 DS treatment in BR5, IR64Sub1 and at 14 DS treatment in IR64Sub1 genotype. Under 14 DS and 21 DS treatment the shoot dry matter was drastically reduced and the root was also very much injured in BR5. As a result, plant became lodged and a few plants did not survive. These might be due to submergence condition where Aminocyclopropane carboxylic acid (ACC) is synthesized inside the root (Taiz and Zeiger, 1998). Then ACC is translocated to shoot and converted into ethylene. The interplay of ethylene, abscisic acid (ABA) and gibberellic acid (GA) regulates shoot elongation in submerged plants (Luo *et al*., 2009). In case of small rice seedlings, the leaves are mainly elongated (Jackson, 2007). Higher shoot elongation under submergence condition is harmful for plant because shoot elongation may be associated with high costs of energy and elongating plants exhaust their energy and carbohydrates reserves for cell division and cell elongation (Voesenek *et al*., 2004; Pierik *et al*., 2009). On the other hand, submergence condition seriously hampers normal respiration leading to decrease in favorable condition for ATP synthesis which is not enough for photosynthate production for elongating plants under submergence condition. Rapid shoot elongation during submergence competes with maintenance respiration for carbon sources leaving less assimilates available to support maintenance required for survival for submergence (Setter and Laureles,

1996; Singh *et al*., 2001). As a result, lodging usually occurs when flood water recedes and submergence injured plant cannot recover them (Ram *et al*., 2002). Finally they cannot survive. So it is clear that excess shoot elongation under submergence condition is very much harmful for plant which is usually observed in submergence sensitive genotype as in BR5. So, it is clear that under submergence the actively growing plants are more susceptible than slowly growing plants. This result has the conformity with the report of Mallik e*t al*. (1995). Sarkar and Bhattacharjee (2011) stated that higher shoot elongation during submergence was associated with intolerance; and slow underwater elongation was considered desirable for submergence tolerance (Singh *et al*., 2014). But the slower stem elongation under submergence condition is beneficial for survival, because by this way they conserve their energy under submergence condition. This is an quiescence strategy. The rice genotypes which were adapted to this type of flooding usually stay dormant or 'quiescent', conserve their energy and maintain their chlorophyll and underwater photosynthesis (Ella *et al*., 2003a and 2003b; Das *et al.*, 2005; Nagai *et al*., 2010; Winkel *et al*., 2013). Plant utilizes this stored energy when flood water recedes and recovers quickly which might help to provide better yield. In the present experiment, slower stem elongation was observed in BRRI dhan52 and FR13A compared to BR5 and IR64Sub1 in all submergence treatments. Under 14 DS and 21 DS treatment, the control and submerged treatment did not differ significantly in BRRI dhan52 but in FR13A, 21 DS treated plant showed significantly lower shoot elongation compared to the control treatment. As a result, their energy consumption was lower during submergence. Due to that suppressing of cell elongation and excess carbohydrate use the tolerance in BRRI dhan52 and FR13A was increased.

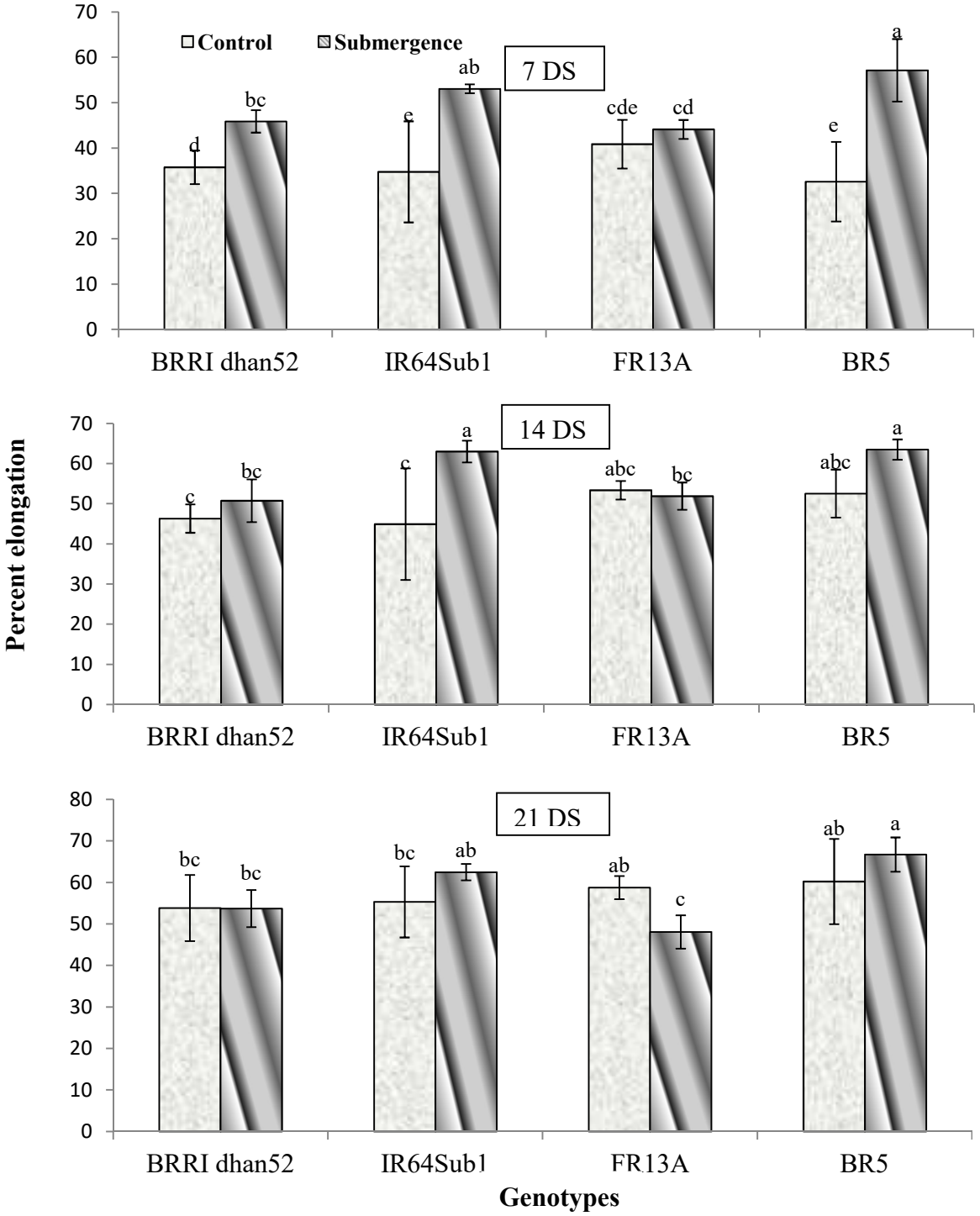


Figure 1. Percent elongation of shoot as influenced by different durations of submergence at seedling stage. Bar represents the standard deviation of the respective treatment. Same letter(s) are not significantly different from each other by LSD at 5% level for each treatment. DS = days of submergence.

4.1.2. Survival percentage

In all the genotypes, different submergence treatments differed significantly among them (Figure 2). Considering all the genotypes and submergence durations, the survival percentage found was the highest at 7 DS treatment and was gradually decreased with the increasing submergence durations. In all the genotypes, the survival percentage in 7 DS treatment was significantly higher than other submergence treatments. In BRRI dhan52, IR64Sub1 and FR13A, the survival percentage due to 7 DS treatment, were as same as the control (100%). But in BR5, the survival percentage was little higher than 70% in 7 DS treated plants. Under 14 DS treatment, the survival percentage of BR5 recorded was the lowest (47.38%). Among the genotypes, the survival percentage was 0% at 21 DS treatment in case of BR5.

The lower shoot elongation under submergence in BRRI dhan52 and FR13A might contribute in higher survival of those genotypes. It was clear that 7 DS treatment did not affect the survivality in BRRI dhan52, IR64Sub1 and FR13A which were statistically similar to control treatment. Though due to further increase in submergence duration, decreased the survivality significantly compared to the control treatment in all the genotypes and a remarkable number of plants were survived in BRRI dhan52, IR64Sub1 and FR13A genotypes. Same results were also observed by Elanchezhian *et al*. (2013) and Singh *et al*. (2014) who stated that submergence up to 14 days caused significant decrease in the survival of different rice genotypes. But in case of BR5, the survivality was significantly lower compared to the control treatment. All the plants were found dead in BR5 at 14 and 21 DS treatment which might be due to death of roots, except in some cases where it was also recorded that some new shoot grew from the base of the dead shoot at 14 DS treatment of BR5; for these reasons the survivility percentage of BR5 at 14 DS treatment found was 47.38% and as such it was possible to collect different data from those plants. Under 21 DS treatment, all the shoots of BRRI dha52, IR64Sub1 and FR13A were found almost dead which was completed after few days of desubmergence; and at the same time, it was also recorded that new shoots grew from the base of the plants when they were in desubmergence condition. Excess under water shoot elongation was occurred in BR5 and after desubmergence due to lack of sufficient stored energy and aerobic shock the plant became lodged and ultimately died. The lower percentage of survival of BR5 due to submergence treatment compared to the other genotypes indicated the submergence susceptibility of this genotype.

The extent of visible injuries caused due to submergence is commonly used as an indicator of their sensitivity. The genotypes give distinctly different response to submergence in terms of visible symptoms and survival (Panda and Sarkar, 2012). Plant survival is an important determinant of grain yield (Singh *et al*., 2009). It was reported that plant survival during submergence is influenced by two factors-(i) limitation of gas diffusion and (ii) limitation of light by shading (Ram *et al*., 2002). As a result, photosynthesis and efficient utilization of carbohydrate are hampered. So, survival during submergence may largely depend on accumulation of high carbohydrate concentrations inside the plants before submergence and maintenance of energy production under oxygen shortage. Therefore, complete submergence can cause a drastic energy and carbohydrate crisis that can threaten plant survival (Voesenek *et al*., 2006; Bailey-Serres and Voesenek, 2008; 2010). Upon recedes of water level after complete submergence plants are subjected to both high light intensity and higher oxygen levels. Submergence becomes more detrimental for plants when water level recedes and becomes a critical factor for seedlings survival (Kawano *et al*., 2009). This is called aerobic shock which occurs after desubmergence.

4.1.3. Relationship between shoot elongation and survival percentage

There was a negative correlation between shoot elongation and survival percentage (Figure 3). The highest survival percentage was observed when shoot elongation was the lowest. Then survival percentage gradually decreased with the increasing shoot elongation in submergence condition. This strong negative association ($r = - 0.784$) of shoot elongation with survival clearly demonstrated that higher survival of different genotypes might be due to restriction on shoot extension during submergence.

Under submergence, plants try to reach the air by elongating their shoot. But after desubmergence, the excess elongated shoot lodged and ultimately died in BR5 due to aerobic shock. As a result, though the shoot elongation was higher in BR5, the survival

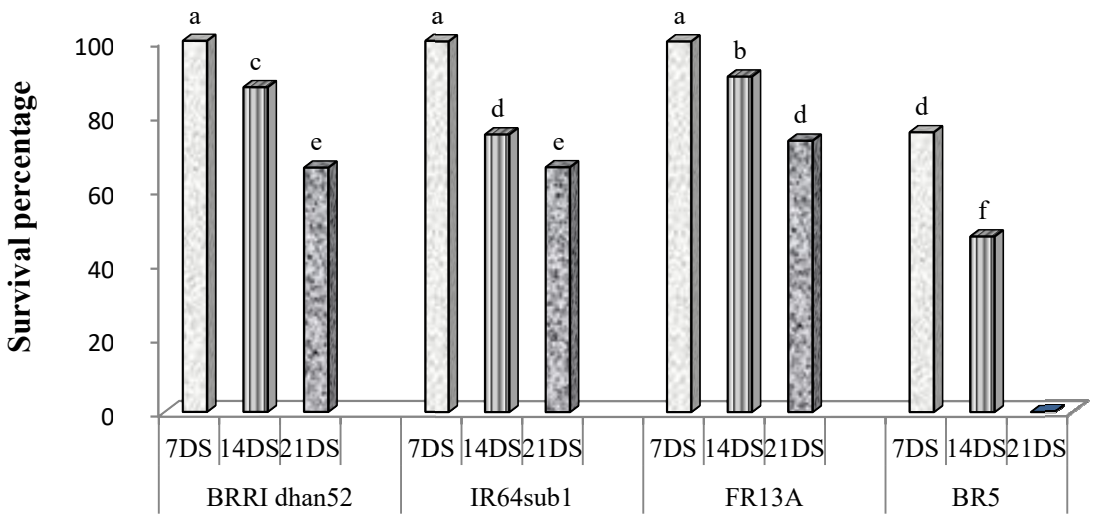


Figure 2. Survival percentage (%) of different rice genotypes under various submergence duration treatments. DS= Days of submergence. Same letter(s) are not significantly different from each other by LSD at 5% level of significance.

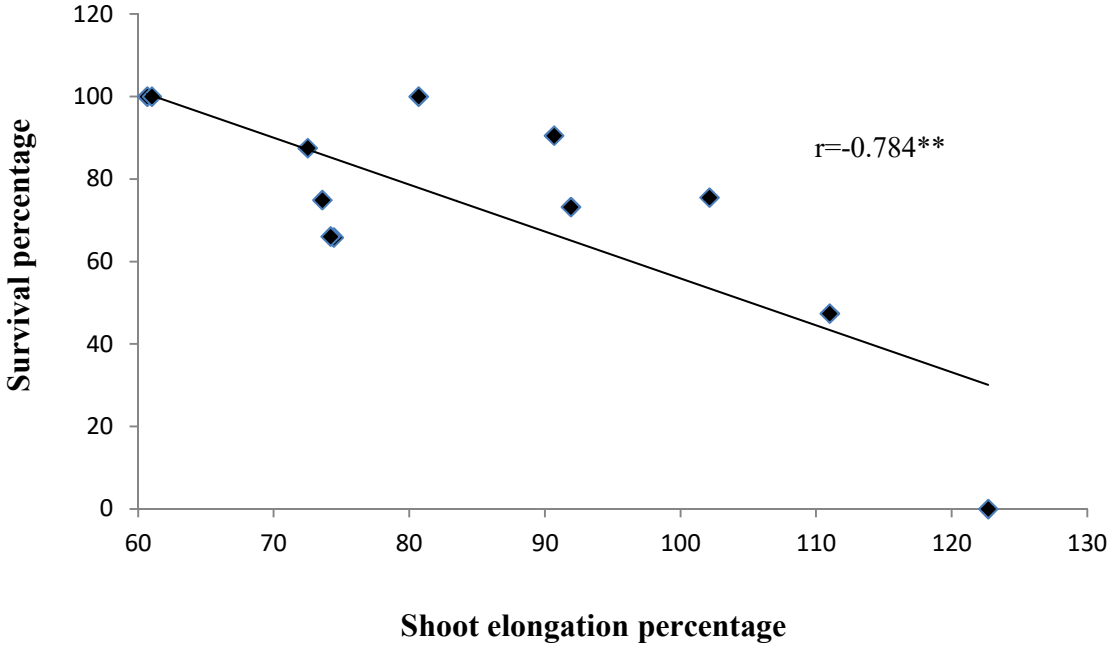


Figure 3. Relationship (r=-0.784**, n=12) between shoot elongation percentage and survival percentage of different rice genotypes under submergence stress.

percentage was the lowest in this genotype. Submergence-induced shoot elongation was negatively correlated with plant survival (Singh *et al*., 2014). Ruanjaichon *et al*. (2008) stated that the effect of plant growth on rapid shoot elongation is highly negatively correlated (r= -0.34*) with the survival data after flooding for 20 days. The relationship between shoot elongation and survival using five rice cultivars, which has been confirmed using the IRRI gene Bank database on 903 cultivars, was reported by Setter and Laureles (1996). The energy and carbohydrates in submerged plants are preserved during submergence, and after desubmergence their growth can be restarted by using this energy (Singh *et al*., 2001). A negative correlation between shoot elongation and survival under complete submergence was also observed by Ram *et al*. (2002).

4.1.4. Tolerance scoring

Tolerance scoring was an eye observation method, which was based on the phenotypic appearance of the genotypes after desubmergence. Tolerance scoring was done from the submergence treated plants at 5 days after desubmergence. The genotype, in which, the tolerance score was higher, suppose to be less tolerance to submergence. In all the genotypes, the lowest score recorded was in 7 DS treatment and the highest score recorded was in 21 DS treatment (Table 3). Under 7 DS treatment, the tolerance score of BRRI dhan52 and FR13A was as same as the control treatment. But 14 and 21 DS treatments of BRRI dhan52 and FR13A differed significantly with control treatment. In rest of the genotypes, all the treated plants differed significantly with control treatment. More submergence duration related with more injury resulted in high tolerance score in all the genotypes. The submergence tolerant genotype normally showed lower tolerance score after imposition of treatment (as in BRRI dhan52 and FR13A). Pervin (2005) measured submergence tolerance score and stated that submergence tolerance increased significantly with increase of seedling age as indicated by lower submergence tolerance score.

Table 3. Tolerance score of different rice genotypes as influenced by various submergence treatments

Genotypes	Days of submergence (DS)	Tolerance score
BRRI dhan52	0	1e
	7	1 e
	14	3 d
	21	3 d
IR64Sub1	0	1 e
	7	3 d
	14	5 c
	21	5 c
FR13A	0	1 e
	7	1e
	14	3 d
	21	3 d
BR5	0	1 e
	7	5 c
	14	7 b
	21	9 a
$LSD_{(0.05)}$		1.42
CV (%)		27.59

Values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.5. Specific leaf weight after desubmergence

Specific leaf weight (SLW) is defined as the mass of leaf dry matter per unit of leaf area. The plant with higher SLW (thick leaf) possesses more mesophyll cells for photosynthesis. The SLW of different rice genotypes was calculated after desubmergence from control and submerged plants (Table 4). The SLW was taken from average of three upper fully expanded leaves. Under control treatment, the SLW found was the highest in IR64Sub1 and the lowest in BRRI dhan52. Due to 7 DS treatment, the submergence treated plants produced lower SLW compared to control treatment in all the genotypes. Among the 7 DS treated plants, the lowest (81.09% of the control) SLW recorded was in BRRI dhan52 and the highest ((92.61% of the control) recorded was in IR64Sub1.

Due to 14 DS treatment, submergence treated plants produced lower SLW compared to control plants in all the genotypes. In this (14 DS) treatment, the highest (81.70% of the control) SLW recorded was in IR64Sub1 and the lowest (75.06% of the control) SLW recorded was in BRRI dhan52. Due to 21 DS treatment, submergence treated plants produced lower SLW compared to control plants in all the genotypes. Among this treatment (21 DS) plants, the highest (75.30% of the control) SLW recorded was in IR64Sub1 also and the lowest (49.69% of the control) in BR5. The SLW was more affected due to submergence in BR5 and less affected in IR64Sub1.

In the present experiment, the SLW recorded was lower than the control in all the genotypes and this was due to increased leaf area and decreased leaf dry matter under submergence condition. But 7 DS treatment in IR64Sub1 and BR5 showed statistically similar SLW as the control treatment. Decrease in SLW means reduction of tissue available in a unit leaf area indicating the sensitivity of a genotype under submergence stress as BR5. Sarkar *et al*. (1996) found that after 9 days of submergence, the reduction of SLW for different varieties of rice was continuous and very high in susceptible varieties (50-51 %) as compared with the tolerant cultivars (12-18 %). As SLW is an index of photosynthesis in rice and chlorophyll is directly related to photosynthesis, it is suggested that better survival of tolerant varieties was due to the capability of synthesizing and maintaining more photosynthates during the period of submergence. Bailey-Serres and Voesenek (2008) also found that leaves of *Rumex palustris* developed under water were 20% thinner with an increased SLA indicating a larger surface area relative to mass.

Table 4. Effect of different submergence treatments on specific leaf weight (SLW) of different rice genotypes

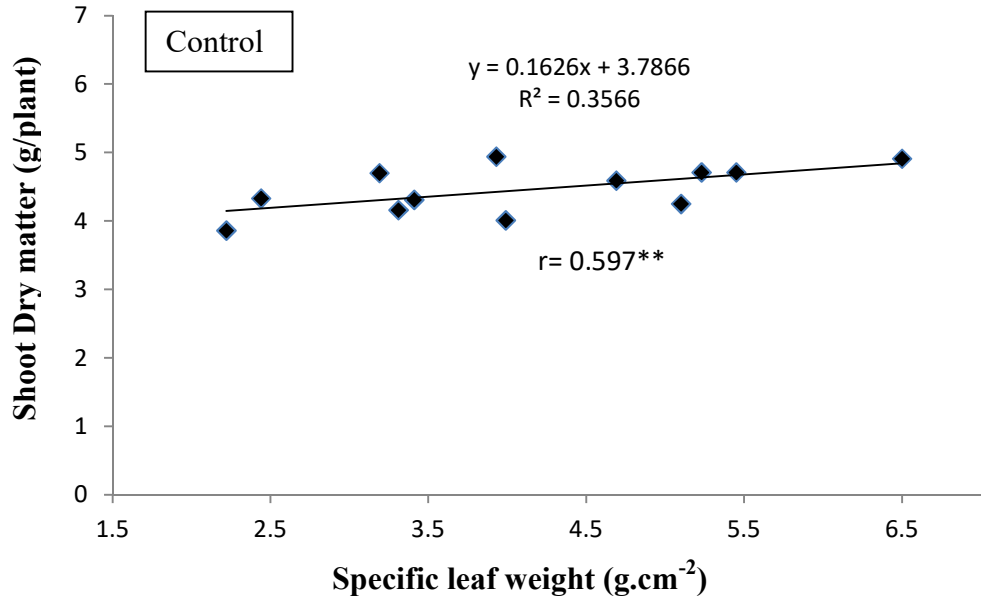
Genotypes	SLW under 7 DS mg/cm^2		SLW under 14 DS mg/cm^2		SLW under 21 DS mg/cm^2	
	C	S	C	S	C	S
BRRI dhan52	3.86 ab	3.13 c (81.09)	4.01 b	3.01 e (75.06)	4.25 b	2.69 d (63.29)
IR64Sub1	4.33 a	4.01ab (92.61)	4.70 a	3.84 b (81.70)	4.94 a	3.72 c (75.30)
FR13A	4.31 a	3.46 bc (80.28)	4.59 a	3.30 d (71.90)	4.71 a	2.49 d (52.87)
BR5	4.16 ab	3.62 abc (87.02)	4.71 a	3.56 c (75.58)	4.91 a	2.44 d (49.69)
$LSD_{(0.05)}$	0.64		0.24		0.31	
CV (%)	11.24		4.03		5.55	

DS= Days of submergence, (C = Control. S= Submergence, Figures inside the parenthesis indicate relative to control), Values followed by same letter(s) are not significantly different from each other by LSD at 5% level for each treatment.

4.1.6. Relationship between specific leaf weight and shoot dry matter after desubmergence

Under control treatment, there was a positive correlation (r = 0.597) between specific leaf weight (SLW) and shoot dry matter (Figure 4). The r value indicated that the relationship was moderately significant. The shoot dry matter was the lowest when the specific leaf weight was also the lowest. The shoot dry matter increased with the increasing specific leaf weight. But under submerged treatment there was a negative correlation (r = -0.656) between specific leaf weight and shoot dry matter (Figure 4). Though the shoot dry matter was increased with the increasing submergence duration, the specific leaf weight as well as the thickness of the leaf was decreased with the increasing submergence duration.

Under control condition, normally the dry matter content of the plant gradually increased with the increasing plant age at vegetative stage. As a result, the SLW was also increased in the present experiment with the increasing shoot dry matter with age. But under submergence condition, though the shoot dry matter was increased with the increasing age; the SLW was decreased and this was due to higher increase in leaf area than leaf dry matter.



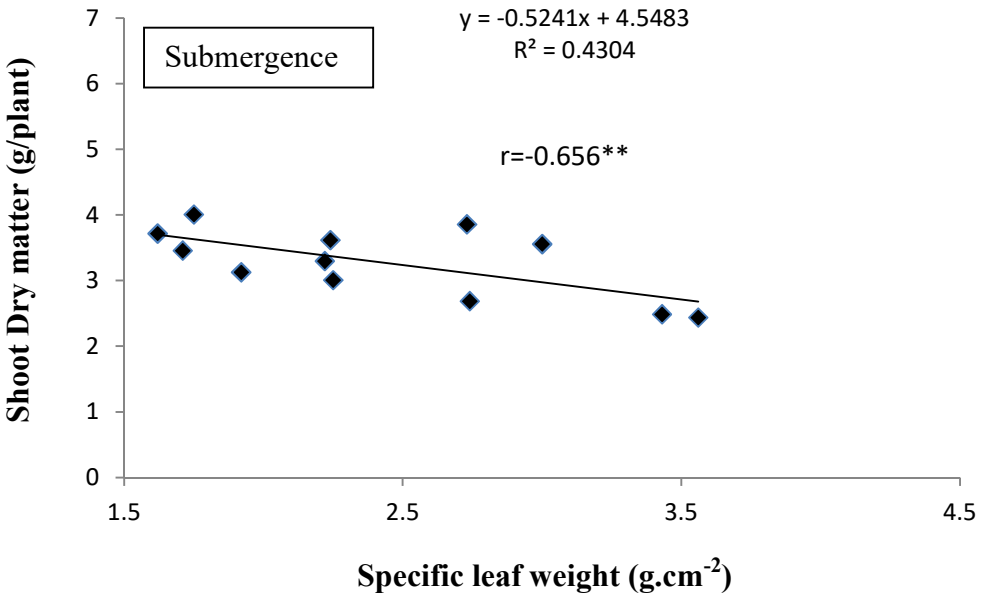


Figure 4. Relationship (n=12) between specific leaf weight and shoot dry matter of different rice genotypes under control and submergence condition.

4.1.7. Shoot dry matter of submerged plants after desubmergence along with control plants

The shoot dry matter recorded was after desubmergence of submerged plants along with the control plants. After 7 DS treatment, the control plants produced significantly higher shoot dry matter than submerged plants in all the genotypes (Figure 5). Among the genotypes, the significantly the highest (1.71g) shoot dry matter recorded was in FR13A at control treatment which was statistically similar to BR5 at control treatment and the lowest (0.88 g) shoot dry matter recorded was in IR64Sub1 at submerged plant which was statistically similar to submerged treatment of BRRI dhan52. But higher reduction in shoot dry matter (DM) under submergence (7 DS) treatment compared to control recorded was in BR5. Lower amount of reduction in shoot DM due to 7 DS treatment recorded was in BRRI dhan52 and in FR13A compared to the others.

After 14 DS treatment, the control plants produced the significantly higher shoot dry matter than the submerged plants in all the genotypes. The control plants of BR5 produced the significantly the highest (2.62 g) shoot DM. The significantly lowest (1.13 g) shoot dry matter recorded was in BRRI dhan52 at submergence treatment. The lowest reduction in shoot DM under 14 DS treatment recorded was in IR64Sub1 which was followed by FR13A.

After 21 DS treatment, control treatment also produced the significantly higher shoot dry matter than the submergence treatment in all the genotypes. Among the genotypes, the highest (4.79 g) shoot dry matter was also recorded in BR5 at control treatment which was significantly higher than any other genotype of this treatment, but a drastic reduction in shoot dry matter occurred in this genotype due to submergence treatment. Considering all the genotypes, the lowest (1.37 g) shoot dry matter recorded was in BRRI dhan52 under submergence treatment which was statistically similar to IR64Sub1 in the same treatment. Lower reduction in shoot DM under 21 DS treatment was also recorded in IR64Sub1 and in FR13A compared to the other.

It is clear from the above result that submergence treated plants produced the lower shoot dry matter than the control plants in all the genotypes. This might be due to higher injury, lower chlorophyll content and also decrease in sugar and starch content under submergence condition. The relative values indicated that the genotypes BRRI dhan52 and FR13A performed better under 7 DS condition; the genotypes IR64Sub1 and FR13A performed better under 14 and 21

DS conditions indicating the tolerant level of those genotypes under respective submergence duration. Lower shoot elongation, the lowest reduction in chlorophyll content with higher carbohydrate (sugar and starch), less membrane injury at 7 DS treatment in BRRI dhan52 and FR13A which finally contributed in higher grain yield in these genotypes at 7 DS treatment. Under 14 and 21 DS treatment, in FR13A, lower elongation, less reduction in chlorophyll content was associated with higher carbohydrate content with less membrane injury might contribute to the better yield. Under 14 and 21 DS treatment, in IR64Sub1, higher elongation, moderate reduction in chlorophyll content was associated with the lower carbohydrate content with higher membrane injury might affect recovery as well as final grain yield.

In the present experiment, a significant difference found was in shoot dry weight between submerged and control treatments in all the genotypes and different submergence durations. These results have the conformity with the results of Elanchezhian *et al*. (2013). Under submerged condition, shoot dry weight decreased was might be due to submerged roots lack sufficient energy to support physiological process on which shoots depend (Jackson and Drew, 1984). Under 7 DS treatment, BRRI dhan52 and FR13A showed relatively higher shoot dry matter than the other genotypes. Under 14 DS treatment, IR64Sub1 and FR13A showed relatively higher shoot dry matter than the other genotypes. Among the genotypes, due to 14 DS and 21 DS treatment, the reduction of shoot dry matter compared to control was comparatively higher in BR5 than the other genotypes. Under submerged condition, the carbohydrate supply was reduced through underwater current photosynthesis. Elongation and maintenance respiration was continued by using the stored sugar and starch in stem. After a certain time, several living tissues were decayed or died that affected plant growth and shoots dry weight was decreased under submergence (Nugraha *et al*., 2012) as in BR5. After desubmergence, plant take much longer time to recover and develop new organs. Consequently these might affect production of assimilate to be translocated to the sink. So, the genotype BR5

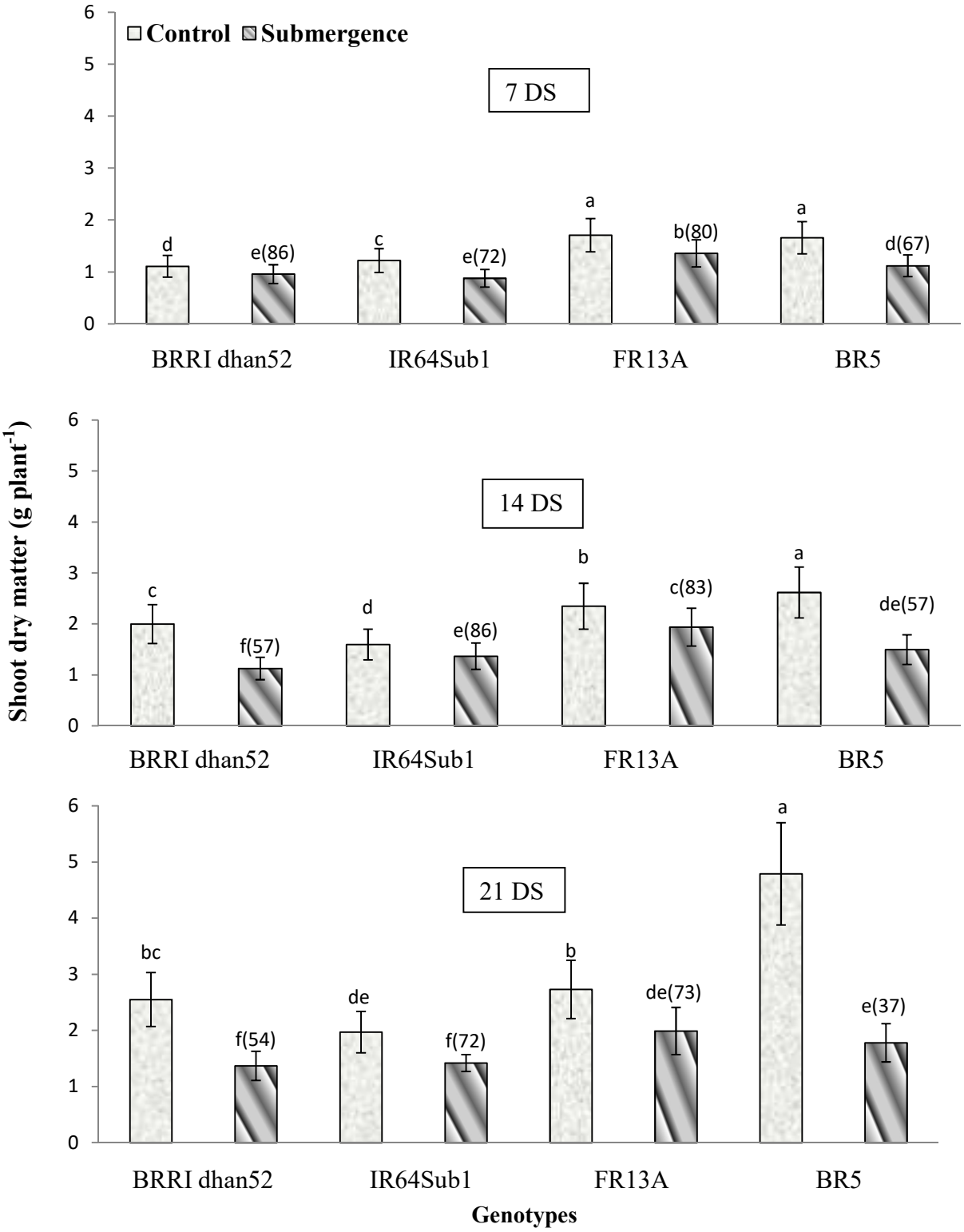


Figure 5. Shoot dry matter content of different rice genotypes after desubmergence of submerged plants along with control plants. Bar represents standard deviation. Values inside the parenthesis indicate relative values.

is considered as sensitive genotype because the reduction in dry matter content was more in susceptible genotype after desubmergence (Sarkar and Bhattacherjee, 2011).

4.1.8. Root dry matter of submergence plants after desubmergence along with control plants

The root dry matter was also recorded after desubmergence of submerged plants along with control plants. Considering all the genotypes and submergence treatments, it recorded was that root dry matter was significantly lower due to submergence treatment compared to control treatment. After 7 DS treatment, control plant produced the significantly higher root dry matter than the submerged plants in all the genotypes (Figure 6). Among the genotypes under 7 DS treatment, the the highest (0.34 g) root dry matter recorded was in FR13A at control treatment which was significantly higher than any other genotype of this treatment. The lowest (0.14 g) root dry matter recorded was in BR5 under submergence treatment which was significantly lower than any other genotype of this treatment. Lower reduction in root DM under 7 DS treatment recorded was in BRRI dhan52 and in FR13A compared to others.

After 14 DS treatment, the control plants produced also the higher root dry matter than the submerged plants in all the genotypes. Due to submergence (14 DS) treatment, a drastic reduction in root dry matter occurred in BR5 compared to the control. Among the genotypes under 14 DS treatment, the highest (0.71 g) root dry matter recorded was in BR5 at control treatment which was significantly higher than any other genotype of this treatment. and the lowest (0.2 g) recorded was in IR64Sub1 at submergence treatment which was statistically similar to 14 DS treatment of BR5. Lower reduction in root DM under 14 DS treatment recorded was in BRRI dhan52 and in FR13A compared to others.

After 21 DS treatment, the control plants produced the higher root dry matter than the submerged plants in all the genotypes. Due to submergence (21 DS) treatment, root dry matter was more affected compared to 7 DS and 14 DS treatment, but a drastic reduction in root dry matter (only 7% of the control) occurred in BR5. Among the genotypes under this treatment (21 DS), the highest (1.51g) root dry matter recorded was in BR5 at control treatment which was significantly higher than any other genotype of this treatment and the lowest (0.1 g) recorded was in the same genotype at submergence treatment which was significantly higher than any other genotype of

this treatment. Lower reduction in root DM under 21 DS treatment was also recorded in BRRI dhan52 and in FR13A compared to others.

Under submergence condition, the submerged root was unable to maintain normal respiration and the root tissues became damaged. In this situation many submerged root was unable to survive. In the present experiment, the relative values indicated that in all the submergence treatment, the root dry matter was less affected in BRRI dhan52 and in FR13A; root dry matter was more affected in BR5 under 14 DS and 21 DS condition compared to the other genotypes, which also indicated the tolerant level of those genotypes under respective submergence duration. In BR5, root dry matter was decreased more due to decaying of roots under anaerobic condition, leading to loss of absorbing area of root. The normal growth and mineral absorption were hampered, consequently, the roots were shorten, and the root number and root weight were decreased (Zhang *et al*., 2015). Finally the plant cannot survive under long days (21 DS) submergence duration.

Zhang *et al*.(2015) found that when the submergence duration was increased, the different treated plants showed decrease in the root dry weight. Rice roots might be more vulnerable for submergence injuries than shoots (Banerjee *et al*., 2015b). Under submerged condition, reduced soil component (Mn^{2+}, Fe^{2+}, S^{2-}) are accumulated to toxic level (Taiz and Zeiger, 1998) in root tissue and different organic acids are accumulated which damage root tissue under submerged condition.

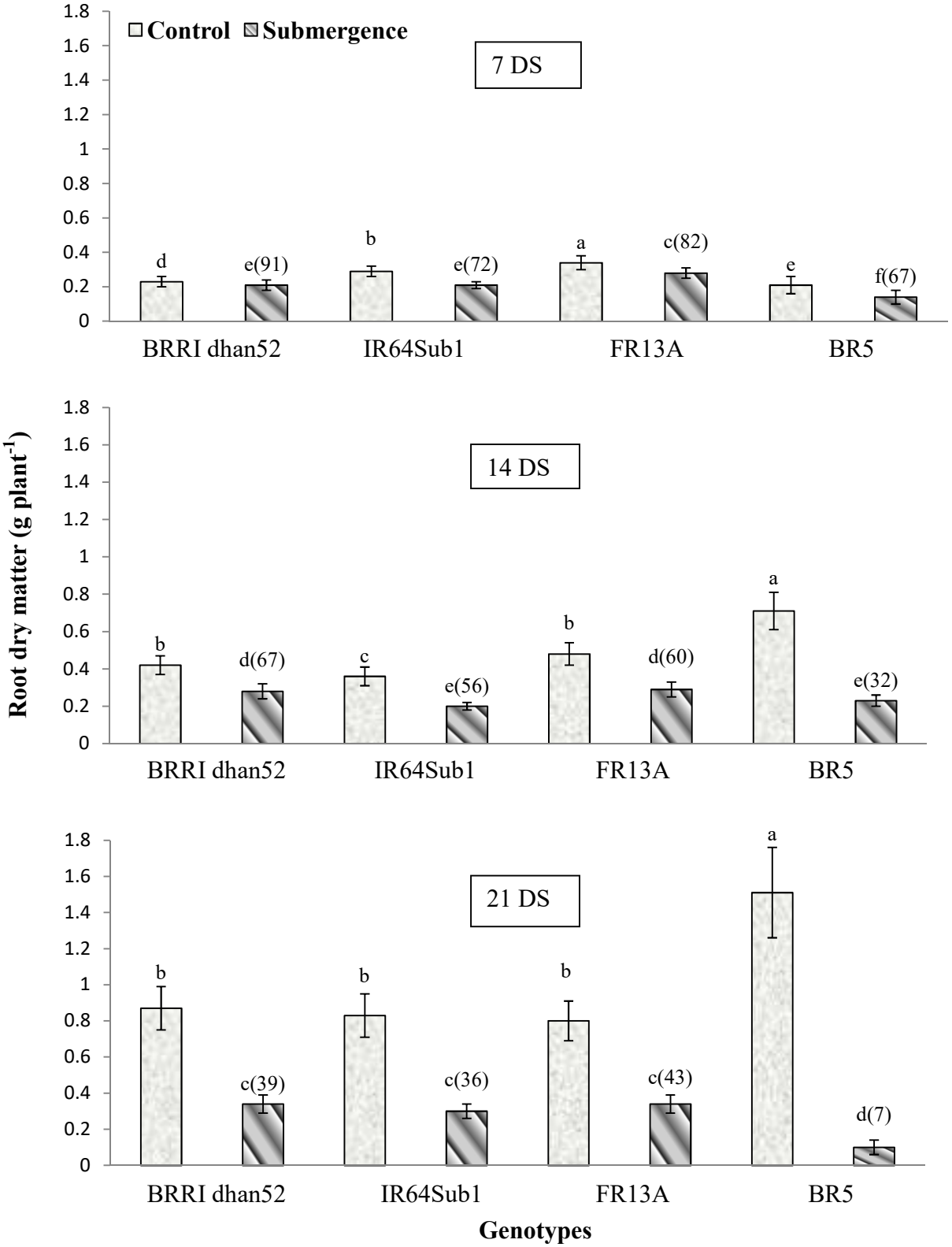


Figure 6. Root dry matter content of different rice genotypes after desubmergence of submerged plants along with control plants. Bar represents standard deviation.

4.1.9. Relative dry matter of shoot and root

The relative value refers to the ratio of the results under submergence condition and control condition. In this section, the relative dry matters of shoot and root have been discussed. The data was collected after desubmergence. Under 7 DS treatment, the relative shoot dry matter recorded was the highest (86%) in BRRI dhan52 which was followed by FR13A (Figure 7). The lowest (68%) relative shoot dry matter recorded was in BR5. Under 7 DS treatment, the relative root dry matter recorded was the highest (91%) in BRRI dhan52 also, which was followed by FR13A. The lowest (67%) relative root dry matter recorded was in BR5 in this treatment.

Under 14 DS treatment, the relative shoot dry matter recorded was the highest (86%) in IR64Sub1 which was followed by FR13A. The lowest (56%) relative shoot dry matter recorded was in BRRI dhan52. Under 14 DS treatment, the relative root dry matter recorded was the highest (66%) in BRRI dhan52 which was followed by FR13A. The lowest (32%) relative root dry matter recorded was in BR5 in this treatment.

Under 21 DS treatment, the relative shoot dry matter recorded was the highest (73%) in FR13A which was closely (72%) followed by IR64Sub1. The lowest (37%) relative shoot dry matter in 21 DS treatment recorded was in BR5. In this treatment, the relative root dry matter was much lower in all genotypes. It was clear from the present experiment that root growth rather than shoot growth was much hampered due to long time submergence. Under 21 DS treatment, the highest (42%) relative root dry matter recorded was in FR13A which was followed by BRRI dhan52. The lowest (7%) relative root dry matter recorded was in BR5 in this treatment. In this submergence level, the root of BR5 found was dead and ultimately the whole plant did not survive any longer. Considering the relative shoot and root dry matter of all the genotypes it was clear that due to submergence treatment, the shoot and root dry matter was less affected in BRRI dhan52, IR64Sub1 and FR13A, whereas BR5 was more affected due to submergence. For this, BR5 might be considered as submergence sensitive type. Zhang *et al*. (2015) found that when the submergence duration increased, different treated plants showed decrease in the aboveground dry weight and root dry weight.

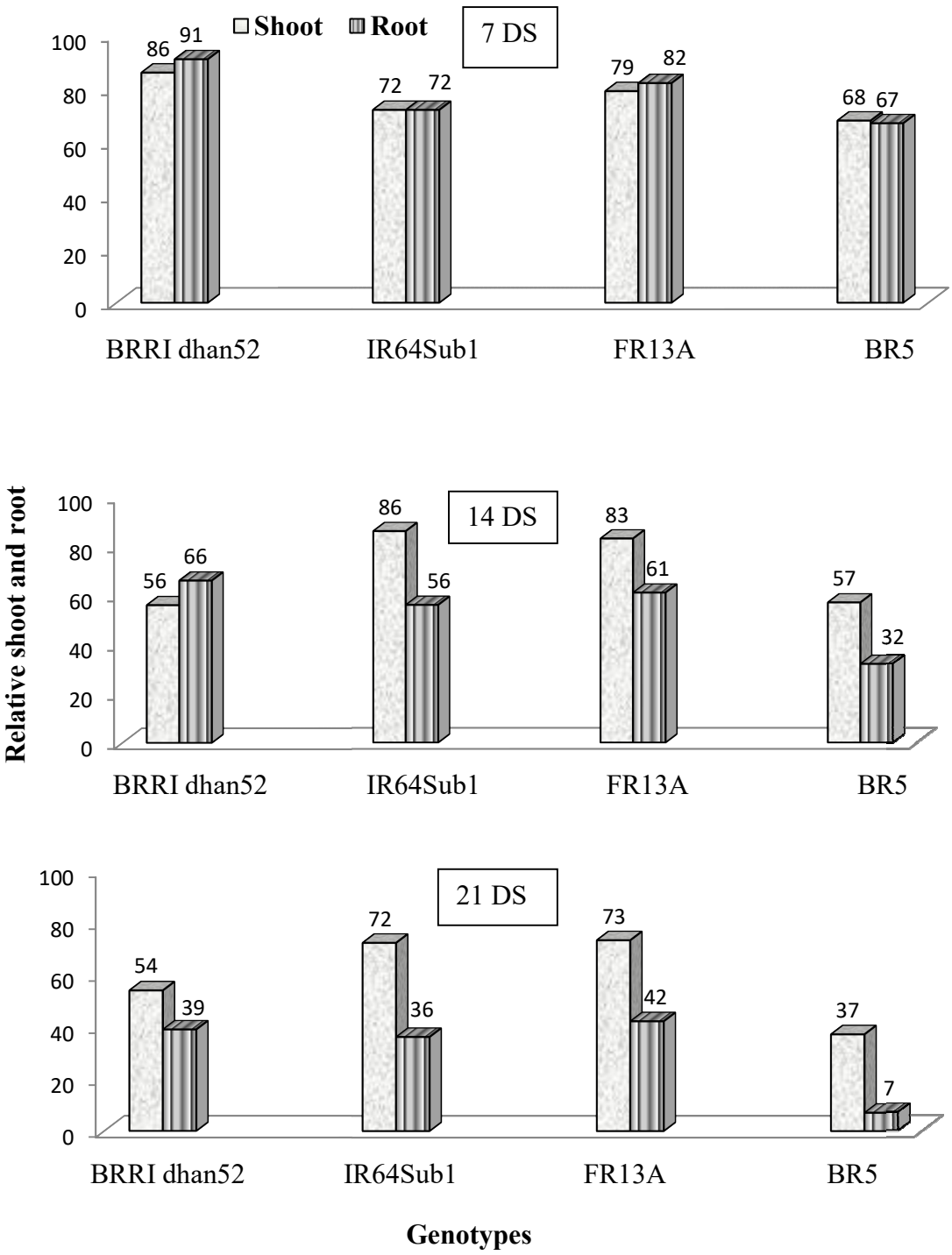


Figure 7. Relative shoot and root dry matter content of different rice genotypes under various submergence treatments. DS= Days of submergence.

4.1.10. Relative water content (RWC) of leaves after desubmergence

The relative water content of leaf was calculated after 2 hours of desubmergence both from control and submerged plants and the results have been shown in figure 8. In all the submergence treatments, it was recorded that the relative water content was significantly the highest at the control treatment compared to submergence treatment. Under 7 DS treatment, the relative water content of leaves was much higher in control treatment than the submerged treatment, in all the genotypes. Among the genotypes under 7 DS treatment, the highest (93.21%) relative water content of leaf recorded was in FR13A at control plant which was statistically similar to IR64Sub1, BRRI dhan52 and BR5 at control treatment and the lowest (64.12%) relative water content of leaf recorded was in BR5 at submerged plant which was significantly lower than any other genotype of this treatment.

Under 14 DS treatment, the relative water content of leaf recorded was higher in control plants than the submerged plants in all the genotypes. Among the genotypes under this treatment, the relative water content of leaf recorded was the highest (93.41%) in FR13A at control plant which was statistically similar to IR64Sub1, BRRI dhan52 and BR5 at control treatment and the lowest (40.11%) recorded was in submerged plants of BR5 genotype which was significantly lower than any other genotype of this treatment.

Under 21 DS treatment, the relative water content of leaf recorded was also higher in control plants than the submerged plants in all the genotypes. Among the genotypes under this treatment, the relative water content of leaf found was the highest (93.51%) in IR64Sub1 at control plants which was statistically similar to BRRI dhan52, FR13A and BR5 at control treatment and the lowest (21.35%) recorded was in BR5 at submerged plant which was significantly lower than any other genotype of this treatment. A drastic reduction in RWC of leaf recorded was in BR5 at 21 DS treatment.

Under 7, 14 and 21 DS treatment the RWC of BRRI dhan52 and FR13A was relatively higher than the other genotypes. In the present experiment, the relative water content (RWC) was decreased significantly in submergence treatment compared to control in all the genotypes and the lowest RWC recorded was in BR5 in all the treatments. This might be due to transpiration rate was increased after desubmergence because of sudden high light and high heat. A sort of

water stress (deficiency) was also created in the affected tissue (Fukao *et al*., 2011). The water stress is related to fall in root hydraulic conductance of the plant. But the lower amount of reduction in root DM under submergence treatment in BRRI dhan52 and in FR13A compared to other might help in higher RWC under submergence. Under submerged condition, the membrane of the root cells were injured and root membrane permeability to water absorption were hampered. The injured root cannot supply enough water to the leaf to meet that transpirational demand. As a result plants fall in drought stress and the RWC of leaf become lower (Banerjee *et al*., 2015b). In this situation, plant try to maintain higher RWC through osmotic regulation by accumulating proline (shown in figure 23). It was also recorded in the present experiment that the RWC of leaf was closely related to the membrane injury level of the leaf in a certain treatment. The RWC of leaf was lower in those treatments where the membrane injury recorded was higher. The severe injured leaf tissues (in BR5 at 21 DS treatment) were unabled to maintain osmotic adjustment under water stress condition. The lowest RWC in submerged leaf of BR5 indicated that this genotype is more susceptible to submergence stress than the other genotypes. In this situation, the genotype (such as FR13A) in which the RWC of leaf remain closer to control by accumulating more proline, is suppose to be tolerant type.

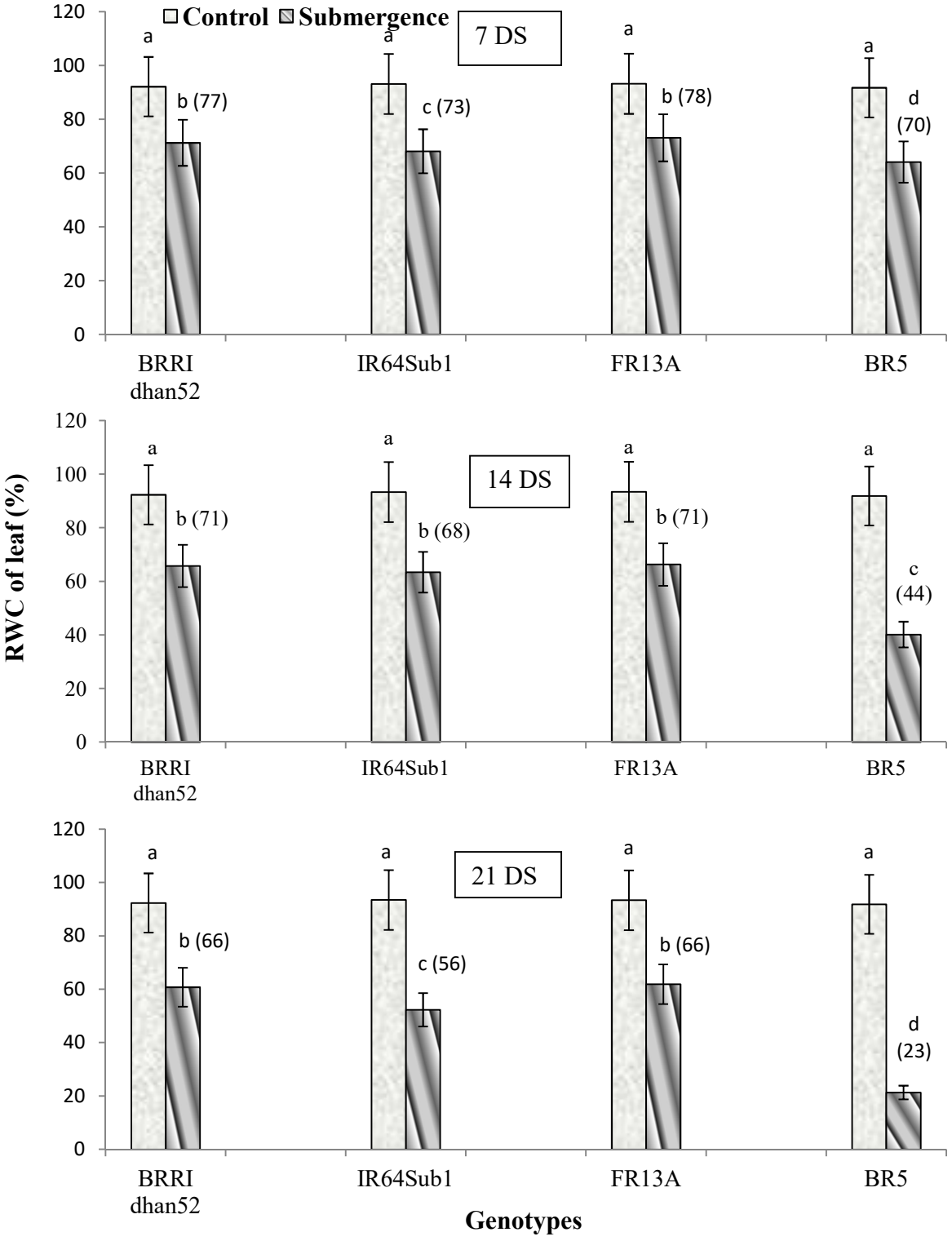


Figure 8. Relative water content of leaf after desubmergence of submerged plants and control plants. Bar represents standard deviation. DS= Days of submergence, figures inside the parenthesis indicate values relative to control.

4.1.11. SPAD values after desubmergence

The SPAD values were recorded from the average of upper three fully expanded leaves. The SPAD values were recorded from the leaves of submerged plants after desubmergence and from non-submerged control plants, in order to compare the greenness as well as the chlorophyll content of the leaves. In all the genotypes, the control plant showed higher greenness than the submerged plants (Figure 9). In BRRI dhan52, IR64Sub1 and FR13A, the SPAD values of control plants were little higher than the SPAD values of submerged plants. The difference between the SPAD value of control plants and submergence plants was much higher under 21 DS treatment in BR5. In the present experiment, due to 7 DS treatment, the plants showed comparatively higher SPAD values. Similar results indicating non-senescent leaves with high content of chlorophyll in all the genotypes was also reported by Siangliw *et al*. (2003). Under 7 DS treatment, the SPAD value of BRRI dhan52 at control and submerged plants was close. Under 21 DS condition, most of the leaves of the rice plant were senescent and the greenness of the plants became lower which might be due to degradation of chlorophyll with the activation of chlorophyllase enzyme. Toojinda *et al.* (2003) measured leaf greenness with SPAD meter and found that submergence-tolerant plants are able to retain green leaves for longer time than the intolerant lines. In the present experiment, BRRI dhan52 and FR13A are supposed to be tolerant type and BR5 is intolerant.

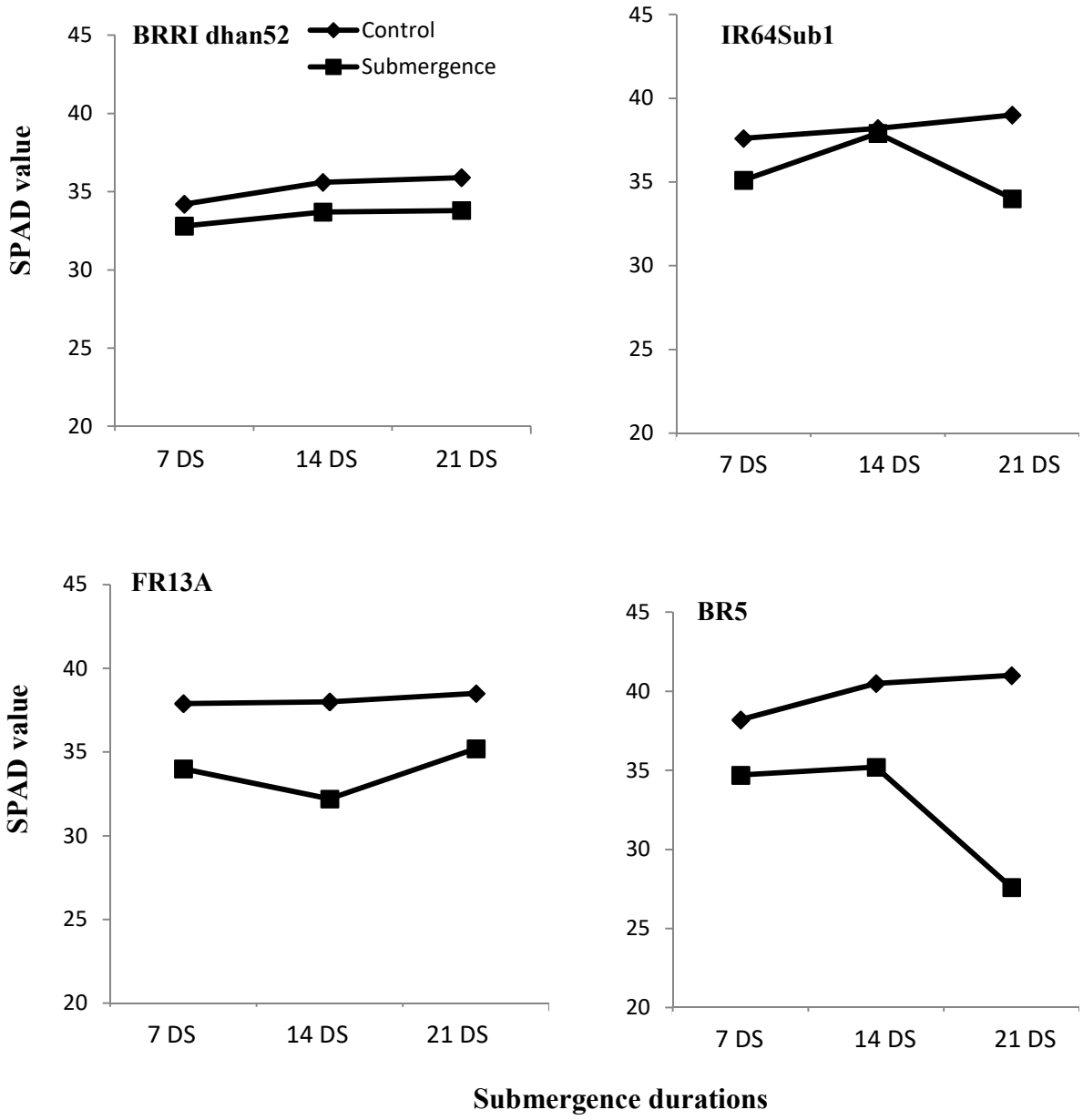


Figure 9. SPAD values of leaf after desubmergence of submerged plants and control plants. DS = Days of submergence.

4.1.12. Stomatal conductance after desubmergence

The stomatal conductance was also recorded from upper three fully expanded leaves of submerged and control plants and average values were recorded. The stomatal conductance found was lower in submerged leaves than control leaves in all the genotypes (Figure 10). Under 7 DS treatment, the stomatal conductance of control and submerged leaves were much closer in all the genotypes. In 21 DS treatment, the difference in stomatal conductance of control and submerged leaves were much higher in all the genotypes. In BR5, the difference in stomatal conductance between control and 21 DS treatment was much higher. This might be due to more injured leaf tissues under 21 DS treatment in BR5.

After desubmergence, plants fall in water stress (deficiency) due to excess water loss from the injured leaf tissues. On the other hand, the injured root was unable to keep pace with the transpiration demand. Finally, the relative water content of the leaf became lower. Possibly the guard cell lost turgidity for which the stomata became closed. Taiz and Zeiger (1998) stated that under water stress condition ABA (Abscisic acid) accumulation might be stimulated in root and movement of ABA to leaves leading to stomatal closure. Debabrata and Kumar (2011) stated that stomatal conductance found to be significantly decreased in both Swarna and Swarna *Sub 1* during submergence as compared to control plants. They also found that after 7days of submergence, the stomatal conductance was significantly more in Swarna *Sub 1* compared to Swarna. In the present experiment, the genotypes BRRI dhan52 and FR13A showed better stomatal conductance than the other genotypes even if they were under submergence treatment.

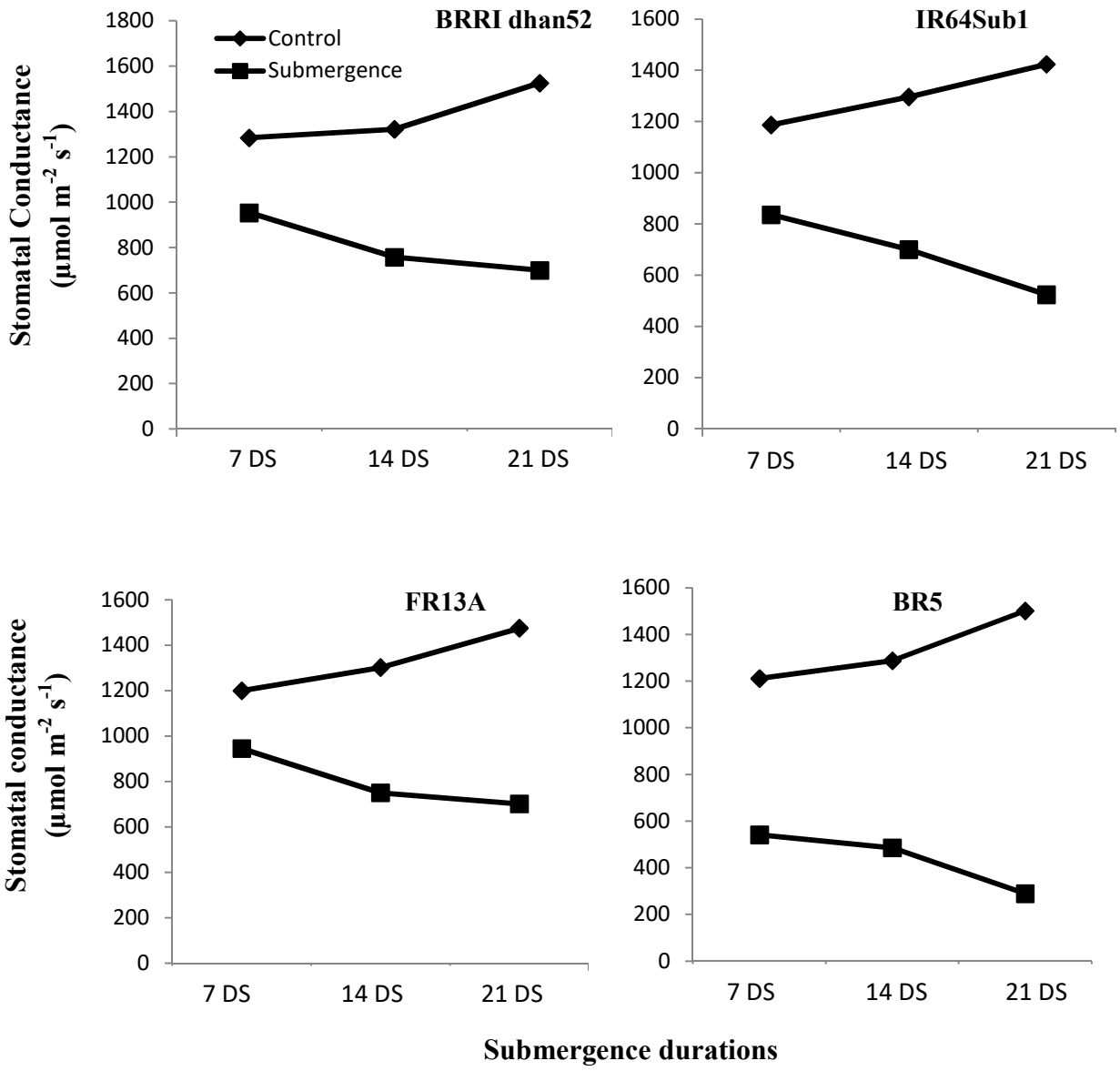


Figure 10. Stomatal conductances of leaf after desubmergence of submerged plants and control plants. DS= Days of submergence

4.1.13. Plant height at maturity

Under control condition, plant height increased with time and reached its maximum near to flowering stage. Considering all the genotypes and submergence durations, the highest (133.13 cm) plant height recorded was in BR5 at 7 DS treatment which was statistically similar to control treatment of BR5 and 7 DS treatment of FR13A (Table 5). The plant height found was the lowest (79.43 cm) in IR64Sub1at 21 DS treatment which was significantly lower than any other treatment. In all the genotypes, the plant height was lower at 21 DS treatment compared to other submergence treatments. In BRRI dhan52, the highest plant height recorded was at control treatment which was gradually decreased with the increase of submergence durations. But in this genotype plant height was less affected due to 21 DS treatment compared to other genotypes. In case of other three genotypes (IR64Sub1, FR13A and BR5), the plant height was slightly higher at 7 DS treatment compared to control treatment and then gradually decreased with the increase of submergence duration. Considering all the genotypes and submergence treatments, the highest relative plant height (118%) recorded was in IR64Sub1 at 7 DS treatment and the lowest relative plant height (79.65%) recorded was in FR13A at 21 DS treatment. 14 DS treatment effect on plant height in IR64Sub1, FR13A was little, but the effect was comparatively higher in case of BR5. All the plants found dead in case of BR5 due to the effect of 21 DS treatment.

In the present experiment, it recorded was that in BRRI dhan52, the plant height was less affected due to submergence treatments compared to other genotypes. It was also recorded that in comparison to control treatment, 7 DS treatment did not create any remarkable effect on plant height in all the genotypes except in IR64Sub1. On the other hand, in BR5, though underwater elongation was much higher due to submergence treatment but finally those plants (of BR5) were appeared with lower relative plant height at maturity. In BRRI dhan52, IR64Sub1 and FR13A at 21 DS treatment and BR5 at 14 DS treatment, their height decreased significantly during recovery period because of senescence and loss of most leaves and new tiller was emerged from the base of the plant during recovery which were also reported by Singh *et al*. (2014). After desubmergence, the plants required energy for recovery in order to compensate in biomass production and

Table 5. Effect of different submergence treatments on plant height at maturity stage of different rice genotypes

Genotypes	Days of submergence (DS)	Plant height (cm)	Plant height relative to control (%)
BRRI dhan52	0	108.00 de	100.00
	7	106.63 ef	98.73
	14	101.95 fg	94.40
	21	99.85 g	92.45
IR64Sub1	0	89.30 h	100.00
	7	105.38 ef	118.00
	14	90.03 h	100.82
	21	79.43 i	88.95
FR13A	0	127.75 bc	100.00
	7	130.13 ab	101.86
	14	123.08 c	96.34
	21	101.75 fg	79.65
BR5	0	131.80 ab	100.00
	7	133.13 a	101.00
	14	111.88 d	84.89
	21	0	0
$LSD_{(0.05)}$		4.78	
CV (%)		3.27	

Values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

attain required plant height. Elanchezhian *et al*. (2013) found that short term flooding significantly affected the growth of all the genotypes. They also stated that significant differences in plant height were observed among different varieties under control and stress treatment. Nugraha *et al*., (2012) found that plant heights among rice genotypes varied, ranged from 91 to 129 cm under submergence. Although there was an excessive elongation on non-*Sub1* lines during submerged conditions, after recovery the final plant heights were not significantly different. Increased in plant height due to submergence treatment was also reported by Singh *et al*. (2014). In the present experiment, the submergence treatments did not create any remarkable effect on final plant height even if the effect was prominent at seedling stage under water.

4.1.14. Effective and total tillers number per plant

The number of effective tillers and total tillers per plant were counted at maturity stages of different rice genotypes from each treatment and replication. In all the genotypes, the highest number of tillers recorded was at control treatment and then gradually decreased with the increase of submergence duration (Table 6). Considering all the genotypes and submergence duration, the total tiller number found was the highest (25.50) in IR64Sub1 at control which was significantly higher than any other treatment. The lowest number (6.0) of tiller recorded was in FR13A at 21 DS treatment which was statistically similar to 14 DS and 21 DS treatment of BRRI dhan52. Due to submergence treatment, the genotype IR64Sub1 produced the higher number of late tillers, which usually failed to reach maturity. Some panicles were very small in size and did not produce any grain.

The number of effective tiller is one of the major yield determinants. In the present experiment, among the genotypes, the highest number of effective tiller were recorded at control treatment and then gradually decreased with the increasing submergence duration. Considering all the genotypes and submergence duration, the number of effective tiller recorded was also the highest (24.25) in IR64Sub1 at control treatment which was significantly higher than any other treatment. The lowest number (5.50) of effective tiller was also recorded in FR13A at 21 DS treatment which was statistically similar to 14 DS and 21 DS treatment of BRRI dhan52. In the present experiment, among the genotypes, the submergence effect on the number of effective tiller was lower in BRRI dhan52 (considering the lettering) compared to other genotypes. The

effective tiller number of BRRI dhan52 at 7 DS and 14 DS treatment was statistically similar to effective tiller number at control treatment. In FR13A, the effective and total tiller at 7 DS and control treatment was statistically same. The genotypes (as in BRRI dhan52 and FR13A), in which the reduction of effective tillers due to submergence is lower are considered as the submergence tolerant because of high tillering ability is desirable for achieving maximum yield. Due to submergence the formation of tiller bud might have been hampered which decreased the total tiller number. It was reported that the plants submerged at seedling establishment increased plant height rapidly as a result of which the tillering was affected adversely (Reddy *et al*., 1985). It was also reported that under low light condition due to submergence, some of the tiller buds may not develop into tillers because of lack of carbohydrate necessary for growth (Yoshida, 1981). Hanada *et al*. (1990) suggested that lack of oxygen for respiration or accumulation of ethylene might inhibit tiller bud formation and growth. Submergence tolerant genotypes produced substantially more tillers than did the sensitive ones after 12 and 17 days of submergence. During the recovery phase, the number of tillers per unit area at maturity correlated positively with survival. However, the loss in tillers per unit area could not be compensated for in the sensitive genotypes because of the drastic decrease (up to 98 %) in survival (Singh *et al*., 2014). Reddy *et al*. (1985) also observed a significant reduction in tiller number in rice genotypes submerged at seedling establishment stages.

Table 6. Effect of different submergence treatments on total tillers and effective tillers number at maturity stage of different rice genotypes

Genotypes	Days of submergence (DS)	Total tiller number per plant	Effective tiller number per plant
BRRI dhan52	0	9.75 d	9.75 d
	7	9.75 d	9.50 d
	14	8.50 de	7.50 def
	21	6.50 e	6.25 ef
IR64Sub1	0	25.50 a	24.25 a
	7	16.50 b	15.00 b
	14	11.25 cd	9.50 d
	21	10.00 cd	9.00 d
FR13A	0	12.75 c	12.25 c
	7	10.50 cd	9.50 d
	14	9.50 d	8.25 de
	21	6.00 e	5.50 f
BR5	0	16.25 b	15.25 b
	7	11.00 cd	9.75 d
	14	10.00 cd	9.74 d
	21	0	0
$LSD_{(0.05)}$		2.55	2.37
CV (%)		16.46	16.52

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.15. Days to flowering and the duration from anthesis to maturity

The days to flowering and the duration from anthesis to maturity was calculated in days from each treatment and replication. Among the genotypes and submergence durations, the longest (123 d) days to flowering recorded was in BRRI dhan52 at 21 DS treatment which was significantly higher than the other treatments and the shortest (88 d) days to flowering recorded was in IR64Sub1 at 0DS or control treatment which was significantly lower than the other treatments (Table 7). In all the genotypes, the shortest days to flowering recorded was at control treatment and was gradually increased with the increasing submergence durations and ultimately the highest days to flowering recorded was at 21 DS treatment.

Considering all the genotypes and submergence duration, the longest (38 d) duration from anthesis to maturity recorded was in BR5 at 14 DS treatment which was statistically similar to 7 DS treatment of BR5 and 21 DS treatment of FR13A. The shortest (30 d) duration from anthesis to maturity recorded was in IR64Sub1 at 21 DS treatment which was statistically similar to the other treatments of this genotype and with all the treatments of BRRI dhan52 and also with all the treatments of FR13A except 21 DS treatment. In BRRI dhan52 and IR64Sub1, the duration from anthesis to maturity, gradually decreased from control to 21 DS treatment and in FR13A and BR5, the duration from anthesis from maturity gradually increased with the increasing submergence durations.

Significant variation found was in the duration from germination to maturity by the interaction effect of submergence duration and rice genotypes (Table 10). Considering all the genotypes and submergence treatments, the duration of life cycle or the duration from germination to maturity found was the highest (155 d) in BRRI dhan52 at 21 DS treatment which was statistically similar to FR13A at 21 DS treatment and with BR5 at 14 DS treatment. The lowest (120 d) life duration recorded was in IR64Sub1 at control treatment, which was significantly lower than any other treatment. In all the genotypes, the life duration recorded was the shortest at control treatment and were gradually increased with the increasing submergence duration. For this reason, the duration from germination to maturity found was the highest at 21 DS treatment in all the genotypes.

In the present experiment, due to submergence treatment, days to flowering was delayed in all the genotypes. This might be due to higher vegetative injury during submergence and the injured plants took longer time for recovery. On the other hand, due to submergence, plants might have not got total required temperature for flowering (degree days). As a result, the days to flowering became longer in submergence treated plants compared to control plants in all the genotypes. The grain filling duration in submergence treated plants was as same as the control plant (at 7 DS treatment in BRRI dhan52, IR64Sub1 and FR13A) which might have positive role in grain yield. So, increased life duration of the submergence treament plant was due to increase in vegetative duration rather than reproductive as well as grain filling duration. Elanchezhian *et al*. (2013) found that short term flooding delayed days to 50 % flowering, however, the responses varied among the genotypes. Days to flowering were varied among the genotypes under submergence, in which the non-*Sub1* were 2-5 days longer compared to that of *Sub1* lines. Under submergence the *Sub1* lines were little delayed in flowering because it had least damage due to submergence (Nugraha *et al*., 2012). Faster recovery of tolerant genotypes was also associated with a shorter delay in flowering and maturity (Singh *et al*., 2009).

In the present experiment, it found was that longer the submergence duration, longer was the duration from germination to maturity. This might be due to the submerged plant did not get enough photoperiod and temperature during submergence period. The growth duration of a variety is highly location and season specific because of interactions between the variety's photoperiod and temperature sensitivity and weather conditions (Yoshida, 1981). As a result, after desubmergence their vegetative period became longer and when they got required amount of temperature and photoperiod, flowering occurred and plant completed their lifecycle. It was also recorded that increased vegetative duration due to submergence treatment mainly contributed in increased life duration and the grain filling duration had little effect on total life duration in this condition.

Table 7. Effect of different submergence treatments on days to flowering, duration from anthesis to maturity and life cycle (germination to maturity) of different rice genotypes

Genotypes	Days of submergence (DS)	Days to flowering	Duration from anthesis to maturity (days)	Duration from germination to maturity (days)	
				Actual	Relative to control (%)
BRRI dhan52	0	100 f	33 cde	133 e	100
	7	107 de	33 cde	140 cd	105
	14	115 b	33 cde	147 b	111
	21	123 a	32 cde	155 a	117
IR64Sub1	0	88 g	32 cde	120 f	100
	7	98 f	32 cde	130 e	108
	14	109 cd	31 de	140 cd	117
	21	112 c	30 e	142 c	118
FR13A	0	106 e	31 de	137 d	100
	7	109 cd	31 de	140 cd	102
	14	115 b	32 cde	147 b	107
	21	116 b	37 ab	153 a	112
BR5	0	111 c	34 bcd	145 b	100
	7	111 c	35 abc	146 b	101
	14	116 b	38 a	154 a	106
	21	0	0	0	
LSD(0.05) CV (%)		2.61 1.79	2.99 6.81	2.94 1.55	

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.16. Dry matter of main stem leaf and stem

The main stem leaf and stem dry matter recorded was from each treatment and replication. The main stem leaf and stem dry matter of each plant was varied significantly by the interaction effect of submergence duration and rice genotypes. All the plants found dead in BR5 genotype at 21 days of submergence (DS) treatment (Table 8). Considering the other three genotypes and their submergence treatments, the leaf dry matter of main stem found was the highest (1.367 g) in FR13A at 0DS treatment, which was statistically similar to 7 DS treatment of BRRI dhan52. The lowest (0.638 g) leaf dry matter of main stem recorded was in IR64Sub1 at 0DS treatment, which was statistically similar to 21 DS treatment of the same genotype and 14 DS treatment of BR5. In BRRI dhan52 and IR64Sub1, 7 DS treatment produced the highest leaf dry matter of main stem, which was followed by 14 DS treatment and then followed by 21 DS treatment. In FR13A and BR5, the control treatment produced the highest leaf dry matter which was followed by 14 DS treatment in FR13A and 7 DS treatment in BR5. So it was clear from the above statement that submergence treatment increased the leaf investment in BRRI dhan52 and in IR64Sub1 compared to control. But in FR13A, the difference in leaf dry matter under control and submergence treatment was very little. Elanchezhian *et al*. (2013) found significant differences in leaf and stem dry weight between submerged and control treatments.

Among the genotypes and submergence treatments, the stem dry matter of main stem recorded was also the highest (3.732 g) in FR13A at 0DS treatment, which was statistically similar to 7 DS and 14 DS treatments of the same genotype. The lowest (2.112 g) stem dry matter was also recorded in IR64Sub1 at 0DS treatment. In BRRI dhan52, the stem dry matter of main stem recorded was the highest at 14 DS treatment which was followed by 7 DS treatment and then followed by 21 DS treatment. In IR64Sub1, the stem dry matter of main stem recorded was the highest at 7 DS treatment which was followed by 14 DS treatment and then followed by 21 DS treatment. In case of FR13A and BR5, the control treatment produced the highest stem dry matter of main stem. Submergence treatment increased the leaf and stem investment in BRRI dhan52 and IR64Sub1 compared to control. But in FR13A, the difference in stem dry matter under control and submergence treatment was very little. As the tiller number was decreased due to submergence treatment, the photosynthetic investment might have increased the main stem dry matter and the vegetative dry matter. Singh *et al*. (2014) found that the Sub1 lines and the

tolerant checks (FR13A and IR49830) showed a significant increase in stem dry weight. The sensitive variety (BR5) showed decrease in stem dry weight under submergence.

Table 8. Effect of different submergence treatments on main stem leaf dry matter and main stem dry matter at maturity stage of different rice genotypes

Genotypes	Days of submergence (DS)	Main stem leaf dry matter (g)	Main stem dry matter (g)
BRRI dhan52	0	0.95 de	2.49 ef
	7	1.30 a	2.98 bcd
	14	1.28 ab	3.02 bc
	21	1.12 bc	2.82 cde
IR64Sub1	0	0.64 g	2.11 f
	7	0.90 ef	2.98 bcd
	14	0.88 ef	2.46 ef
	21	0.71 g	2.14 f
FR13A	0	1.37 a	3.73 a
	7	1.25 ab	3.59 a
	14	1.28 ab	3.37 ab
	21	1.07 cd	2.58 de
BR5	0	1.27 ab	3.37 ab
	7	1.09 cd	3.11 bc
	14	0.76 fg	2.49 ef
	21	0	0
$LSD_{(0.05)}$		0.15	0.37
CV (%)		10.75	9.74

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.17. SPAD reading from anthesis to maturity

The SPAD (Soil Plant Analysis Development) value represents the greenness of the leaf. The SPAD value has been well recognized as a mean of determining the onset of senescence process in a leaf (Rajcan *et al*., 1999). The sharp decrease in SPAD value indicates the onset of leaf senescence. In the present experiment, the SPAD value recorded was from the flag leaves of all tillers and the average value was taken during the grain filling period after 5 days interval from anthesis to maturity. In the present study, sharp decrease in SPAD value to around 20 was an indication of the onset of flag leaf senescence. In BRRI dhan52, the SPAD values of submerged leaf were found lower than control leaves (Figure 11). During anthesis period the SPAD values recorded were ranged from 40 to 45 in BRRI dhan52. After anthesis, the SPAD value slightly increased and then gradually decreased with advancement towards maturity. In BRRI dhan52, longer the submergence duration lower was the SPAD value recorded. The control treatment maintained stronger trend of SPAD values from anthesis to maturity compared to submerged treatment. At maturity stage, the SPAD values recorded were around 20 in both the control and submerged treatment. Rajcan *et al*. (1999) reported that the leaf senescence of maize occurred at a SPAD reading between 25 and 30. In an experiment, Hasan (2009) recognized the SPAD values ranging from 25 to 30 as an indicator of flag leaf senescence in wheat.

In IR64Sub1, the submerged plants showed lower SPAD values compared to control plant during grain filling (Figure 12). During anthesis period, the SPAD values recorded were ranged from 45 to 50. Then slightly increased during active grain filling period and then gradually decreased with advancement towards maturity. Here control treatment also maintained stronger trend of SPAD values from anthesis to maturity (about 30 to 35 days after anthesis) than the submerged treatments.

In FR13A, the SPAD values in control treatment were much higher than submerged treatments (Figure 13). During anthesis period the SPAD values recorded were ranged from 35 to 45. Then slightly increased during active grain filling period and then gradually decreased with advancement towards maturity (about 30 to 35 days after anthesis).

In BR5, the submerged plants SPAD values recorded were also lower than the control plants (Figure 14). During anthesis period the SPAD value of BR5 found was around 50. Then slightly

increased during active grain filling period and then sharply decreased with the advancement towards maturity. Higher SPAD values as well as higher chlorophyll content in control plant contributed to higher photosynthesis and consequently total dry matter content in BR5 was increased.

The degradation of chlorophyll increases with the age towards maturity, as a result the SPAD values gradually decreased from anthesis to maturity in all the genotypes. Plants showing high SPAD values indicate non-senescent leaves with high content of chlorophyll (Siangliw *et al*., 2003). Toojinda *et al*. (2003) measured leaf greenness with SPAD meter and found that submergence-tolerant plants are able to retain green leaves for longer than intolerant lines.

4.1.18. Stomatal conductance from anthesis to maturity

The stomatal conductance recorded was from the flag leaf of all the tillers and the average value was taken during the grain filling period with an interval of 5 days from anthesis to maturity. The stomatal conductance recorded was upto maturity when the SPAD value recorded was around 20. In BRRI dhan52, the stomatal conductance of submerged plants recorded was below the stomatal conductance of control plants (Figure 15). The stomatal conductance recorded were the highest during anthesis (ranged from 750 to 1200 µmol m^{-2} s^{-1}) both in control and submerged plants and then gradually decreased towards maturity (about 30 to 35 days after anthesis).

In IR64Sub1, the stomatal conductance recorded was the highest during anthesis (ranged from 1000 to 1200 µmol m^{-2} s^{-1}) both in control and submerged plants and then gradually decreased towards maturity (about 30 to 35 days after anthesis). The control treatment showed higher stomatal conductance than the submerged treatments throughout the grain filling period (Figure 16).

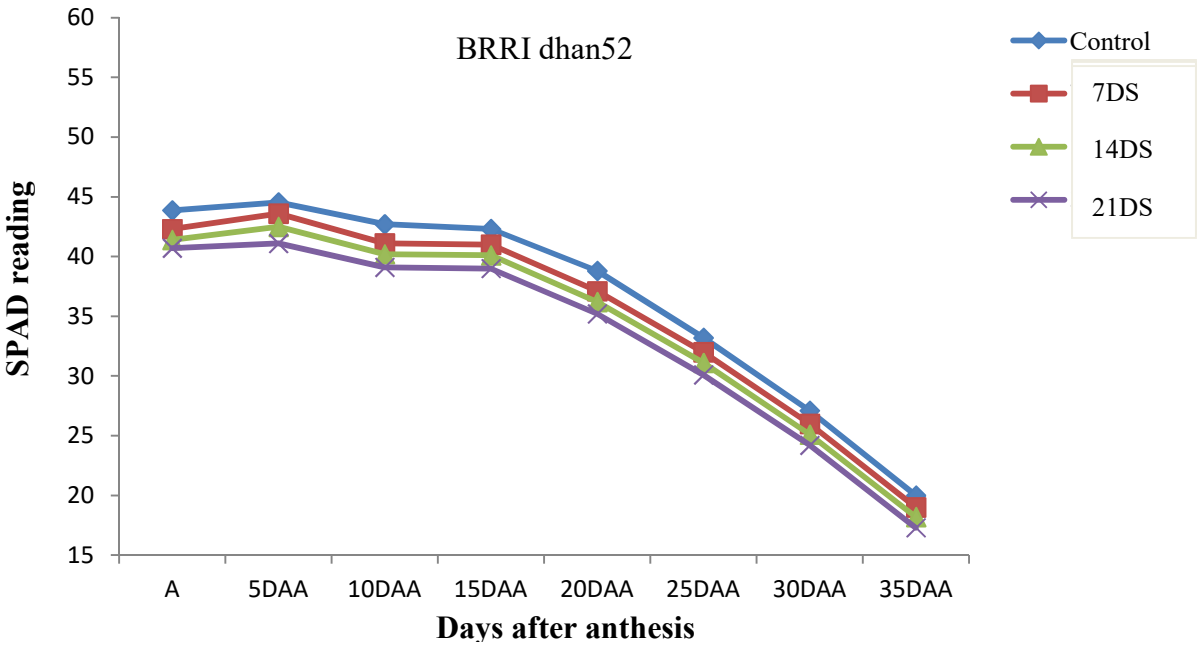


Figure11. SPAD reading from anthesis to maturity of BRRI dhan52 under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

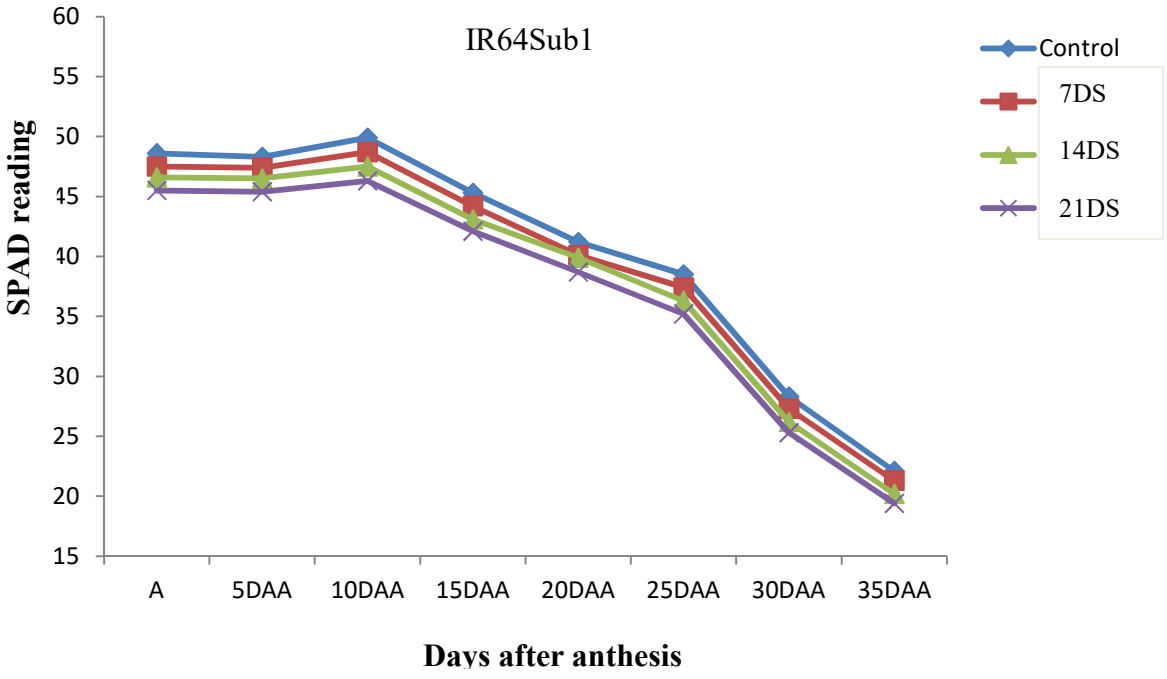


Figure12. SPAD reading from anthesis to maturity of IR64Sub1 under different submergence treatments , DAA=Days after anthesis, A= Anthesis.

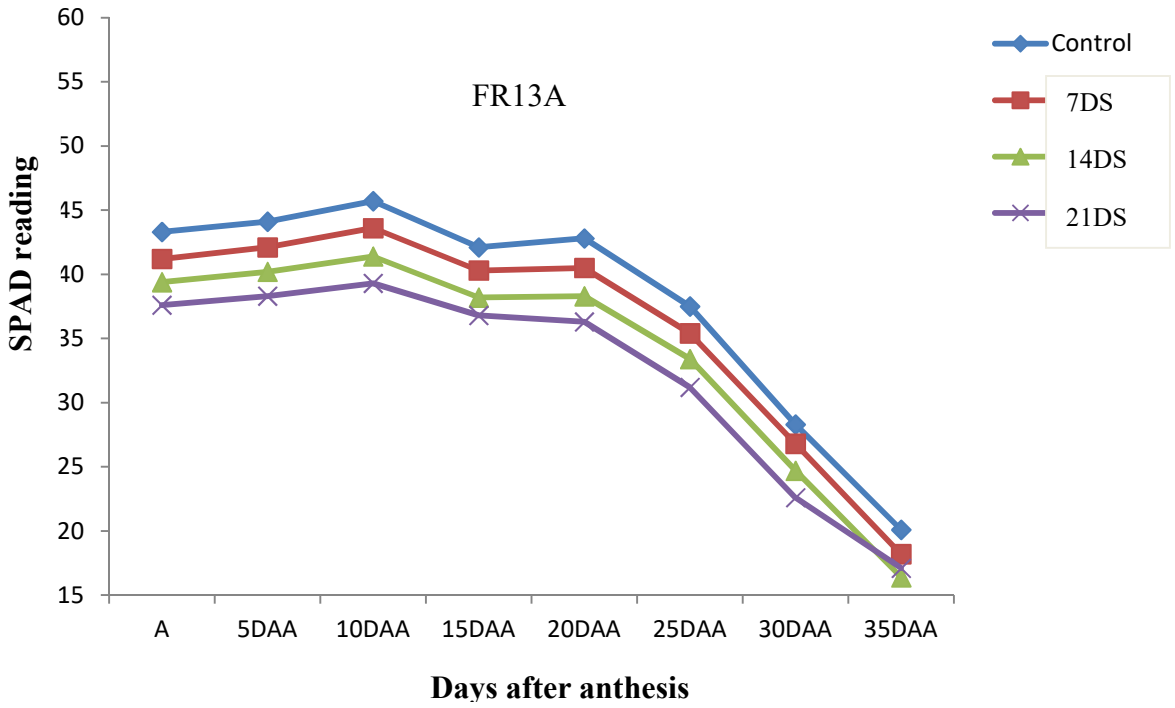


Figure13. SPAD reading from anthesis to maturity of FR13A under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

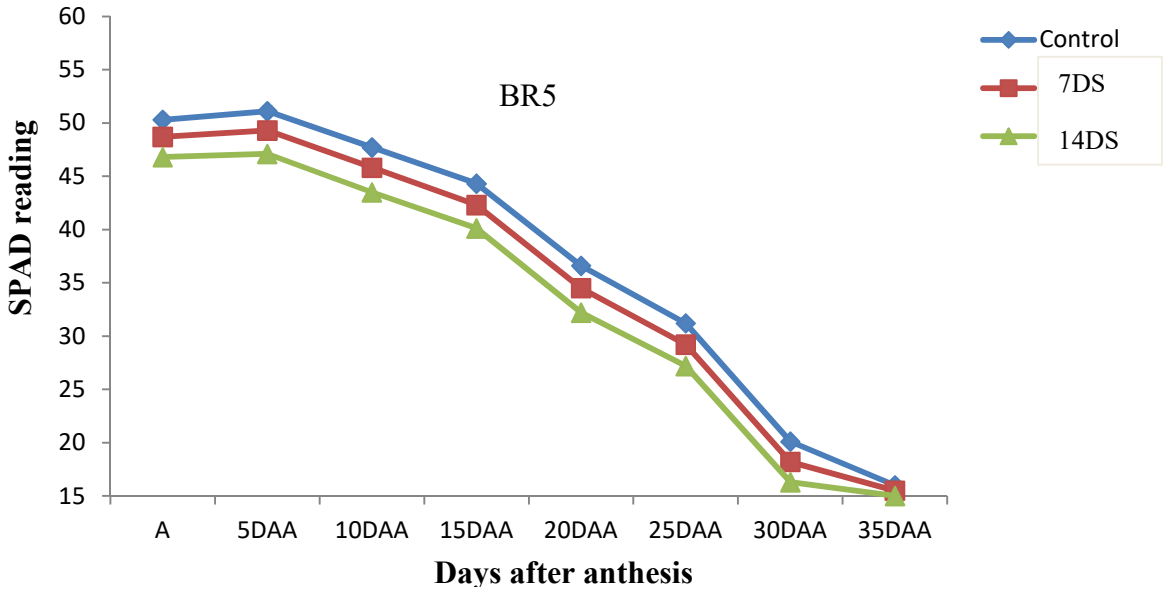


Figure 14. SPAD reading from anthesis to maturity of BR5 under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

In FR13A, the stomatal conductance recorded was the highest during anthesis (ranging from 800 to 1000 µmol m^{-2} s^{-1}) both in control and submerged plant and then gradually decreased toward maturity (about 30 to 35 days after anthesis) and the results are shown in (Figure 17). The stomatal conductance of control treatment was much higher than submerged treatments throughout the grain filling period.

In BR5, the stomatal conductance recorded was the highest during anthesis (ranging from 700 to 900 µmol m^{-2} s^{-1}) and then gradually decreased towards maturity (about 30 to 35 days after anthesis). The stomatal conductance of submerged plants were much lower than the control plants in this genotypes (Figure 18).

The lower stomatal conductance in submerged plants compared to control might be due to lower chlorophyll content and higher leaf injury due to submergence in all the genotypes. Relatively higher chlorophyll content of IR64Sub1 in submerged treated plant might contributed in relatively higher stomatal conductance in this genotype. The chlorophyll content gradually decreased towards maturity which might contribute in gradual decrease in stomatal conductance from anthesis to maturity. The changes in CO_2 assimilation under flooding was attributed to the stomatal and non stomatal limitation (Pezeshki, 2001), which include reduction of RuBP generation, down regulation of RuBP carboxylase and ethylene mediated chlorosis (Jackson and Ram, 2003; Ella *et al*., 2003c).

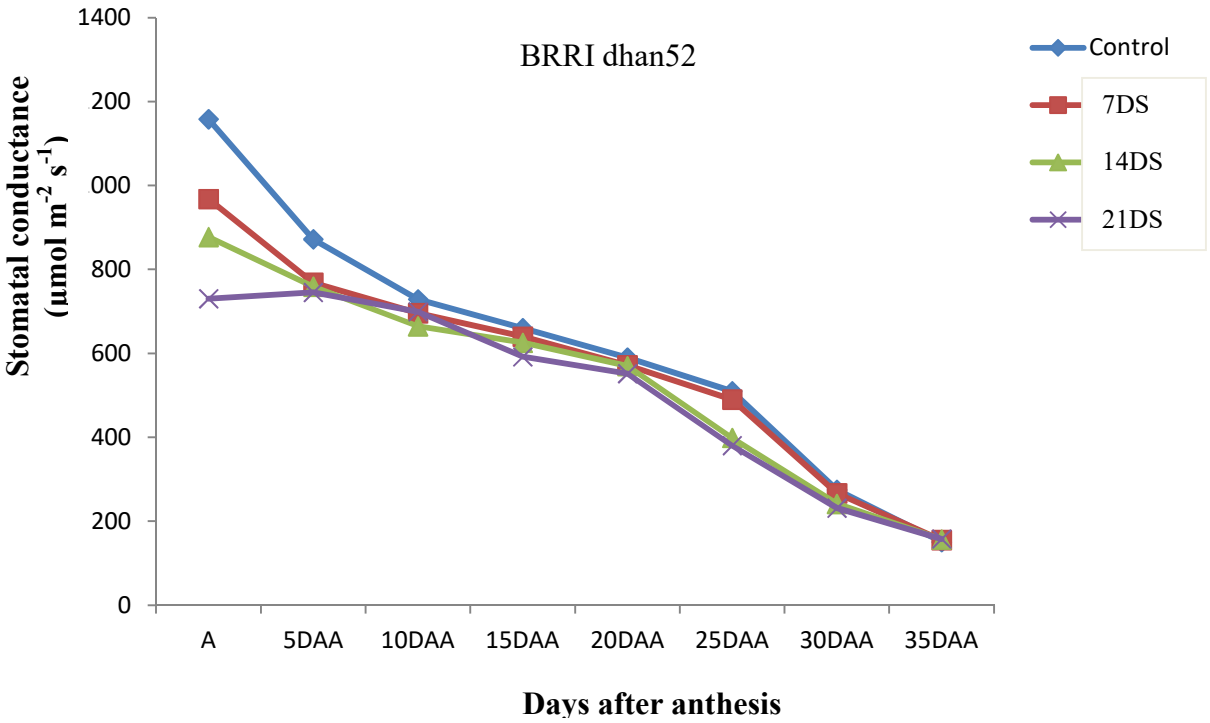


Figure 15. Stomatal conductance from anthesis to maturity of BRRI dhan52 under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

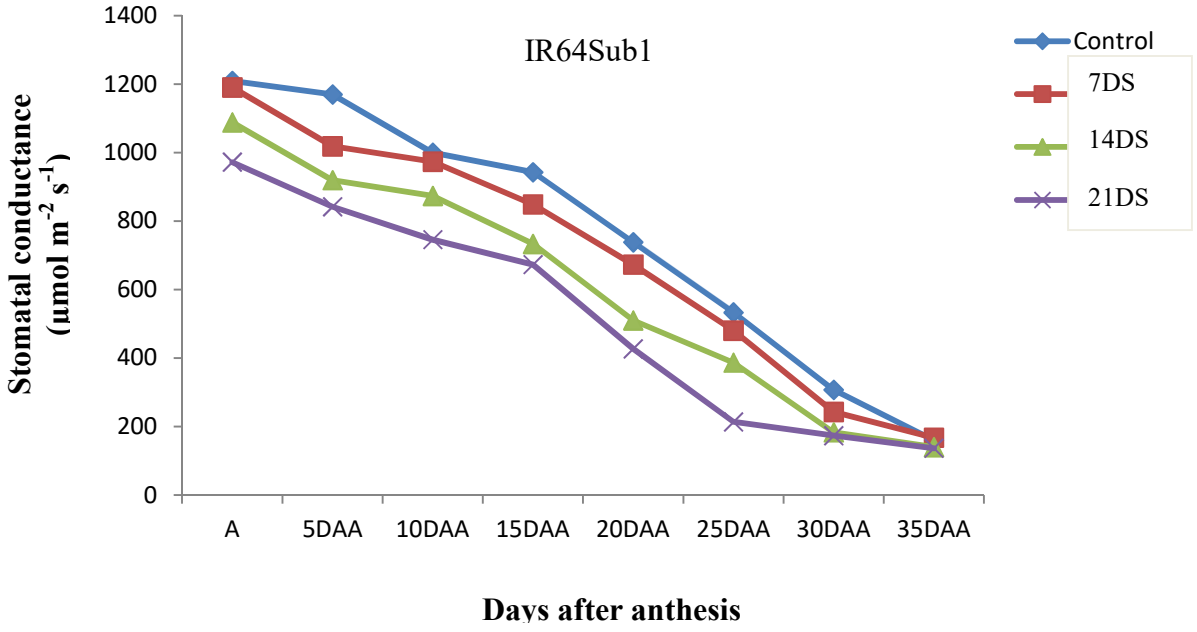


Figure 16. Stomatal conductance from anthesis to maturity of IR64Sub1 under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

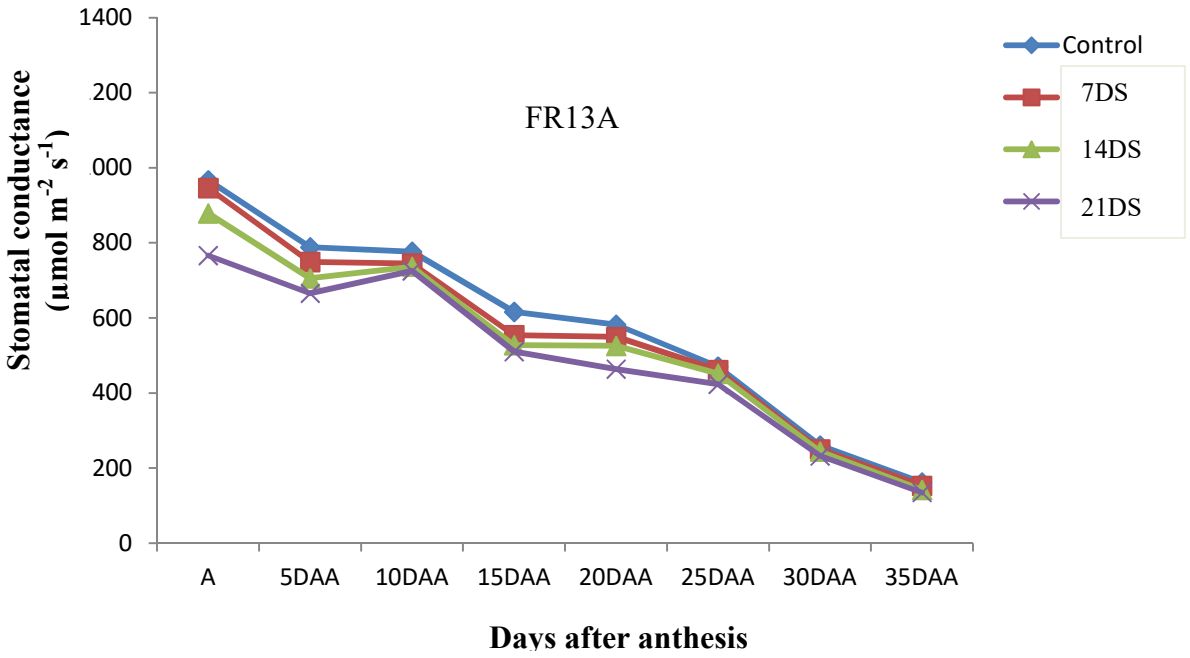


Figure 17. Stomatal conductance from anthesis to maturity of FR13A under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

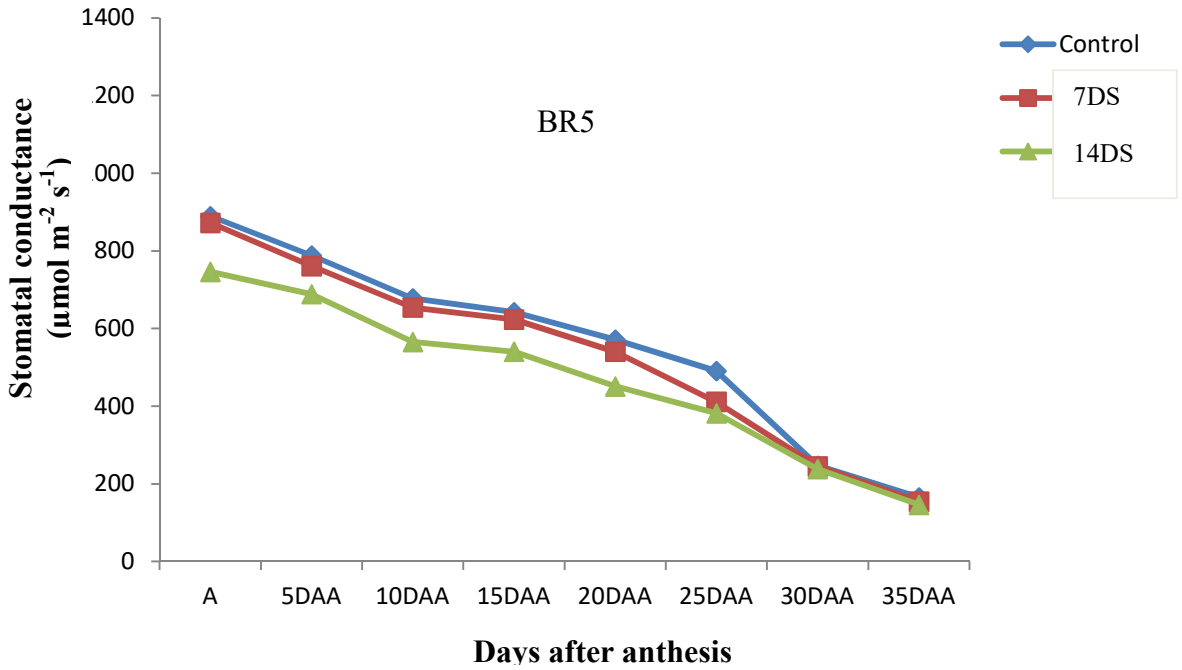


Figure 18. Stomatal conductance from anthesis to maturity of BR5 under different submergence treatments, DAA=Days after anthesis, A= Anthesis.

4.1.19. Stomatal conductance and transpiration rate during grain filling stage

The major role of the stomata is to allow entry of CO_2 into the leaf for photosynthesis while at the same time preventing excessive water loss. The stomatal conductance and transpiration rate in flag leaf of main stem were measured by gas exchange meter during grain filling period and the average values were taken for analysis.

In all the genotypes, the stomatal conductance of control treatment recorded was higher compared to submergence treatment. The stomatal conductance gradually decreased with the increasing submergence duration in all the genotypes. Considering all the genotypes and submergence durations, the stomatal conductance recorded was the highest (533 µmol m^{-2} s^{-1}) in BRRI dhan52 at 0DS treatment which was statistically higher than any other treatment (Table 9). The lowest (130 µmol m^{-2} s^{-1}) stomatal conductance recorded was in IR64Sub1 at 21 DS treatment which was significantly lower than any other treatment. Debabrata and Kumar (2011) found that stomatal conductance was significantly decreased in both Swarna and Swarna *Sub1* during the progression of submergence as compared to the control plant. They also found that after 7days of submergence, stomatal conductance was significantly higher in Swarna *Sub 1* compared to Swarna.

The transpiration rate in FR13A recorded was the highest (7.425 mmol m^{-2} s^{-1}) at control treatment then gradually decreased with the increase of submergence duration. But in rest of the genotypes the transpiration rate recorded was little higher at 7 DS treatment than the control and then gradually decreased with the increasing submergence durations. Among the genotypes and submergence levels, the transpiration rate recorded was the highest (7.560 mmol m^{-2} s^{-1}) in BRRI dhan52 at 7 DS treatment which was statistically similar to those at 0DS treatment in the same genotype. The lowest (4.790 mmol m^{-2} s^{-1}) transpiration rate recorded was in BR5 at 14 DS treatment which was statistically similar to IR64Sub1 at 21 DS treatment. In BRRI dhan52, IR64Sub1 and in BR5, the maximum transpiration recorded was at 7 DS treatment. In FR13A, the maximum transpiration recorded was at control treatment.

Table 9. Average of stomatal conductance and transpiration rate of flag leaf during grain filling of different rice genotypes as influenced by different submergence treatments

Genotypes	Days of submergence (DS)	Stomatal conductance (µmol m^{-2} s^{-1})	Transpiration rate (m mol m^{-2} s^{-1})
BRRI dhan52	0	533 a	7.56 a
	7	340 b	7.56 a
	14	323 b	7.24 bc
	21	242 cd	7.29 abc
IR64Sub1	0	330 b	6.56 d
	7	275 c	7.01 c
	14	275 c	6.20 e
	21	130 e	4.91 g
FR13A	0	250 cd	7.43 ab
	7	245 cd	6.49 d
	14	237 cd	6.06 e
	21	220 d	6.31 de
BR5	0	265 cd	5.72 f
	7	245 cd	5.73 f
	14	217 d	4.79 g
	21	0	0
$LSD_{(0.05)}$		45.04	0.278
CV (%)		10.47	3.20

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

In the present experiment, the stomatal conductance of FR13A at 7 DS treatment was statistically similar to the control treatment which might help this genotype to perform better. The decrease in stomatal conductance and transpiration rate in submerged treated plant compared to control plant might be due to decrease in chlorophyll content and increase in leaf injury. At physiological level, flooding modifies water relations and plants carbon fixation. Closing of stomata, with or without leaf dehydration, reduction of transpiration and inhibition of photosynthesis, are responses that can occur in hours or days, depending on the tolerance to flooding of each plant species (Bradford and Hsiao, 1982; Else *et al*., 1996; Insausti *et al*., 2001; Striker *et al*., 2005; Mollard *et al*., 2008; 2010).

4.1.20. Intercellular CO_2 concentration and net assimilation rate

The intercellular CO_2 concentration and net assimilation rate of flag leaf of main stem were also measured by gas exchange meter during grain filling period and the average values were recorded for analysis. Considering all the genotypes and submergence durations, the intercellular CO_2 concentration found was the highest (354.75) μmol m^{-2} s^{-1}) in FR13A at 7 DS treatment (Table 10). The lowest (307 μ mol m^{-2} s^{-1}) intercellular CO_2 concentration recorded was in BRRI dhan52 at 0DS treatment. In BRRI dhan52, the highest intercellular CO_2 concentration was recorded at 21 DS treatment which was significantly higher than control treatment. In IR64Sub1, the highest intercellular CO_2 concentration recorded was at 14 DS condition which was statistically similar to the other treatments of the same genotype. In FR13A, the highest intercellular CO_2 concentration recorded was at 7 DS which was statistically similar to the other treatments of the same genotype and in BR5, the highest intercellular CO_2 concentration recorded was also in 14 DS treatment, which was significantly higher than the control and 7 DS treatment.

Net assimilation rate is a useful measure of the photosynthetic efficiency of plants. Net assimilation rate is the rate of increase of dry weight per unit of leaf area. Considering all the genotypes and submergence durations, the highest (14.078 μ mol m^{-2} s^{-1}) net assimilation rate recorded was in BRRI dhan52 at 0DS treatment which was statistically similar to 7 DS treatment in the same genotypes, where the intercellular CO_2 concentration was in the lowest level. Among the genotypes and submergence levels, the lowest (4.957 μ mol m^{-2} s^{-1}) net assimilation rate

recorded was in IR64Sub1 at 21 DS treatment which was significantly lower than any other treatment.

In all the genotypes, submerged plants showed lower net assimilation rate than control plant, though the 7 DS treatment of BRRI dhan52, 7 and 14 DS treatment of BR5, showed statistically similar NAR with control treatment. The intercellular CO_2 concentration was increased with the increasing submergence duration in all the genotypes; exhibiting the lower CO_2 fixation rate. Lower net assimilation rate and higher intercellular CO_2 concentration under submergence condition indicated that photosynthetic enzyme such as RuBP carboxylase might had some limitation to fix CO_2. So the lower stomatal conductance and higher intercellular CO_2 concentration might contributed in lower net assimilation rate in submergence treated plants. Panda and Sarkar (2012) stated that due to submergence three functional steps of photosynthetic reaction center, namely absorption of light energy, trapping of the excitation energy and the conversion of excitation energy to electron transport were affected, which ultimately affected the photosynthesis as well as net photosynthesis. They also stated that both donor and acceptor sides of PS II were damaged, electron transport perturbed, connectivity between the antennae of PS II lost which resulted in the fall of CO_2 photo-assimilation rate. The structural and functional damage of PS II was more prominent in susceptible cultivars. Sakagami *et al*. (2009) found that complete submergence adversely affected leaf area and photosynthesis.

Table 10. Average of intercellular CO_2 concentration and net assimilation rate of flag leaf during grain filling of different rice genotypes as influenced by different submergence treatments

Genotypes	Days of submergence (DS)	Intercellular CO_2 concentration (μ mol mol^{-1})	Net assimilation rate (μ mol m^{-2} s^{-1})	
			Actual	Relativeto control (%)
BRRI dhan52	0	307.00 g	14.08 a	100
	7	320.75 defg	13.75 a	98
	14	320.75 defg	10.31 c	73
	21	337.25 bcd	8.26 e	59
IR64Sub1	0	325.25 cdef	11.93 b	100
	7	325.50 cdef	10.60 c	89
	14	333.50 bcde	10.285 c	86
	21	325.75 cdef	4.96 f	42
FR13A	0	340.25 abc	12.46 b	100
	7	354.75 a	10.30 c	83
	14	347.75 ab	9.78 cd	79
	21	340.50 abc	9.11 de	73
BR5	0	314.00 fg	10.72 c	100
	7	325.00 cdef	10.66 c	99
	14	347.50 ab	9.84 cd	92
	21	0	0	0
$LSD_{(0.05)}$		15.23	1.068	
CV (%)		3.43	7.59	

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.21. Relative growth rate (RGR)

The relative growth rate of different rice genotypes under various submergence durations was calculated and shown in figure 19. In all the genotypes, control treatment showed the highest relative growth rate which was significantly higher than any submergence treatment. After control treatment, the RGR gradually decreased with the increasing submergence durations in all the genotypes. After control treatment a drastic reduction of RGR occurred in BR5. Considering all the genotypes and submergence treatment, the highest (16.75 g g^{-1} day^{-1}) RGR recorded was in IR64Sub1 at control treatment which was significantly higher than any other treatment. The lowest (5.02 g g^{-1} day^{-1}) RGR was recorded in FR13A at 21 DS treatment which was significantly lower than any other treatment.

Due to submergence, as the injury occurred, the recovery cost was higher in all the genotypes. On the other hand, the chlorophyll content was lower and the gas exchange was also lower in submergence treated plants due to lower stomatal conductance, which ultimately affected the RGR. The effect of submergence on RGR of BRRI dhan52 and FR13A was lower. They also performed better under 14 and 21 DS treatments considering their RGR, which indicated that these two rice genotypes were more submergence tolerant type compared to other. Yu *et al*. (2011) found that submergence significantly reduced relative growth rates (RGRs) in different grass plants, with greater reduction in the sensitive genotypes compared to tolerant genotypes.

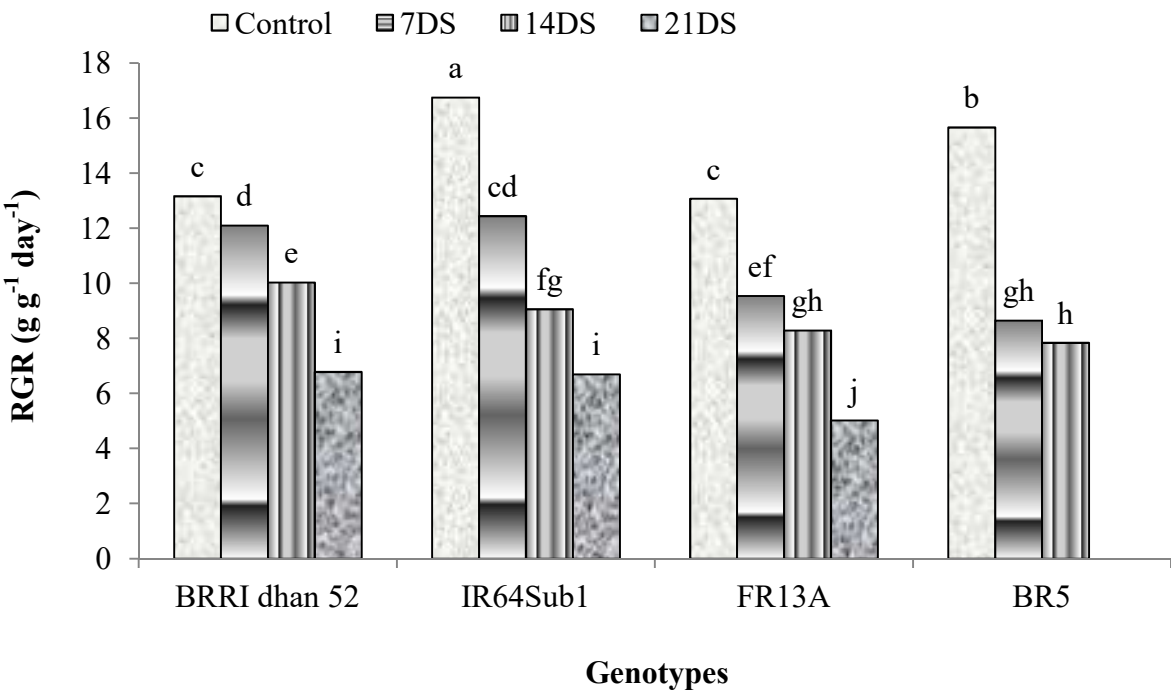


Figure 19. Relative growth rate (RGR) of different of rice genotypes under various submergence treatments. Values followed by same letter(s) are not significantly different from each other by LSD at 5% level.

4.1.22. Absolute grain growth rate (AGR)

The absolute grain growth rate depends on the rate of current photosynthesis and photosynthates supply towards developing grain. Grain growth rate is also depends on the amount of stem reserve translocation. In the present experiment, the absolute grain growth rate (mg/grain/day) was calculated from the developing grain. The absolute grain growth rate of different rice genotypes varied significantly by the interaction effect of the genotypes and submergence durations and the results were shown in figure 20. The genotype BRRI dhan52 showed the highest (1.8 mg/grain/day) absolute grain growth rate at control treatment which was statistically similar to 7 DS treatment. Then gradually decreased with the increasing submergence duration.

In IR64Sub1, the highest (1.8 mg/grain/day) absolute grain growth rate was also recorded at control treatment and was gradually decreased with the increasing submergence duration. As a result, the lowest (1.3 mg/grain/day) absolute grain growth rate, in this genotype, recorded was in 21 DS treatment which was significantly lower than the control treatment.

In the genotype FR13A, the absolute grain growth rate found was also the highest (2.0 mg/grain/day) at control treatment which was gradually decreased with the increasing submergence durations. But the AGR of 7 DS treatment was statistically similar to control treatment in this genotype. The lowest (1.2 mg/grain/day) absolute grain growth rate recorded was at 21 DS treatment which was significantly lower than the other treatments.

In BR5, the absolute grain growth rate was much lower in all the treatments including control treatment. The highest (1.2 mg/grain/day) absolute grain growth rate recorded was at control treatment which was significantly higher than any other treatment. Then a drastic reduction in AGR recorded was in both 7 and 14 DS treatments. The AGR of 7 DS and 14 DS treatment of BR5 was statistically similar.

The overall results indicated that the AGR recorded was the highest at control treatment and then gradually decreased with the increasing submergence duration in all the genotypes. But BRRI dhan52 and FR13A produced more than 90% relative AGR under 7 DS treatment. The 14 DS treatment of FR13A also showed 80% relative AGR; indicated the tolerant levels of these genotypes. Due to submergence treatment, decrease in chlorophyll content, stomatal

conductance, transpiration rate, RGR occurred, which ultimately affected the current photosynthesis and NAR and decreased assimilates supply towards developing grains. Sarkar and Bhattacharjee (2011) found that absolute growth rate was greater in control plants compared to the 14 days and 20 days of submerged plants.

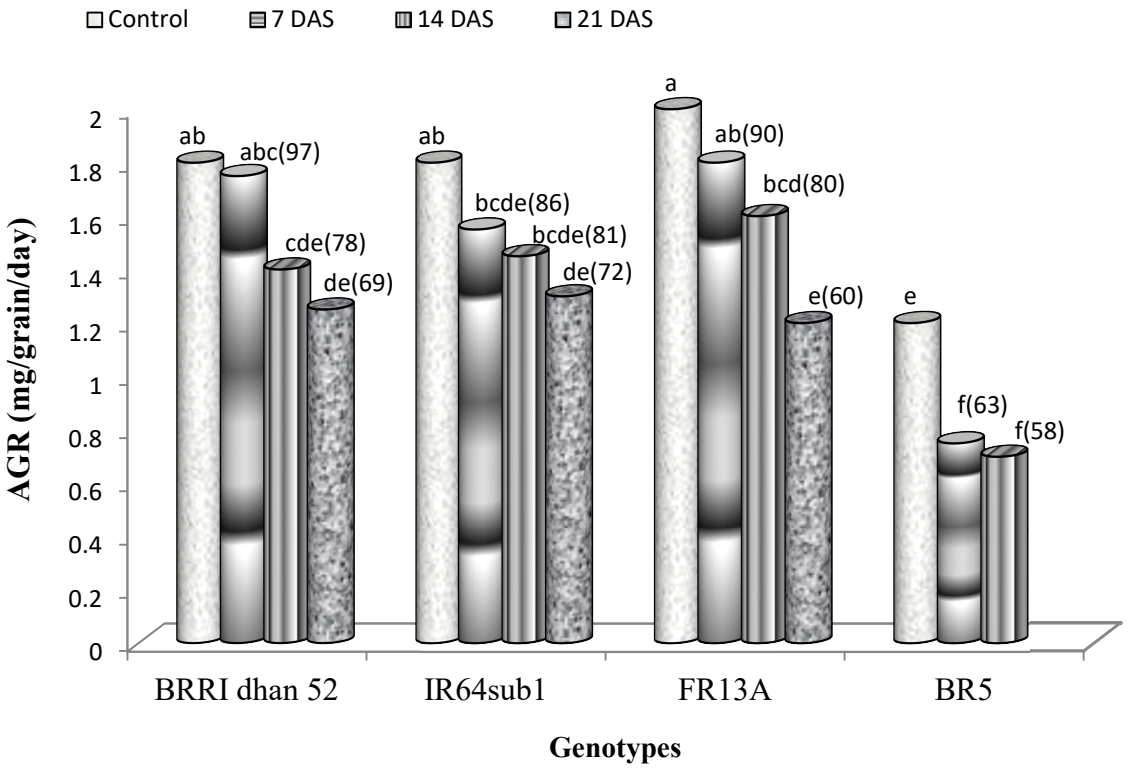


Figure 20. Absolute grain growth rate (AGR) of different rice genotypes under various submergence treatments. Values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance. Figures inside the parenthesis indicate relative to control.

4.1.23. Leaf, stem, and root dry matter content per plant

Leaf, stem and root dry matter content per plant recorded was at maturity stage. The dry matter content of plant was decreased due to submergence treatment. Considering all the genotypes and submergence durations, the leaf dry matter content found was the highest (12.70 g) in BR5 at control treatment which was significantly higher than any other treatment (Table 11). The lowest (4.00 g) leaf dry matter recorded was in FR13A at 21 DS treatment which was statistically similar to 21 DS treatment of BRRI dhan52 and IR64Sub1. In all the genotypes, the highest leaf dry matter was recorded from control treatment and then gradually decreased with the increasing submergence duration, with an exception in BRRI dhan52, where 7 DS treatment produced the highest leaf dry matter. A drastic reduction in leaf dry matter due to submergence recorded was in BR5.

Considering all the genotypes and submergence durations, the stem dry matter content recorded was the highest (36.78 g) in BR5 at control treatment which was significantly higher than any other treatment. The lowest (12.15 g) stem dry matter recorded was in FR13A at 21 DS treatment which was statistically similar to 21 DS treatment of BRRI dhan52. In all the genotypes, the highest stem dry matter were recorded at control treatment and then gradually decreased with the increasing submergence duration. A drastic reduction in stem dry matter due to submergence recorded was in BR5. But in case of other three genotypes the reduction was comparatively lower than BR5.

Considering all the genotypes and submergence durations, the root dry matter content recorded was the highest (9.88 g) in BR5 at 0DS or control treatment which was statistically similar to control treatment of BRRI dhan52 and FR13A. The lowest (2.56 g) root dry matter recorded was in the same genotype (BR5) at 14 DS treatment which was statistically similar to 14 and 21 DS treatment of other genotypes. In all the genotypes, the root dry matter content recorded was the highest at control treatment and was gradually decreased with the increasing submergence duration. Consequently, the lowest root dry matter per plant recorded was at 21 DS treatment in all the genotypes except in BR5 where the roots were found dead after few days of desubmergence.

Due to submergence, as the SLW was decreased, the leaf DM was also recorded lower compared to control in all the genotypes. Higher injury, lower chlorophyll content, and all the physiological processes were hampered due to submergence. As a result, the dry matter content of different organs of the plant became lower compared to control. The plants were unable to attain the complete recovery of that submergence injury and back to their normal state after desubmergence till maturity. The 7 DS treatment created little effect on dry matter content in BRRI dhan52 (statistically similar to control) and in FR13A. But a remarkable reduction in dry matter content occurred at 14 and 21 DS treatment in all the genotypes. Singh *et al*. (2014) found that the *Sub1* lines and the tolerant checks (FR13A and IR49830) showed higher stem dry weight, the sensitive variety showed decrease in stem dry weight under submergence. They also found that in general, the trends in leaf dry weight were similar to those of stem dry weight. Significant genotypic differences were observed in above ground total dry matter accumulation both under normal and submerged conditions (Sarkar and Bhattacharjee, 2011). They also found that total above ground dry matter contents were greater under control condition followed by 14 and 20 days of submergence. They also observed that the reduction in dry matter content was more than 90% in susceptible genotypes after 14 days of submergence. Nugraha *et al*. (2012) stated that the sensitive genotypes lose their biomass, leaves and tillers and take much longer time to recover and to develop new organs; these affect the production of assimilate as well as the translocation to the sink.

Table11. Effect of different submergence treatments on leaf, stem and root dry matter content per plant of different rice genotypes

Genotypes	Days of submergence (DS)	Leaf dry matter per plant (g)	Stem dry matter per plant (g)	Root dry matter per plant (g)
BRRI dhan52	0	7.54 de	20.88 c	7.70 abc
	7	7.72 de	20.49 c	6.33 bcde
	14	7.64 de	19.45 c	5.33 cdef
	21	4.38 gh	12.50 e	3.64 def
IR64Sub 1	0	8.99 c	30.70 b	7.04 abcd
	7	8.26 cd	29.44 b	6.74 abcde
	14	6.47 f	21.67 c	4.95 cdef
	21	4.68 gh	15.57 d	3.38 ef
FR13A	0	10.78 b	31.12 b	9.55 ab
	7	7.66 de	21.15 c	6.16 bcde
	14	6.79 ef	20.85 c	5.33 cdef
	21	4.00 h	12.15 e	3.66 def
BR5	0	12.70 a	36.78 a	9.88 a
	7	6.64 f	20.69 c	3.78 def
	14	5.20 g	18.81 c	2.56 fg
	21	0	0	0
$LSD_{(0.05)}$		0.84	2.53	3.02
CV (%)		8.61	8.55	39.44

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.24. Root dry matter, shoot dry matter and root shoot ratio per plant

In BRRI dhan52, the highest root dry matter recorded was at control treatment, which was gradually decreased with the increasing submergence duration (Table 12). Considering all the submergence treatments, IR64Sub1, FR13A and BR5 produced the highest root dry matter at control treatment which was gradually decreased with the increasing submergence duration. It was clear that root dry matter was severely affected due to submergence in all the rice genotypes and BR5 was one of the most (submergence) sensitive genotype.

Shoot dry matter was also recorded lower due to submergence treatment compared to control. In all the genotypes, the control treatment produced the highest shoot dry matter which was gradually decreased with the increasing submergence duration. Considering all the genotypes and submergence durations, the highest (80.042 g) shoot dry matter recorded was in BR5 at control treatment which was statistically similar to control treatment of IR64Sub1. The lowest (31.638 g) shoot dry matter recorded was in FR13A at 21 DS treatment which was significantly lower than any other treatment.

In BRRI dhan52, the root shoot ratio recorded was the highest at control treatment, which was gradually decreased with the increase of submergence duration, but the difference was not significant between control and submergence treatments. In IR64Sub1, the highest root shoot ratio recorded was in 7 DS treatment and the lowest recorded was at control, but the difference was not significant. In FR13A, the highest root shoot ratio recorded was at control treatment and the lowest root shoot ratio recorded was at 14 DS treatment, but the difference was not significant. In BR5, the highest root shoot ratio recorded was at control condition which was gradually decreased with the increasing submergence duration and the lowest root shoot ratio recorded was at14 DS treatment which was significantly lower than the control treatment of BRRI dhan52, FR13A and BR5, 21 DS treatment of FR13A.

Decreased root shoot ratio due to submergence stress indicated that root dry matter rather than shoot dry matter was more affected due to submergence. Relatively lower reduction

Table 12. Effect of different submergence treatments on root dry matter, shoot dry matter and root shoot ratio of different rice genotypes

Genotypes	Days of submergence (DS)	Root dry matter per plant (g)	Shoot dry matter per plant (g)	Root shoot ratio per plant	
				Actual	Relative to control (%)
BRRI dhan52	0	7.70 abc	58.21 d	0.13 a	
	7	6.33 bcde	58.15 d	0.11 ab	83
	14	5.33 cdef	52.48 e	0.10 ab	76
	21	3.64 def	37.00 h	0.10 ab	76
IR64Sub1	0	7.04 abcd	77.38 a	0.09 ab	
	7	6.74 abcde	64.75 c	0.11 ab	119
	14	4.95 cdef	51.32 e	0.10 ab	111
	21	3.38 ef	38.81 h	0.09 ab	102
FR13A	0	9.55 ab	72.15 b	0.13 a	
	7	6.16 bcde	54.24 e	0.11 ab	86
	14	5.33 cdef	50.74 e	0.11 ab	81
	21	3.66 def	31.64 i	0.12 a	88
BR5	0	9.88 a	80.04 a	0.12 a	
	7	3.78 def	45.91 f	0.09 ab	69
	14	2.562 fg	42.405g	0.060b	49
	21	0	0	0	0
		3.02 8.55	3.32 4.57	0.045 34.59	

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

in root-shoot ratio due to submergence recorded was in FR13A which was followed by BRRI dhan52 compared to BR5. Increased root-shoot ratio (higher than control) in IR64Sub1 indicated that shoot dry matter rather than root dry matter was more affected due to submergence in this genotype. The lowest root-shoot ratio at 14 DS treatment of BR5 indicated the susceptibility of this genotype at this treatment.

4.1.25. Leaf weight ratio (LWR)

The leaf weight ratio was also recorded from each plant at maturity. The LWR was significantly influenced by the interaction effect of rice genotypes and submergence durations in this experiment. Among the genotypes, the highest (0.158) LWR recorded was in BR5 at 0DS treatment which was significantly higher than any other treatment (Table 13). The lowest (0.117) LWR recorded was in IR64Sub1 at 0DS condition which was statistically similar to BRRI dhan52 at 21 DS treatment. In BRRI dan52, the highest LWR recorded was at 14 DS treatment. In IR64Sub1, the highest LWR recorded was at 7 DS treatment. In FR13A and BR5, the highest LWR recorded was at control treatment. In BRRI dhan52 and FR13A the lowest LWR were recorded 21 DS treatment. In BRRI dhan52 the LWR was increased with the increasing submergence duration up to 14 DS treatment. In case of FR13A and BR5, the LWR was decreased with the increasing submergence duration. But a drastic reduction of LWR recorded was in BR5 in 14 DS treatment compared to control treatment.

Due to submergence treatment, the genotype, where the LWR was more affected compared to control, might be considered as sensitive type (such as BR5). The statistically similar LWR at 7 DS treatment of BRRI dhan52 with control indicated that 7 DS treatment had little effect on this genotype. Zhang *et al*. (2015) stated that as the submergence duration increased, different treatment groups showed decreases in above ground dry weight and root dry weight, but short-term submergence increased aboveground dry weight and root dry weight.

Table 13. Effect of different submergence treatments on leaf weight ratio of different rice genotypes

Genotypes	Days of submergence (DS)	Leaf weight ratio (LWR)
BRRI dhan52	0	0.13 e
	7	0.13 e
	14	0.15 b
	21	0.12 h
IR64Sub1	0	0.12 h
	7	0.13 f
	14	0.13 f
	21	0.12 g
FR13A	0	0.15 b
	7	0.14 d
	14	0.13 e
	21	0.13 f
BR5	0	0.16 a
	7	0.14 c
	14	0.12 g
	21	0
$LSD_{(0.05)}$		0.0014
CV (%)		9.04

Values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.1.26. Stem reserve translocation (SRT)

Grain filling is a deposition of starch from two sources: current photosynthate (60–100%) and remobilization (rest) from the reserve pool (Yang and Zhang, 2010; Yoshida, 1981 and Samonte *et al*., 2001). In the present experiment, the stem reserve translocation to the developing grain differs significantly due to interaction effect of different genotypes and submergence duration and results have been shown in figure 21. In BRRI dhan52, all the submergence treatments showed higher stem reserve translocation than the control treatment. The highest (17.36%) SRT of this genotype recorded was at 21 DS treatment which was significantly higher than the control and 14 DS treatment. The lowest (8.15%) stem reserve translocation of this genotype recorded was in control treatment. The SRT of IR64Sub1 was much lower in all the treatments including control treatment. Here the lowest (5.07%) stem reserve translocation recorded was in control treatment which was statistically similar to other treatments. In this genotype the stem reserve translocation found was the highest (8.04%) in 14 DS treatment which was followed by 7 DS treatment.

In FR13A, the highest (26.48%) stem reserve translocation recorded was in 14 DS treatment which was significantly higher than any other treatment of this genotype. The second the highest SRT recorded was in 7 DS treatment. The lowest (17.83%) stem reserve translocation of this genotype found was in control treatment which was statistically similar to 7 and 21 DS treatments.

In all the genotypes including BR5, the control treatment showed lower stem reserve translocation than submergence treatment. The highest (31.1%) stem reserve translocation of BR5 genotype recorded was in 7 DS treatment which was significantly higher than any other treatment. The lowest (15.17%) stem reserve translocation recorded was in control treatment which was statistically similar to 14 DS treatment.

When the current photosynthetic source is inhibited, grain filling becomes more dependent on mobilized stem reserves (Blum, 1988). So, stored assimilates in stem is an important source for grain filling in rice. In BR5, the current photosynthesis might be lower in submerged treated plants due to higher membrane injury, lower chlorophyll content, lower gas exchange and lower absolute grain growth rate. So higher stem reserve translocation in BR5 at 7 DS treatment might

have contributed in better grain yield in this genotype. But at higher (14 and 21 days) submergence treatments, the SRT was better in BRRI dhan52, IR64Sub1 and in FR13A compared to BR5. Improper grain filling under 10 days of submergence was reported to be due to reduction in carbohydrate content and less translocation of the same to sink (Palada and Vergara, 1972; Yoshida, 1972).

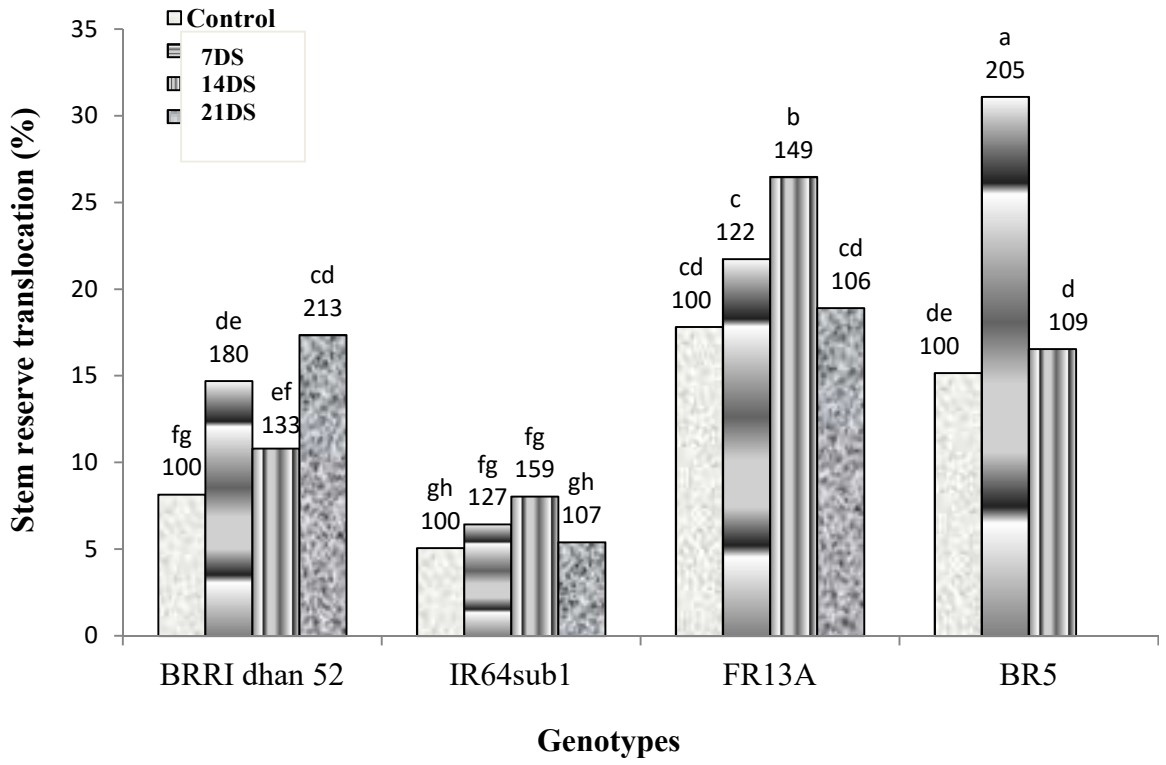


Figure 21. Stem reserve translocation (SRT) of different rice genotypes under various submergence treatments. Values followed by the same letters are not significantly different from each other by LSD at 5% level of significance, values on the bar indicate the relative (to control) value.

In the present experiment, studying the different morphological and physiological characters of *aman* rice genotypes due to submergence treatment indicated that different morphological and physiological processes were hampered due to submergence and there existed genotypic variations also. The overall results also indicated that lower stem elongation and less affected root dry matter help plants to survive under submergence condition. After desubmergence less affected tiller number and relatively higher physiological activities (such as net assimilation rate, stomatal conductance, relative growth rate and absolute grain growth rate) contributed in higher relative TDM (Total dry matter). Considering the above characters, the genotypes BRRI dhan52 and FR13A showed more submergence tolerant compared to the other two genotypes (IR64Sub1 and BR5). It is also important to know the effect of different submergence durations on yield characters.

4.2. Experiment-II: The yield and yield parameters responses of rice genotypes under different submergence durations

In the present experiment, different yield components of four rice genotypes were studied under different submergence durations to assess the relatively submergence tolerant rice genotypes considering their yield attributes.

4.2.1. Filled, unfilled and total grain number of main stem

The data of main stem represents most of the characters of the whole plant. The main stem has the major contribution to grain yield in rice (*Martinez-Eixarch et al., 2015)*. For this reason, in the present experiment, different yield data of main stem were also recorded. The filled grain, unfilled grain and total grain number of main stem of each plant were varied significantly by the interaction effect of submergence duration and rice genotypes (Table 14). The filled grain number of main stem was gradually increased with the increasing submergence duration in BRRI dhan52 and in FR13A. But in IR64Sub1 and BR5, the filled grain number of main stem was lower at submergence treated plants compared to control plants. Considering all the genotypes and submergence treatments, the highest (281) filled grain number of main stem recorded was in BR5 at 0DS treated plants compared to other three genotypes, which was statistically similar to 7 DS and 14 DS treatment of the same genotype. The lowest (138.50) filled grain number of main stem recorded was in IR64Sub1 at 21 DS treated plants. In BRRI dhan52, the highest filled grain number of main stem recorded was at 21 DS treated plants. But in IR64Sub1, the control 7 DS and 14 DS treated plants produced the similar number of filled grain. In FR13A, the highest number of filled grain recorded was at 14 DS treated plants. Nugraha *et al.* (2012) found that under submergence condition the numbers of filled and unfilled grains per panicle were similar to normal conditions.

The unfilled grain number of main stem was gradually increased with the increasing submergence duration in all the genotypes and the higher increases recorded was in BR5 compared to other three genotypes. Considering all the genotypes and submergence treatments, the highest (111.75) unfilled grain number of main stem was also recorded in BR5 at 14 DS treated plants. So, it was clear that BR5 was highly affected due to submergence treatment compared to other three genotypes. For this reason, BR5 could be considered as submergence

sensitive genotype. The lowest (10) number of unfilled grain of main stem, recorded was in IR64Sub1 at control plants, which was statistically similar to FR13A at control plants also. In BRRI dhan52, the highest number of main stem unfilled grain recorded was at 14 DS treated plants and the lowest recorded was at control plants. In IR64Sub1, the highest number of unfilled grain recorded was at 21 DS treated plants and the lowest recorded was at control plants. In FR13A, the lowest number of unfilled grain recorded was at control plants and the highest recorded was at 14 DS treated plants. In all the genotypes including BR5, the lowest unfilled grain number recorded was at control treatment.

The total number of grain of main stem was also gradually increased with the increasing submergence duration in all the genotypes except in IR64Sub1, in which 21 DS treatment produced lower total number of grain of main stem compared to other treatments of the same genotype. Considering all the genotypes and submergence treatments, the total number of grain of main stem recorded was also the highest (381) in BR5 at 14 DS treatment, but the lowest number (165.50) of total grain of main stem found was in IR64Sub1 at 21 DS treatment, which was statistically similar to other DS treatments of the same genotype and also similar to those of all DS treatments of FR13A. 21 DS treatments of all the genotypes and 14 DS treatment in IR64Sub1 and BR5 produced higher total number of main stem grain, compared to control.

In the present experiment, due to decrease in tiller number, the main stem got higher environmental facilities and also got higher assimilates for its reproductive development. As a result, the filled grain number of main stem recorded was higher in submerged (7, 14 and 21 DS) plant compared to control plant in BRRI dhan52 and FR13A. Increase in total grain number in submergence treated plants compared to control was mainly due to increase in unfilled grain number. In BR5, though the filled grain number decreased, the unfilled grain number was much increased, which contributed in increased total grain number in submergence treated plants. Haque *et al*. (2015) found that total grains per panicle were significantly influenced by the interaction effect of water levels

Table 14. Effect of different submergence treatments on filled grain number, unfilled grain number and total grain number of main stem of different rice genotypes

Genotypes	Days of submergence (DS)	Filled grain number of main stem	Unfilled grain number of main stem	Total grain number of main stem
BRRI dhan52	0	196.00 bcd	39.50 de	235.50 d
	7	199.75 bc	60.50 bc	260.25 cd
	14	207.50 b	71.00 b	278.50 bc
	21	213.75 b	66.00 b	279.75 bc
IR64Sub1	0	161.25 cde	10.00 gh	171.25 e
	7	161.0 cde	16.25 fgh	177.25 e
	14	161.25 cde	19.50 fg	180.75 e
	21	138.50 e	27.00 efg	165.50 e
FR13A	0	156.00 de	11.75 gh	167.75 e
	7	158.25 cde	27.50 efg	185.75 e
	14	165.50 cde	31.00 def	196.50 e
	21	159.50 cde	24.50 efg	184.00 e
BR5	0	281.00 a	30.25 def	311.25 b
	7	263.50 a	45.75 cd	309.25 b
	14	269.25 a	111.75 a	381.00 a
	0	0	0	0
$LSD_{(0.05)}$		37.19	16.16	37.52
CV (%)		14.45	30.65	12.10

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

and seedling number hill^{-1}. Zhang *et al*. (2015) observed that short term (1-5 days) submergence exhibited a significantly increased seed-setting rate (2.5%). They also observed an increased panicle number and decreased grain number per panicle and seed-setting rate as the submergence duration increased.

4.2.2. Filled, unfilled and total grain weight of main stem

Significant variation was observed in grain dry weight per main stem panicle by the interaction effect of submergence duration and rice genotypes (Table 15). The filled grain weight of main stem was increased with the increasing submergence durations in BRRI dhan52 and FR13A but decreased in BR5 and IR64Sub1 genotypes. Considering all the genotypes and submergence durations, the filled grain weight of main stem recorded was the highest (5.47 g) in BRRI dhan52 at14 DS treatment which was statistically similar to 0, 7 and 21 DS treatments and the lowest (2.95 g) recorded was in BR5 at 14 DS treatment. This lowest value was statistically similar to other DS treatment of BR5 and 21 DS treatment of IR64Sub1. In BRRI dhan52 and FR13A, the lowest filled grain weight recorded was at control plants. In IR64Sub1, the filled grain weight found was lowest at 7 DS treatment and in BR5, the filled grain weight recorded was lowest at 14 DS treatment.

The unfilled grain weight of main stem was increased with the increasing submergence duration in all the genotypes. Considering all the genotypes and submergence durations, the main stem unfilled grain weight recorded was the highest (0.36 g) in BRRI dhan52 at 14 DS treatment which was significantly higher than any other treatments. The lowest (0.05 g) unfilled grain weight recorded was in R64Sub1 at 0DS or control treatment, which was statistically similar to FR13A at 0DS treatment.

The total grain weight of main stem was gradually increased with the increasing submergence duration from 0 DS to 14 DS condition, in all the genotypes except in BR5, where the total grain weight of main stem gradually decreased with the increasing submergence duration. 21 DS treatment also showed higher total grain weight of main stem than the control treatments in BRRI dhan52 and FR13A. Considering the interaction effect of submergence durations and genotypes, the highest (5.83 g) total grain weight of main stem was recorded in BRRI dhan52 at 14 DS treatment which was significantly higher than any other treatments. The lowest (3.08 g)

total grain weight of main stem was recorded in BR5 at 14 DS treatment which was significantly lower than any other treatments.

In submergence (7, 14 and 21 DS) treated plants, filled grain number and weight of main stem were increased in BRRI dhan52 and FR13A; this might be due to decrease in number of panicle bearing tillers and for the reduction in competition among the sinks for assimilates. In this condition the main stem also got higher environmental facilities. In IR64Sub1 and in BR5, the total grain weight of main stem was increased compared to control and this was mainly due to increase in unfilled grain number and weight. In BR5, though the total grain number was increased and the total grain weight was decreased in submergence treated plants and this was due to increase in unfilled grain number and decrease in 100-grain weight. Shan *et al*. (2000) stated that super-compensatory effects are a universal phenomenon in biology and are normally caused by stress and damage as a self-accommodation behavior in response to harmful environments. They also stated that for some crops, the compensatory effects caused by moderate stress are even better than the effects of normal growth conditions, resulting in increased production or minor underproduction.

Table 15. Effect of different submergence treatments on filled grain weight, unfilled grain weight and total grain weight of main stem of different rice genotypes

Genotypes	Days of submergence (DS)	Filled grain weight of main stem (g)	Unfilled grain weight of main stem (g)	Total grain weight of main stem (g)
BRRI dhan52	0	5.08 abc	0.19 cd	5.28 c
	7	5.19 ab	0.26 bc	5.44 bc
	14	5.47 a	0.36 a	5.83 a
	21	5.27 ab	0.27 b	5.53 b
IR64Sub1	0	4.25 de	0.05 hi	4.14 f
	7	4.10 e	0.09 gh	4.19 f
	14	4.13 de	0.10 fgh	4.23 f
	21	3.36 f	0.14 defg	3.51 g
FR13A	0	4.38 de	0.06 hi	4.43 e
	7	4.55 cde	0.14 defg	4.69 d
	14	4.73 bcd	0.17 de	4.72 d
	21	4.71 bcd	0.16 def	4.67 d
BR5	0	3.50 f	0.08 gh	3.58 g
	7	3.16 f	0.12 efgh	3.28 h
	14	2.95 f	0.25 bc	3.08 i
	21	0	0	0
$LSD_{(0.05)}$		0.53	0.064	0.17
CV (%)		9.25	30.54	9.24

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.3. Panicle length and rachis weight of main stem

The panicle length and rachis dry weight were measured from main stem panicle after harvest. Significant variation found was in the panicle length and rachis dry weight of main stem by the interaction effect between submergence duration and rice genotypes (Table 16). The panicle length was increased gradually with the increasing submergence duration in FR13A and IR64Sub1 except in 21 DS treatment. In IR64Sub1 and FR13A the highest panicle length was observed at 7 DS treatment. The panicle length was the highest at control treatment in BRRI dhan52 and BR5 and decreased with the increasing submergence duration in these genotypes. Considering all the submergence durations, panicle length of main stem recorded was the highest (28.58 cm) in FR13A at 7 DS treatment which was statistically similar to 14 DS treatment of the same genotype and was also similar to 7 DS treatment in IR64Sub1. The panicle length recorded was lowest (21.60 cm) in BR5 at 7 DS treatment. Under control treatment, the highest panicle length found was in BRRI dhan52 and the lowest panicle length found was in BR5. Normally, in the same genotype, the longer panicle bears more grains and which ultimately contribute to the higher grain yield.

The rachis weight of main stem was increased under submergence treatment compared to control treatment in all the genotypes except in IR64Sub1. In BRRI dhan52 and FR13A, 7 DS treated plants showed higher rachis weight compared to other treatments. The rachis weight found was the highest at control treatment in IR64Sub1 and decreased with the increasing submergence duration. Considering all the genotypes and submergence durations, the rachis weight of main stem recorded was the highest (0.323 g) in BRRI dhan52 at 7 DS treatment and the lowest (0.210 g) recorded was in BR5 at 7 DS treatment. Control treatment of IR64Sub1 and 14 DS treatment of BR5 showed higher rachis weight compared to other treatments.

In IR64Sub1 and in BR5 the rachis length and weight of main stem was increased in submergence (7 and 14 DS) treated plants compared to control. But their filled grain number and weight was deceased in submergence treated plants compared to control plants, which was not desirable and also responsible for lower grain yield.

Table 16. Effect of different submergence treatments on panicle length and rachis weight of main stem at maturity stage of different rice genotypes

Genotypes	Days of submergence (DS)	Panicle length of main stem (cm)	Rachis weight of main stem (g)
BRRI dhan52	0	26.83 ab	0.270 d
	7	25.85 bc	0.323 a
	14	24.38 cde	0.315 b
	21	25.15 bcd	0.300 c
IR64Sub1	0	26.03 bc	0.255 f
	7	28.50 a	0.242 h
	14	27.23 ab	0.240 i
	21	25.20 bcd	0.215 k
FR13A	0	25.75 bc	0. 217 j
	7	28.58 a	0.257 e
	14	28.30 a	0.255 f
	21	27.25 ab	0.245 g
BR5	0	23.13 def	0.215 k
	7	21.60 f	0.210 l
	14	22.45 ef	0.242 h
	21	0	0
$LSD_{(0.05)}$		2.026	0.0014
CV (%)		5.89	9.15

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.4. Grain sterility percentage and 100-grain weight of main stem

The grain sterility percentage and 100-grain weight of main stem were measured from the main stem panicle of control and submerged plants in all the genotypes. Significant variation was observed in 100-grain weight and in grain sterility percentage by the combined effect of submergence duration and rice genotypes (Table 17). Considering all the genotypes and submergence durations, the grain sterility percentage recorded was the highest (29%) in BR5 at 14 DS treatment which was significantly higher than any other treatment and the lowest (6%) recorded was in IR64Sub1 at control treatment. Grain sterility was increased with the increasing submergence duration in all the genotypes. In BRRI dhan52, FR13A and in BR5, the highest grain sterility recorded was at 14 DS treatment, where as in IR64Sub1, the highest grain sterility recorded was at 21 DS treatment. This might be because of the prolonged submergence would have reduced the amount of photosynthesis as well as stem reserve, thus decreased the rate of translocation of assimilate to the reproductive development after desubmergence.

100-grain weight was differed significantly due to combine effect of submergence durations and rice genotypes (Table 17). Considering all the genotypes and submergence treatments, the highest (2.88 g) 100-grain weight recorded was in FR13A at 7 DS treatment which was statistically similar (2.86 g) to that of 14 DS treatment in the same genotype and the lowest (1.10 g) 100-grain weight recorded was in BR5 at 14 DS treatment. Under control treatment, the highest (2.81g) 100-grain weight recorded was in FR13A and the lowest (1.24 g) 100-grain weight recorded was in BR5. These deviations in 100-grain weight among the rice genotypes at control were due to their genetic variation. Due to submergence treatment, the 100-grain weight decreased significantly compared to control in BR5. In every genotype, the 21 DS treatment produced the lowest 100-grain weight than the other treatments. In IR64Sub1 and BR5, the highest 100-grain weight recorded was at control treatment and in BRRI dhan52 the highest 100-grain weight recorded was at 14 DS treatment and in FR13A the highest 100-grain weight recorded was at 7 DS treatment.

The 7 and 14 DS treatment of BRRI dhan52 and FR13A produced higher 100-grain weight compared to control, might be due to decrease in tiller number. Because the rest of the panicle including main stem got better environmental facilities as well as higher photosynthates for grain

filling. Moreover, as the number of unproductive tiller became lower due to submergence, the maintenance cost also decreased and assimilates partitioning toward grain increased. But decreased 100-grain weight in IR64Sub1 and in BR5 might be due to improper grain filling. Nugraha *et al*. (2012) stated that the lowest 1,000-grain weight under submergence conditions was due to improper grain filling and uneven filling stage, therefore, at harvest the grains had different maturity stages thus lowered seed weight. Zhang *et al*. (2015) did not found any significant difference between control and submergence treated plant considering their 1000-grain weight. Zhang *et al*. (2015) observed a decreased seed-setting rate as the submergence duration increased. Nugraha *et al*. (2012) observed that different rice genotypes had low panicle fertility when exposed to flooding and under this condition, the panicle fertility ranged from 44 to 81%. Increased sterile spikelet due to submergence was also recorded by Reddy and Mittra (1985).

Table 17. Grain sterility percentage and 100-grain weight of main stem of different rice genotypes as influenced by various submergence treatments

Genotypes	Days of submergence (DS)	Grain sterility percentage (%)	100-grain weight (g)
BRRI dhan52	0	17 c	2.59 de
	7	23 b	2.60 d
	14	25 b	2.64 c
	21	24 b	2.47 g
IR64Sub1	0	6 h	2.63 c
	7	9 fg	2.55 f
	14	11 ef	2.56 ef
	21	16 c	2.43 h
FR13A	0	7 gh	2.81 b
	7	15 cd	2.88 a
	14	16 c	2.86 a
	21	13 de	2.80 b
BR5	0	10 f	1.24 i
	7	15 cd	1.20 j
	14	29 a	1.10 k
	21	0	0
$LSD_{(0.05)}$		2.303	0.306
CV (%)		10.96	0.97

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.5. Filled grain number per plant

The total filled grain number per plant of all the genotypes differed significantly under different submergence treatments compared to control treatment (Table 18). Considering all the genotypes and submergence treatments, the total filled grain number per plant counted was the highest (2174.25) in BR5 at control treatment and the lowest (490) in FR13A at 21 DS treatment. In all the genotypes, the filled grain number per plant recorded was the highest in control treatment, which was gradually decreased with the increasing submergence treatments. But the relative values indicate that the filled grain number per plant was severely affected due to submergence treatment in BR5 compared to the other genotypes. The filled grain number per plant was less affected due to submergence in BRRI dhan52 and FR13A.

Due to submergence, the vegetative dry matter became lower. The injury level of the submerged plants was higher and as a result, their maintenance cost was also higher, RGR was lower; which ultimately affected the reproductive development. On the other hand, the chlorophyll content and gas exchange was lower; which might have affected the current photosynthesis. As a result, all the spikelets did not get sufficient photosynthates and finally the filled grain number became lower. But in BRRI dhan52, the filled grain number per plant was less affected in every submergence treatment (7, 14 and 21 DS) compared to other genotypes; indicating its submergence tolerant character. Decreased in filled grain number due to submergence was also recorded by Adak *et al*. (2011), Nugraha *et al*. (2012) and Zhang *et al*. (2015).

Table 18. Filled grain number per plant of different rice genotypes under various submergence treatments

Genotypes	Days of submergence (DS)	Filled grain number per plant	
		Actual	Relative to control (%)
BRRI dhan52	0	992.25 d	100
	7	985.75 d	99
	14	809.75 ef	82
	21	696.00 gh	70
IR64Sub1	0	1174.25 c	100
	7	872.50 e	74
	14	767.25 fg	65
	21	618.25 h	53
FR13A	0	983.75 d	100
	7	781.75 efg	79
	14	711.75 gh	72
	21	490.00 i	50
BR5	0	2174.25 a	100
	7	1334.50 b	61
	14	1332.50 b	61
	21	0	0
$LSD_{(0.05)}$ CV (%)		89.75 6.85	

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.6. Total filled grain weight per plant, total panicle number per plant and filled grain weight per panicle

Total filled grain weight per plant, total panicle number per plant and filled grain weight per panicle of all the genotypes were varied significantly due to submergence treatments and the results have been presented in the table 19. Considering all the genotypes and submergence treatments, the total filled grain weight per plant recorded was the highest (30.92 g) in IR64Sub1 at control treatment which was significantly higher than any other treatment and the lowest (13.70 g) total filled grain weight per plant was recorded in FR13A at 21 DS treatment which was statistically similar to IR64Sub1 at 21 DS and BR5 at 14 DS treatment. In all the genotypes, the total filled grain weight per plant found was the highest at control treatment and the values were decreased with the increasing submergence durations. The lowest filled grain weight per plant recorded was at 21 DS treatment in every genotype.

The reduction in grain yield due to submergence was a cumulative effect. The chlorophyll content, gas exchange, NAR and AGR was decreased due to submergence compared to control. But the reduction in grain yield due to submergence conditions could be attributed to the degree of injury experienced by each genotype, depending on the level of tolerance. The higher the genotypes tolerance to flooding conditions, the higher was the yield achieved. Less affected different physiological processes and increased duration of anthesis to maturity might help BRRI dhan52 and FR13A to perform better after desubmergence and to give better yield.

Considering all the genotypes and submergence treatments, the total number of panicles per plant recorded was the highest (24.25) in IR64Sub1 at control plants which was significantly higher than any other treatment and the lowest (5.50) number of total panicle per plant was recorded in FR13A at 21 DS treatment which was statistically similar to BRRI dhan52 at 14 DS and 21 DS treatment. In all the genotypes, the highest number of total panicle per plant were recorded at control treatment and then gradually decreased with the increasing submergence durations. Among the genotypes, the submergence effect on total number of panicles per plant, was lower in BRRI dhan52 which was followed by FR13A compared to other two genotypes (IR64Sub1 and BR5).

Due to submergence, the injury level was higher and their maintenance cost was also higher, RGR was lower; which ultimately affected the reproductive development. The genotype BRRI dhan52 and FR13A, in which the reduction of total number of panicles per plant due to submergence was lower; were considered as submergence tolerant because high panicle initiation ability is desirable for achieving higher yield. The lower number of panicles was also probably due to the compensation for increase in plant height due to submergence. The limited biomass produced under submergence condition caused the plant to choose whether to produce more panicle bearing tillers or to elongate the stem. Because the elongation was more important for survival, then the number of panicles bearing tillers were reduced (Nugraha *et al*., 2012). Decreased in number of panicle due to submergence was also recorded by Adak *et al*. (2011).

In BRRI dhan52, the average filled grain weight per panicle found was the highest at 14 DS treatment, which was followed by 21 DS treatment. In IR64Sub1, the 14 DS treatment also produced the highest average filled grain weight per panicle which was followed by 21 DS treatment also. In FR13A, 21 DS treatment produced the highest average filled grain weight per panicle which was followed by 14 DS treatment. But in BR5, the control treatment produced the highest average filled grain weight per panicle which was gradually decreased with the increasing submergence durations.

It was observed from the above results that submerged treatment increased the average filled grain weight per panicle in all the genotypes except in BR5. So it was clear that decreased grain yield per plant under submergence treatment mainly due to reduction of panicle bearing tiller number rather than grain weight per panicle. Due to reduction in panicle number, the rest of the panicle got better environmental facilities and the flagleaf could give sufficient food supply to its own panicle. Decreased in filled grain weight per panicle due to submergence was also recorded by Nugraha *et al*. (2012).

Table 19. Total filled grain weight per plant, total panicle number per plant and filled grain weight per panicle of different rice genotypes as influenced by various submergence treatments

Genotypes	Days of submergence (DS)	Total filled grain weight per plant (g)	Total panicles per plant	Average filled grain weight per panicle (g)
BRRI dhan52	0	25.72 cd	9.75 d	2.68 abc
	7	25.58 d	9.50 d	2.72 ab
	14	21.34 ef	7.50 def	2.93 a
	21	17.26 h	6.25 ef	2.80 ab
IR64Sub1	0	30.92 a	24.25 a	1.30 f
	7	22.20 e	15.00 b	1.49 ef
	14	19.66 g	9.5 d	2.08 cde
	21	15.00 ij	9.0 d	1.68 def
FR13A	0	27.62 b	12.25 c	2.27 bcd
	7	22.47 e	9.5 d	2.71 ab
	14	20.31 fg	8.25 de	2.49 abc
	21	13.70 j	5.50 f	2.63 abc
BR5	0	27.04 bc	15.25 b	1.81 def
	7	16.05 hi	9.75 d	1.69 def
	14	14.63 ij	9.74 d	1.54 ef
	21	0	0	0
$LSD_{(0.05)}$		1.38	2.37	19.06
CV (%)		4.84	16.52	0.5571

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.7. Panicle dry weight per plant

The total panicle dry weight per plant of all the genotypes was differed significantly under different submergence treatments compared to control treatment (Table 20). In BRRI dhan52, the panicle dry weight per plant found was the highest (29.94 g) in 7 DS treatment which was statistically similar to control treatment, then gradually decreased with the increasing submergence duration and the 14 DS treatment produced 87% relative panicle dry weight. In IR64Sub1, control treatment produced the highest (37.69 g) panicle dry weight per plant which was significantly higher than any other submergence treatment and gradually decreased with the increasing submergence duration. In FR13A, the control treatment also produced the highest (30.25 g) panicle dry weight per plant which was significantly higher than any other treatment of the same genotype and gradually decreased with the increasing submergence duration. In BR5, the control treatment produced the highest (30.57 g) panicle dry weight per plant which was significantly higher than any other treatment of the same genotype and gradually decreased with the increasing submergence duration.

The reduction in panicle dry matter per plant after desubmergence was mainly due to reduction in chlorophyll content, gas exchange, NAR and AGR compared to control. The panicle dry weight per plant is one of the main yield determining character. But in IR64Sub1 and BR5, a drastic reduction in panicle dry weight per plant occurred due to submergence treatment which indicated that these two genotypes are more submergence sensitive than other two genotypes. As the reduction of panicle dry weight per plant was lower in BRRI dhan52 and FR13A, indicated that this two genotypes are more submergence tolerant. Sarker and Das (2003) stated that panicle weight of different rice genotypes were reduced due to 14 days of submergence.

Table 20. Panicle dry weight per plant of different rice genotypes as influenced by various submergence treatments

Genotypes	Days of submergence (DS)	Panicle dry weight per plant (g)	
		Actual	Relative to control (%)
BRRI dhan52	0	29.30 b	100
	7	29.94 b	102
	14	25.39 d	87
	21	20.11 f	69
IR64Sub1	0	37.69 a	100
	7	27.05 c	72
	14	23.19 e	62
	21	18.56 g	49
FR13A	0	30.25 b	100
	7	25.43 d	84
	14	23.10 e	76
	21	15.50 h	51
BR5	0	30.57 b	100
	7	18.59 g	61
	14	18.40 g	60
	21	0	0
$LSD_{(0.05)}$		1.37	
CV (%)		4.14	

Values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.8. Filled, unfilled and total grain dry weight per plant

Significant variations were observed in filled, unfilled and total grain dry weight per plant by the interaction effect of submergence duration and rice genotypes and the results have been shown in the table 21. In all the genotypes, the filled grain dry weight per plant recorded was the highest at control treatment which was gradually decreased with the increasing submergence duration. The 7 DS treatment of BRRI dhan52 produced the highest (25.58 g) filled grain weight per plant which was significantly higher than 14 and 21 DS treatment of the same genotype as well as the 7, 14 and 21 DS treatments of other genotypes. Due to submergence treatment, the highest (99%) relative filled grain weight per plant recorded was in BRRI dhan52 at 7 DS treatment which was followed by 14 DS treatment (83%) of the same genotype. But the reduction in filled grain weight per plant due to submergence was much lower in BRRI dhan52 and FR13A compared to others. A drastic reduction in filled grain weight per plant recorded was in IR64Sub1 and in BR5 due to submergence treatment. Less affected filled grain weight in BRRI dhan52 and FR13A due to submergence indicated that these two genotypes are more submergence tolerant.

Due to submergence, the number of filled grain or seed setting rate decreased. Though the filled grain weight per panicle increased, the panicle number decreased and the grain yield became lower. Compared to control treatment the 100-grain weight was also lower in 21 DS treatment of all genotypes, 7 and 14 DS treatment of IR64Sub1 and BR5. Decreased shoot and root dry matter, chlorophyll content, RGR, AGR, NAR and gas exchange occurred due to submergence. All of above effect ultimately affected the grain yield per plant.

Considering all the genotypes and submergence durations, the highest (2.15 g) weight of unfilled grain recorded was in 14 DS treatment of BRRI dhan52 and the lowest (0.53 g) weight of unfilled grain recorded was in control treatment of FR13A. In BRRI dhan52, the highest unfilled grain weight per plant recorded was at 14 DS treatment, which was followed by 7 DS treatment. The lowest unfilled grain weight per plant recorded was at 21 DS treatment in BRRI dhan52. In IR64Sub1, the highest unfilled grain weight per plant recorded was at 21 DS treatment, which was followed by 7 DS treatment. The lowest unfilled grain weight per plant recorded was at 14 DS treatment in IR64Sub1. In FR13A, the highest unfilled grain weight per plant recorded was at 14 DS treatment which was followed by 7 DS treatment. The lowest unfilled grain weight per

plant recorded was at control treatment in FR13A. In BR5, the lowest unfilled grain weight per plant recorded was at 7 DS treatment, which was followed by control treatment and the highest unfilled grain weight per plant recorded was at 14 DS treatment.

Due to submergence injury, the current photosynthesis became lower; moreover the maintenance cost was also higher. As a result, all the spikelets did not get sufficient assimilates and ultimately, the number and weight of unfilled grains became higher.

Considering all the genotypes and submergence durations, the highest (32.87 g) weight of total grain per plant was recorded in control treatment of IR64Sub1 and the lowest (14.41 g) weight of total grain recorded was in 21 DS treatment of FR13A. In BRRI dhan52, the total grain dry weight per plant recorded was the highest at 7 DS treatment which was statistically similar to control treatment. In IR64Sub1, the total grain weight per plant recorded was the highest at control treatment which was significantly higher than any other treatments and then gradually decreased with the increasing submergence duration. In FR13A and BR5, the total grain dry weight per plant recorded was the highest at control treatment which was gradually decreased with the increasing submergence duration. A drastic reduction in total grain dry weight per plant due to submergence treatment compared to control treatment recorded was in IR64Sub1 and in BR5. But in BRRI dhan52 and FR13A, the reduction of total grain dry weight per plant was much lower due to submergence treatment compared to other genotypes.

Due to submergence, the plants became highly injured. As a result, their maintenance cost was higher. Different physiological processes were hampered due to submergence. The chlorophyll content was drastically reduced due to submergence; which might affect the current photosynthesis. As a result, all the spikelets did not get sufficient photosynthates and finally the filled grain number became lower which ultimately affected the total grain weight per plant. BRRI dhan52 performed better compared to other genotypes in every submergence (7, 14 and 21 DS) treatment; indicated its submergence tolerant character. Decreased in total filled grain weight due to submergence was also recorded by Adak *et al*. (2011), Nugraha *et al*. (2012) and Elanchezhian *et al*. (2013).

Table 21. Filled, unfilled and total grain weight per plant of different rice genotypes under various submergence treatments

Genotypes	Days of submergence (DS)	Filled grain weight per plant (g)		Unfilled grain weight per plant (g)	Total grain weight per plant (g)
		Actual	Relative (%)		
BRRI dhan52	0	25.72 cd	100	1.49 f	27.21 b
	7	25.58 d	99	1.92 cd	27.50 b
	14	21.34 ef	83	2.15 a	23.49 cd
	21	17.26 h	67	1.34 g	18.59 e
IR64Sub1	0	30.92 a	100	1.95 c	32.87 a
	7	22.20 e	72	2.0 b	24.20 c
	14	19.66 g	64	1.71 e	23.88 c
	21	15.00 ij	49	2.02 b	17.02 e
FR13A	0	27.62 b	100	0.53 l	28.15 b
	7	22.47 e	81	1.02 i	23.49 cd
	14	20.31 fg	74	1.11 h	21.42 d
	21	13.70 j	50	0.71 k	14.41 f
BR5	0	27.04 bc	100	0.96 j	28.00 b
	7	16.05 hi	59	0.93 j	16.98 e
	14	14.63 ij	54	1.90 d	16.53 e
	21	0	0	0	0
$LSD_{(0.05)}$		1.38		0.045	1.996
CV (%)		4.84		2.71	6.52

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.9. Total biological yield, total grain yield and harvest index

Total biological yield, total grain yield and harvest index of all the genotypes were varied significantly due to the interaction effect of different rice genotypes and submergence treatments and the results have been presented in the table 22. In all the genotypes, the total biological yield found was the highest at control treatment, which was gradually decreased with the increasing submergence durations. A drastic reduction (around 50% of the control treatment) in total biological yield due to submergence treatment recorded was in BR5. But in other genotypes, the relative total biological yield were much higher than BR5 considering 7 and 14 DS treatments. Considering all the genotypes, the highest (89.92 g) total biological yield recorded was in BR5 at control treatment which was significantly higher than any other treatments of the same and other genotypes and the lowest (35.29 g) recorded was in FR13A at 21 DS treatment.

At 7, 14 and 21 DS treatment, the relative total biological yield recorded was much higher (98.59%, 88.39% and 62.12% at 7,14 and 21 DS treatments respectively) in BRRI dhan52 which was followed by IR64Sub1and FR13A. The 99% of the relative total biological yield in BRRI dhan52 at 7 DS treatment might be due to 99% of the relative grain yield mainly.

In all the genotypes, the total grain yield per plant recorded was the highest at control treatment, which was gradually decreased with the increasing submergence duration and the difference between control and submergence treatment were significant in all the genotypes except in BRRI dhan52 at 7 DS treatment. But the relative total grain yield indicated that the reduction in total grain yield per plant due to submergence treatment was much lower in BRRI dhan52 and FR13A (compared to IR64Sub1 and BR5). The relative total grain yield per plant due to submergence treatment recorded was much lower in IR64Sub1 and BR5 (around 50-70%). Less affected total grain yield in BRRI dhan52 (above 80% of the control both at 7 and 14 DS treatment) and in FR13A (above 80% of the control at 7 DS treatment) due to submergence, indicated that this two genotypes are more submergence tolerant than the others. Considering the combined effect of the genotypes and submergence duration, the highest (30.92 g) total grain yield recorded was in IR64Sub1 at control treatment which was significantly higher than any other treatment and the lowest (13.70 g) recorded was in FR13A at 21 DS treatment. Elanchezhian *et al*. (2013) found

that the range of grain yield in control condition was 2.65–6.14 t ha^{-1}, whereas it varied from 0.13 to 3.18 t ha^{-1} under submergence stress.

Reduction in grain yield due to submergence could be due to injury, experienced by submergence treatment, as well as tolerant level of various genotypes. The higher the genotypes tolerant to flooding conditions, the higher was the yield can be produced. The sensitive genotypes would have lost their biomass, leaves and tillers and took much longer time to recover and to develop new organs, suggesting that the sensitive genotypes were mostly source limited during grain filling. These affected the translocation of assimilates to the sink as well as the developing grain. A significant decrease in panicles number and filled grain number per plant, increase in number of sterile spikelet, were the main causes of yield decline due to submergence treatment. After longer submergence period the rice plants were very much injured. The injured plants took a longer time for recovery. As a result, the panicle initiation stage of the treated plant was delayed. After that when panicle initiation and panicle differentiation occurred, at the same time, recovery of submerged injury was also going on. After that, the panicle differentiation was hampered due to lack of sufficient food and energy supply. Consequently, it was also observed that some panicles never emerged completely from the boot in 21 DS treated plants. The spikelet inside the boot was unable to produce grain because anthesis did not occur. This tendency found was in panicles other than main stem panicle. Ultimately, total grain weight per plant was much lower in 21 DS treated plants compared to control plant.

The 99% of the relative grain yield in 7DS treatment of BRRI dhan52 might be due to less affected vegetative dry matter, effective tiller number, LWR and root dry matter in this treatment. The grain filling duration in this treatment was as same as the control. The 100-grain weight and transpiration rate at 7DS treatment recorded was higher than control. Here the panicle number, NAR and AGR was statistically similar to control. Increased main stem contribution to yield compared to control and increased proline content after desubmergence helped this genotype to early recovery and to provide better yield at 7DS treatment. So, it may be concluded that if we be able to maintain the effective tiller number and the filled grain number per plant as same as control by management practices; the 7DS treatment of BRRI dhan52 may produce better yield.

In the present experiment, the submergence effect on BR5 was much higher compared to other genotypes which contributed in lower yield. This was due to reduced the number of filled grain and reduced weight of filled grain, lower 100-grain weight, higher grain sterility, higher unfilled grain number and weight, lower main stem contribution, higher under water elongation and injury, higher reduction in root and shoot dry matter, lower RWC after desubmergence, lower tiller number, drastic reduction of SPAD value near maturity. Different physiological activities such as stomatal conductance, RGR, AGR were also hampered in BR5 due to submergence. The percent reduction of non structural carbohydrate (sugar and starch) was also higher in BR5. All of these were responsible for the minimum yield in BR5 due to submergence stress.

Considering all the genotypes and submergence treatments, the harvest index recorded was the highest (0.43) in BRRI dhan52 at 21 DS treatment, which was significantly higher than any other treatments and the lowest (0.30) harvest index recorded was in BR5 at control treatment which was significantly lower than any other treatment. In BRRI dhan52 and FR13A, the harvest index was higher at 21 DS treatment compared to other treatments. In IR64Sub1, the harvest index recorded was the highest at control condition. In BR5, the harvest index value recorded was the highest at 14 DS treatment which was followed by 14 DS treatment. Increased harvest index due to submergence, indicated that the magnitude of reduction in reproductive dry matter was lower than that of vegetative dry matter in all the genotypes except in 14 DS treatment of BRRI dhan52 and all the treatments of IR64Sub1. Haque *et al*. (2015) found that the harvest index was varied for different submergence levels. Nugraha *et al*. (2012) found that flooding reduced above ground dry matter weight (AGDMW) on different rice genotypes. They also found that *Sub1* lines produced high biomass compared to the non- *Sub1* lines and the reduction in AGDMW due to 15 days of submergence did not lower the harvest index (HI), even it was higher than that in normal condition. The result of the HI in the present experiment was dissimilar to that of Elanchezhian *et al*. (2013) who found lower HI due to submergence treatment compared to control.

Table 22. Total biological yield, total grain yield and harvest index of different rice genotypes under various submergence treatments

Genotypes	Days of submergence (DS)	Total biological yield (g plant^{-1})		Total grain yield (g plant^{-1})		Harvest index
		Actual	Relative (%)	Actual	Relative (%)	
BRRI dhan52	0	65.41 d	100	25.72 cd	100	0.40 b
	7	64.49 d	98.59	25.58 d	99.48	0.40 b
	14	57.82 e	88.39	21.34 ef	82.97	0.37 d
	21	40.63 g	62.12	17.26 h	67.13	0.43 a
IR64Sub1	0	84.42 b	100	30.92 a	100	0.37d
	7	71.48 c	84.68	22.20 e	71.80	0.31 k
	14	56.27 e	66.65	19.66 g	63.59	0.35 g
	21	42.19 g	49.98	15.00 ij	48.51	0.36 f
FR13A	0	81.70 b	100	27.62 b	100	0.34 h
	7	60.40 de	73.93	22.47 e	81.35	0.37 d
	14	56.07 e	68.63	20.31fg	73.55	0.36 e
	21	35.29 h	43.20	13.70 j	49.60	0.39 c
BR5	0	89.92 a	100	27.04bc	100	0.30 l
	7	50.95 f	56.66	16.05 hi	59.34	0.32 j
	14	44.97 g	50.01	14.63 ij	54.09	0.33 i
	21	0	0	0	0	0
$LSD_{(0.05)}$		4.90		1.38		0.0045
CV (%)		6.11		4.84		5.95

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.2.10. Dry matter partitioning percentage per plant to different organs

Dry matter partitioning percentage to leaf, stem, filled grain, unfilled grain and root were calculated after harvest. Dry matter distribution to different vegetative and reproductive organs varied significantly due to submergence treatment. Different genotypes also showed variability in this regard and the results were shown in table 23. In BRRI dhan52, the dry matter partitioning to leaf gradually increased from control to 14 DS treatment then decreased in 21 DS condition, which was significantly lower than 14 DS treatment. Dry matter partitioning to stem found was the highest at 14 DS treatment in BRRI dhan52, but the difference between 14 DS with other treatments was not significant. Dry matter partitioning to filled grain found was the highest at 21 DS treatment which was significantly higher than any other treatment and 7 DS treatment also showed increased dry matter distribution to filled grain compared to control in BRRI dehan52. The dry matter distribution to unfilled grain was increased in submergence treatments compared to control treatment. The dry matter distribution to root found was the highest at control treatment and gradually decreased from control to 21 DS treatment in this genotype; though the difference was not significant.

In IR64Sub1, the dry matter partitioning to leaf increased in submergence treatment than control treatment; but the difference between control and submerged treatment was not significant. Dry matter partitioning to stem also increased under submergence treatment compared to control treatment, but significant difference found was only with 7 DS treatment. Dry matter partitioning to filled grain decreased due submergence treatment compared to control which was significantly lower in 7 DS treatment compared to control. But the dry matter partitioning to unfilled grain and root increased in submergence treatment compared to control treatment in this genotype.

In FR13A, the dry matter partitioning to leaf and stem were lower in submergence treatment compared to control treatment. The dry matter partitioning to filled grain increased under submergence treatment compared to control treatment and partitioning was significantly higher in 21 DS treatment compared to control treatment. The dry matter partitioning to unfilled grain and to root also decreased under submergence treatment compared to control treatment in this genotype.

In BR5 genotype, the dry matter partitioning to leaf gradually decreased from control to 14 DS treatment and significant difference with control and 14 DS treatment was observed. But there were no remarkable difference (non significant) in partitioning of dry matter to stem among the treatments in this genotype. The dry matter distribution to filled grain and unfilled grain increased in submergence treatment compared to control treatment. A drastic reduction in dry matter partitioning to root occurred in submergence treatment compared to control treatment in BR5.

The grain yield is the ultimate goal for rice production. So, the genotypes in which dry matter distribution to yield remain higher due to submergence treatment, suppose to be submergence tolerant type; as in BRRI dhan52 and FR13A in the present experiment. Increased root investment due submergence in IR64Sub1 is not desirable. Lower reduction in TDM in BRRI dhan52 at 7 DS treatment was mainly due to higher panicle investment in the same treatment. Increased dry matter investment to filled grain at 7 DS treatment compared to control treatment recorded was in all the genotypes except IR64Sub1. Increased dry matter investment to filled grain at 14 DS treatment compared to control treatment recorded was in FR13A and BR5, Increased dry matter investment to filled grain at 21 DS treatment compared to control treatment recorded was in BRRI dhan52 and FR13A; all these were desirable under respective submergence treatment. Sarkar and Bhattacharjee (2011) found that total above ground dry matter contents were greater under control condition followed by 14 and 20 days of submergence. They also found that the reduction in dry matter content was more than 90% in susceptible genotypes after 14 days of submergence.

Table 23. Dry matter distribution percenage to different organs of rice genotypes as influenced by various submergence treatments

Genotypes	Days of submergence (DS)	Total dry matter (g plant^{-1})	% dry matter distribution to				
			Leaf	Stem	Filled grain	Unfilled grain	Root
BRRI dhan52	0	65.41	11.52 cd	32.00 de	39.40 b	2.28 g	11.61 a
	7	64.49 (98.59%)	11.96 bcd	31.79 de	39.67 b	2.98 e	9.82 ab
	14	57.82 (88.39%)	13.22 ab	33.65 cde	36.90 bcde	3.72 c	9.22 ab
	21	40.63 (62.12%)	10.80 d	30.75 e	42.45 a	3.30 d	8.95 ab
IR64Sub1	0	84.42	10.65 d	36.34 bc	36.67 bcde	2.31 g	8.32 ab
	7	71.48 (84.68%)	11.55 cd	41.17 a	31.10 gh	2.80 f	9.39 ab
	14	56.27 (66.65%)	11.52 cd	38.36 ab	35.03 def	3.05 e	8.81 ab
	21	42.19 (49.98%)	11.08 cd	36.94 bc	35.58 de	4.79 a	7.96 ab
FR13A	0	81.70	13.19 ab	38.19 ab	33.91 efg	0.65 k	11.48 a
	7	60.40 (73.93%)	12.69 abc	35.03 bcd	37.20 bcd	1.69 i	10.18 ab
	14	56.07 (68.63%)	12.16 bcd	37.08 bc	36.30 cde	1.99 h	9.47 ab
	21	35.29 (43.20%)	11.35 cd	34.35 cde	38.84 bc	2.02 h	10.36 a
BR5	0	89.92	14.18 a	41.00 a	30.09 h	1.07 j	10.78 a
	7	50.95 (56.66%)	13.37 ab	41.63 a	32.29 fgh	1.87 h	7.61 ab
	14	44.97 (50.01%)	11.59 cd	41.92 a	32.46 fgh	4.23 b	5.64 b
	21	0	0	0	0	0	0
$LSD_{(0.05)}$		4.90	1.40	6.80	2.74	0.16	3.86
CV (%)		6.11	8.73	3.329	5.72	4.54	31.06

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance. Figures inside the parenthesis indicate relative to control environment.

4.2.11. Relative total dry matter (TDM) per plant

Different submergence durations exerted a significant effect in dry matter accumulation in leaf, stem, root and yield, which ultimately affects the total dry matter content of plant (Figure 22). Generally, TDM production recorded was the highest in control treatment and then gradually decreased with the increasing submergence duration in all the genotypes. The genotype BRRI dhan52 produced higher relative TDM (98.75%) under 7 DS condition, which was followed by 14 DS treatment. In other three genotypes, 7 DS treatment also produced higher relative TDM compared to other submergence treatments. All plants found were dead in BR5 at 21 DS treatment.

Dry matter accumulation; in other way express the photosynthetic activity of the plant which is dependent upon stress tolerance of the genotypes. So, larger relative TDM under submergence stress in the present experiment, therefore, suggests the level of tolerance against submergence of those genotypes. The genotypes BRRIdhan52 and FR13A showed better adaptation to submergence by producing higher relative TDM under 7 and 14 DS treatment. Higher relative TDM in BRRI dhan52 at 7 DS treatment mainly due to higher panicle DM in the same treatment. A considerable decrease in aboveground biomass under submergence stress as compared to control condition was also reported by Elanchezhian *et al*. (2013).

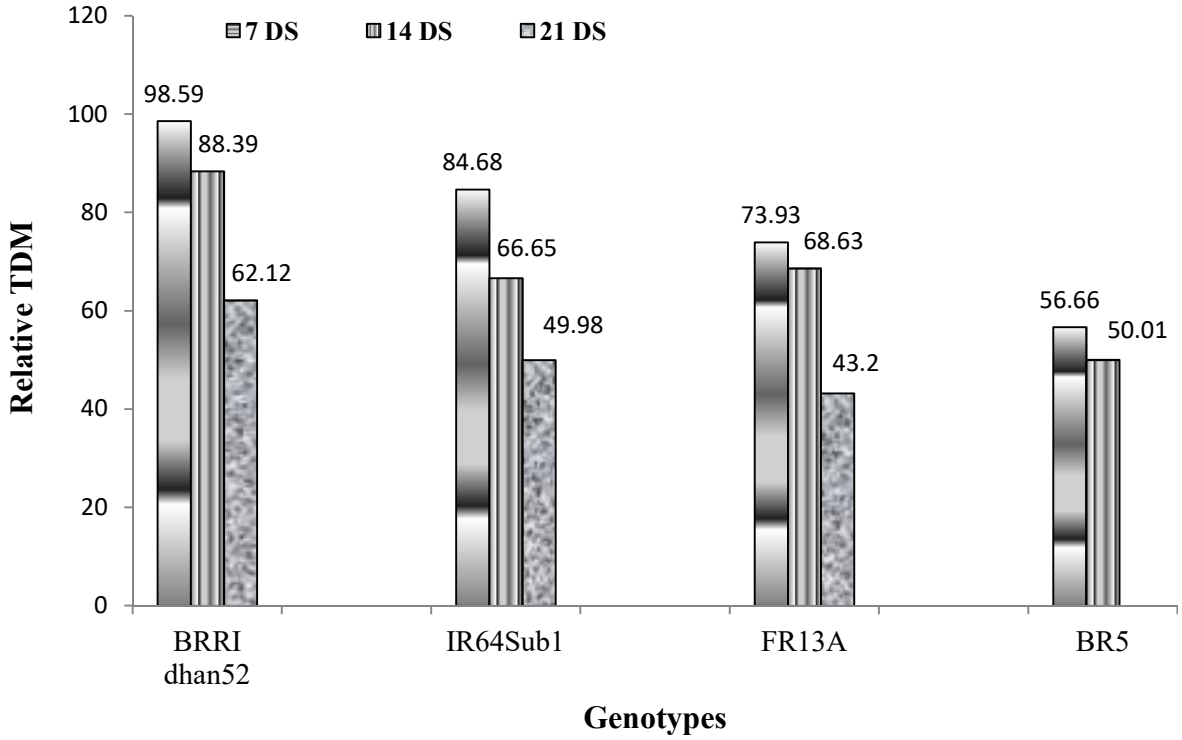


Figure 22. Relative total dry matter (TDM) content of different rice genotypes under various submergence treatments.

In the second experiment, the genotypic variation among the rice genotypes was also found considering their yield components due to submergence treatments. Considering the different yield attributes (such as number of panicles per plant, number of filled grain per panicle and 100-grain weight) the genotypes BRRI dhan52 and FR13A showed more submergence tolerant compared to the other two genotypes (IR64Sub1 and BR5). For further confirmation of the results of experiment 1 and 2, different physiological and anatomical parameters were recorded subsequently.

4.3. Experiment-III: The anatomical and physiological responses of rice genotypes under different submergence durations

The objective of this study was to assess the anatomical and physiological response of different rice genotypes under different submergence duration.

4.3.1. Proline content of leaf

Proline content of leaf was determined both from control and submerged (7, 14 and 21 DS) plants after 2 hrs of desubmergence and the results have been presented in the figure 23. Considering all the genotypes and submergence treatments, it recorded was that proline content was significantly higher in submerged plants compared to control plants except in IR64Sub1, where submerged and control treatment did not differ significantly corresponding to their proline content. Due to 7 DS treatment, all the treated plants showed little higher proline content than control plants in every genotype. Considering all the genotypes, the highest (3.15 µmole/g FW, which was 117% of the control) proline content was observed in FR13A at 7 DS treated plants which was statistically similar to 7 DS treatment of BR5 and the lowest (1.3 µmole/g FW) proline content recorded was in BRRI dhan52 at control plants which was significantly lower than any other genotypes of this (7 DS) treatment.

Due to 14 DS treatment, the proline content of submerged plants were also higher than control plants in all the genotypes. Among the genotypes, the highest (3.2 µmole/g FW, which was 119% of the control) proline content recorded was in FR13A in submerged plant which was statistically similar to submerged plant of BR5 and the lowest (1.25 µmole/g FW) proline content recorded was in BRRI dhan52 in control plants which was significantly lower than any other genotypes of this (14 DS) treatment.

Due to 21 DS treatment, the leaves of BR5 were found very much injured and lodged. In BRRI dhan52 and FR13A, the proline content was much higher in submerged (21 DS) plants than the control plants. But in IR64Sub1 and BR5, the proline content was little higher in submergence treated plants than the control plants. Among the genotypes, the highest (4.67 µmole/g FW, which was 172% of the control) proline content was also recorded in FR13A at submerged (21 DS) plants which were significantly higher than any other genotypes of this (21 DS) treatment and the lowest (1.28 µmole/g FW) content recorded was in BRRI dhan52 at control plants which

was significantly lower than any other genotypes of this (21 DS) treatment. In all the genotypes, proline content increased with the increasing submergence durations.

Due to submergence injury, the plant faced aerobic shock after desubmergence. The root was also injured after desubmergence and was unable to absorb sufficient water. As a result, the desubmerged plant fall on water stress and the RWC of leaf became lower. In this situation proline accumulation occurred for osmotic adjustment. Higher proline accumulation in BRRI dhan52 in all (7, 14 and 21 DS) treatments might help this genotype in early recovery of submergence injury and finally gave better yield. The proline accumulation under 7 and 14 DS treatment was also higher in BR5 and in FR13A. But in 21 DS treatment, proline content was decreased significantly in BR5 genotype. This might be due to that, under 21 DS treatment the cells of BR5 were very much injured and physiological function of the cell was seriously hampered. After desubmergence the injury level was lower in IR64Sub1. The leaf dehydration was also lower. As a result, the proline content was also recorded lower in IR64Sub1. This low level of proline production might be due to the genetic potentiality of this variety, which has the ability to overcome submergence stress without increasing the proline level too much. On the other hand, the submergence tolerant mechanism of BRRI dhan52 and FR13A was related to relatively higher accumulation of proline and increased relative water content. Banerjee *et al*. (2015b) found that proline content was significantly induced by submergence, though variably in the varieties. A significant rise in the proline content in different rice varieties could establish the osmotic adjustment for developed water stress in the leaf tissues. Proline has also been found to serve as a substrate for respiration and as a source of nitrogen and other metabolites (Steward and Boggess, 1978); thus increased proline content help in early recovery from stress injury. Proline might have a protecting role of stabilizing membrane and protein (Upadhyay *et al*., 2009).

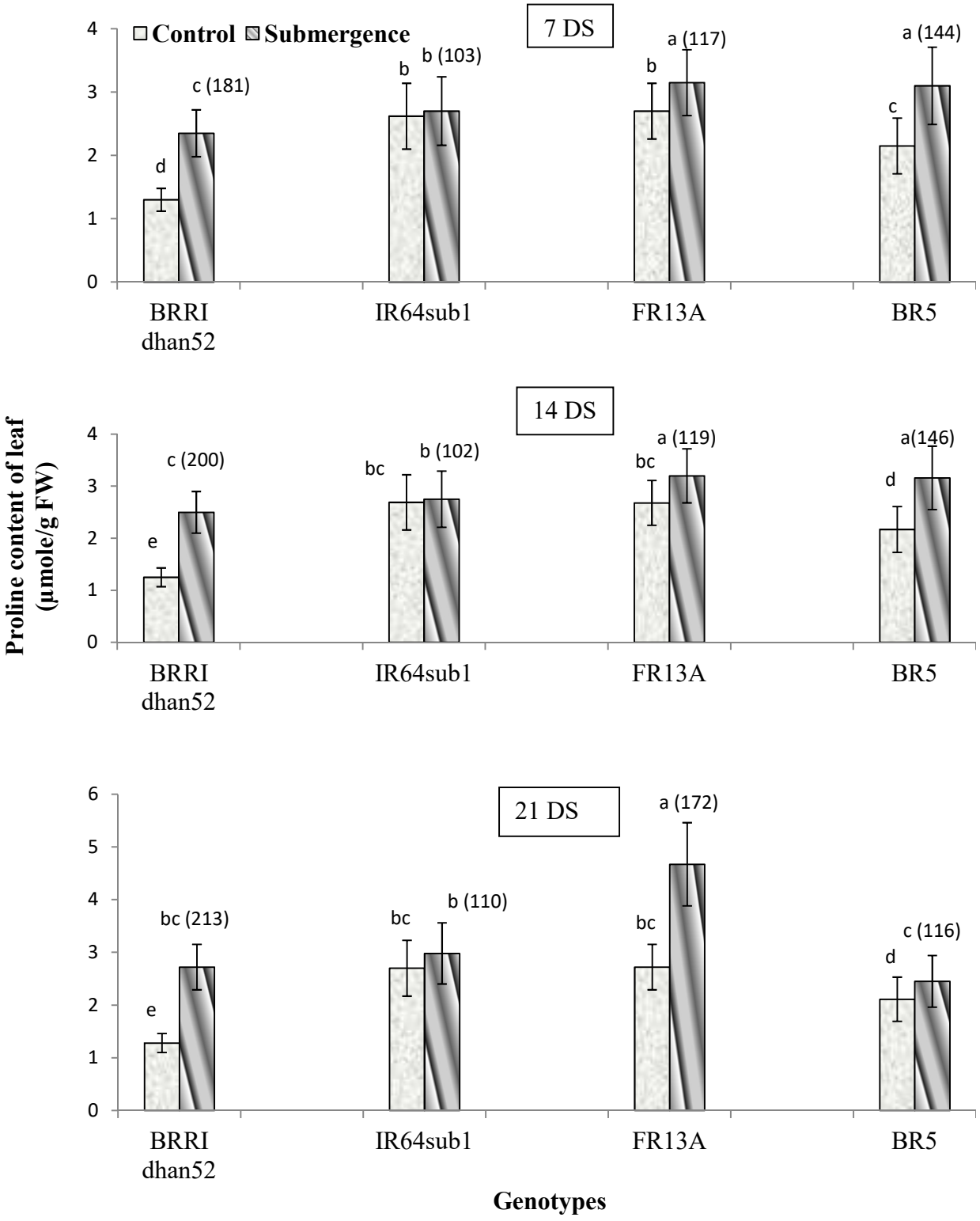


Figure 23. Proline content of leaf under various submergence treatments of different rice genotypes. Bar represents standard deviation for the respective treatment. Figures inside the parenthesis indicate relative to control. DS= days of submergence.

4.3.2. Relative injury of cell membrane

In the present experiment, the relative injury was determined by the amount of cell electrolytes leakage through the leaf cell membrane. The ion leakage due to membrane injury was caused by oxygen depletion due to submergence. The relative membrane leakage of fully expanded leaf cells of all the genotypes recorded was both from control and treated plants after desubmergence and the results have been shown in the figure 24. Due to 7 DS treatment, the relative leakage recorded was higher in treated plants than control plants in all the genotypes. Among the genotypes, the highest (82.05%) leakage recorded was in BR5 in treatment (7 DS) plant which was significantly higher than any other genotype in 7 DS treatment and the lowest (61.06%) leakage recorded was in BR5 also at control plant which was significantly lower than any other genotype.

Due to 14 DS treatment, the relative leakage was also recorded higher in treated plants than control plants in all the genotypes. Among the genotypes, the highest (93.39%) leakage was also recorded in BR5 at treatment (14 DS) plant which was significantly higher than any other genotype and the lowest (63.08%) leakage was also recorded in BR5 at control condition which was statistically similar to control treatment of BRRI dhan52.

Due to 21 DS treatment, the relative leakage was much higher in treatment (21 DS) plants than the control plants. Considering all the genotypes, the highest (96.3%) relative leakage recorded was in BR5 at treatment (21 DS) plants which were significantly higher than any other genotype and the lowest (57.22%) relative leakage recorded was in BR5 at control a plant which was significantly lower than any other genotypes in this treatment.

The difference in relative leakage between control and submergence treated plant was lower in BRRI dhan52, IR64Sub1 and in FR13A and higher in BR5. The proline content of FR13A after 21 DS treatment was much higher compared to control treatment which might be due to lower membrane injury in this genotype at 21 DS treatment. In all (7, 14 and 21) DS treatments, the relative injury of BR5 was the highest; indicated the submergence susceptibility of this genotype. The lowest injury in IR64Sub1 at 7 and 21 DS treatment was desirable, though their yield level was not up to the mark in those treatments. In the present experiment, the treatment (7, 14 and 21 DS) plant showed significantly higher relative injury compared to control plant in all the

genotypes. But there existed genotypic variation also. In BR5, the relative injury level was significantly higher compared to other genotype under all submergence conditions. In BRRI dhan52, the relative injury level was significantly lower compared to other genotype under 7 and 14 DS treatment. Under stress condition, higher proline content might contribute in lower membrane injury (Hasan, 2009). Oxygen shortage becomes higher due to submergence inhibiting respiration and ATP formation. Under submergence, when ATP formation is reduced, the oxidation-reduction state between cell membranes becomes unbalanced and membrane permeability is increased. Hence, the solute leakage and electrical conductivity are increased, deteriorating cell membrane (Kawano *et al*., 2009). Upadhyay *et al*. (2009) revealed that submergence stress increased the ion leakage due to membrane damage, as is evident from increased value of electrical conductivity.

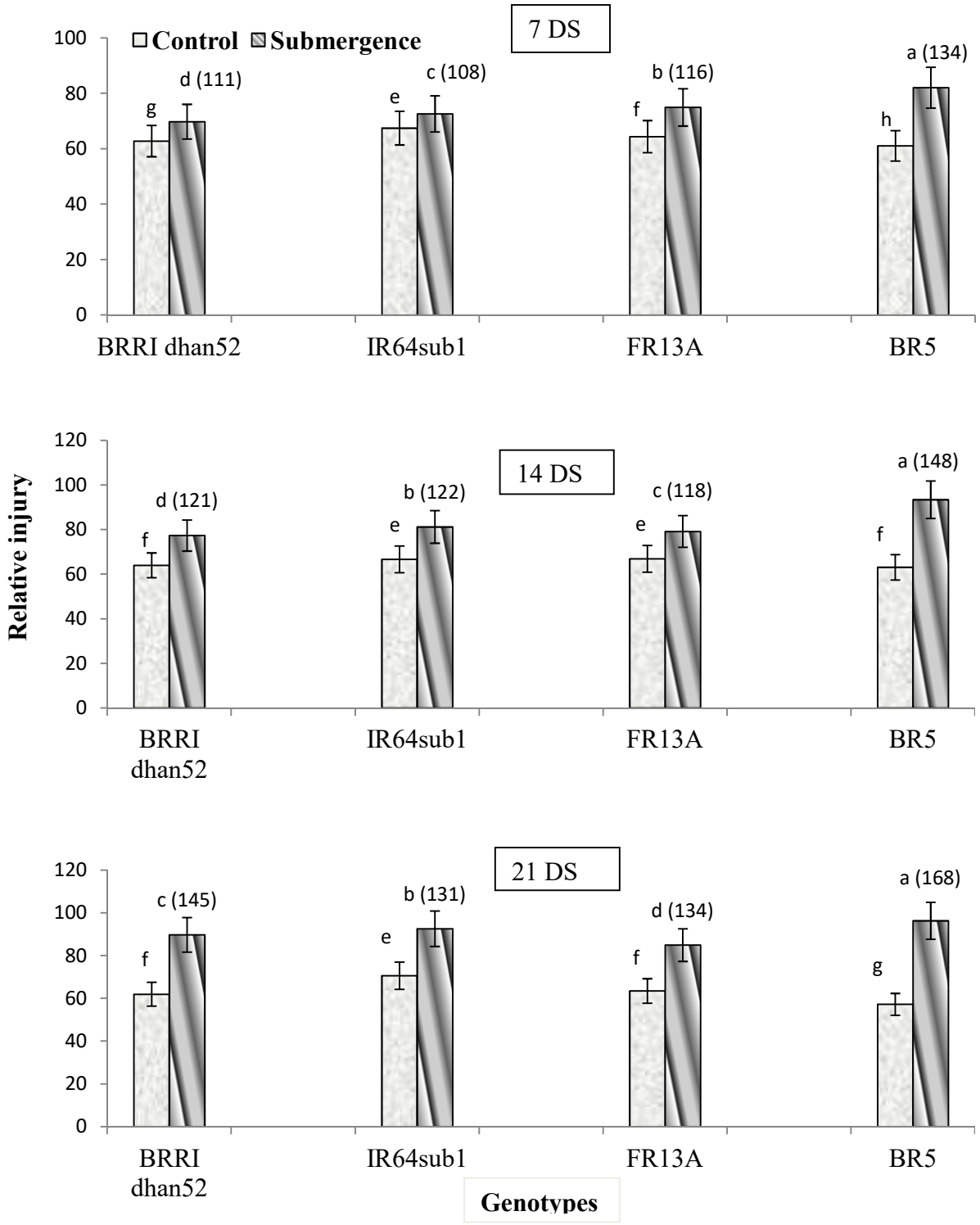


Figure 24. Relative injury of leaf tissue of different rice genotypes under various submergence treatments. Bar represents standard deviation for each respective treatment. Figures inside the parenthesis indicate relative to control. DS= days of submergence.

.4.3.3. Chlorophyll content after desubmergence

The chlorophyll a content of upper fully developed leaves of different rice genotypes was determined from both the control and submergence treated plants after desubmergence. The chlorophyll a content found was significantly higher in control plants than the submergence treated plants in all the genotypes (Figure 25). But the magnitude of reduction was lower in FR13A and higher in BR5. Due to submergence treatment, chlorophyll a content gradually decreased with the increasing submergence durations. At 7 DS treatment, the highest (3.12 mg g^{-1}) chlorophyll a recorded was in IR64Sub1 at control which was statistically similar to control of BR5 and the lowest (1.68 mg g^{-1}) chlorophyll a content recorded was in BR5 of treated plant. In case of 14 DS treatment, the lowest (1.25 mg g^{-1}) chlorophyll a recorded was in BRRI dhan52 and in BR5 at treated plants and the highest (3.66 mg g^{-1}) chlorophyll a content recorded was in IR64Sub1 at control, which was statistically similar to control of BR5. At 21 DS treatment, the highest (3.93 mg g^{-1}) chlorophyll a recorded was in BR5 at control which was statistically similar to control of IR64Sub1 and the significantly lowest (0.57 mg g^{-1}) chlorophyll a content recorded was in BR5 of treated plant.

The chlorophyll b and total chlorophyll content of leaf of different rice genotypes were also determined from both the control and submerged treated plants and the results were shown in figure 26 and 27. The chlorophyll b content found was significantly higher in control plants than the submergence treated plants in all the genotypes. Due to submergence treatment, chlorophyll b content was gradually decreased with the increasing submergence durations. Under 7 DS treatment, the significantly higher (0.96 mg g^{-1}) chlorophyll b content recorded was in IR64Sub1 at control treatment and significantly lowers (0.62 mg g^{-1}) chlorophyll b content recorded was in BRRI dhan52 at submergence treatment. Under 14 DS treatment, the significantly higher (1.06 mg g^{-1}) chlorophyll b content recorded was in IR64Sub1 at control treatment and significantly lowers (0.39 mg g^{-1}) chlorophyll b content recorded was in BR5 at submergence treatment. But in 21 DS treatment, the highest (1.15 mg g^{-1}) and the lowest (0.2 mg g^{-1}) chlorophyll b content recorded was in BR5 at control and submergence condition respectively. Pourabdal *et al*. (2008) found that the amount of chlorophyll a and chlorophyll b in the leaves of flooding treated maize plants was significantly lower than control.

In case of total chlorophyll, the control treatment always produced significantly higher total chlorophyll than the submerged treatment in all the genotypes. At 7 DS treatment, the highest (6.14 mg g^{-1}) total chlorophyll recorded was in IR64Sub1 at control and the lowest (3.38 mg g^{-1}) total chlorophyll content recorded was in BR5 of treated plants. In case of 14 DS treatment, the lowest (2.47 mg g^{-1}) total chlorophyll recorded was in BR5 at treated plants which were statistically similar to treated plants of BRRI dhan52 and the highest (6.61 mg g^{-1}) total chlorophyll content recorded was in IR64Sub1 at control. At 21 DS treatment, the highest (6.87 mg g^{-1}) total chlorophyll recorded was in IR64Sub1 at control which was statistically similar to control of FR13A, BR5 and the significantly lowest (1.04 mg g^{-1}) total chlorophyll content recorded was in BR5 of treated plant which was statistically similar to treated plant of BRRI dhan52.

A drastic reduction of total chlorophyll content in relation to higher injury in BR5 at all submergence treatments compared to control, indicated that the genotype BR5 might more submergence susceptible than other genotypes. Reduction of chlorophyll content under submergence stress is probably due to fast destruction of the chlorophyll pigment. Elanchezhian *et al*. (2013) stated that submergence caused a greater reduction of chlorophyll content, which was also reported by Sarkar and Bhattacherjee (2011). Increasing ethylene concentrations during submergence is a possible reason for chlorophyll degradation (Fukao and Serres, 2008). Submergence stress also increases the enzymatic activity of chlorophyllase, the key enzyme of chlorophyll degradation pathway (Ella *et al*., 2003b). This reduces the capacity for CO_2 fixation during and after submergence. Stability of chlorophyll concentration during submergence stress might have helped the genotypes in maintaining the optimum light energy utilization for photosynthesis and provided carbon reserves needed for maintenance metabolism (Ella *et al*., 2003b). The sensitive and tolerant genotypes have similarly high leaf chlorophyll concentrations before submergence but, when submerged, the tolerant genotypes maintained more chlorophyll than the intolerant genotypes (Singh *et al*., 2014). Considering the above statement, in the present experiment, the genotype FR13A might

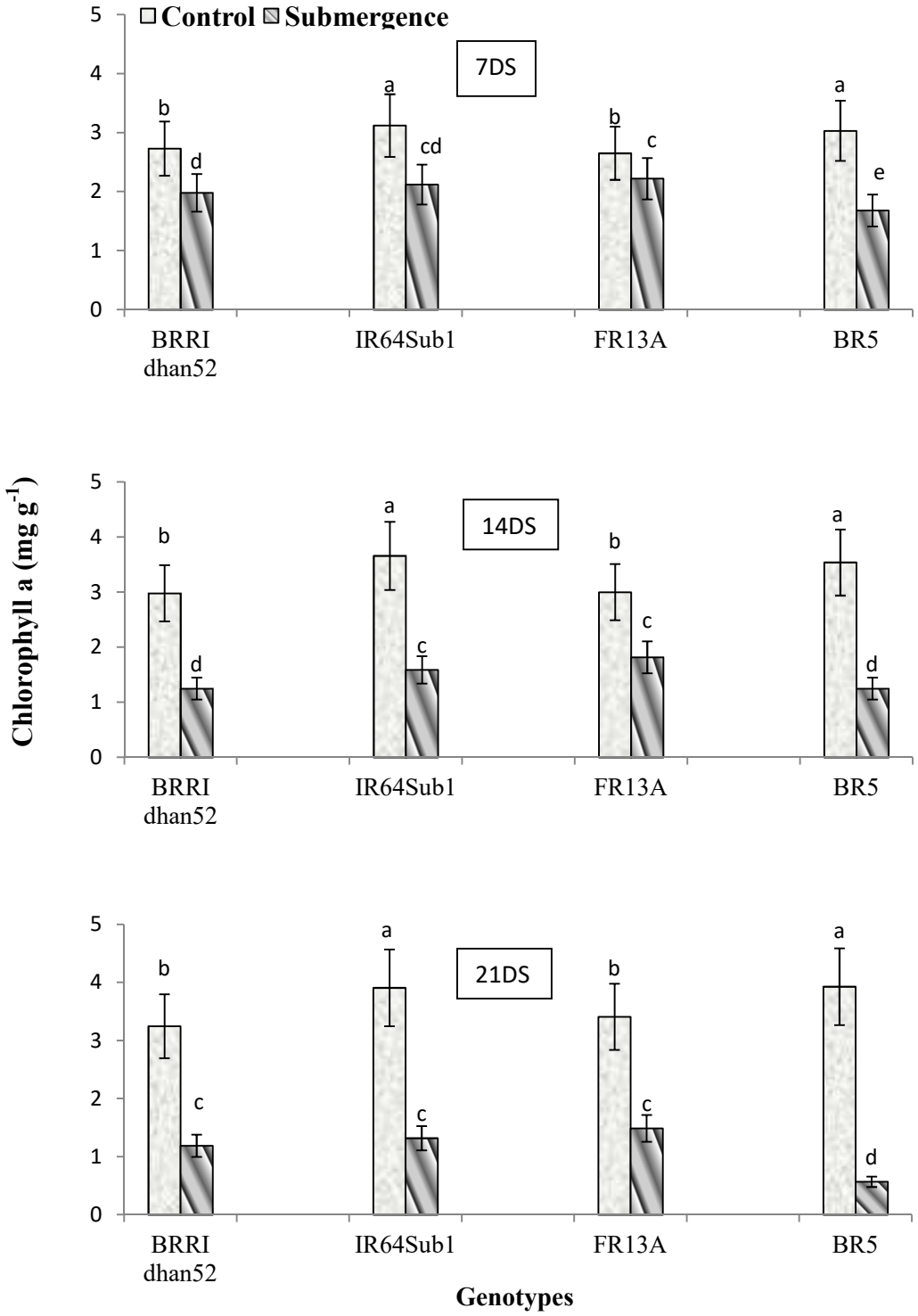


Figure 25. Chlorophyll a content of leaf tissue of different rice genotypes after desubmergence of submerged plants and control plants. Bar represents standard deviation for each respective treatment. DS= days of submergence.

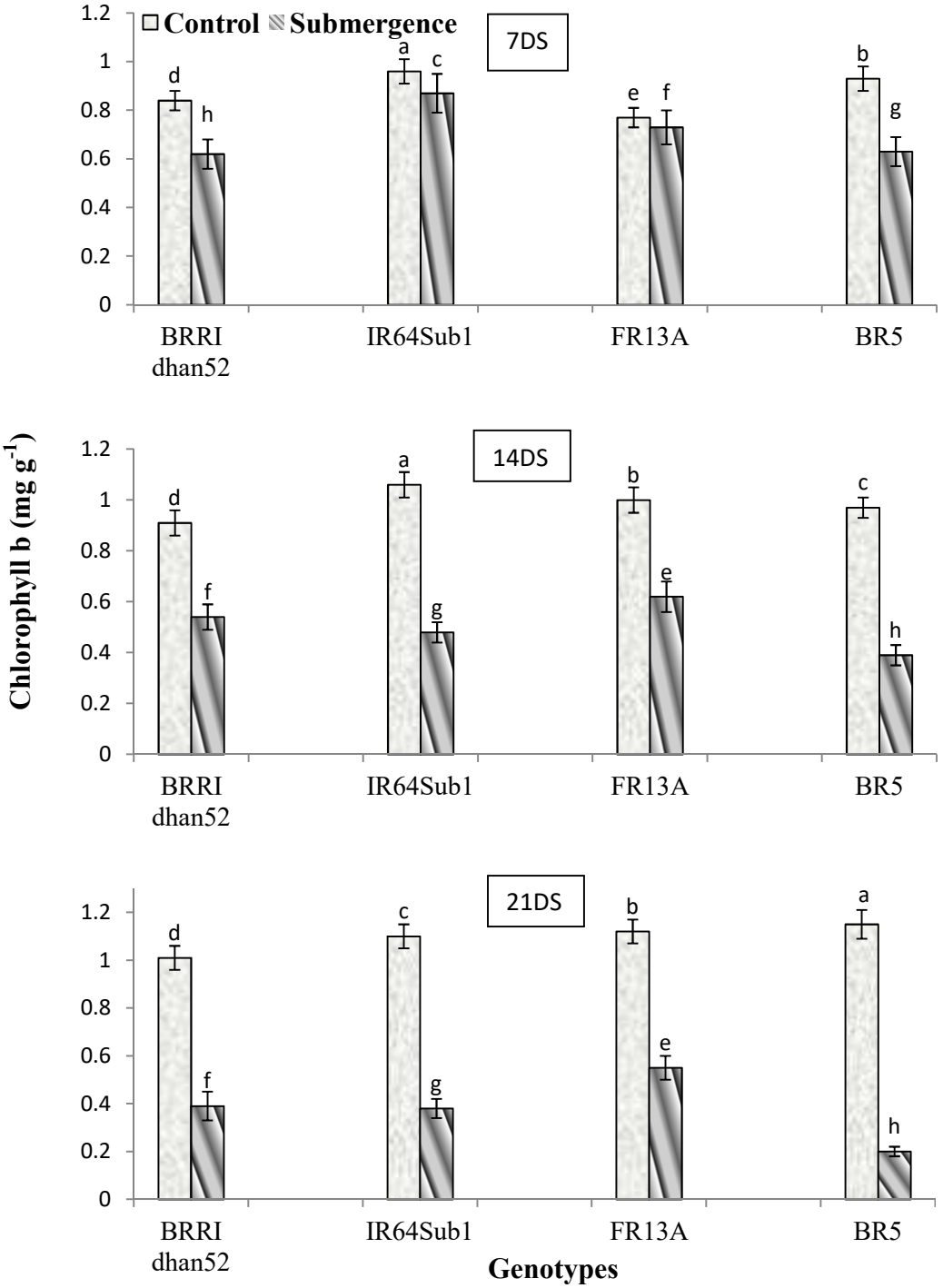


Figure 26. Chlorophyll b content of leaf tissue of different rice genotypes after desubmergence of submerged plants and control plants. Bar represents standard deviation for each respective treatment. DS= days of submergence.

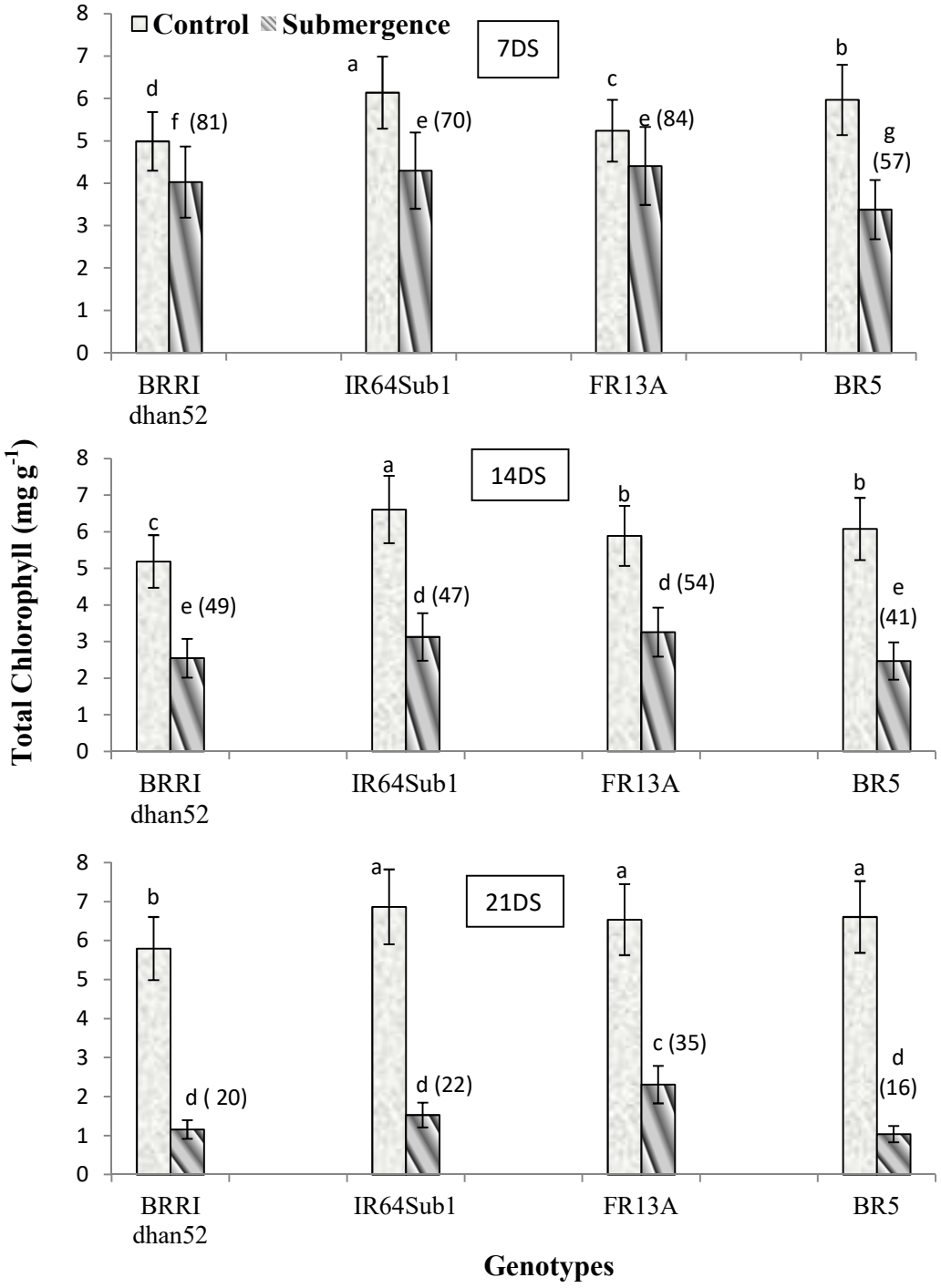


Figure 27. Total chlorophyll content of leaf tissue of different rice genotypes after desubmergence of submerged plants and control plants. Bar represents standard deviation for each respective treatment, figures inside the parenthesis indicate relative to control. DS= days of submergence.

consider as more submergence tolerant genotype which was followed by BRRI dhan52 and IR64Sub1; the genotype BR5 might consider as intolerant genotype. Relatively higher chlorophyll content in FR13A after desubmergence might contribute in higher survivality.

4.3.4. Chlorophyll a/b ratio after desubmergence from control and submerged plant

In all the rice genotypes, the submergence treated plants showed lower chlorophyll a/b ratio than control plants (Figure 28). Lower chlorophyll a/b ratio under submergence in all the genotypes, indicated that chlorophyll b content of leaf was less affected and chlorophyll a content was more affected due to submergence. These results also suggest that chlorophyll a was degraded more than chlorophyll b under submergence, resulting in a significant decrease in chlorophyll a/b ratio after submergence. The chlorophyll b is a constituent of light harvesting complex of chloroplast, so under shaded condition due to submergence, the chlorophyll b helps plants to harvest more light. In BRRI dhan52 and FR13A, the chlorophyll a/b ratio was decreased more under submerged condition compared to control than the other genotypes. The lowest chlorophyll a/b ratio might help those genotypes to perform better under submergence condition by harvesting more light under this condition. Reduction in chlorophyll a/b ratio was also observed by Ella and Ismail (2006) in senescing leaves of rice.

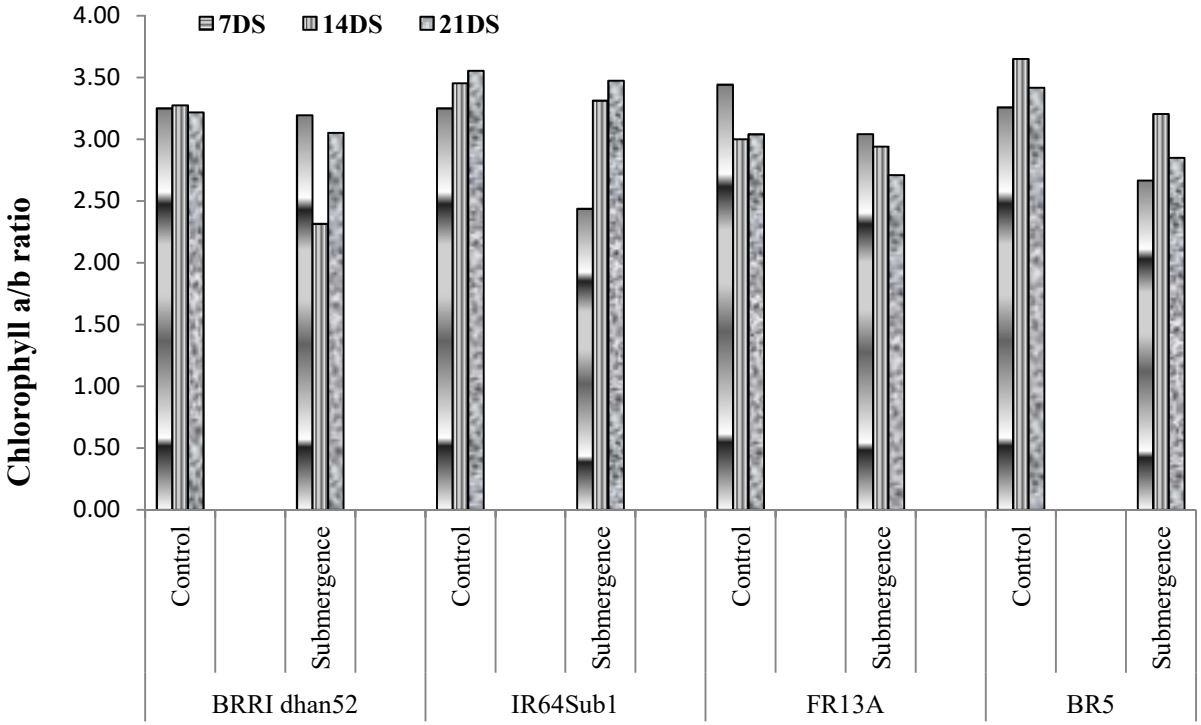


Figure 28. Chlorophyll a/b ratio of different rice genotypes after desubmergence of submerged plants and control plants. DS= days of submergence.

4.3.5. Percent reduction of total chlorophyll content

In the present experiment, percent reduction of total chlorophyll content was measured after desubmergence (Figure 29). After 7 DS treatment, the reduction of total chlorophyll recorded was the lowest (8%) in BRRI dhan52 and the highest (37.17%) in BR5. After 14 DS treatment, the reduction of total chlorophyll recorded was the highest (54.09%) in BR5 and the lowest (36.38%) in FR13A. After 21 DS treatment, the reduction of total chlorophyll recorded was the lowest (54.08%) in FR13A and the highest (81%) in BR5. Higher depletion in total chlorophyll content due to submergence indicated the susceptibility of those genotypes to submergence as in BR5. In BR5, the percent reduction of total chlorophyll was much higher compared to other genotypes in every submergence treatments. But in case of FR13A, the percent reduction of total chlorophyll was comparatively lower compared to other genotypes in every submergence treatment (7, 14 and 21 DS) indicated that FR13A might be tolerant to comparatively longer period of submergence. Lower under water reduction of chlorophyll might help these genotypes in early recovery and to give better yield. Sarkar *et al*. (1996) stated that due to submergence for 9 days, the maximum reduction of chlorophyll was observed in susceptible cultivars (45-66 %) as compared with tolerant varieties (10-17 %). Debabrata and Kumar (2011) found that submergence resulted in significant reduction of chlorophyll content both in Swarna and Swarna *Sub1*. After 7days of submergence the %reduction in chlorophyll content was greater in Swarna (76%) than Swarna *Sub1* (56%) compared to the respective control plant.

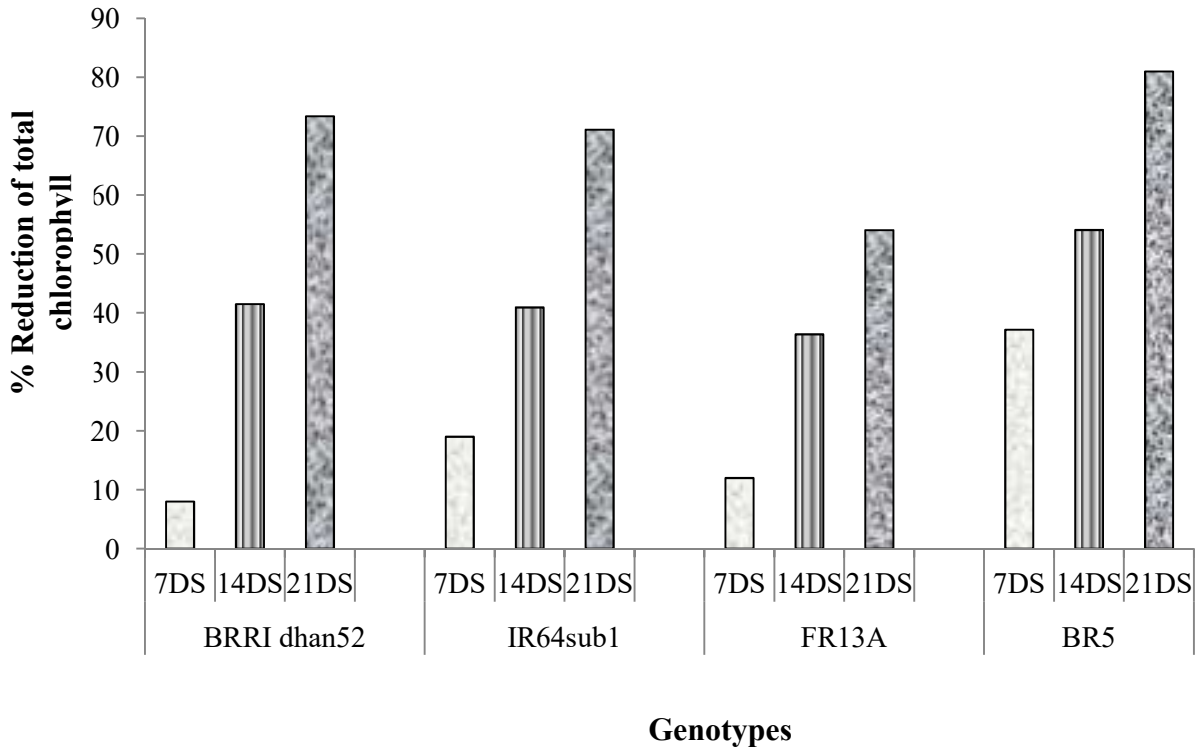


Figure 29. Percent reduction of total chlorophyll of different rice genotypes after desubmergence. DS= days of submergence.

4.3.6. Chlorophyll content at anthesis

The chlorophyll a, chlorophyll b and total chlorophyll content were also determined at anthesis from the flag leaf of all treated plants and was differed significantly by the interaction effect of different submergence duration and rice genotypes (Table 24). The chlorophyll a content of flag leaf recorded was the highest in control plants and was gradually decreased with the increasing submergence durations in all the genotypes. Considering all the genotypes and submergence treatments, the highest chlorophyll a content recorded was in IR64Sub1 at control plants which was significantly higher (3.08 mg g^{-1}) than any other treatment and the lowest (1.75 mg g^{-1}) chlorophyll a content recorded was in BRRI dhan52 at 21 DS treated plants which was statistically similar to 7 DS, 14 DS and 21 DS treatment of FR13A and with14 DS treatment of BR5.

The chlorophyll b content was gradually decreased with the increasing submergence durations in all the genotypes. Considering all the genotypes and submergence treatments, the highest (1.02 mg g^{-1}) chlorophyll b content recorded was in IR64Sub1at control plant which was significantly higher than any other treatment and the lowest (0.52 mg g^{-1}) chlorophyll b content recorded was at 14 DS treatment of BR5 which was statistically similar to 21 DS treatment of BRRI dhan52 and with all the submergence treatments of FR13A and with 21 DS treatment of IR64Sub1.

In all the genotypes, control treatment showed significantly higher total chlorophyll which was gradually decreased with the increasing submergence duration. Considering all the genotypes and submergence treatments, the total chlorophyll content found was also the highest (6.11 mg g^{-1}) in IR64Sub1 at control plant which was significantly higher than any other treatment and the lowest (3.49 mg g^{-1}) in FR13A at 21 DS treated plants which was statistically similar to 14 DS treatment of the same genotype, with 21 DS treatment of BRRI dhan52 and with 14 DS treatment of BR5. It recorded was that after desubmergence the chlorophyll (a, b and total) content was reduced due to submergence shown in previous figures. After anthesis period, when the plant almost recovered their stress injury, it found was that chlorophyll content was also lower in submergence treated plants compared to control plants in all the genotypes.

Lower chlorophyll content at submerged plant was due to underwater degradation of chlorophyll and all the treated plants were unable to attain the 100% recovery of chlorophyll or same as the

control up to maturity period. Considering all the genotypes and submergence treatments, the percent reduction of total chlorophyll recorded was the lowest in BRRI dhan52 and FR13A which was followed by IR64Sub1. The chlorophyll content after anthesis indicated the recovery status of those genotypes. Lower the percent reduction of total chlorophyll indicated, the higher was recovery status as in BRRI dhan52, FR13A and IR64Sub1. Singh *et al*. (2014) found that chlorophyll concentration in leaves decreased under submergence and with the increasing submergence duration from 12 to 17 days. They also observed that the sensitive and tolerant genotypes had similarly high leaf chlorophyll concentrations before submergence but, when submerged, the tolerant genotypes maintained more chlorophyll than the intolerant genotypes. Ella and Ismail (2006) stated that the relative concentration of both the chlorophyll a and b, measured 3 days after submergence, decreased significantly, with a greater decrease in chlorophyll a than in chlorophyll b in both cultivars.

Table 24. Effect of different submergence treatments on chlorophyll a, chlorophyll b and total chlorophyll content during anthesis stage of different rice genotypes

Genotypes	Days of submergence (DS)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	
				Actual	Relative (%)
BRRI dhan52	0	2.66 b	0.88 b	5.28 b	100
	7	2.62 b	0.87 b	5.19 b	98
	14	2.27 d	0.75 c	4.51 d	85
	21	1.75 g	0.54 f	3.51 g	66
IR64Sub1	0	3.08 a	1.02 a	6.11 a	100
	7	2.70 b	0.88 b	5.33 b	87
	14	2.50 c	0.84 b	4.96 c	81
	21	1.93 ef	0.60 def	3.80 e	62
FR13A	0	1.97 e	0.64 cd	3.91 e	100
	7	1.86 efg	0.57 def	3.67 f	94
	14	1.81 fg	0.55 ef	3.56 fg	91
	21	1.78 g	0.53 f	3.49 g	89
BR5	0	2.30 d	0.69 c	4.52 d	100
	7	1.96 e	0.62 de	3.86 e	86
	14	1.79 g	0.52 f	3.50 g	77
	21	0	0	0	0
$LSD_{(0.05)}$		0.13	0.06	0.13	
CV (%)		4.67	6.92	2.27	

In a column, values followed by same letter(s) are not significantly different from each other by LSD at 5% level of significance.

4.3.7. Soluble sugar content (mg g^{-1}sample)

The soluble sugar content of leaf of different rice genotypes was estimated after 7, 14 and 21 days of submergence both from control and submerged plants and the results were expressed as mg g^{-1} shoot dry weight which have been presented in the figure 30. The control treatment showed significantly high sugar content than submerged plants in all the genotypes. But in FR13A, the soluble sugar content was relatively (relative to control) higher in submergence treatment compared to other genotypes, which was followed by BRRI dhan52. Due to 7 DS treatment, the control plants showed significantly higher sugar content than submerged plants in all the genotypes. Among the genotypes, the soluble sugar content found was the highest (26.55 mg g^{-1}) in FR13A at control plants and the lowest (9.96 mg g^{-1}) soluble sugar content recorded was in BR5 at submerged plants. But FR13A showed higher (84%) relative soluble sugar content than any other genotype.

Due to 14 DS treatment, the soluble sugar content found was also significantly higher in control plants than the submerged plants. Among the genotypes, the highest soluble sugar content recorded was in FR13A (29.04 mg g^{-1}) at control plants which was statistically similar to control plants of BRRI dhan52 and the lowest (8.2 mg g^{-1}) soluble sugar content recorded was in BR5 at submerged plants. FR13A also showed higher (70%) relative soluble sugar content than any other genotype.

Due to 21 DS treatment, the control plants also showed higher soluble sugar content than submerged plants in all the genotypes. Among the genotypes, the highest (32.22 mg g^{-1}) soluble sugar content recorded was also in FR13A at control plant which was significantly higher than any other genotypes at this treatment and the lowest (8.42 mg g^{-1}) soluble sugar content recorded was in BR5 at submerged plant which was significantly lower than any other genotype at this treatment. Here FR13A also showed higher (59%) relative soluble sugar content than any other genotype

Higher degradation of chlorophyll, higher injury and lower light under submergence might be responsible for lower soluble sugar content. In every treatment, the relative soluble sugar content was better in FR13A which was followed by IR64Sub1 and BRRI

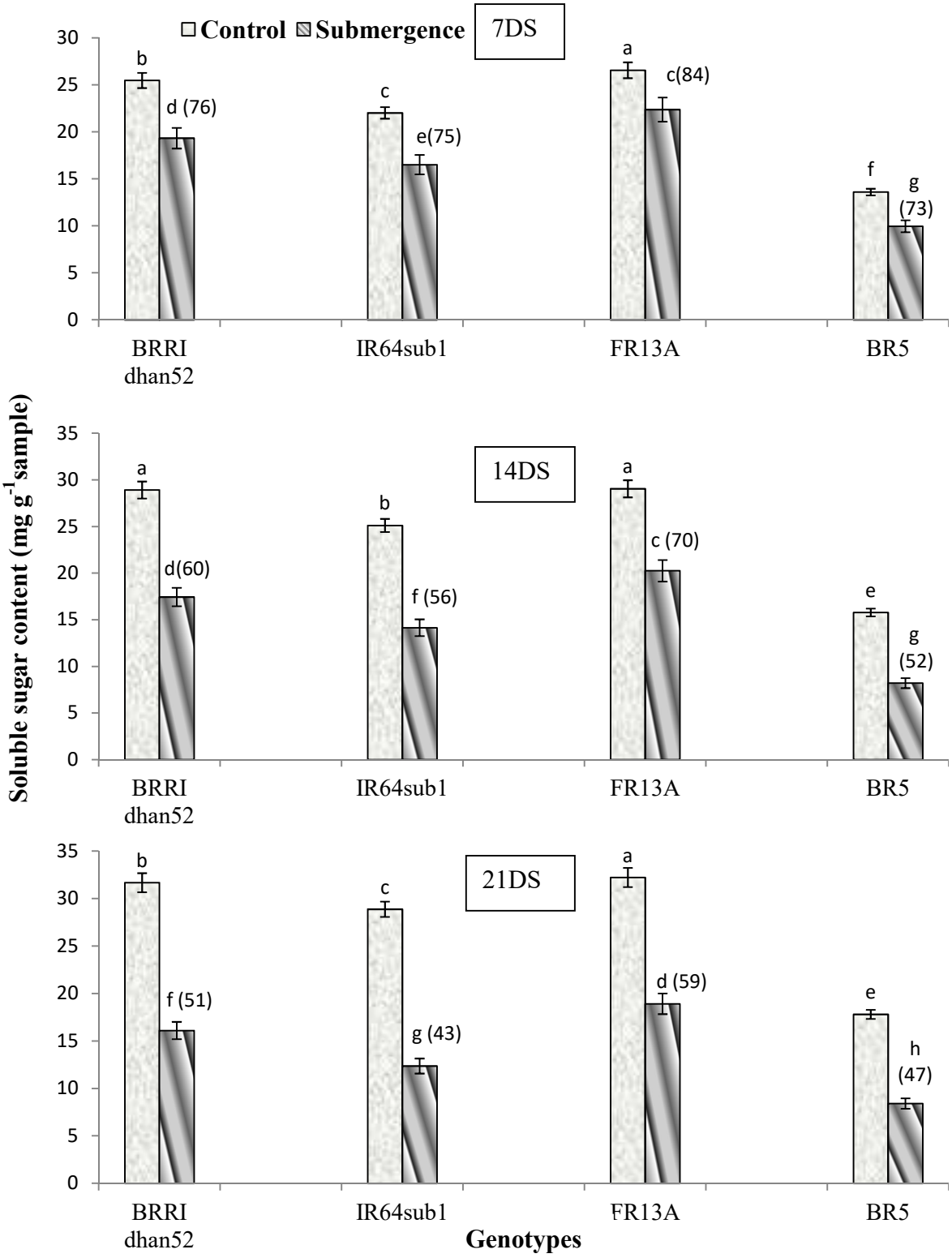


Figure 30. Soluble sugar content of leaf of different rice genotypes under control and various submergence treatments. Bar contains standard deviation, figures inside the parenthesis indicate relative to control. DS= days of submergence.

dhan52. The BBRI dhan52 and FR13A genotypes, those are able to maintain higher non-structural carbohydrate (NSC) during submergence, develop new leaves more quickly and accumulate greater biomass after desubmergnce (Singh *et al*., 2014 ; Panda *et al*., 2008b; Sarkar and Bhattacharjee, 2011). In an experiment, Pervin *et al*. (2010) estimated the soluble sugar content in four varieties of rice separately at different ages of seedling. They found that the sugar level of plant tissue was generally higher before submergence which decreased markedly due to submergence.

4.3.8. Starch content

The starch content was also measured at the same time of soluble sugar estimation. All the treated plants of every genotype showed lower starch content than control plants (Figure 31). Due to 7 DS treatment, the starch content was significantly higher in control plants than the submerged plants in all the genotypes. The relative starch content recorded was the highest (91%) in FR13A which was followed by BRRI dhan52 (86%). The lowest (66%) relative starch content recorded was in IR64Sub1 which was followed by BR5 (70%). Among the genotypes, the highest (39.78 mg g^{-1}) starch content recorded was in FR13A at control plant which was significantly higher than any other genotype and the lowest (22.12 mg g^{-1}) starch content recorded was in BR5 at submerged plant which was statistically similar to submerged treatment of IR64Sub1.

Due to 14 DS treatment, the starch content was significantly lower in submerged plants compared to control plants in all the genotypes. The highest (45.67 mg g^{-1}) starch content recorded was in FR13A at control plant and the lowest (11.07 mg g^{-1}) starch content recorded was in BR5 at submerged plant. The relative starch content recorded was the highest (77%) in FR13A which was followed by BRRI dhan52 (68%). The lowest (30%) relative starch content recorded was in BR5.

A drastic reduction of starch content was also observed at 21 DS treated plants compared to control plants in all the genotypes. The relative starch content recorded was the highest (58%) in FR13A which was followed by BRRI dhan52 (47%) then IR64Sub1 (45%). The lowest (11%) relative starch content recorded was in BR5. Among the genotypes, the highest (51.69 mg g^{-1}) starch content recorded was in FR13A at control plant which was significantly higher than any

other genotype and the lowest (4.48 mg g^{-1}) starch content recorded was in BR5 at submerged plant which was significantly lower than any other genotype.

Higher degradation of chlorophyll, higher injury and lower light under submergence might be responsible for lower starch content. In every treatment the relative starch content was better in FR13A which was followed by IR64Sub1 and BRRI dhan52. In BR5, the soluble sugar content, due to submergence, found was to be less affected but starch content was more affected in the same condition which indicated that under submergence the increased soluble sugar was supplied by the breakdown of stored starch. This stored carbohydrate gives mechanical rigidity of the culm and leaf sheath which contribute to the stiffness of the culm (Banerjee *et al*., 2015b). But in BR5, due to lack of this stored carbohydrate, the genotype became lodged after desubmergence. The starch content of submerged plant in FR13A was comparatively higher than the other genotypes which were followed by BRRI dhan52. Carbohydrate status in culm is most important criteria for submergence tolerance. Under submergence condition, due to limitation of gas diffusion and reduced light by shading, the production of carbohydrate is hampered. So, the genotype that maintained higher carbohydrate before submergence or less depletion of carbohydrate during submergence are able to maintain residual starch concentrations above the minimum threshold, they develop new leaves more quickly and accumulated greater biomass during recovery (Singh *et al*., 2014). Thus, a rice variety, maintaining higher stored carbohydrates as well as carbohydrate constituents in the stems and with greater ability to conserve it during submergence, is supposedly proved more stress tolerant, even under oxidative exposure also (Nagai *et al*., 2010). The level of carbohydrate remaining after submergence is more critical for survival. Non-structural carbohydrate (total soluble sugar and starch) concentrations in shoot have long been associated with genotypic differences in ability to tolerate submergence in rice (Das *et al*., 2009). These non-structural carbohydrates are utilized during submergence to supply energy for growth and maintenance metabolism (Sarker *et al*., 1996). Singh *et al*. (2014) found that the reduction in total soluble sugar and starch under submergence was about 60-70% in tolerant genotypes and about 80-90% in the sensitive ones but there was no

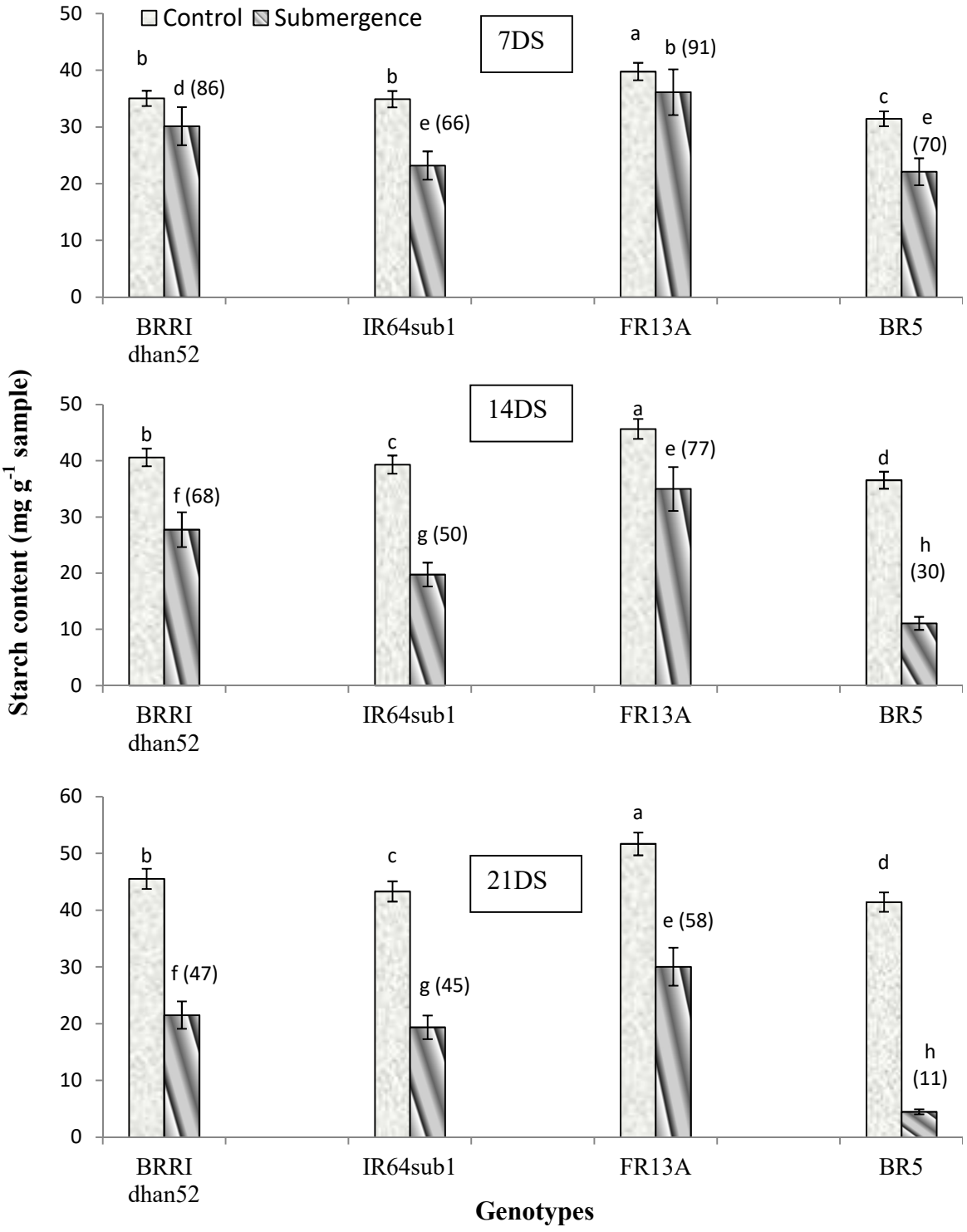


Figure 31. Starch content of leaf of different rice genotypes under control and various submergence treatments. Bar contains standard deviation. Figures inside the parenthesis indicate relative to control. values followed by same letter(s) are not significantly different from each other by LSD at 5% level. DS= days of submergence.

specific trend in differences between tolerant and sensitive genotypes in their stem sugar and starch concentrations before submergence. Under low O_2 condition, the activity of α-amylase and sucrose synthase is increased which are involved in starch and sucrose metabolism (Nishiuchi *et al*., 2012).

4.3.9. Percent depletion of non structural carbohydrate (NSC)

In the present experiment, the total amount of soluble sugar and starch was considered as non structural carbohydrate content. The total amount of non structural carbohydrate decreased due to submergence. Percent depletion of non structural carbohydrate in rice seedlings due to submergence were measured after desubmergence from all the control and submerged (7, 14 and 21 days of submergence) plants. After desubmergence, the 7 DS treated plants showed the lowest (7.60%, 20.43%, 5.38% and 15.31% in BRRI dhan52, IR64Sub1, FR13A and in BR5 respectively) percent depletion of non structural carbohydrate of all the genotypes and then the depletion was gradually increased with the increasing submergence durations (Figure 32). In every genotype, the highest depletion (30.29%, 36.42%, 20.83% and 65.95% in BRRI dhan52, IR64Sub1, FR13A and in BR5 respectively) was recorded in 21 DS treated plants. Among the genotypes, the percent depletion of non structural carbohydrate due to submergence was much higher in BR5 compared to other genotypes. The lowest depletion due to submergence recorded was in FR13A which was followed by BRRI dhan52.

The depletion of total non structural carbohydrate might be due to higher injury and lower gas diffusion under submergence. This lowest depletion of total non structural carbohydrate in FR13A and BRRI dhan52 recorded was in all submergence treatments; and was suppose to be considered as tolerant character. The level of depletion of carbohydrates was lower in tolerant cultivars compared to the susceptible cultivar. The rate of depletion of stored carbohydrate very rapidly for susceptible varieties makes the plants feeble to stress in subsequent duration (Banerjee *et al*., 2015b). Submergence tolerance is related to high carbohydrate supply during submergence through energy supply which is needed for maintenance process under submerged condition. So, factor affecting carbohydrate supply and depletion during submergence may also influence submergence tolerance. The cultivars that maintained a higher carbohydrate content at the end of submergence were found to develop new leaves very quickly and accumulated greater

biomass during reemergence (Sarker and Bhattacharjee, 2011). Banerjee *et al*. (2015b) also found the variation of total carbohydrate depletion in the plants under submergence as compared to control.

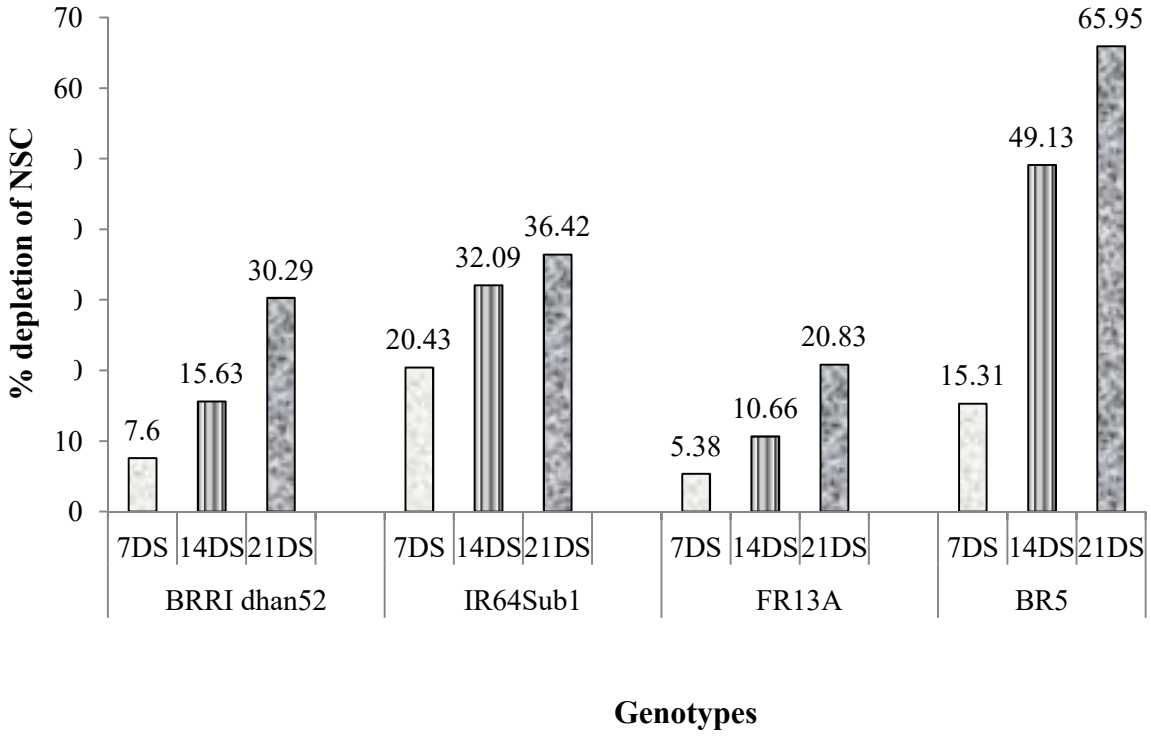


Figure 32. Percent depletion of non structural carbohydrate (soluble sugar and starch) due to submergence in different rice genotypes. DS= days of submergence.

4.3.10. Relationship between non-structural carbohydrate and survival percentage

There was a positive correlation between non-structural carbohydrate (NSC) content and survival percentage (Figure 33). The lowest survival percentage was observed when NSC content was also lowest. Then survival percentage gradually increased with the increasing NSC content. Under control condition the relationship was very poor ($r= 0.143^{NS}$) between survival percentage and non-structural carbohydrate content and the relationship was significant ($r= 0.852^{**}$) under submerged condition. The positive relationship between amount of carbohydrate in plant parts and submergence tolerance was also reported by many researchers (Panda *et al.* 2008a, Chaturvedi *et al*. 1993).

The carbohydrates remaining after submergence is more important than that of before submergence. Total nonstructural carbohydrate (sugar & starch) contents after submergence showed highly significant positive association with survival percentage. The maintenance of greater quantities of NSC at the end of submergence depended on the level of NSC contents before submergence and their low consumptive use during submergence. The carbohydrate content of plants found was to be significantly and positively associated with re-generation growth. This statement was also supported by Singh *et al*. (2014) ; Sarkar and Bhattacharjee (2011) and Panda *et al*. (2008a).

4.3.11. Decreased in cell number and aerenchyma formation

The cell number of leaf tissue (leaf sheath and leaf blade) was observed from control and 21DS treatment plant after desubmergence. It is clear from the following photographs that cell number was decreased and aerenchyma formation was accelerated due to submergence compared to control in all the genotypes. There were genotypic variations also. Under control condition, the genotype BRRI dhan52 showed seven layers of parenchymatous cell and under submergence (21days) condition this genotype showed three layers of parenchymatous cell in leaf sheath. In IR64Sub1, the control treatment produced five layers and the submergence treatment produced only three layers of parenchymatous cell. In case of FR13A, control treatment produced seven layers and the

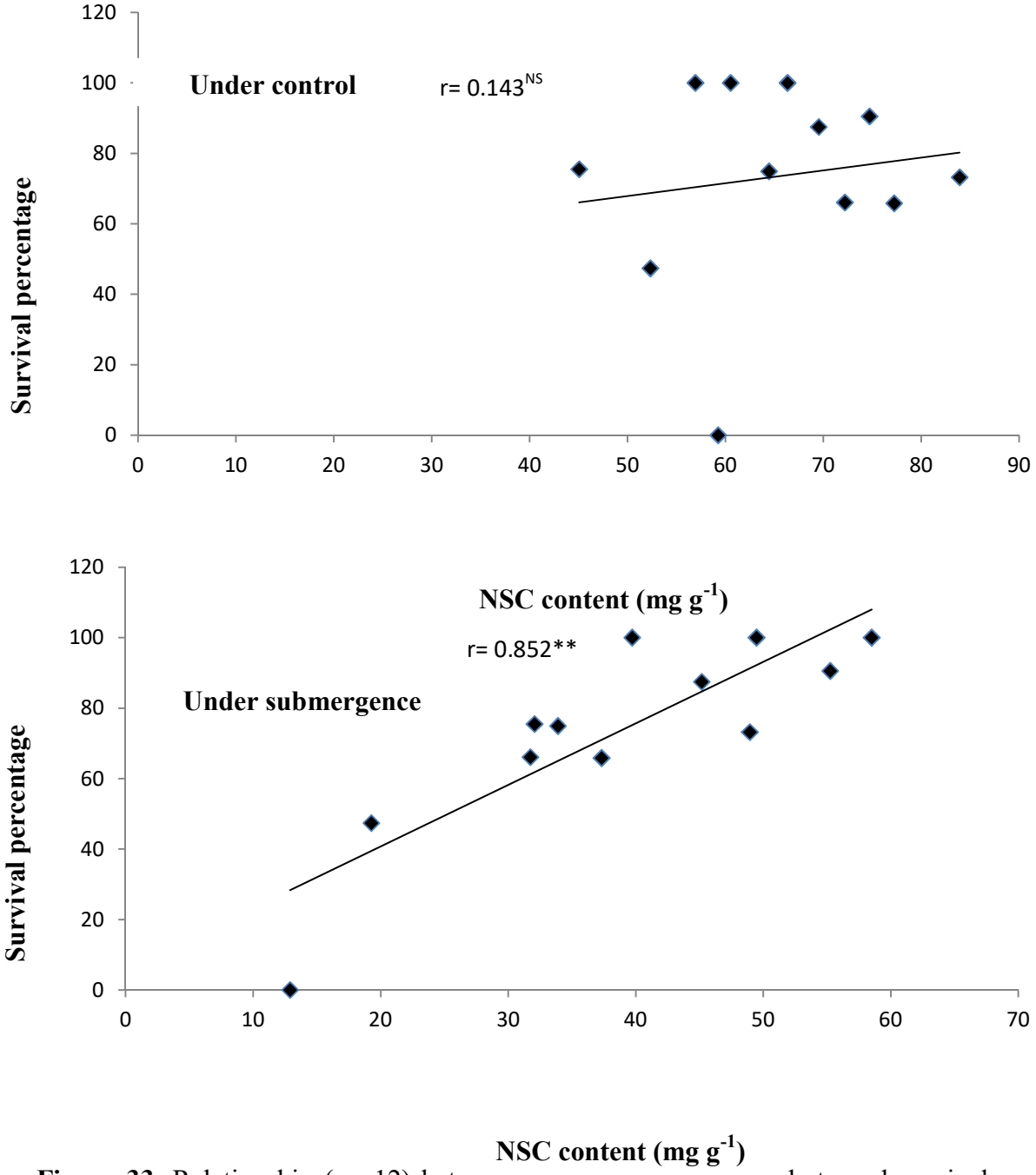


Figure 33. Relationship (n= 12) between non-structural carbohydrate and survival percentage under control and submergence condition.

submergence treatment produced four layers of parenchymatous cell. five layers of parenchymatous cell was recorded in the leaf sheath of BR5 in the control treatment but the parenchymatous cell layers in leaf sheath appeared disintegrated and almost dead under 21DS

treatment. So it is clear from the following photographs that parenchymatous cell layers as well as cell numbers of leaf sheath decreased due to 21DS treatment compared to control. The leaf blade also became thinner due to submergence in all the genotypes.

Submerged condition promotes biosynthesis and accumulation of ethylene. Under submerged condition ethylene accelerated the ROS (reactive oxygen species) accumulation in the cortex of root and shoot, which activates the subsequent process of programmed cell death and lysis of cortical cells, finally lysigenous aerenchyma is formed in rice plant (Nishiuchi et al.,2012). Aerenchyma is very important in submerged conditions, allowing the plant to access the alternative O_2 sources. During photosynthesis oxygen is produced and stored in underwater organs or retained by the leaf surface gas film (Colmer and Pedersen, 2008a; Pedersen et al., 2009). This aerenchyma might help a photosynthetic benefit by concentrating CO_2 from root respiration and surrounding environment. Larger aerenchyma formation (in order to O_2 storage and O_2 transportation from shoot to root during submergence) and stronger arrangement of the parenchyma cell in FR13A gave better adaptation of this genotype under submergence. The genotypes BRRI dhan52 and IR64Sub1 also showed better anatomical features under submergence compared to BR5.

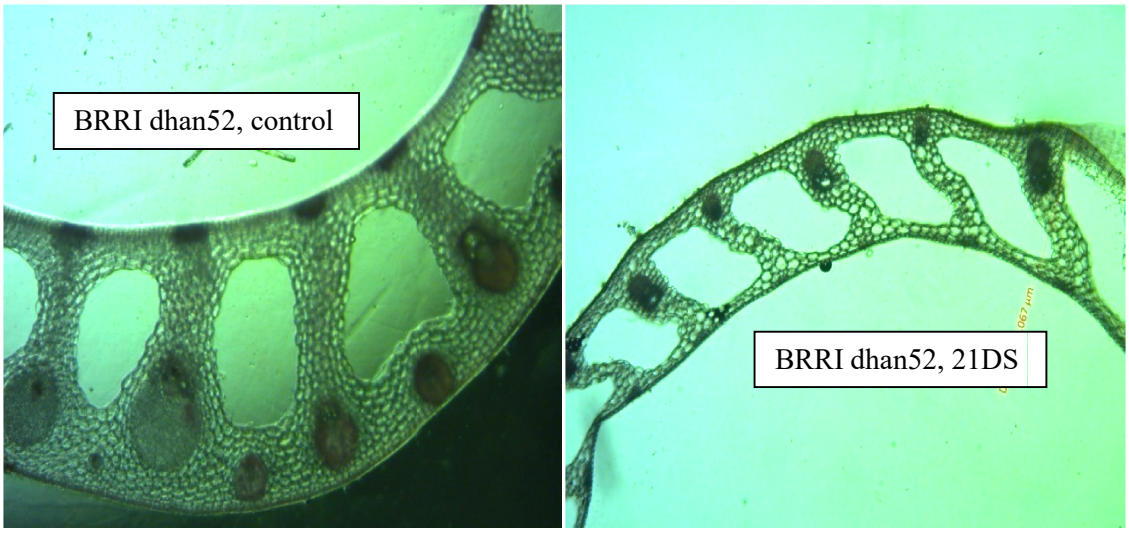


Figure 34. Leaf sheath (×10) of BRRI dhan52 under 21 DS treatment from treated and control plants.

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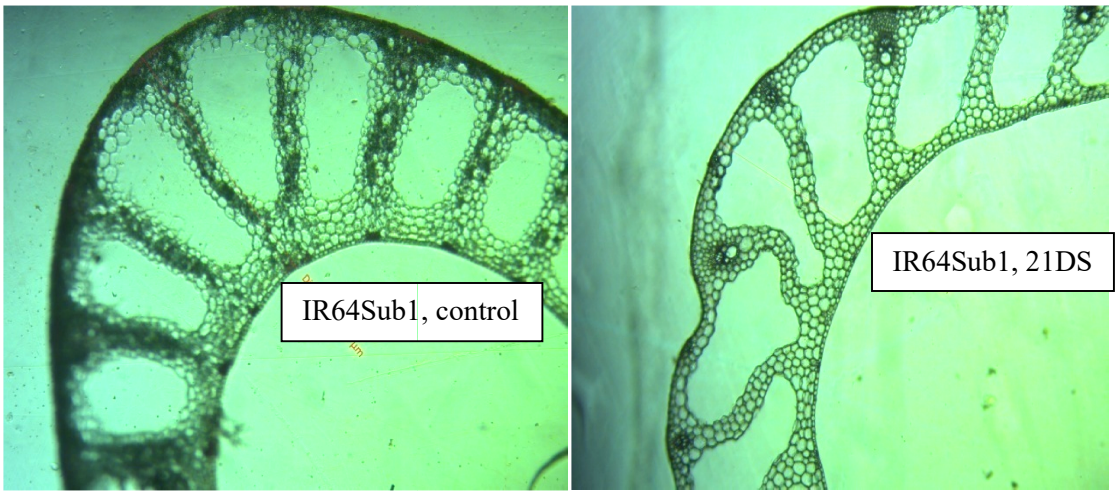

Figure 35. Leaf sheath (×10) of IR64Sub1 under 21 DS treatment from treated and control plants.

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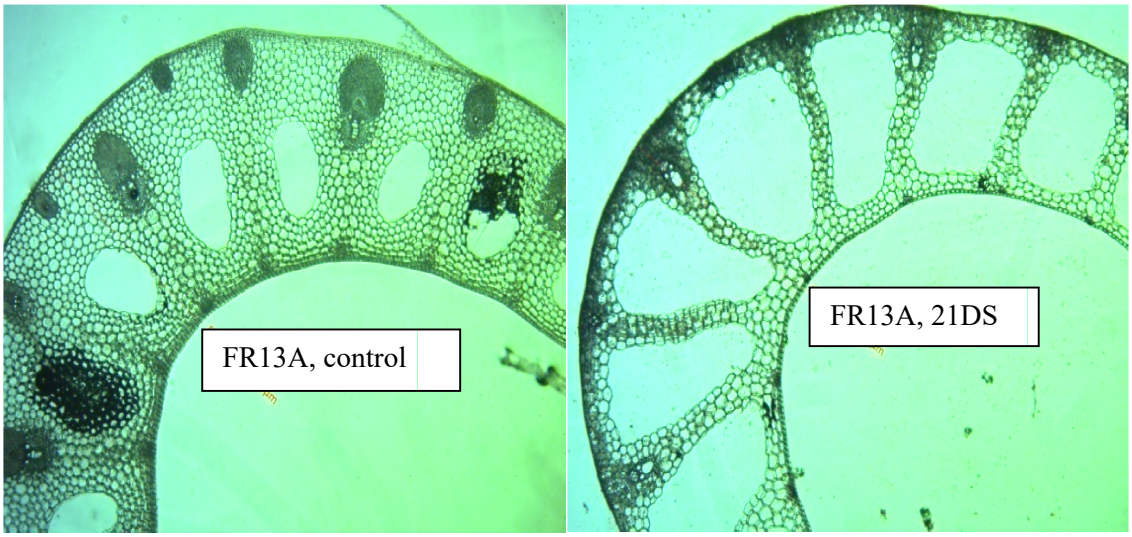

Figure 36. Leaf sheath (×10) of FR13A under 21 DS treatment from treated and control plants.

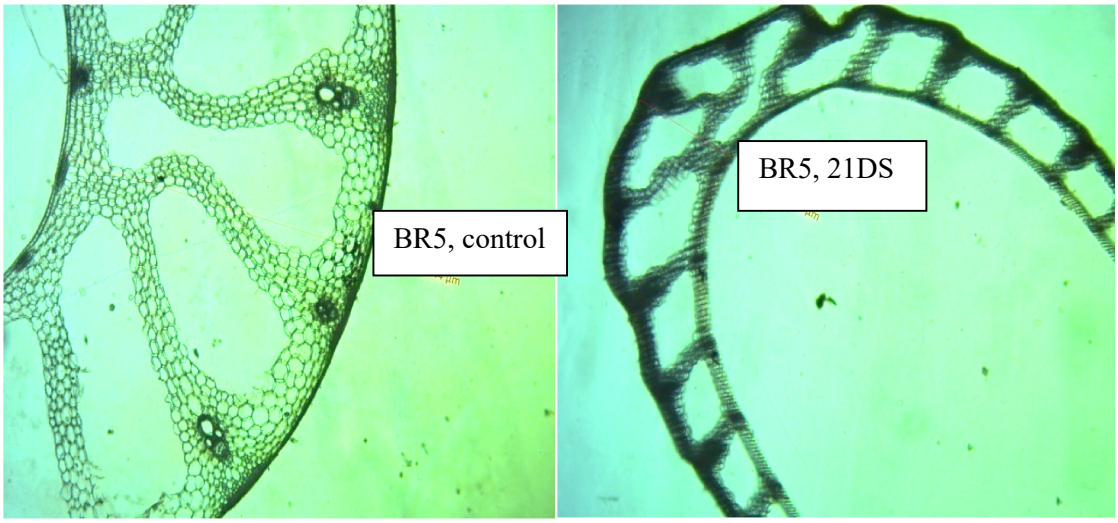

Figure 37. Leaf sheath (×10) of BR5 under 21 DS treatment from treated and control plants.

Figure 38. Leaf blade (×10) of BRRI dhan52 under 21 DS treatment from treated and control plants.

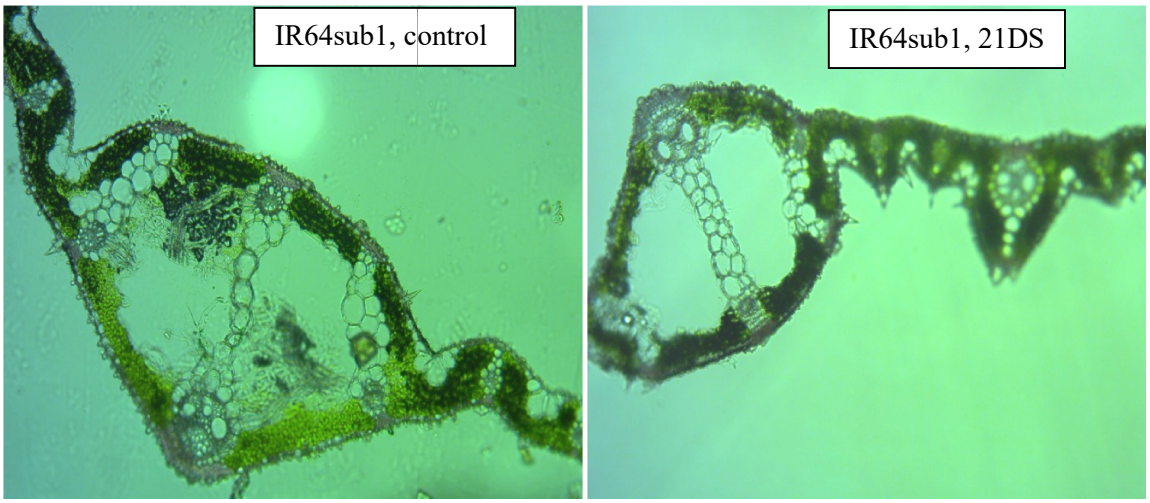

Figure 39. Leaf blade (×10) of IR64sub1 under 21 DS treatment from treated and control plants.

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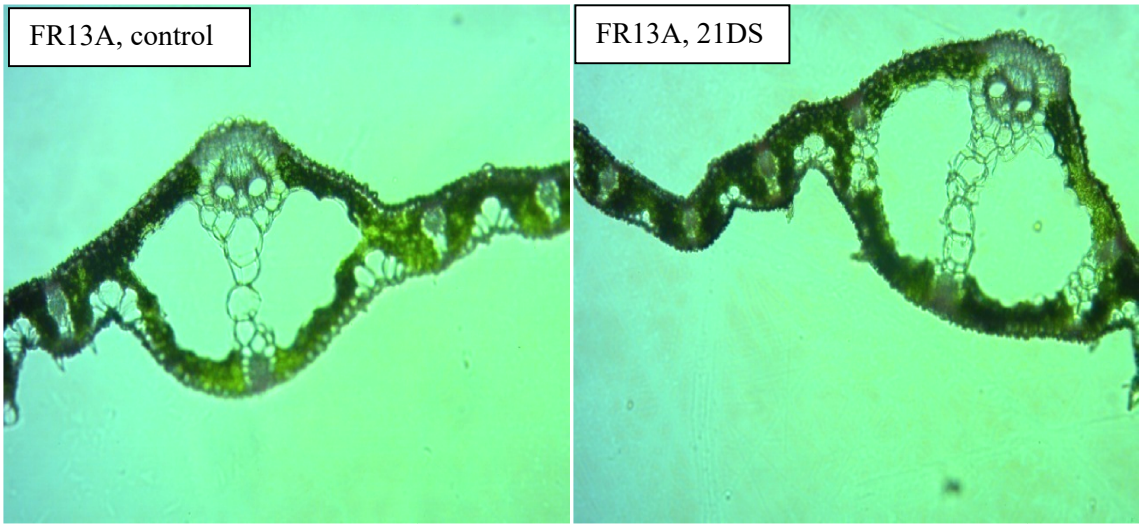

Figure 40. Leaf blade (×10) of FR13A under 21 DS treatment from treated and control plants.

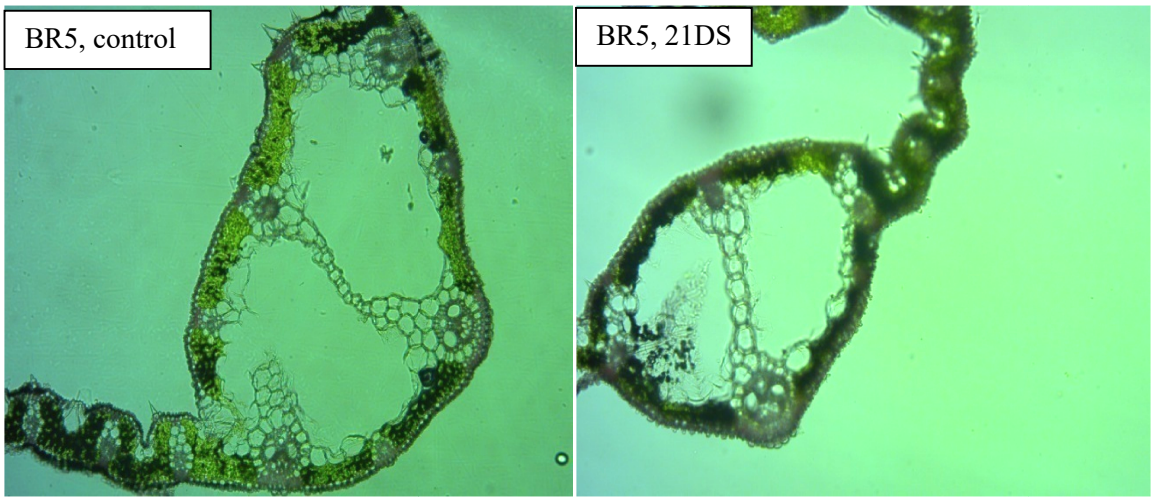

Figure 41. Leaf blade (×10) of BR5 under 21 DS treatment from treated and control plants.

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In the third experiment, different genotypes showed various anatomical and physiological responses due to submergence treatment. Larger aerenchyma formation and stronger arrangement of parenchyma cell in FR13A gave better adaptation of this genotype under submergence. Lower membrane leakage, higher proline content, higher stem carbohydrate and lower chlorophyll depletion play important roles in submergence tolerance.