School of Agriculture, Policy and Development

Thesis

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Seed priming approaches to increase salt tolerance of rice (*Oryza sativa* L.) accessions from Mozambique and improve their yield and grain nutritional quality

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Declaration:

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

In Mozambique, rice is one of the staple crops and is among the main sources of carbohydrates for most of the population. Most rice production occurs along the coastal areas. Rice crop is sensitive to salt stress and sea salt intrusion severely limits growth, development and yield of rice in Mozambique. However, scientific evidence on the specific effects of salinity on rice varieties cultivated in Mozambique and the mechanisms that could increase salt tolerance are still scarce. To fill this gap, laboratory experiments in hydroponic system in a growth cabinet were conducted to investigate rice salt tolerance, the effect of priming treatments with inorganic salts ($CaCl_2$, KCl, and KNO_3) in mitigating salt stress, and the physiological mechanisms that confer higher salt tolerance. The following parameters were assessed in twelve rice accessions: plant growth (shoot dry weight, percent germination, root and shoot lengths), plant physiology (Na-ions and K-ions), grain yield and grain composition (starch, amylose, protein concentrations). Indica Mozambique (landrace) rice accessions are moderately tolerant and the indica improved (IRRI Lines) are tolerant to salt stress. $CaCl_2$ and KNO_3 priming treatments showed potential to alleviate salt stress effects on Indica Mozambique (landrace) by increasing shoot dry weight and salt tolerance, altering Na-ions and K^+/Na^+ ratios, and increasing grain protein concentrations. However, these changes were not translated in the increase of grain yield, thus there is a need for further studies to identify the ideal priming inorganic salt concentration and duration suitable for each rice accession, stage of crop growth, level and duration of salinity stress, including the evaluation of more physiological parameters (e.g. Ca-ions, osmolytes, antioxidants, hydrolases). Thereafter, priming approaches with positive significant effects may be adopted by the smallholder farmers to increase rice grain yield and quality, hence contributing to food and nutritional security in Mozambique.

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To my parents, **Evaristo Mondlane** and **Vitória Mathe**, for all sacrifice they have done to educate me.

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*"You must find the place inside yourself where nothing is impossible."(By **Deepak Chopra)***

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mM NaCl – **G1,** and Gaza KNO_3 60 mM NaCl – **G2**). Across different lanes bands were selected (framed) and quantified Lane 2 and 3 correspond to water extracts (Albumins - **A**); lane 4 and 5 correspond to salt extracts (Albumins/Globulins - **A/G**), lane 6 and 7 correspond to water extracts (Water - **W**), lane 8 and 9 correspond to alcohol extracts (Cysteine Poor Prolamins - **CPP**), lane 10 and 11 correspond to alcohol extracts (Cysteine Rich Prolamins - **CRP**), lane 12 and 13 correspond to SDS-extracted residue (Glutelins - **Gt**) all from **G1** and **G2**, respectively. **B) & C)** indicating the selection and quantification of SDS extracted protein in the lanes 12 and 13 in **A)**. 131

Figure 5.6: Densitometry graphic from sequential protein extract of the rice accession IRGC 7546 Gaza under two treatments of salinity (0mM NaCl and 60mM NaCl) and KNO_3 priming treatment, indicating the relative band density of storage protein (Albumins, Albumins/Globulins (A/G), Prolamins, and Glutelins). Data are shown as mean value +/- SEM of two individual replications (n=2). The bars with * are significantly different (p- value < 0.05). The error bars represent SEM. 133

CHAPTER 1. Literature Review

1.1 Rice (Oryza Sativa L.): Global Perspective, Taxonomy, Production

Rice belongs to the genus Oryza in the family Poaceae (or Gramineae) (Linares, 2002, Khush, 2005, Vaughan et al., 2008), and there are currently more than twenty identified Oryza species. However, only two are widely cultivated, namely: *Oryza sativa* L. and *Oryza glaberrima* Steud. *O sativa* is the most widespread and commonly cultivated rice in Asia, whereas *O glaberrima* is an African rice, mainly grown in African countries (West Africa) (Linares, 2002, Vaughan et al., 2008). *O sativa* is subdivided in two main subspecies: Indica (including glutinous, non-glutinous, aromatic basmati and aromatic jasmine types) and Japonica rice types (Awan et al., 2017). Based on the grain colour, rice may be categorized as white, black, red and green, the first type being the most commonly grown (Graham et al., 1999).

Rice is one of the most cultivated cereal crops worldwide, being grown in different ecologies, which vary from dry upland ecosystems to flooded rainfed low-land and irrigated soils and spanning latitudes from 50° of Northern China to 35° in South Australia and Argentina (Ismail and Horie, 2017). The subspecies *O sativa* Indica rice is grown in subtropics and tropics of the world (particularly tropical Asia), in submerged and lowland conditions; whereas, subspecies *O sativa* Japonica rice is cultivated at high elevation (South Asia), dry fields (temperate East Asia), upland areas (Southeast Asia) and in temperate and cooler zones of the subtropics (Awan et al., 2017). Overall, rice is grown in 114 of the 193 total global countries and covering the six continents of Asia, Africa, Australia, Europa, Latin America, and North America (Virmani and Ilyas-Ahmed, 2007, Hoang et al., 2016). Currently, rice is grown in a global area of about 162,427 million hectares. The total annual production is

about 501,201 million metric tons (MMT) of milled rice, from which 497,694 MMT (about 99%) is for domestic consumption (INDEXMUNDI, 2021a). More than 90 % of global rice production and consumption is accounted in Asia (Khush, 2005, Virmani and Ilyas-Ahmed, 2007). China and India have the highest annual production of 147 MMT and 120 MMT, respectively. Major producers outside of Asia are Brazil, followed by United States of America (**Figure 1.1**). In Africa, Nigeria (West Africa), with 5,040 MMT of annual production, has the highest annual rice production, followed by Egypt (North Africa), Madagascar (East Africa), Mali (West Africa), and Tanzania (East Africa) (**Figure 1.1**).

Among the major rice producing countries China and Japan have the highest yields, at 7 metric tons per hectare (MT/ha), while Nigeria has the lowest at 2 MT/ha. (INDEXMUNDI, 2021a). Therefore, the lowest yields are observed in Africa.

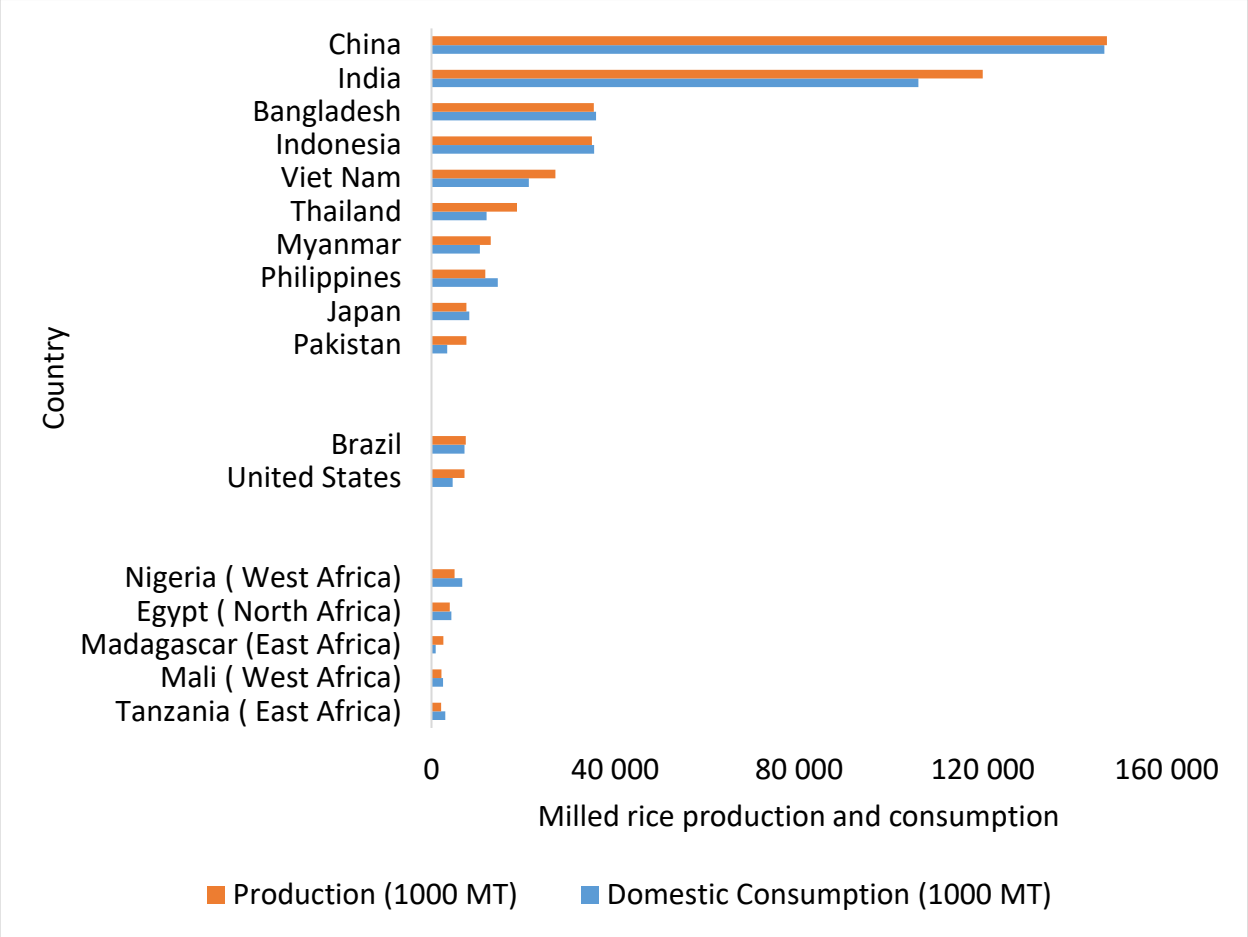


Figure 1.1: The major rice producing countries worldwide. Annual production and domestic consumption (in MMT – million metric tons) in Asian countries, Brazil, United States of America and Africa (INDEXMUNDI, 2021a).

1.2 Rice Importance and Consumption

1.2.1 Rice Grain Composition

The rice grain (rough rice) consists of an edible portion (caryopsis) and its covering structure called hull (vestige of the bracts within which the caryopsis developed). Removal of the covering structures results in caryopsis or brown rice. Brown rice comprises the bran (pericarp, tegmen, aleurone layers, and embryo), polish (subaleurone with or without small part of starchy endosperm), and milled white or polished rice (Juliano, 1972, Champagne et al., 2004). The subaleurone has relatively smaller cells, higher protein bodies , lower starch

compared to the starchy endosperm (Champagne et al., 2004). Milled white or polished rice grain consists of starch, proteins, water, various minerals and vitamins (Chen et al., 2012, Calingacion et al., 2014). Starch is the main component, accounting for about 80% of the milled rice grain (**Table 1.1**) (Champagne et al., 2004), and it is formed by two types of glucose polymers, amylose and amylopectin. Amylose is a linear polymer of D-glucose and represents between 15-30% of rice starch, while amylopectin is a highly branched polymer of glucose and constitutes 70-85% of the starch in the rice grain (Umeda et al., 1991). Proteins are the second largest component of rice grain and represent 5-12% of the total grain weight (Villareal and Juliano, 1978, Chen et al., 2012, Calingacion et al., 2014). Based on their solubility, proteins are classified into four groups, namely: water soluble albumin, salt soluble globulin, alkaline soluble glutelin, and alcohol soluble prolamin (Chen et al., 2012). The latter two types are the most prevalent in rice grain. The alkaline soluble glutelin make up to 80%, whereas the alcohol soluble prolamin account for about 20% of the total rice grain proteins (Yamagata et al., 1982, Chen et al., 2012). Water typically composes 12% of the harvest-mature rice grain (Calingacion et al., 2014). Although in relatively smaller quantities, rice grain contains several minerals such as N, P, K, Ca, Mg, and trace elements such as Fe, Cu, Zn, Se and Mn, which are predominantly found in the bran layer (**Table 1.1**) (Champagne et al., 2004, Heinemann et al., 2005, Razzaq et al., 2020). Rice grain, particularly brown rice, contains vitamin B complex, namely: Thiamine (B1), Riboflavine (B2), Niacin (B3), Pantothenic acid (B5), and Vitamin B6 (**Table 1.1**) (Champagne et al., 2004, Chen et al., 2012, Ghosh et al., 2016).

Table 1.1: Organic fractions of rough, brown and milled rice grain and the respective bran.

Nutrient (% of organic fractions at 14% moisture)	Rice grain type			
	Rough (raw)	Brown (unpolished)	Milled (polished)	Bran
Protein (N*5.95)	5.8 - 7.7	4.3 - 18.2	4.5 - 10.5	11.3 - 14.9
Carbohydrates	64 - 73	73 - 87	77 - 89	34 - 62
Starch	53.4	66.4	77.6	13.8
Macroelements (mg/g at 14% moisture)				
Nitrogen	1.0 - 1.3	0.7 - 3.1	0.8 - 1.8	1.9 - 2.5
Calcium	0.1 - 0.8	0.1 - 0.5	0.1 - 0.3	0.3 - 1.2
Magnesium	0.6 - 1.5	0.2 - 1.5	0.2 - 0.5	5 - 13
Phosphorus	1.7 - 3.9	1.7 - 4.3	0.8 - 1.5	11 - 25
Potassium	1.5 - 3.7	0.6 - 2.8	0.7 - 1.3	10 - 20
Silicon	10.8	0.6 - 1.4	0.1 - 0.4	3 - 5
Sulphur	0.4 - 1.6	0.3 - 1.9	0.8	1.7
Microelements (mg/g at 14% moisture)				
Copper	2 - 11	1 - 6	2 - 3	9 - 34
Iron	14 - 60	2 - 52	2 - 28	86 - 430
Manganese	17 - 94	2 - 36	6 - 17	95 - 230
Sodium	53 - 810	17 - 340	5 - 86	71 - 335
Zinc	1.7 - 31	6 - 28	6 - 23	43 - 258
Vitamins (mg/g at 14% moisture)				
Thiamine (B1)	2.6 - 3.3	2.9 - 6.1	0.2 - 1.1	12 - 24
Riboflavin (B2)	0.6 - 1.1	0.4 - 1.4	0.2 - 0.6	1.8 - 4.3
Niacin (B3)	29 - 56	35 - 53	13 - 24	267 - 499
Pantothenic acid (B5)	7 - 12	9 - 15	3 - 7	20 - 61
Vitamin (B6)	4 - 7	5 - 9	0.4 - 1.2	9 - 28

Adapted from tables 1, 2 and 3; pages 88 and 89 (Champagne et al., 2004).

1.2.2 Rice Grain Quality

The quality of rice grain is determined based on storage, milling and marketing properties, physical appearance of the caryopsis, cooking and eating quality, and nutritional value; these in turn are determined by rice grain composition (Juliano et al., 1964b, Yang et al., 2007, Sun et al., 2011, Chen et al., 2012, Calingacion et al., 2014).

1.2.2.1 Milling and Marketing Properties

Milling quality is measured in terms of head rice recovery (HRR), which indicates the amount of whole grain obtained from the removal of bran and polish components with milling. It is expressed as the ratio of milled grain to rough rice (Cruz and Khush, 2000). Therefore, it determines the final yield, which may range from 25% to 65% (Khush et al., 1978, Chen et al., 2012). Starch, and to a lesser extent proteins, play a prominent role on rice grain quality (Muramatsu et al., 2006, Sun et al., 2011, Chen et al., 2012, Calingacion et al., 2014, Thitisaksakul et al., 2015). Rice grains with lower amylose content are characterized by having less densely packed starch granules, thus as being more susceptible to breakage during milling (Juliano, 1979, Cruz and Khush, 2000). Rice grains with a lower protein content also display a higher propensity to breakage during milling (Leesawatwong et al., 2004, Lee et al., 2009).

1.2.2.2 Physical Appearance of the Caryopsis

Physical appearance is related to the size, shape and visual attributes of milled rice grain. Regarding the visual attributes, rice grain may be chalky or translucent (Cruz and Khush, 2000). A chalky grain results from incomplete grain filling, caused by adverse environmental conditions, which lead to endosperm opacity (Chen et al., 2012). Rice grain endosperm may be opaque on the dorsal, ventral, or central side; resulting respectively in a white back, white belly or white centre (Cruz and Khush, 2000). Grain translucency is also influenced by the amylose content. In general, a translucent rice grain contains a greater amount of amylose than a chalky grain (Rani and Bhattacharya, 1989, Lisle et al., 2000, Singh et al., 2003).

Consumers have different preferences in the size and shape of rice grain, but translucent grain is the most preferred (Cruz and Khush, 2000).

1.2.2.3 Cooking and Eating Quality

Cooking and eating quality describe the easiness of cooking, firmness and stickiness of rice grain upon cooking (Chen et al., 2012). This is influenced by the ratio of amylose and amylopectin in the starch, which govern starch properties, and by the content of protein (Rao et al., 1952, Cruz and Khush, 2000, Chen et al., 2012). The properties of starch are gelatinization temperature, amylose content and gel consistency (Cruz and Khush, 2000, Chen et al., 2012) (**Table 1.2**). The gelatinization temperature determines the time required for 90% of the starch granules to dissolve irreversibly in hot water. Therefore, it defines the time required for cooking the rice grain (Cruz and Khush, 2000, Chen et al., 2012). The gelatinization temperature varies from 55 ^{o}C to 79 ^{o}C, hence it is subdivided into three categories which are: low (gelatinization temperature 55 ^{o}C to 69 ^{o}C), intermediate (gelatinization temperature 70 ^{o}C to 74 ^{o}C), and high (gelatinization temperatures higher than 74 ^{o}C) (**Table 1.2**). In laboratory experiments, the alkali spreading value is used to evaluate the gelatinization temperature of rice starch granule: an alkaline solution (1.7% KOH) is used to test the degree of dispersion value of individual milled rice grain (Cruz and Khush, 2000). Grains with low gelatinization temperature show complete disintegration of starch granules, intermediate gelatinization temperature show partial disintegration of starch granules, while high gelatinization temperature the starch granules remain unaffected (Cruz and Khush, 2000). Traditional tropical varieties are in general intermediate (Juliano et al., 1964a) (**Table 1.2**).

The amylose content determines the ability of rice grain to absorb water and expand its volume during cooking, and the texture of cooked rice (Juliano, 1979, Rao et al., 2013). It is therefore considered a highly and distinctly relevant parameter in the determination of the grain quality of milled rice (Juliano, 1979, Rao et al., 2013). Regarding the proportion of amylose in the starch, rice grain may be grouped into five classes namely: waxy (0-2%), very low (3-9%), low (10 - 19%); intermediate (20-25%) and high (25% or more amylose content in the grain) (Kumar and Khush, 1986). Waxy rice grains almost do not contain amylose in the starch, thus do not expand in volume, are glossy and sticky, cook moist and remain firm after cooking. High amylose content rice grains exhibit a high volume of expansion, high degree of flakiness, cook dry, are less tender and become harder upon cooling. While, rice grain varieties with intermediate amylose content cook moist and tender and do not harden when cool. The class of intermediate amylose content is the most preferred in many rice producing regions (Cruz and Khush, 2000) (**Table 1.2**). There is no strict correlation between low gelatinization temperature with any class of amylose content in the grain, thus it is freely recombined, but intermediate gelatinization temperature is associated with either intermediate or high amylose content, whereas high gelatinization temperature is related with low amylose content (Cruz and Khush, 2000) (**Table 1.2**). The gel consistency property indicates the trend of cooked rice to harden on cooling, thus it may be hard, medium, and soft. These classes are based on the length of the gel (flow characteristics of milled rice gel in 0.2 M KOH) which are < 40 mm for hard, 40 – 61 mm for medium, and > 61 mm for soft gel consistency. These intervals exhibit respectively very flake, flake and soft rice grains (Juliano, 1979, Chen et al., 2012), with each of these classes also differing in terms of cohesiveness, tenderness, colour and gloss of the cooked grain. Among them, the soft gel consistency is the most preferred, because it provides tender cooked rice grain. Moreover,

soft gel consistency is generally combined with amylose content lower than 25% (Rao et al., 2013) (**Table 1.2**).

Table 1.2: Characteristics of different rice varieties based on the properties of the starch in the rice grain endosperm (Juliano et al., 1964a, Cruz and Khush, 2000, Rao et al., 2013, Awan et al., 2017)

Gelatinization temperature (defines time required for cooking for cooking the rice grain**)**				
Categories	**Gelatinization temperature**	**Degree of dispersion starch granules**	**Amylose content**	**Rice grain type**
Low	55 °C to 69 °C	complete disintegration	no strict correlation	_____
Intermediate	70 °C to 74 °C	partial disintegration	intermediate/high	traditional tropical varieties Indica (Basmati rice)
High	> 74 °C	starch granules unaffected	low	_____
Amylose content (determines rice grain water absorption, expansion and texture during cooking**)**				
Classes	**Amylose content**	**Characteristics of rice grains**		
Waxy	0-2%	➢ do not expand in volume ➢ are glossy and sticky ➢ cook moist ➢ remain firm after cooking		Japonica rice Indica (glutinous, jasmine rice)
Very low	3-9%	_____		_____
Low	10 - 19%	_____		_____
Intermediate	20-25%	➢ cook moist and tender ➢ do not harden when cool		most preferred Indica (Basmati rice)
High	≥ 25%	➢ exhibit a high volume of expansion ➢ high degree of flakiness, cook dry ➢ are less tender ➢ become harder upon cooling		_____
Gel consistency (indicates trend of rice grain to harden on cooling**)**				
Classes	**Length of the gel**	**Characteristics of rice grains**	**Amylose content**	
Hard	< 40 mm	very flake,	_____	_____
Medium	40 – 61 mm	flake	_____	_____
Soft	> 61 mm	soft rice grains	< 25%	most preferred Indica (Basmati rice)

1.2.2.4 Nutritional Value

Rice grain is among the major staple foods and part of the diet for more than 50% of global population (Linares, 2002, Khush, 2005, Vaughan et al., 2008). It is commonly consumed in the form of polished or milled rice grain without the embryo and the bran layers, which are removed during the grain processing (Yang et al., 2019). Currently, rice grain is prepared in several forms from boiled, steamed, fried or parched rice grain, and in a wide range of rice based food, such as cake, noodle, dumpling, glutinous rice flour, snacks, brewed beverages, and rice bran oil for use in religious events (Graham et al., 1999, Ghosh et al., 2016, Razzaq et al., 2020). Therefore, the nutritional value of rice grain is a fundamental quality parameter for the consumers, particularly in many developing countries, where rice is among the top staple foods (Chen et al., 2012).

Rice is predominantly a source of carbohydrates, in the form of endosperm starch, and provide more than 21% of the human calorific requirements (Fitzgerald et al., 2009, Ghosh et al., 2016, Yang et al., 2019). The nutritional value of rice grain is also related to the content of protein, minerals (e.g. Ca, Mg, Cu, Fe, Zn, Mn) and vitamins, which are essential for the human diet (Juliano, 1979, Heinemann et al., 2005, Liu et al., 2017). Proteins of rice grain are highly digestible (about 88%), thus considered of high nutritional value (Ghosh et al., 2016). Minerals have a substantial role in the control of immunological functions for adults and contribute for the development of intelligence in children (Belder et al., 2005, Boonchuay et al., 2013). Lower dietary intake of minerals may lead to negative effects on human health, such as anaemia (Shan, 2006). However, deficient intake of Fe and Zn is a problem that affects about one third of the global population, and this is severe in developing countries (Boonchuay et al., 2013). Significant proportion of vitamins and minerals of the rice grain are concentrated in the bran (brown rice), hence effort should be

made to increase the quality of milled rice in consuming countries with particular attention to developing countries.

1.3 Rice Production in Mozambique

1.3.1 Harvested Area, Producing Regions and Growth Conditions

In Mozambique, rice has been cultivated for more than 500 years. Although approximately 900,000 ha are suitable for the production of rice, only 365,000 ha are currently used for rice production (NRDS, 2009). The production of rice occurs along the coastal zone in all regions of Mozambique: north, centre and south, and this is concentrated in four niches: Cluster 1: Xai-xai, Cluster 2: Beira, Cluster 3: Quelimane and Cluster 4: Nampula. A significant proportion of rice, about 90%, is produced under rainfed lowland conditions in clusters 2 and 3 (**Figure 1.2**). Rice in Mozambique is predominantly cultivated during the rainy season by small landholder farmers, with an average total land area of less than 0.5 ha/farmer. Therefore, rice is direct seeded from November to January and harvested from May to June, with an average yield of about 1.2 ton/ha(EDA, 2005).

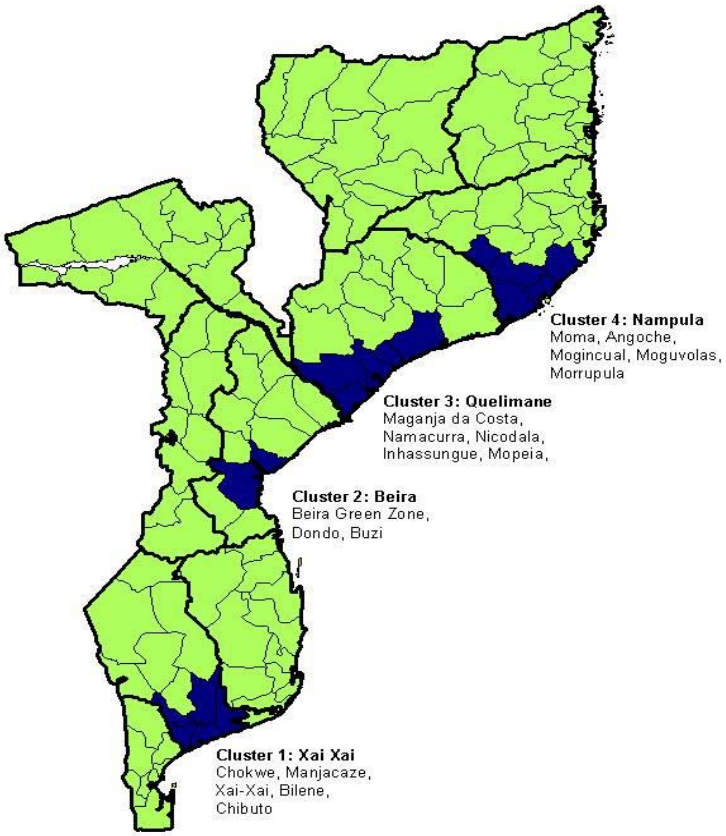


Figure 1.2: Regions with higher concentration of rice production in Mozambique(EDA, 2005).

1.3.2 National Importance

Rice is mainly produced for domestic consumption and only small quantities of surplus are sold in local markets. Rice plays an important dietary role for more than 90% of Mozambican population, because it is a staple food and one of the major sources of daily intake of carbohydrates. Currently, rice is the third source of calories after cassava and maize, contributing to about 10.5% of total consumption of calories, which is equivalent to nearly 23 kg of milled rice per person a year (NRDS, 2009). Rice consumption has increased tenfold, while production has increased sevenfold in the last 30 years. Therefore, national rice production remains below consumption (**Figure 1.3**). The current rice consumption is about

1,000,000 tons/year, but only 300,000 tons/year is covered by national production and the remaining 700,000 tons/year rely on imports from Asia (**Figure 1.3**) (INDEXMUNDI, 2021b).

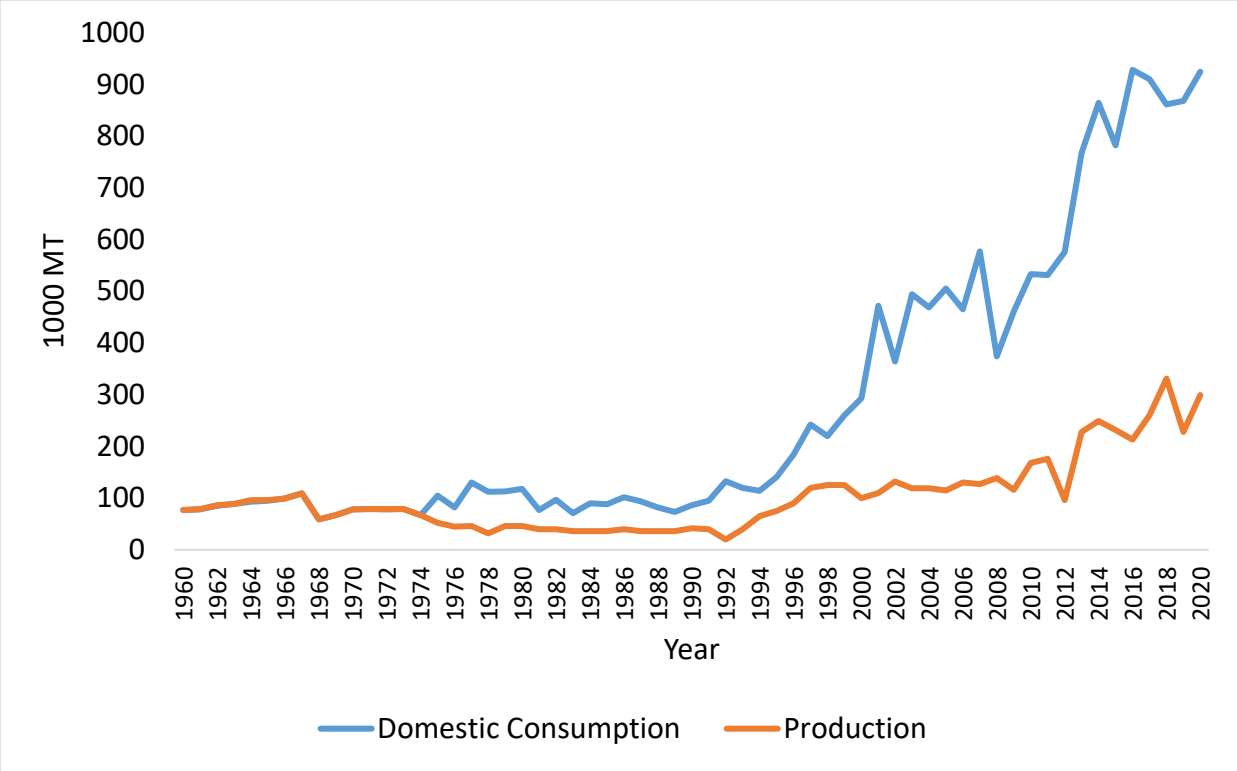


Figure 1.3: Annual Production and Domestic Consumption of Milled Rice in Mozambique (INDEXMUNDI, 2021b).

1.3.3 Constraints of Rice Production

There are many factors contributing to the limited use of land for rice production as well as to the low yield. The most prominent barriers which restrict the production of rice in Mozambique are low availability of technologies such as tractors, mechanized ploughs, irrigations systems, fertilizers and access to good seed. In addition, there are climatic adversities such as floods, drought and salinity, which negatively impact rice production (EDA, 2005). Rice cultivation in Mozambique occurs along the coastal zone and during the rainy season, thus crops are prone to salinity stress, in particular due to salt intrusion originating from storms. Therefore, a better understanding of the impact of soil salinity on

rice genotypes from Mozambique, and the identification of appropriate husbandry practice which may enhance salt tolerance in rice, could contribute to mitigate the impact of salinity, increase yield, and hence increase food security in Mozambique.

1.4 Soil Salinity

1.4.1 Definition of Soil Salinity

Soil salinity is one of the major abiotic stresses that threatens agricultural productivity worldwide (Shabala and Munns, 2017). Currently, more than 6% (830 million hectares) of the global total land area and more than 20% of cultivated land is affected by salinity, which results mainly from natural processes and to a lesser extent from human activities. More than half of global land area affected by salinity is found in Asia, the Pacific and Australia, with 444 million hectares (**Figure 1.4**) (Munns, 2005, Smajgl et al., 2015, Hoang et al., 2016).

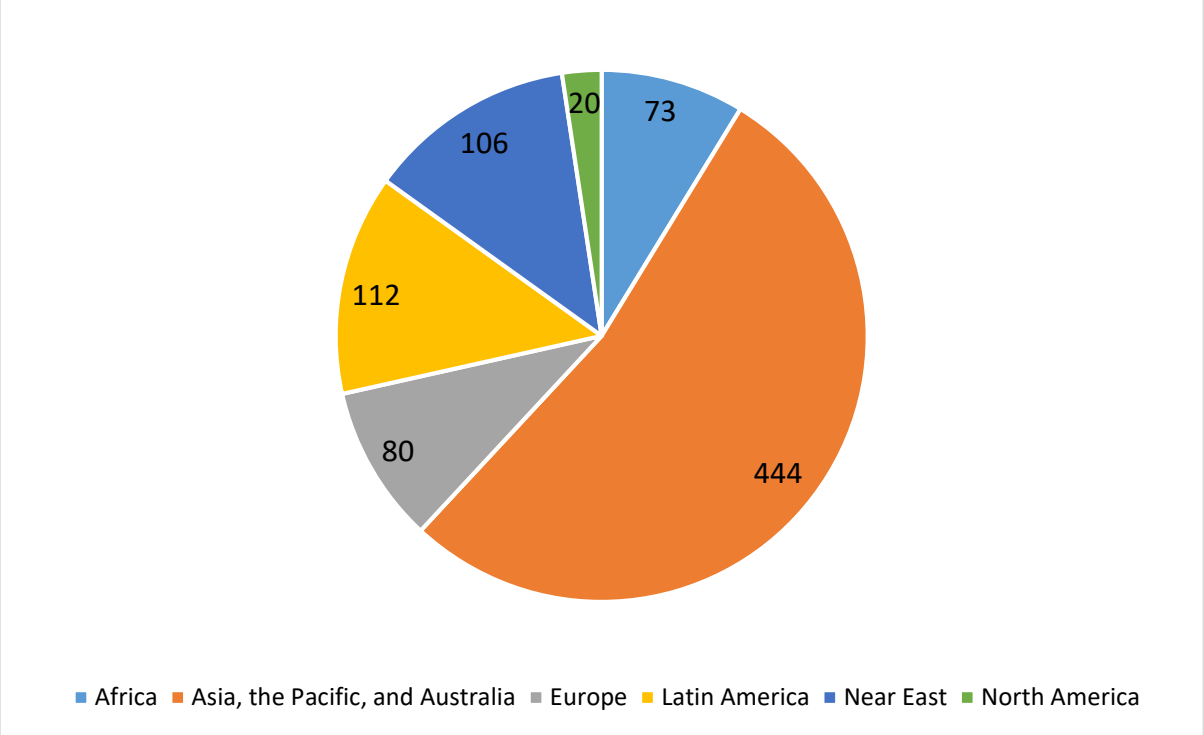


Figure 1.4: Distribution of global total land area affected by salinity (million hectares) (Hoang et al., 2016).

Weathering of parental rocks releases soluble salts such as chlorides of sodium, calcium, magnesium, sulphates and carbonates, which accumulate in the soils over time. Among the most common salts, sodium chloride is the most predominant (Munns and Tester, 2008). Rainwater and wind can also transfer salts from oceans to the soils (Rengasamy, 2006), and salt intrusion originating from storms and consequent rise of sea level also contributes to the salinization of coastal soils (Smajgl et al., 2015, Shabala and Munns, 2017). Human activities such as clearing of land and irrigation can also contribute to the increase of salt concentrations in the soil by increasing water table and subsequent concentration of soluble salts in the root zone (Rengasamy, 2006, Smajgl et al., 2015).

A soil is considered saline when the Electrical Conductivity of its saturated paste extract (ECe) is more than 4 ds/m, which is equivalent to 40 mM NaCl and creates osmotic pressure of 0.2 MPa (Richards, 1969, Munns and Tester, 2008). This concept of salinity corresponds to the concentrations that impair growth and development of the majority of crops (Munns and Tester, 2008, Shabala and Munns, 2017). However, a soil in the field is rarely saturated, thus the concentration of salts in the root zone may be higher than indicated by the saturated paste extract (Rengasamy, 2006). Therefore, soil with an ECe of 4 ds/m may have concentrations of salts of approximately 80 -100 mM NaCl and have a negative impact on plant growth and productivity, significantly reducing the yield of most crops, including rice (Munns and Tester, 2008, Shahbaz and Ashraf, 2013, Shabala and Munns, 2017). In many countries such as the United States of America, Australia, China, India and Pakistan, the impact of salinity in the agricultural sector is significant, with an annual loss of about US$27.3 billion (Qadir et al., 2014).

1.4.2 Effect of Soil Salinity on Plant Physiology

Salinity affects cereals crop morphology, physiology and biology (Qin et al., 2020). The predominant changes at the morphological levels are suppressed root growth, leaf rolling, reduction of plant height and the number of tillers, spikelet sterility, and eventual yield loss (Chang et al., 2019, Razzaq et al., 2020). At physiological and biological levels, the rate of photosynthesis and water content decrease, the metabolism is disturbed, the concentration of Na-ions in the shoot rise, and those of K, Zn and P-ions decrease (Lekklar et al., 2019, Tsai et al., 2019, Razzaq et al., 2020). Therefore, focusing on physiological changes, soil salinity prevents growth and development of plants by causing osmotic stress, ion toxicity and nutritional imbalance, such as limited uptake of the K-ions from the soil, which ultimately lead to growth inhibition and reduced crop yield (Greenway and Munns, 1980, Ismail et al., 2007, Munns and Tester, 2008).

1.4.2.1 Osmotic Stress

Concentrations of salts above the threshold (4 ds/m) in the root zone create an osmotic pressure that reduces the ability of plants to take water from the soil (Munns, 2002, Munns and Tester, 2008). Low water availability for plants reduces the rate of cell elongation, the stomatal aperture and conductance of CO_2 in shoot tissues, and consequently results in slower formation of photosynthetic area and limited rate of photosynthesis. These effects on photosynthesis reduce the movement of assimilates to the meristematic and growing tissues in both leaves and roots of a plant, but this effect is more prominent in leaves (Greenway and Munns, 1980, Shabala and Munns, 2017). The adverse effects of osmotic

stress in cellular and metabolic processes are similar to the effects of drought on plants (Munns, 2002).

1.4.2.2 Ion Toxicity

The inability of plants to exclude salts from the transpiration stream results in the accumulation of salts to toxic concentrations, which cause disruption of metabolic processes and injury to the photosynthetic cells in transpiring leaves (Greenway and Munns, 1980, Ismail et al., 2007, Munns and Tester, 2008). Toxic concentrations of Na-ions in the transpiration stream accelerate the senescence of old leaves, resulting in premature death of plants (Munns, 2005). While Cl-ions may follow the same pathway of Na-ions, they rarely accumulate to reach toxic concentrations (Munns and Tester, 2008). This is because Na-ions have the same physico-chemical properties as the K-ions, specifically the ionic radius and ion hydration energy. Therefore, the accumulation of salts leads to competition between the Na-ions and K-ions for the major binding sites in fundamental metabolic processes in the cytoplasm, including many enzymatic reactions, protein synthesis and ribosome functions (George et al., 2012). More than 50 enzymes in the cytoplasm are activated by K-ions, thus the competition between the Na-ions and K-ions leads to severe disturbance of metabolism in the leaves as well as in the roots (Shabala and Munns, 2017). Asch et al. (2000) and Haq et al. (2014) emphasize that the concentration of Na-ions in shoot tissues is associated with the reduction of the concentration of K-ions , which results in the decrease of K^+/Na^+ ratios. The reduction of K^+/Na^+ ratio is commonly linked with the reduction of yield, and can be used to estimate salt induced yield reduction from 60 days after sowing in rice (Asch et al., 2000).

1.4.3 Physiological Adaptation to Salinity Stress

Plants physiologically respond to soil salinity stress in three distinct ways: osmotic adjustment, sodium exclusion and potassium retention in the cytosol.

1.4.3.1 Osmotic Adjustment

Plants may counteract effect of salinity through osmotic adjustment by accumulating compatible solutes or organic osmolytes, biosynthesis of compatible solutes or increase in the accumulation of Na, Cl and K-ions from the soil (ion homeostasis and compartmentation)(Shabala and Shabala, 2011, Hoang et al., 2016). The most common compatible solutes are sugars, polyols, amino acids and quaternary ammonium compounds (Delauney and Verma, 1993). However, accumulation of organic osmolytes requires high energy, which may lead to yield reduction, and the biosynthesis of compatible solutes is a slow process. Therefore, increased accumulation of Na, Cl and K-ions is the ideal adaptation strategy to salinity for plants (Shabala and Munns, 2017).Flowers et al. (1986) observe that a small amount of ions accumulated in the leaves may contribute to osmotic adjustment, but excessive amount of Na and Cl-ions may cause toxicity in the leaves or reproductive tissues. Therefore, the accumulation of Na and Cl-ions should be essentially accompanied by efficient compartmentation of these ions in vacuoles to avoid toxicity (Shabala and Munns, 2017).

1.4.3.2 Na^+ Exclusion

Under high salt conditions there is a unidirectional influx of Na-ions, which is thermodynamically passive. This influx of Na-ions is common in most glycophytic plants,

which are non-native flora of saline soils, and this process is poorly controlled (Tester and Davenport, 2003). After a prolonged exposure to salt, plants, through the transpiration stream, accumulate Na-ions in the old leaves, since there is no longer an increase of leaf area, which would lead to the dilution of the salt deposited (Munns and Tester, 2008).The exclusion of Na-ions from growing leaves is an essential strategy for the survival of plants under salt stress, because it prevents salt accumulation at toxic levels (Munns, 2005, Munns and Tester, 2008). Most glycophytic plants rely on mechanisms that maintain low concentrations of Na-ions to survive under salinity stress, and this is attained by the balance between Na-ions exclusion and Na-ions sequestration in the vacuoles (Munns, 2005, Haq et al., 2014). Translocation of Na-ions from the leaves to the roots through the phloem would be an alternative for Na^+ exclusion in the shoots (Munns, 2005, Munns and Tester, 2008). However, this approach appears to be unfeasible, because salt concentrations remain in the leaves after salt solution removal in the root zone, revealing a low movement of salt from leaves to roots via the phloem (Munns, 2005). Therefore, Na^+ exclusion in the shoots is a process predominantly controlled by the net delivery of Na-ions to the root xylem (Munns and Tester, 2008). The net delivery of Na-ions to the xylem is divided into four distinct components: (1) influx into cells in the outer half of the root; (2) efflux back out from these cells to the soil solution; (3) efflux from cells in the inner half of the root to the xylem; and (4) influx back into these cells from the xylem before the transpiration stream delivers the Na-ions to the leaf blade (Munns and Tester, 2008). Approximately 98% of Na-ions in the transpiration stream should be excluded at the root zone and only 2% should flow in the xylem to avoid toxic concentrations (Munns, 2005). For this reason, the accumulation of Na-ions in leaves has been used as an indicator of salt tolerance of crop plants (Ashraf and Harris, 2004, Munns et al., 2006).

1.4.3.3 K^+ Retention and K^+/Na^+ Discrimination

The ability of a plant to survive under salt stress is not determined only by the concentration of Na-ions, but by the ratio of K^+/Na^+ in the cytosol (Shabala and Cuin, 2008). Therefore, in addition to the exclusion of Na-ions, it is essential that K-ions are retained in the cytosol, to increase the ratio of K^+/Na^+ (Shabala and Munns, 2017)

1.5 Impact of Soil Salinity on Rice Production

1.5.1 Salt Stress on Rice Growth, Physiology and Yield

Rice is widely grown in different ecologies, thus the production of rice may be observed in diverse environments, including saline soils in coastal tropical regions, where salinity is dynamic and seasonal, and inland saline and sodic soils, where salinity remains all year (Ismail and Tuong, 2009).

Among major cereals (maize, wheat , rice , barley), rice is the most sensitive to salinity stress, with a low threshold of 19-30 mM NaCl (Grattan et al., 2002). Salt concentrations that exceed 30 mM NaCl considerably affect growth, grain yield and grain quality of rice. Maas and Hoffman (1977) observed that under a salt concentration of about 60mM NaCl, the yield of rice is reduced by 50%. Other authors have observed rice to be more sensitive, and assert that lower salt concentrations of 20 mM NaCl and 40 mM NaCl cause a yield reduction of 1/3 (33%) and 1/2 (50%) respectively (Lutts et al., 1995, Abdullah et al., 2001, Grattan et al., 2002, Baxter et al., 2011). Shabala and Munns (2017) highlight that in field conditions where the concentrations of salts are about 100 mM NaCl, rice plants will not survive to maturity. However, the response to saline stress varies according to the stage of growth and development, severity, and the duration of stress (Zeng et al., 2001, Ismail and Horie, 2017).

The impact of salinity on rice is greater during the seedling stages, panicle initiation, flowering and pollination than in germination, tillering, grain filling and maturity (Lutts et al., 1995, Roessner and Beckles, 2012, Ismail and Horie, 2017). However, the negative effect of salinity at seedling stages may be compensated by the higher percentage of production of new leaves, earlier panicle initiation, higher inflorescence formation and increased rate of grain filling (shorter period of grain filling) (Lutts et al., 1995, Munns, 2005).

The effect of salinity during the reproductive stage has a direct effect on yield, because at this final stage, the plant has few adaptation mechanisms and this can lead to a high number of sterile florets, partially filled or unfilled grains and, ultimately, lower yield (Roessner and Beckles, 2012, Thitisaksakul et al., 2012, Shavrukov, 2013, Beckles and Thitisaksakul, 2014).

Moreover, the sensitivity to salinity varies with genotype (Lee et al., 2003, Kurotani et al., 2015). Lee et al. (2003) observe that japonica rice is more sensitive to salinity than indica rice. Regarding, the physiological effect of salinity stress, rice plants are poor in excluding Na-ions from saline soil, being able to exclude up to 94%, which is relatively low compared to the ideal 98% (Munns, 2005). Munns et al. (2006) recommend Na^+ exclusion as a parameter to evaluate salt tolerance in rice. Under salt stress, salt tolerant varieties of rice retain lower concentrations of Na-ions in the leaves than salt sensitive varieties (Flowers and Yeo, 1981, Haq et al., 2009, Haq et al., 2014).

Platten et al. (2013) observed a strong correlation between the concentration of Na-ions and salt tolerance in many rice accessions of different rice species. Generally, the Indica rice subspecies has higher ability to exclude Na-ions in the leaves and retain K-ions and consequently increase K^+/Na^+ ratio in the cytosol than the Japonica rice subspecies (Gregorio et al., 1997). Previous studies indicate that Indica rice accumulate 75% less Na-ions, and has

27% higher K^+/Na^+ ratio in the shoots than Japonica rice. Therefore, Na^+ exclusion and higher K^+/Na^+ ratio were the key mechanisms that conferred higher salt tolerance of Indica variety compared to Japonica variety (Haq et al., 2009, Haq et al., 2014).

1.5.2 Effect of Soil Salinity on Rice Grain (Nutritional) Quality

Rice grain composition and quality are defined by several developmental processes. These processes are influenced by genetic factors as well as the growth environment (Chen et al., 2012). In general, rice is consumed in the form of whole milled grain with very little processing (without adding several ingredients to the end-product), hence the effect of growth environmental conditions is prominent in the quality of the raw material (Yamakawa et al., 2007, Sun et al., 2011, Beckles and Thitisaksakul, 2014). Rice grain yield and grain quality are both affected by abiotic stresses, including soil salinity; (Peiris et al., 1988, Siscar-Lee et al., 1990, Lutts et al., 1995, Munns and Tester, 2008, Pattanagul and Thitisaksakul, 2008, Chen et al., 2012, Razzaq et al., 2020). Although soil salinity has significant impact on rice grain quality, knowledge of the specific effects on composition, processing and visual attributes are still limited (Siscar-Lee et al., 1990, Baxter et al., 2011, Chen et al., 2012, Beckles and Thitisaksakul, 2014). Previous research indicates that rice production under salt stress results in reduced grain size, grain weight, head rice recover, starch and amylose concentrations, grain translucence and gel consistency; on the contrary, the grain protein concentration is increased as consequence of reduced grain size and starch concentration (Siscar-Lee et al., 1990, Rao et al., 2013, Thitisaksakul et al., 2015).

Siscar-Lee et al. (1990) investigated the response of four rice varieties with different degrees of salt tolerance under EC = 5/6 dS/m and found reduced caryopsis weight, starch

concentration, less translucent grain, and decrease in length, width and thickness compared to the control (normal soil). However, there was no effect on gel consistency (the length of the gel based on the consistency of a cold 4.4% milled rice paste in 0.2 M KOH); and the brown rice protein concentration (higher nutritional value) was higher compared to the control (normal soil). Rao et al. (2013) tested nineteen genotypes categorized as tolerant, semi-tolerant and sensitive, under EC = 4 and 8 mS/cm salinity stress, in five different agro-ecological regions representative of semi-arid sub-tropical India, with climatic conditions of hot and dry summers and cold winters. They observed a reduction in grain dimensions, head rice recover, amylose concentration, starch concentration and gel consistency, with effects more prominent in the salt sensitive varieties. Conversely, Thitisaksakul et al. (2015) observed that when the rice cultivar Nipponbare was grown under EC = 2 and 4 ds/m salinity stress, applied either at seedling stage or at anther appearance, in greenhouse conditions, there was an increase of starch concentrations in the caryopsis. This increase, compared to control (EC = 0 ds/m), was 32.6% under EC = 4 ds/m applied at seedling stage and 39% under EC = 2 ds/m applied at anther appearance. However, there was no significant change in the concentration of amylose, protein or grain weight. This suggests that under these two levels of salinity treatments, there was a stimulatory increase in the concentrations of starch, and the concentrations of starch and protein were differently structured (i.e. the effect of salt stress may have changed some characteristics of starch biosynthesis: the starch granule initiation and amylose biosynthesis). In contrast, for EC = 4 ds/m salinity stress imposed at anthesis stage there was no significant effect on starch concentrations, the grain protein concentration increased by 20.1 % and the grain weight reduced by 28.8 %. The increase of grain protein concentration was mainly due to the increase of the glutelin followed by prolamin. It was speculated that salt stress may have led to difference in the allocation and

portioning of grain reserves. From these results it can be deduced that the effect of salinity stress on rice grain quality is influenced by the concentration of salt, timing or the stage of growth and development, and the rice genotype.

In addition, the stress of salinity alters the mineral composition of rice (Verma and Neue, 1984, Saleethong et al., 2013). Salt tolerant and salt sensitive rice varieties were grown in culture solution in the greenhouse, salt concentrations equivalent to 2.5 dS/m, 5.6 dS/m, and 8.7 dS/m salinity stress were applied to 21-day-old seedlings and up to maturity, and it was observed that increasing salinity stress resulted in the increase of rice grain Na, Fe and Zn concentrations, and the reduction of rice grains Mn concentration. The uptake of N, Mg, Cu, K, and Ca were not affected in either variety (Verma and Neue, 1984).

When rice varieties Pokkali (salt-tolerant) and KDML105 (salt-sensitive) were exposed to 25 mM NaCl salinity stress from booting stage up to maturity , there was a reduction in the content of macronutrients N, P, K, and Mg accompanied by the increase of Ca in brown rice grains. This effect was more evident in salt sensitive varieties (Saleethong et al., 2013).

1.6 Seed Priming

1.6.1 The Concept of Seed Priming

Priming is a process of seed enhancement or invigoration, which consists of a controlled hydration of seeds in water, solution or other conventional priming agent. This process stimulates pre-germination physiological and biochemical activities prior to sowing, enhancing germination, uniformity of crop establishment and in some cases tolerance to environmental stresses (**Figure 1.5**) (Taylor et al., 1998, Dutta, 2018). To achieve this, seeds are immersed in water or solution for specific period of time and re-dried to their original

weight and moisture content (Khan, 1992, Taylor et al., 1998, Afzal et al., 2016, Dutta, 2018). A viable and non-dormant seed requires water, oxygen and optimum temperature to initiate the germination process. This process comprises three distinct phases of water uptake, namely: imbibition stage, activation or lag stage and the growth stage (Bewley and Black, 1994, Bewley, 1997). The phase I, or imbibition stage, is characterized by the rapid water uptake resulting from the lower water potential inside the seeds compared to its surrounding environment. Low metabolic activity is observed during this first stage. The phase II, or activation stage, corresponds to the beginning of metabolic activities, i.e. mitochondria and DNA repairing processes and protein synthesis, which lead to the modification of proteins, lipids and fats (mobilization of nutrient reserves) into compounds required for germination. All through the activation phase there is a reduction of water uptake and the seed fresh weight is constant. Phase III or growth stage is distinguished by the seed recovering the ability to quickly uptake water, which is accompanied by its fresh weight increase. The growing processes associated with cell elongation that results in radicle protrusion begin at this stage (**Figure 1.5**) (Bewley and Black, 1994, Bewley, 1997). The three different phases are controlled by the availability of water and seeds tolerate desiccation during phases I and II (Taylor et al., 1998). Therefore, priming is a traditional technique that allows seeds to imbibe water, initiate the metabolic activities and undergo the physiological and biochemical changes (the increase of energy metabolism, advanced mobilization of seed reserves, embryo expansion and endosperm breakdown) required for germination before sowing, improving the performance of seed (Bray, 1995, Pandita et al., 2007, Theerakulpisut et al., 2017). During the priming procedure, seeds experience the imbibition phase and partially the activation phase (Bray, 1995, Theerakulpisut et al., 2017); however, the pre-germination activities occur up to the level where they are still reversible and the seeds may

exhibit only a slight radicle protrusion (**Figure 1.5**). Thus the seed priming process is discontinued before complete germination is achieved (Bray, 1995).

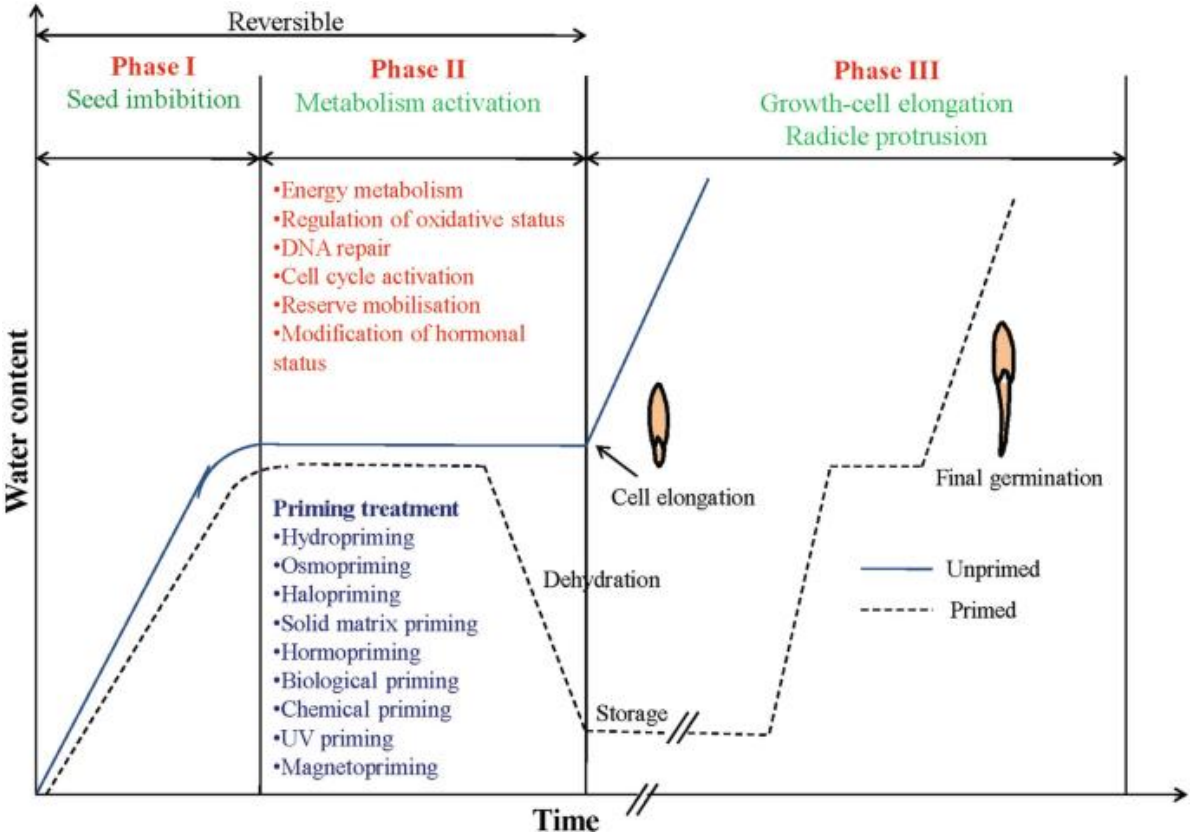


Figure 1.5: Seed hydration process and germination stages of un-primed and primed seeds (Thakur et al., 2019).

1.6.2 Beneficial Effects of Seed Priming

The process of priming reduces the length of germination, increases seed vigor, promotes the germination rate and synchronizes the germination, which results in uniform crop establishment and improved yield. These effects of seed priming may be observed in an extensive range of environments including (biotic or abiotic) stressed conditions. In addition, the priming technique is simple, effective and affordable with minimum environmental risks (Taylor et al., 1998, Farooq et al., 2006c, Jisha et al., 2013, Paparella et al., 2015, Dutta, 2018). McDonald (2000) highlights that priming triggers positive changes at cellular,

subcellular and molecular levels promoting germination and seed vigour of various plant species, under diverse environmental constraints, including salinity stress. Rehman et al. (2011) state that seed priming is a practical strategy to improve the performance of direct seeded rice. Therefore, seed priming is a strategy that enables plants to promptly and effectively respond to various environments, particularly under stressful conditions.

1.6.3 Types of Seed Priming

Currently there are several conventional categories of seed priming, based on the priming solution. Therefore, seed priming can be classified as: (a) hydropriming, if only water is used to prime the seeds; (b) halopriming, when the seeds are soaked in solutions of inorganic salts; (c) osmopriming, where an osmotic substance of lower water potential such as Polyethylene Glycol (PEG), Glycerol, Sorbitol, or Mannitol is used in the priming solution; (d) chemopriming ,which includes the application of chemicals; (e) hormopriming, based on the use of hormones; (f) solid matrix priming, in which seeds are immersed in an inert substance of identified matrix potential; (g) biopriming, which involves priming of seeds through inoculation of beneficial organisms; (h) nutriopriming, described as the use of micronutrients (e.g. Zn, B, Mo, Mn, Cu, Co) to prepare the seeds; and (i) thermopriming, where the heat is employed to prime the seeds (Afzal et al., 2016, Dutta, 2018, Lal et al., 2018). These priming techniques have been employed in several horticultural crops, as well as in cereals such as wheat, maize and latterly in rice (Khan, 1992, Farooq et al., 2006e, Jisha et al., 2013, Paparella et al., 2015, Dutta, 2018).

1.6.3.1 Hydropriming

Hydropriming is a pre-sowing treatment that involves soaking the seeds in water to a moisture level very close to radicle protrusion, followed by drying before sowing (Khan, 1992, Afzal et al., 2015, Bakhtavar et al., 2015). Hydropriming may be carried out either under aerated or not aerated conditions (Thornton and Powell, 1992). Throughout this priming process, no chemicals are involved, hence it is harmless for the environment, (Farooq et al., 2009). There are considerable number of studies carried out to improve the production of rice under optimal and non-optimal environments through seed hydropriming. Farooq et al. (2006b) evaluated the effect of hydropriming for 12, 24, 36, 48 and 60 h on the germination and seedling vigour of fine (i.e. long or medium grain - indica rice) and coarse (i.e. bold short grain - japonica rice) rice under unstressed conditions in growth chamber. Except for the 60 h hydropriming treatment, improvements were observed in all treatments, and the 48 h priming treatment provided the best results ,with the 36 h treatment second best. The effect of 48 h hydropriming treatment in both fine and coarse rice was subsequently assessed in lab and field experiments, and better emergence, seedling establishment, growth, yield and grain quality were observed (Farooq and Basra, 2006, Farooq et al., 2006d, Mahajan et al., 2011). The impact of 48 h hydropriming treatment was also examined on fine and coarse transplanted rice and better growth of seedling in the nursery, which led to better growth, yield and quality in the field (Farooq et al., 2007a, Farooq et al., 2007b). Farooq et al. (2009) emphasizes that priming seeds with water for a duration of up to 48 h may be a practical approach to improve the performance of both direct seeded and transplanted rice. Janmohammadi et al. (2008) observed that hydropriming for 36 h alleviated the effect of salinity and drought on maize plants by improving the germination and seedling growth. Hussain et al. (2017) observed that

hydropriming effectively improved crop establishment, growth, polyphenols, flavonoids and antioxidants activities of pigmented and non-pigmented rice under drought conditions. Lal et al. (2018) highlights that the beneficial effect of hydropriming may be observed in both non-salt and salt stressed conditions. Rice plants germinated in a solution of different salt mixture exhibited quicker germination compared to the non-primed control (Chang, 2002, Ashraf and Foolad, 2005). However, hydropriming may lead to irregular hydration and non-uniform germination (Lal et al., 2018).

1.6.3.2 Halopriming

Halopriming is the hydration of seeds with an osmotic solution of lower water potential to the level of constant moisture. The reduction of water potential is achieved through addition of one or more solutes (inorganic salts) to the priming solution (Khan, 1992, Janmohammadi et al., 2008). Among the diverse osmotic solutes, potassium nitrate (KNO_3), sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium sulphate ($MgSO_4$), calcium sulphate ($CaSO_4$), potassium orthophosphate (KH_2PO_4), and potassium phosphate (K_3PO_4) are the most commonly applied to decrease the water potential of the priming solution (Khan, 1992, Taylor et al., 1998, Afzal et al., 2016, Lal et al., 2018). The duration, osmotic potential and temperature required for a successful priming process vary with plant species. Therefore, the duration of seed priming may last from 2 to 24 days, the osmotic potential of a solution may be maintained between 0.8 to 1.6 MPa and the temperature range from 15 to 20^{o} C (Khan, 1992). Previous studies indicate that priming seeds with inorganic salts improves crop growth under non-saline conditions and also reduce effectively the negative effect of salinity stress. The reduction of salinity impact is due to the

improvement of the growth parameters and considerable change in the concentrations of Na-ions and K-ions, which lead to higher ability to osmotic adjustment (Cayuela et al., 1996, Iqbal and Ashraf, 2007, Afzal et al., 2008). Farooq et al. (2006a) observed that priming coarse rice seed with (20.74 g/l) KCl or (22.2 g/l) $CaCl_2$ salt solutions of Ψ_s= -1.25 MPa osmotic potential for 48 h at 27 $^oC \pm 3\ ^oC$ increased germination, the emergence of seedlings, number of fertile tillers, yield and the harvest index. Moreover, priming altered the grain quality attributes, thus salt solution of KCl or $CaCl_2$ increased the percentage of crude protein and decreased the amylose concentration of direct seeded rice grain in farmer rice growing belt (Pakistan) under non-saline soils. Priming with KCl provided better results that priming with $CaCl_2$. Rehman et al. (2011) also noticed that priming fine rice seed with (22.2 g/l) $CaCl_2$ salt solution of Ψ_s= -1.25 MPa osmotic potential increased crop establishment, number of fertile tillers, yield, harvest index and quality parameters of direct seeded rice, under normal conditions in farmer fields. Afzal et al. (2012) reported that halopriming with 2.2 % (22.2 g/l) $CaCl_2$ or 2.2 % (20.74 g/l) KCl salt solutions of Ψ_s= -1.25 MPa osmotic potential, for 36 h at 25 $^oC \pm 2\ ^oC$ room temperature, improved the tolerance of both salt tolerant and sensitive fine aromatic rice cultivars under moderate salinity stress of 40 and 80 mM NaCl. This was the result of improved germination ability, faster germination, increased root length, shoot length, shoot dry weight, decreased Na-ions and increased K-ions concentrations in the leaves. Theerakulpisut et al. (2017), on the other hand, primed rice seeds with 0.25%, 0.50% or 0.75% KNO_3, 200 mM (22.2 g/l) $CaCl_2$ or 200 mM (14.91 g/l) KCl for 48 hours at room temperature and evaluated their effect on the alleviation of 150 mM NaCl salinity stress. KNO_3 priming treatment was found to alleviate the negative impact of salinity on germination and growth of young rice seedlings; under 150 mM NaCl salt treatment, there was an increase in shoot length by 69 % to 83 %, of shoot dry weight by 25 to 31.15%, while

the ratio Na^+/K^+ was relatively low compared to the non-primed control. However, priming with $CaCl_2$ and KCl did not affect shoot dry weight, which may be due to both rice genotype and concentration of priming solution applied in this study.

1.7 Research Overall Aim

The improved crop performance and the alleviation of the negative effect of salinity stress through seed priming indicate that there is scope to improve rice production, increase grain yield and grain (nutritional) quality under non-saline and saline conditions in Mozambique. Therefore, this research aimed to:

- To determine if and which seed priming approaches could be effective in increasing salt tolerance of rice (*Oryza sativa* L.) accessions from Mozambique and improve their yield and grain composition (grain nutritional quality).

1.7.1 Specific Objectives

- To determine the salinity tolerance of rice accessions from Mozambique;
- To evaluate changes in salt tolerance of rice accessions from Mozambique following different seed priming treatments, and establish the physiological mechanisms that confer higher salt tolerance;
- To quantify the impact of salt stress and different priming treatments on germination, plant growth and development of rice accessions from Mozambique;

- To determine the effect of salinity stress and evaluate the impact of different priming treatments on grain yield and grain composition (grain nutritional quality) of rice accessions from Mozambique.

CHAPTER 2. Salinity Tolerance of Rice Accessions from Mozambique

2.1 Introduction

The aim of this chapter was to determine the salinity tolerance of rice accessions from Mozambique, under high salinity stress. It was hypothesized that high salinity stress will decrease shoot dry weight, salt tolerance, K^+ retention and K^+/ Na^+ discrimination, while increasing shoot Na^+ concentrations. Salt tolerance was calculated as the percentage of shoot dry weight production under salt stress in relation to the respective control. The ratios of K^+/Na^+ were calculated based on the means of respective tissue Na and K concentrations.

2.2 Materials and Methods (Experiment I - 2018)

2.2.1 Plant Material

Rice accessions were obtained from the International Rice Research Institute (IRRI), in the Philippines (**Table 2.1**). The majority of these accessions are representative of those grown in the main rice producing regions and the main ecosystems of Mozambique. Those not from Mozambique where selected based on previous studies on salinity stress to represent salt tolerant and sensitive accessions controls (**Table 2.1**).

Table 2.1: Rice accessions screened for salt tolerance. These accessions were obtained from the International Rice Research Institute (IRRI), Manila, Philippines, and the majority of them represent the rice accessions grown in main rice producing regions of Mozambique.

Rice Varieties	Accession number	Subspecies	Grown	Ecosystem
Gaza	IRGC 7546	Indica	Mozambique (landrace)	Rainfed lowland/upland
Chincherica	IRGC 7547	Indica	Mozambique (landrace)	Rainfed lowland/upland
Chibica	IRGC 7548	Indica	Mozambique (landrace)	Rainfed lowland/upland
Moroberekan	IRGC 12048	Japonica	Guinea (West Africa)	Upland
IR 46	IRGC 32695	Indica	IRRI line in Mozambique	Rainfed lowland
CO 39	IRGC 51231	Indica	India	Rainfed lowland
IR 54	IRGC 53435	Indica	IRRI line in Mozambique	Rainfed lowland/Irrigated
IR 54	IRGC 55969	Indica	IRRI line in Mozambique	Rainfed lowland/Irrigated
IR 64	IRGC 66970	Indica	IRRI line in Mozambique	Irrigated
Moroberekan	IRGC 101363	Japonica	Côte D'Ivoire (West Africa)	Upland
IR 64	IRGC 116793	Indica	IRRI line in Mozambique	Irrigated
IR 52	IRGC 126505	Indica	IRRI line in Mozambique	Rainfed lowland

2.2.2 Seed Surface Sterilization and Pre-germination

Seeds of rice accessions were surface sterilized and pre-germinated prior to sowing. A total of 120 seeds per accession were selected, placed in 50 mL conical centrifuge tubes containing 30 mL of 0.8% sodium hypochlorite (NaClO) for twenty minutes, then rinsed thrice with autoclaved distilled water. The sodium hypochlorite solution was prepared from commercial bleach 5%NaClO (Bado et al., 2016). Germination was carried out on a set of three HOSTESS 230 mm x 310 mm paper towels laid on top of one another, wetted with autoclaved distilled water and wrung to remove the excess of liquid. In each set of three paper towels, 40 sterilized seeds were uniformly arranged in three lines, leaving a space of about 2 cm around the edges, and covered with a fourth wetted and wrung HOSTESS 230 mm x 310 mm paper towel. This arrangement of paper towels and seeds was then rolled loosely. Each of the twelve accessions had three sets of rolled papers, which were placed in labelled plastic bags and incubated in the LEEC plant growth incubator (Models PL2, PL3, and PL33 with JUMO dTRON 316 CONTROL) at 34/11°C day/night with 16hrs light/8 hrs dark for eight days (Ellis et al., 1985, Ueno and Miyoshi, 2005).

2.2.3 Hydroponic System for Rice Screening: Hydroponic System Setup, Seed Establishment and Salt Treatment

After eight days in the incubator, seedlings were transferred to a hydroponic system constructed following the IRRI protocol (Gregorio et al., 1997, Bado et al., 2016), within a glasshouse chamber in the control environment laboratory (CEL) at School of Agriculture Policy and Development(SAPD), University of Reading, United Kingdom.

2.2.3.1 Hydroponic System Setup

Eight grey food grade polypropylene tanks (REF: 3-6413-13-CASE GREY RANGE EURO CONTAINER CASE - 26 LITRES (600 X 400 X 155MM) - http://www.plastor.co.uk/), were placed on level benches. Lids of the test tanks were drilled to obtain 48 holes of 2 cm diameter in each. Two air pumps, feeding plastic tubes with aeration stones at the bottom of the tanks, insured aeration of the nutrient solution (**Figure 2.1**).

Figure 2.1: Experiment setup: tanks containing nutrient solution, with aeration stone at the bottom and lids with 48 holes (white sponge strips). In each tank, seedlings of the twelve rice accessions were randomly planted in white sponge strips, with four replicates per tank in groups of three seedlings per sponge strip.

The nutrient solution was prepared following Yoshida et al. (1976), with modifications subsequently made by Gregorio et al. (1997). Therefore, six stock solutions, containing the first five supply macroelements and the sixth microelement, were prepared in small amount of 5 L to simplify the handling during rice growth (**Table 2.2**). Each of the stock solution was shaken, and either 25 mL or 150 mL was added to autoclaved distilled water, to prepare 20 L or 120 L of working solution, respectively. For this study, 120 L working solution was predominantly prepared. The pH of the working solution was adjusted in the drum to 5.0

with 6 N NaOH. A submersible pump was attached to the drum to aid in mixing, aeration and homogenization of the working solution, as well as its distribution to the tanks. pH in the tanks was measured thrice a week and adjusted to 5.0 with either 1 N HCl or 6 N NaOH; solution in the tanks was renewed every week (Bado et al., 2016).

Table 2.2: Nutrient composition of the six stock solutions and the chemical amounts required for five litres, for rice production in hydroponic system.

Stock no.	Chemical	Amounts/5 L
1	NH_4NO_3	457 g
2	$NaH_2PO_4H_2O$	201.5 g
3	K_2SO_4	357 g
4	$CaCl_2$	443 g
5	$MgSO_4$ $7H_2O$	1 620 g
6	$MnCl_2$ $4H_2O$	7.5 g
	$(NH_4)_6$ Mo_7O_{24} $4H_2O$	0.37 g
	H_3BO_3	4.67 g
	$ZnSO_4$ $7H_2O$	0.175 g
	$CuSO_4$ $5H_2O$	0.155 g
	$FeCl_3$ $6H_2O$	38.5 g
	$C_6H_8O_7$ H_2O	59.5 g
	$1MH_2SO_4$	250 mL

2.2.3.2 Seed Establishment

Pre-germinated seeds with the longest roots (approximately 4 cm length) were selected, grouped in three, rolled in sponge strips of about 10x2x1 cm and placed in the holes of the eight tanks randomly. Therefore, all twelve accessions, with four replicates in groups of three seedlings per sponge strip, were represented in each tank. Seedlings were kept in the glasshouse, with no supplemental light and the average photoperiod was about 15 h of daylight during the experiment. Not all seedlings survived the transplantation process, hence after three weeks from transplant, surviving plants were selected and rearranged

among the tanks, to obtain six tanks with plants uniform in size and vigor. Three tanks were used for salt treatment and three for control.

2.2.3.3 Salt Treatment

Salt treatment was introduced after the seedlings were allowed to establish in the hydroponic system for four weeks and had been rearranged among the tanks (see previous paragraph). Therefore, dry NaCl was added up to the desired molarity in a drum with nutrient solution sufficient for the three tanks, dissolved and mixed with the submersible pump. To avoid seedling shocking, salt treatment was introduced in two increments of 50 mM NaCl to reach the final concentration of 100 mM NaCl over two days. The 100 mM NaCl salt concentration is commonly used for rice crop testing (Bado et al., 2016) The level of salinity was measured with a portable waterproof conductivity meter (Multi-Parameter Testr 35 Series). Water volume was checked and autoclaved distilled water was added in each tank thrice a week, to replace water lost from evaporation and transpiration and maintain the original volume**.**

2.2.4 Data Collection

2.2.4.1 Shoot Dry Weight Production

Shoot dry weight was measured after 4 weeks of salt treatment. In each tank, two samples of three shoots /accession (six plants/accession) were collected. These samples were separately washed with autoclaved distilled water, dried with tissue paper and placed in paper bags. Samples were then dried in an oven at 80^{o}C for 4 d. Shoot dry weight was then

measured. The average shoot dry weight per plant for each rice accession was calculated. The remaining plants were kept in the tanks for further analysis.

2.2.4.2 Na^+ Exclusion and K^+/Na^+ Discrimination

One set of the two replicates per accession in each tank was ground on FRITSCH Rotor Speed Mill. A 0.5 g subsample of the respective powder was mixed with 2 mL ultra-pure water and 8 mL Trace Element Grade concentrated nitric acid, and digested at 200 °C for 30 min on MARS 6 microwave digestion system (under plant material settings). After digestion, samples were cooled for 15 min and filtered (with Whatman filter paper 9.0 cm) into Fisherbrand Falcon 50 mL Conical Centrifuge Tubes. Ultra-pure water was added to each centrifuge tube up to a weight of 50 g (excluding the weight of the empty centrifuge tube) and tubes were then kept in the refrigerator. These samples were further diluted by adding 2.5 mL of sample solution to 7.5 mL of ultra-pure water and submitted to ICP analysis for the measurement of tissue Na and K concentrations. Samples were measured for Na and K radially on ICP-OES (Inductively coupled plasma - optical emission spectrometry) (Optima 7300 dual view, Perkin Elmer, Nebuliser type: Meinhard), looking at the plasma sideways on and the wavelengths used were: Na 589.592nm and K 766.490nm. The plasma parameters for the Meinhard Nebuliser were: plasma flow 15 L/min, auxiliary flow 0.2 L/min, nebuliser flow 0.40 L/min, RF power 1500W, viewing distance 15.0mm, and sample flow rate 1ml/min.

2.2.5 Statistical Data Analysis

Two-way analysis (salt treatment*rice accession) using a Linear Mixed Model (p-value < 0.05) was used in the statistical package GenStat 18th Edition, to assess the differences in the means of shoot dry weight, and tissue Na and K concentrations. Salt tolerance was calculated as the percentage of shoot dry weight production under salt stress compared to the respective control. The ratios of K^+/Na^+ were calculated based on the means of respective tissue Na and K concentrations. The assumptions of ANOVA (Shapiro-Wilk Test for Normality and Test of Homogeneity) were checked and data were Log_{10} transformed to improve the normality of the data.

2.3 Results

2.3.1 Effects of Salt Stress on Shoot Dry Weight Production

A reduction in shoot dry weight was observed in the majority of accessions in the salt treatment compared to control treated plants (**Figure 2.2**). However, in some accessions the reduction in shoot dry weight was higher than in others, hence some accessions were more sensitive to salinity than others.

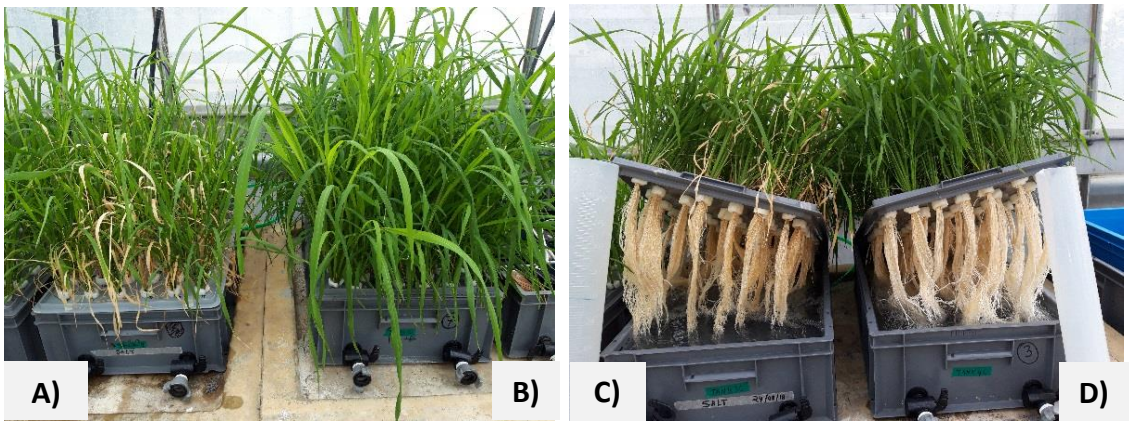


Figure 2.2: Effect of salt stress in 53-day old seedlings after 24 days of salt treatment. Twelve rice accessions were grown in a hydroponic system, in the greenhouse and salt stress was imposed on 29-day old seedlings. **A)** and **C)** Salt treatment (100 mM NaCl); **B)** and **D)** Control (0 mM NaCl).

Mean shoot dry weight was significantly different between the two salt treatments (p-value < 0.001) and the twelve rice accessions (p-value < 0.001), and there was a significant interaction between salt treatments and rice accessions (p-value =0.002) (**Appendix: Chapter 2**). The 0 mM NaCl salt treatment (control) exhibited a higher mean shoot dry weight (0.68 g/plant) and the 100 mM NaCl salt treatment showed a lower mean shoot dry weight (0.45 g/plant). Overall 100 mM NaCl salt treatment reduced shoot dry weight by 34%. Under 100 mM NaCl salt treatment, the highest shoot dry weight was observed in the rice accession IRGC 7547 (Chincherica) (1.03 g/plant), followed by IRGC 7548 (Chibica) (0.62 g/plant), and IRGC 66970 (IR64) (0.53 g/plant); whereas, the lowest shoot dry weight was

observed in the rice accessions IRGC 12048 (Moroberekan) (0.29 g/plant), followed by IRGC 101363 (Moroberekan) (0.30 g/plant), and IRGC 126 505 (IR52) (0.32 g/plant) **(Figure 2.3)**. However, compared to control, the highest reduction in shoot dry weight under 100 mM NaCl salt treatment was observed in the rice accession IRGC 12048 (Moroberekan) (74%), followed by IRGC 101363 (Moroberekan) (70%), IRGC 7548 (Chibica) (49%), IRGC 7547 (Chincherica) (39%); and the lowest reduction in shoot dry weight was observed in the rice accession IRGC 53435 (IR54) (7%), followed by IRGC 66970 (IR64) (9%). Moreover, under 100 mM NaCl salt treatment, there was an increase in shoot dry weight in the rice accessions IRGC 32695 (IR46) (17%) and IRGC 55969 (IR54) (9%) (**Figure 2.3**). However, this change was statistically significant only for the rice accessions IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan) (**Appendix: Chapter 2**).

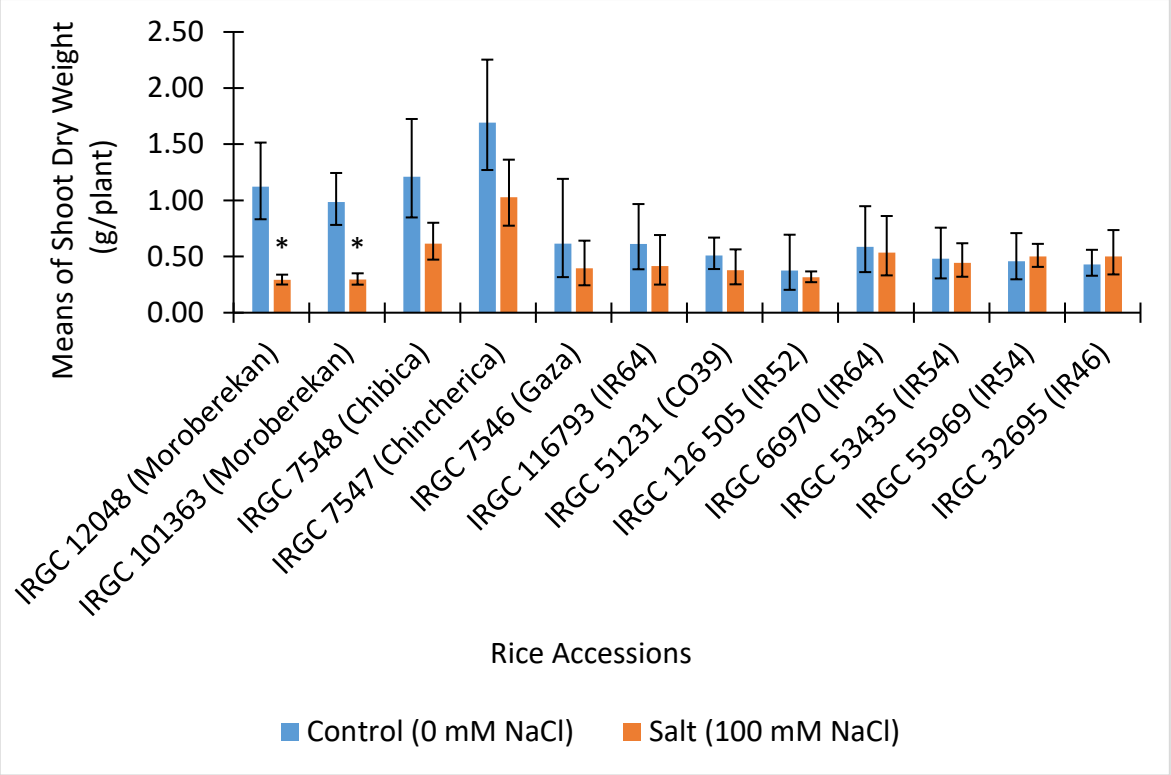


Figure 2.3: Effects of salt treatment in shoot dry weight of 60-day old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. Data are shown as mean value +/- SD of eighteen individual replications (n=18) and * mark show significant differences (p- value < 0.05). Error bars represent 95% CI.

2.3.2 Salt Tolerance of Rice Accessions

There were large variations in salt tolerance among the twelve rice accessions, ranging from 26% to 117% after four weeks of salt treatment. The rice accessions were classified into three different categories of salt tolerance from sensitive (0 - 30%), moderate (31 - 70%), and tolerant (more than 70%). The sensitive category included rice accessions IRGC 12048 (Moroberekan) (26%) and IRGC 101363 (Moroberekan) (30%); the moderate category included the rice accessions IRGC 7548 (Chibica), IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 116793 (IR64) which showed salt tolerances of 51%, 61%, 64% and 68% respectively; and the tolerant category included the rice accessions accessions IRGC 51231

(CO39), IRGC 126 505 (IR52), IRGC 66970 (IR64), IRGC 53435 (IR54), IRGC 55969 (IR54) and IRGC 32695 (IR46) which showed at least 70% salt tolerance (**Figure 2.4**).

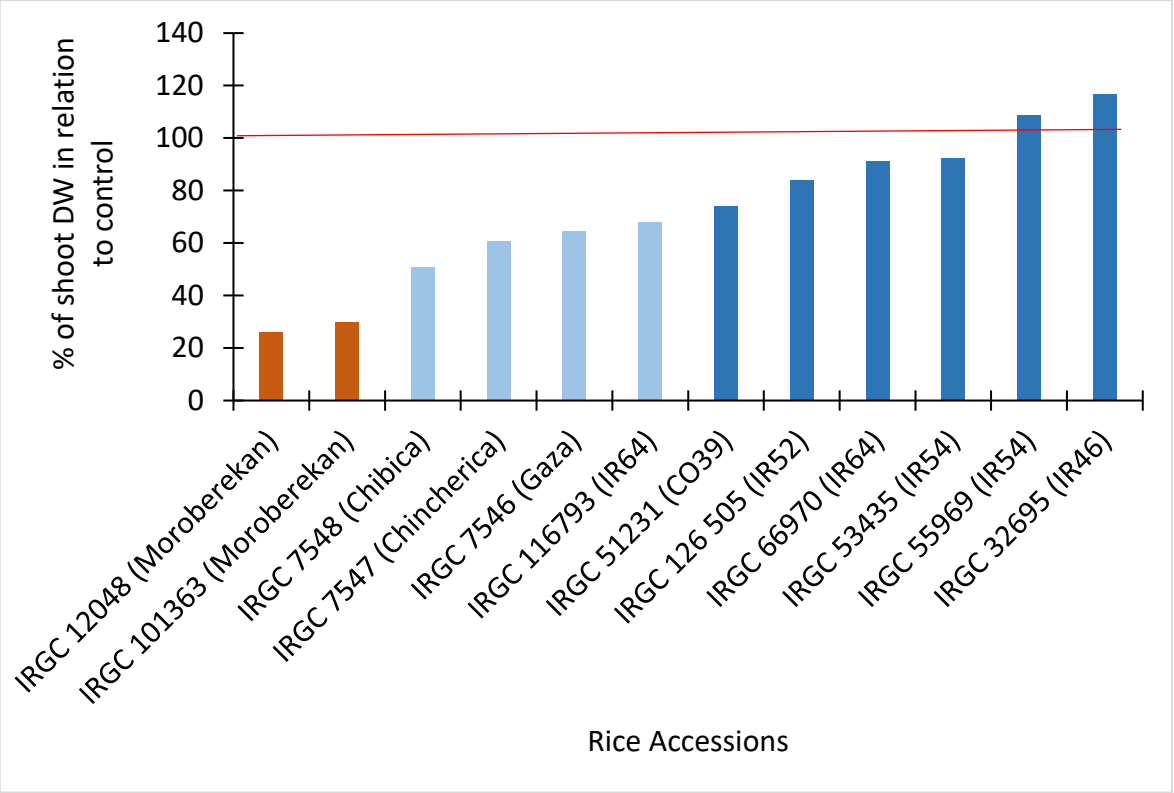
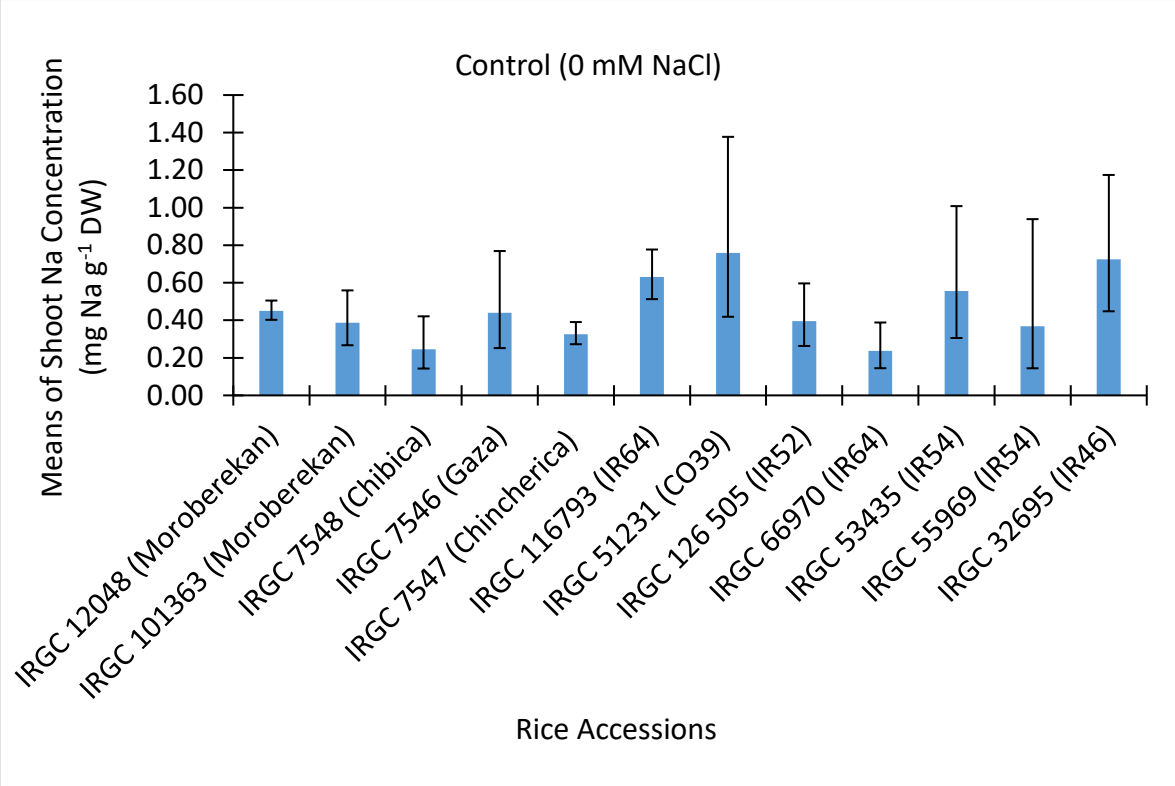


Figure 2.4: Salt Tolerance of 60-day old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. Data are shown as percentage of the means of shoot dry weight under salt stress in relation to control. The red line indicate salt tolerance of 100%. The bar colours: - sensitive, - moderate, and - tolerant rice accessions.

2.3.3 Na^+ Exclusion

Mean shoot Na concentrations were significantly different between the two salt treatments (p-value < 0.001) (**Appendix: Chapter 2**). The control treatment (0 mM NaCl) had a mean shoot Na concentration of 0.43 mg Na g^{-1} DW and the 100 mM NaCl salt treatment had a mean shoot Na concentration of 30.16 mg Na g^{-1} DW. There was a significant difference in the mean shoot Na concentrations among the twelve rice accessions (p-value < 0.001), and

a significant interaction between salt treatments and rice accessions (p-value < 0.001) (**Appendix: Chapter 2**). The variation in shoot Na concentration among the rice accessions was lower (ranging from to 0.24 mg Na g^{-1} DW to 0.76 mg Na g^{-1} DW) under the control salt treatment, but under the 100 mM NaCl salt treatment large variation (ranging from to 7.38 mg Na g^{-1} DW to 67.89 mg Na g^{-1} DW) was observed (**Figure 2.5**). Under the 100 mM NaCl salt treatment, the highest shoot Na concentration was observed in IRGC 12048 (Moroberekan) (67.89 mg Na g^{-1} DW), followed by IRGC 101363 (Moroberekan) (64.21 mg Na g^{-1} DW), IRGC 51231 (CO39) (45.01 mg Na g^{-1} DW), and IRGC 126 505 (IR52) (43.84 mg Na g^{-1} DW), while the lowest shoot Na concentration was recorded in IRGC 55969 (IR54) (26.48 mg Na g^{-1} DW),followed by IRGC 116793 (IR64) (24.99 mg Na g^{-1} DW), IRGC 66970 (IR64) (15.16 mg Na g^{-1} DW), and IRGC 53435 (IR54) (7.38 mg Na g^{-1} DW) (**Figure 2.5**). However, compared to control, the highest shoot Na concentration accumulation due to salt stress was observed on the rice accessions IRGC 12048 (Moroberekan) (99.3%), followed by IRGC 101363 (Moroberekan) (99.4%), IRGC 7548 (Chibica) (99.2%), and IRGC 126 505 (IR52) (99.1%); and the lowest shoot Na concentration accumulation in the rice accession IRGC 51231 (CO39) (98.3%), followed by IRGC 116793 (IR64) (97.5%), IRGC 32695 (IR46) (97.4%), and IRGC 53435 (IR54) (92.5%). Therefore, the increase of shoot Na concentrations under 100 mM NaCl salt treatment were higher in the sensitive rice accessions IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan), and lower in the tolerant rice accessions IRGC 53435 (IR54), IRGC 32695 (IR46) and moderate IRGC 116793 (IR64). The rice accessions IRGC 12048 (Moroberekan), and IRGC 101363 (Moroberekan) had 2% to 7% higher shoot Na concentrations compared to the salt tolerant rice accessions IRGC 32695 (IR46) and IRGC 53435 (IR54), respectively.



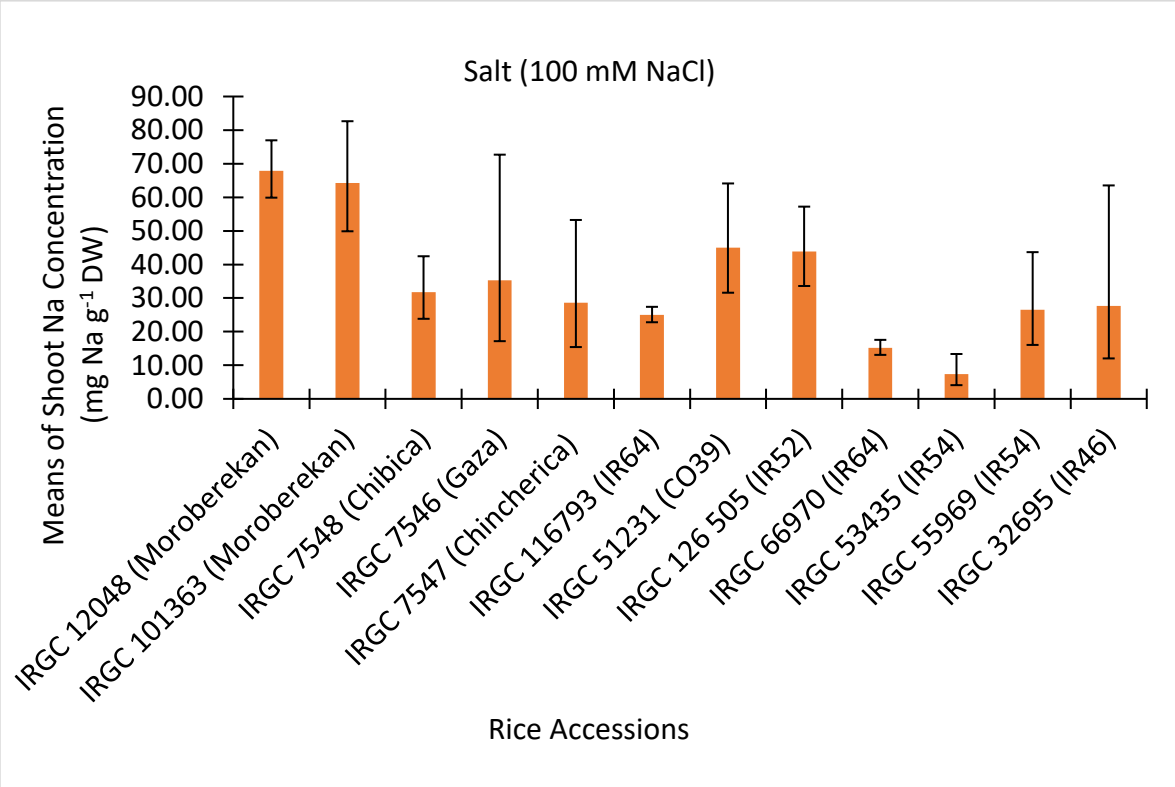


Figure 2.5: Mean shoot Na concentration of 60-day old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. Data are shown as mean value + SD of nine individual replications (n=9).The error bars represent 95% CI.

2.3.4 K^+ Retention

Mean shoot K concentrations were significantly different between the two salt treatments (p-value < 0.001) (**Appendix: Chapter 2**). The control treatment had a mean shoot K concentration of 55.85 mg K g^{-1} DW and the 100 mM NaCl salt treatment had a mean shoot K concentration of 38.28 mg K g^{-1} DW. There was significant difference in the mean shoot K concentrations among the twelve rice accessions (p-value < 0.001), and a significant interaction between salt treatments and rice accessions (p-value < 0.001) (**Appendix: Chapter 2**). The variation in shoot K concentration among the rice accessions was lower under the control treatment than under 100 mM NaCl salt treatment (**Figure 2.6**). The largest and significant reduction in shoot K concentration between control and 100 mM NaCl salt treatment within an accession was approximately 50 %, and was recorded in IRGC 12048 (Moroberekan) (29.94 mg K g^{-1} DW), followed by IRGC 101363 (Moroberekan) (26.44 mg K g^{-1} DW); while, the lowest and not significant reduction in shoot K concentration was of approximately 15 %, recorded in IRGC 55969 (IR54) (8.03 mg K g^{-1} DW), followed by IRGC 53435 (IR54) (6.79 mg K g^{-1} DW). All the other eight rice accessions exhibited intermediate shoot K concentration reductions, ranging from 37.9% - 20% (22.64 – 11.64 mg K g^{-1} DW) (**Figure 2.6**, **Appendix: Chapter 2**). The shoot K concentration decreases observed between control and salt 100 mM NaCl treatment were contrasting to the shoot Na concentration within the sensitive and tolerant rice accessions. There was increase of shoot Na concentrations and reduction of shoot K concentrations from control to 100 mM NaCl salt treatment in the sensitive rice accessions IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan); and in tolerant rice accession IRGC 53435 (IR54).

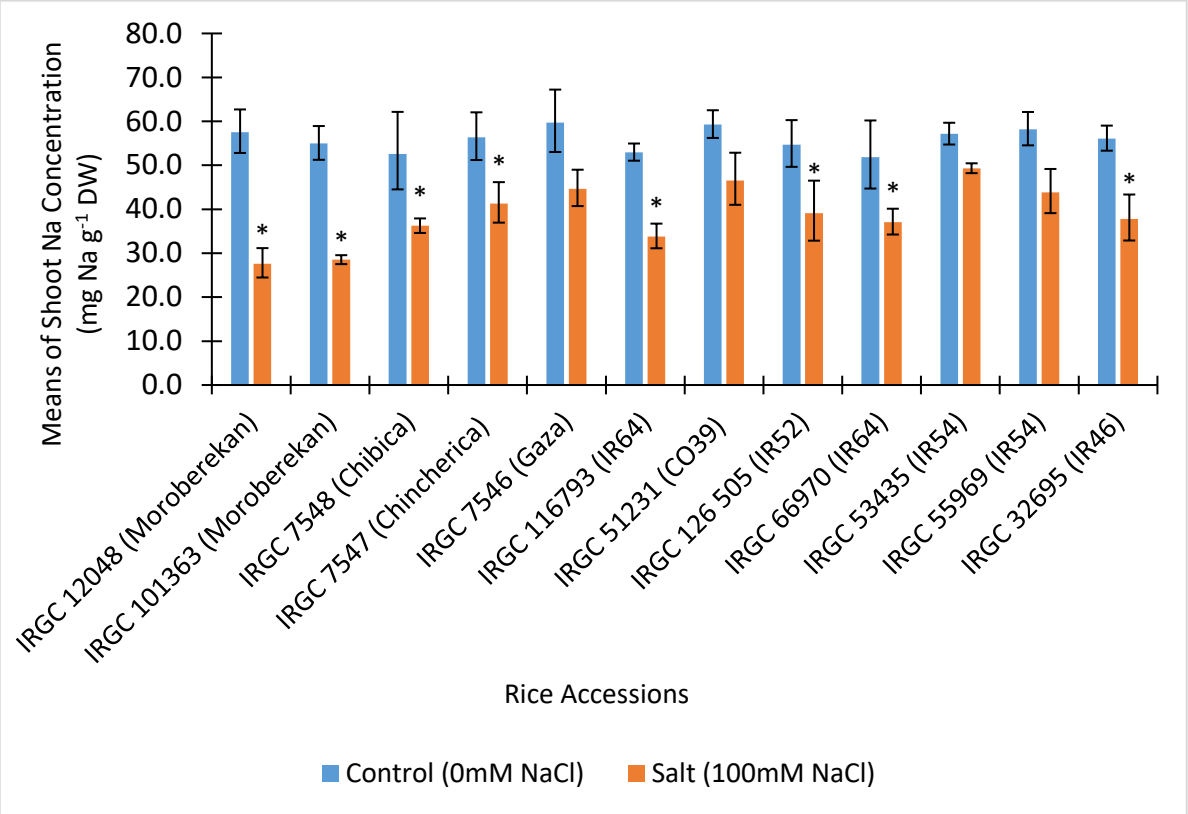


Figure 2.6: Means of shoot K concentration of 60-day old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. Data are shown as mean value + SD of nine individual replications (n=9) and * mark show significant differences (p-value < 0.05).Error bars represent 95% CI.

2.3.5 K^+/Na^+ Discrimination

The ratio of K^+/Na^+ discrimination were calculated from the means of shoot Na concentration and shoot K concentration under the control and 100 mM NaCl. The K^+/Na^+ ratio was lower under salt stress (salt treatment 100 mM NaCl K^+/Na^+ ratio mean = 1.68) compared to control (K^+/Na^+ ratio mean = 137.69) (**Figure 2.7 & Figure 2.8**). However, under the 100 mM NaCl salt treatment, the twelve rice accessions exhibited variation in the reduction of K^+/Na^+ ratio. The lowest value of K^+/Na^+ ratio was observed in the rice accession IRGC 12048 (Moroberekan) (K^+/Na^+ ratio = 0.41), followed by IRGC 101363 (Moroberekan)

(K^+/Na^+ ratio = 0.44), and IRGC 126 505 (IR52) (K^+/Na^+ ratio = 0.89); whereas, highest value of K^+/Na^+ ratio was observed in the rice accession IRGC 55969 (IR54) (K^+/Na^+ ratio = 1.66), followed by IRGC 66970 (IR64) (K^+/Na^+ ratio = 2.44), and IRGC 53435 (IR54) (K^+/Na^+ ratio = 6.68) **(Figure 2.8**). The increase in shoot Na concentration and the decrease in shoot K concentration due to the stress of salinity caused the lower K^+/Na^+ ratio in the sensitive rice accessions IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan) compared to the salt tolerant rice accessions IRGC 53435 (IR54) and IRGC 66970 (IR64).

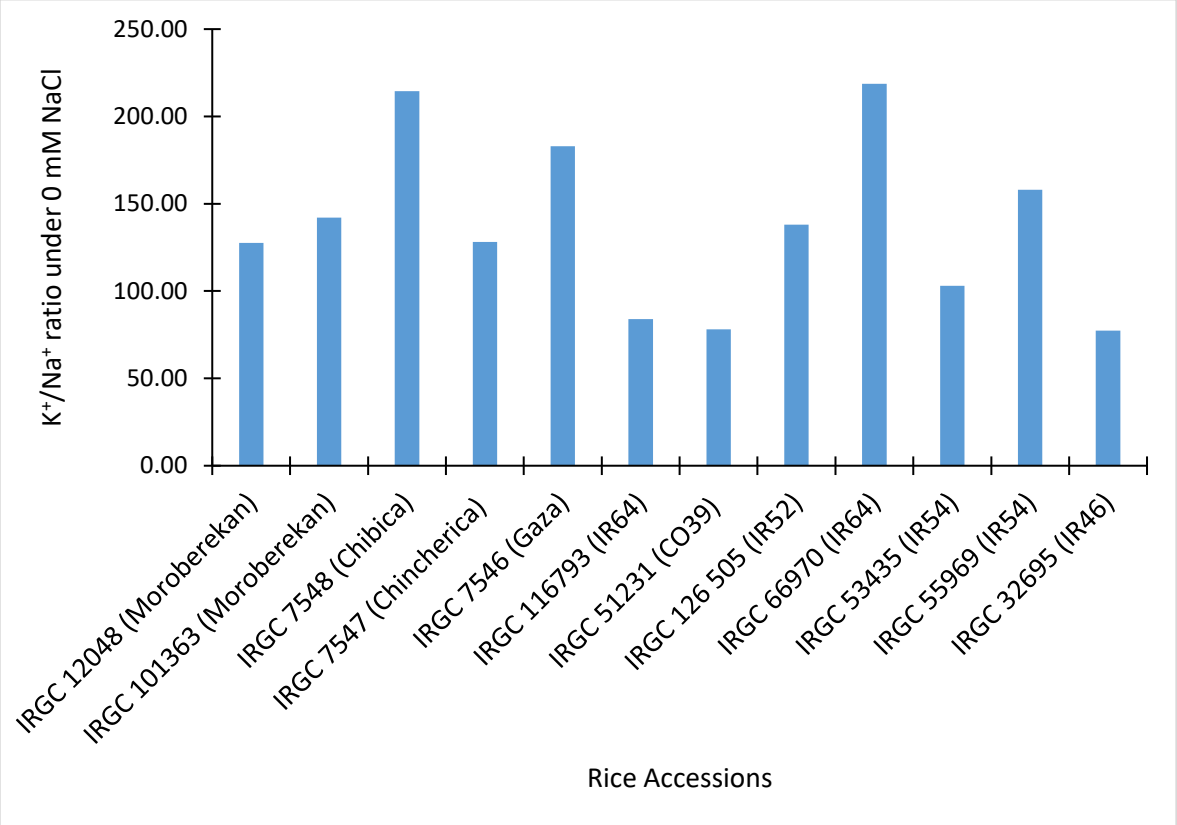


Figure 2.7: K^+/Na^+ Discrimination of 60-day old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. Data are shown as ratio of the means of shoot K concentration and shoot Na concentration under the control treatment.

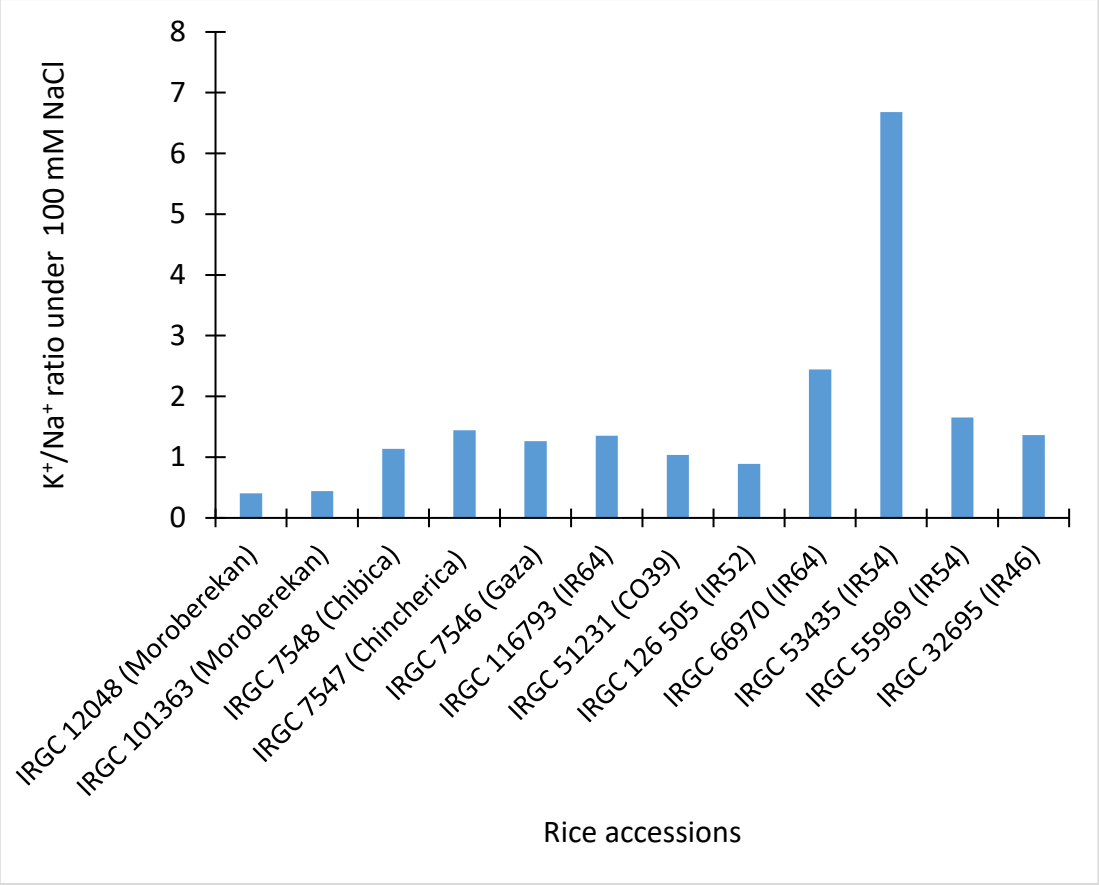


Figure 2.8: K^+/Na^+ Discrimination of 60-days old seedlings. Twelve rice accessions were grown under control (0mM NaCl) and salt treatment (100mM NaCl) in hydroponic system in a glasshouse. Salt stress was imposed for four weeks. The data are shown as ratio of the means of shoot K concentration and shoot Na concentration under the salt treatment 100 mM NaCl.

2.3.6 Relationship between Shoot Dry Weight, Salt tolerance and Shoot Na and K Concentrations

Correlation coefficient among salt tolerance, shoot Na and K concentrations, and shoot K^+/Na^+ ratio were analysed. A high and significant negative correlation between salt tolerance and shoot Na concentration (r = - 0.720), significant positive correlation between shoot K concentration (r = 0.612), and no significant positive between salt tolerance and shoot K^+/Na^+ ratio (r = 0.426) were observed (**Table 2.3**).

Table 2.3 Association coefficient among salt tolerance, shoot Na and K concentrations, and shoot K^+/Na^+ discrimination in rice accessions (n=12), ***** represents significant correlation.

Variable	Means	Std.Dev.	Salt Tolerance	Na^+	K^+	K^+/Na^+
Salt Tolerance	72.290	28.341	1.000			
Na^+	34.868	17.956	-0.720*	1.000		
K^+	38.813	6.769	0.612*	-0.625	1.000	
K^+/Na^+	1.677	1.665	0.426	-0.717	0.590	1.000

2.4 Discussion

2.4.1 Shoot Dry Weight Production Responses to Salt Stress vary widely between Rice Accessions

High concentrations of salt in the soil negatively affects plant growth and productivity, and significantly decreases yield of staple foods including rice (Munns and Tester, 2008, Shahbaz and Ashraf, 2013). The adverse effects of salinity stress were observed in the majority of rice accessions tested in this study. On average, 100 mM NaCl salt treatment significantly reduced shoot dry weight by 34% **(Figure 2.3)**. Greenway and Munns (1980) and Shabala and Munns (2017) observe that under salt conditions, there is a reduction of the movement of assimilates to the meristematic and growing tissues in both leaves and roots of a plant and this effect is more evident on leaves. Gerona et al. (2019) reported a decrease in shoot dry weight by 45% on average when salt stress in the form of 100 mM NaCl was applied to six rice genotypes contrasting in salt tolerance (sensitive, moderate, and tolerant). However, the magnitude was influenced by the genotype, thus there was large variation fluctuating from 14% to 79%; the tolerant genotypes showed an average reduction of 18%; whereas, the sensitive genotypes showed on average a reduction of 75% in shoot dry weight. In this study, a similar response was observed, since there were large variations in salt tolerance among the twelve rice accessions, ranging from 26% to 117%. On average, sensitive rice accessions showed a decrease in shoot dry weight by 70 %, moderately tolerant rice accessions a decrease by 40 %; while, tolerant rice accessions decrease by less than 20% **(Figure 2.4)**.

2.4.2 Na^+ Exclusion

From a physiological point of view, salt stress reduces the rate of photosynthesis and plant water content, disturbs plant metabolism, increases shoot Na concentrations and decreases shoot K, Zn, and P concentrations (Lekklar et al., 2019, Tsai et al., 2019, Razzaq et al., 2020). The addition of 100 mM NaCl to the hydroponic solution increased shoot Na concentration from 0.43 mg Na g^{-1} DW (control plants) to 30.16 mg Na g^{-1} DW (shoot Na concentration increased 98.6% on average). Exclusion of Na-ions in the leaves is a fundamental strategy for salt tolerance (Munns and Tester, 2008). The inability of plant to exclude Na from the transpiration stream may lead to toxic concentrations, causing disruptions to metabolic processes and injury of the photosynthetic cells in transpiring leaves (Greenway and Munns, 1980, Ismail et al., 2007, Munns and Tester, 2008). There was large variation in shoot Na concentrations among the twelve rice accessions. The salt sensitive rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan showed the highest accumulation of shoot Na concentrations of 99.3% and 99.4% respectively; while, the tolerant rice accessions IRGC 32695 (IR46) and IRGC 53435 (IR54) showed the lowest accumulation of 97.4% and 92.5% respectively, from non-saline to saline treatments (**Figure 2.5).** These results are in concordance with the view that under salt stress, salt sensitive varieties of rice accumulate higher concentrations of Na-ions in the leaves than salt tolerant varieties (Flowers et al., 1986, Haq et al., 2009, Haq et al., 2014, Gerona et al., 2019). Commonly, most glycophytic plants (non-native flora of saline soils) rely on strategies that maintain lower concentrations of Na-ions to survive under saline stress, and this is achieved through Na^+ exclusion and/or Na-ions sequestrations in the vacuoles (Munns, 2005, Haq et al., 2014). Therefore, Na^+ exclusion may have been the mechanism underpinning the higher percentages of salt tolerance of rice accessions IRGC 32695 (IR46), and IRGC 53435 (IR54) compared to

accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan. Platten et al. (2013) reports that there is a strong correlation between the concentrations of Na-ions and salt tolerance in many species of rice. In this study, strong negative correlation between salt tolerance and shoot Na concentration, and strong positive correlation between shoot K concentration and salt tolerance were observed (**Table 2.3**). This indicate that salt tolerance decrease with increase of shoot Na concentration, and increase with increase of shoot K concentration. Under 100 mM NaCl salt treatment, the rice accession IRGC 126 505 (IR52) (99.1%) also showed the highest accumulation of shoot Na concentration, following IRGC 12048 (Moroberekan) (99.3%), IRGC 101363 (Moroberekan) (99.4%), and IRGC 7548 (Chibica) (99.2%), but it was among the salt tolerant rice accessions (**Figure 2.5)**.Flowers et al. (1986) observe that a small amount of Na-ions accumulated in the leaves may contribute to osmotic adjustment, and Shabala and Munns (2017) emphasize that increased accumulation of Na- ions combined with its efficient compartmentation in the vacuoles is an effective mechanism for the survival of plants under salinity stress. This indicate that the rice accessions IRGC 126 505 (IR52) may have the ability to effectively compartmentalized Na-ions in the vacuoles and maintain high tolerance to salinity. Rice accession IRGC 126 505 (IR52) showed relatively high shoot Na concentration, but produced more than 70% shoot dry weight of the control, under salinity stress. This high shoot Na concentration was combined with relatively moderate shoot K concentrations (reduction in saline condition = 34.1% /20.21 mg K g^{-1} DW) (**Figure 2.6**) and low K^+/Na^+ ratio (0.89) (**Figure 2.8**), which means that the rice accession IRGC 126 505 (IR52) may have accumulated Na-ions in the leaves for osmotic adjustment, combined with effective compartmentation of Na-ions in the vacuoles and thus increased its tolerance to salt.

The sensitivity to salt stress is influenced by the genotype. Japonica rice subspecies are more sensitive to salt than Indica rice subspecies (Lee et al., 2003). In this study, the Japonica rice subspecies IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan) were the less tolerant to salt with the highest shoot Na concentration compared to the other ten Indica rice subspecies. Previous studies, Haq et al. (2009), Haq et al. (2014), indicate that rice cultivar Moroberekan, accumulated about 75% more Na-ions than the rice cultivar C039 and was less tolerant to salinity, with concentrations of Na and K being measured after 42 d of salt application and plants grown in flood bench system. In our study, the shoot concentrations of Na and K were measured 4 weeks (30 d) after the beginning of salt applications and rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan accumulated about 30% more of Na-ions than the rice IRGC 51231 (CO39) accession, and were the accessions most sensitive to salinity stress. The duration of salt exposure or the growth system may be the reason why rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan accumulated less than 75% more Na-ions. Gregorio and Senadhira 1993 reported that Indica rice subspecies have a higher ability to exclude Na-ions and retain K-ions than Japonica rice; in agreement with this report, in our study Indica rice accessions were more efficient at excluding Na-ions and taking up more K-ions to maintain higher K^+/Na^+ ratio than Japonica rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan.

2.4.3 K^+/Na^+ Discrimination

The Na-ions have the same physico-chemical properties as the K-ions, hence the accumulation of salts result in competition between Na-ions and K-ions for the major

binding sites in fundamental metabolic processes in the cytoplasm (George et al., 2012). Accumulation of Na-ions in the shoots is usually accompanied by the decrease of K-ions in the cytosol, which in turn decreases the K^+/Na^+ ratios and this is generally connected with the reduction of yields (Asch et al., 2000, Haq et al., 2014). In this study, sensitive rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan showed the highest Na-ions increase and the highest K-ions reduction and lowest K^+/Na^+ ratios (**Figure 2.5 - Figure 2.8**). The survival of plants, under salt stress is not determined only by the concentrations of Na-ions, but the K^+/Na^+ ratio (Shabala and Cuin, 2008). Shabala and Munns (2017) assert that in addition to the ability to exclude Na-ions, it is crucial that the K-ions are retained in the cytosol to ensure high K^+/Na^+ ratio and increase the survival under salt stress. The rice accessions IRGC 53435 (IR54), IRGC 66970 (IR64), IRGC 55969 (IR54) showed the lowest Na-ions accumulation and the lowest K-ions reduction and highest K^+/Na^+ ratios, which lead to higher shoot dry weight production and higher tolerance to salinity stress.

2.5 Conclusions

Salinity has negative effect on growth and development of rice crops. On average, salinity stress decreased shoot dry weight of the twelve rice accessions by 34%. However, rice accessions responded differently to the stress of salinity, with some accessions being more tolerant than others. The japonica rice subspecies IRGC 12048 (Moroberekan) and IRGC 101363 (Moroberekan) were the most sensitive, followed by the indica Mozambique (landrace); IRGC 7548 (Chibica), IRGC 7547 (Chincherica), and IRGC 7546 (Gaza), which were moderately tolerant to salinity. Sodium exclusion from the transpiration stream, K- ions retention in the cytosol and increased K^+/Na^+ ratio are the key physiological mechanisms for survival of plants under salt stress. The salt sensitive rice accessions IRGC 12048 Moroberekan and IRGC 101363 Moroberekan were poor at excluding Na-ions, maintain K-ions and increase K^+/Na^+ ratio, compared to the IRRI Mozambican lines IRGC 126 505 (IR52), IRGC 66970 (IR64), IRGC 53435 (IR54), IRGC 55969 (IR54) and IRGC 32695 (IR46). Most tolerant varieties rely on efficient exclusion of Na-ions from the transpiration stream and increase in the uptake of K-ions to maintain growth and cope with salinity stress. Some varieties, combining exclusion of Na-ions from the transpiration stream with effective sequestration of Na-ions in the vacuoles. The IRRI Mozambican line IRGC 126 505 (IR52) appears to have combined Na-ions exclusion and Na-ions sequestrations in the vacuoles, because it showed high shoot Na concentrations and low K^+/Na^+ ratio, but it was among tolerant rice accession, with 84% salt tolerance.

Among the rice genotypes grown in the main rice producing regions of Mozambique, the Indica Mozambique (landraces) are moderately tolerant, while the Indica IRRI Lines in Mozambique are tolerant to high salinity stress at seedling stage. Indica IRRI Lines would be recommended for rice production under salt affected soils in Mozambique.

CHAPTER 3. Seed Priming Approaches to Increase Salt Tolerance of Rice Accessions from Mozambique

3.1 Introduction

We have showed in the previous chapter that the indica landraces rice accessions, representatives of those used in the rice producing regions and ecosystems of Mozambique, are relatively sensitive to salinity and showed a reduction of shoot dry weight of up to 50%, when exposed to 100 mM NaCl salt concentration during the seedling stage in a controlled environment experiment (**Chapter 2**). Therefore, there is a need to identify strategies to ameliorate the impact of salinity that are accessible also to smallholders farmers. The aim of this chapter was to evaluate changes in tolerance to medium-high salinity application (80 mM NaCl) of rice accessions from Mozambique following different seed priming treatments, and establish the physiological mechanisms that confer higher salt tolerance It was hypothesized that priming treatments (hydropriming and halopriming -$CaCl_2$, KCl, and KNO_3) alleviate the negative effect of salinity by increasing mean shoot dry weight and salt tolerance; and altering shoot Na and K concentrations of rice accessions from Mozambique.

3.2 Materials and Methods (Experiment II - 2019)

3.2.1 Plant Material - Selection of Rice Accessions

The most salt sensitive rice accessions were selected from previous experiment, carried out in 2018 (**details in chapter 2**). Therefore, rice accessions for this study represent the five most sensitive accessions (the score was less than 70% salt tolerance) and the most tolerant accession (with 117% of salt tolerance), which was included as standard among tolerant accessions (**Table 3.1**).

Table 3.1 Rice Accessions Assessed for Salt Tolerance under different Priming Treatments

Rice Varieties	IRRI Accession Number	Sub-species	Grown	Salt Tolerance (%)
Moroberekan	IRGC 12048	Japonica	Guinea (West Africa)	26
Chibica	IRGC 7548	Indica	Mozambique (landrace)	51
Chincherica	IRGC 7547	Indica	Mozambique (landrace)	61
Gaza	IRGC 7546	Indica	Mozambique (landrace)	64
IR 64	IRGC 116793	Indica	IRRI line in Mozambique	68
IR 46	IRGC 32695	Indica	IRRI line in Mozambique	117

* **Salt Tolerance (%)**: score derived from experiments described in Chapter 2.

3.2.2 Seed Surface Sterilization and Priming Treatments

For each rice accession and respective priming treatment, 40 seeds were placed in a 15 mL falcon tube and surface sterilized by soaking them in 10 mL 0.8% sodium hypochlorite solution, prepared from commercial bleach 5% NaClO, for 20 minutes over tube rotator (Stuart Scientific CO. LTD; Made in UK). Seeds were then rinsed thrice with autoclaved distilled water, dried with tissue paper and placed in a fresh 15 mL falcon tube (Bado et al., 2016)

Priming treatments, namely: hydropriming, halopriming and non-priming (control) were applied to the disinfected seeds in the falcon tubes. Seeds exposed to hydropriming treatment were soaked in 5 mL of autoclaved distilled water. Seeds exposed to halopriming treatments were immersed in 5 mL of one of the three solutions: (1) 200mM calcium chloride ($CaCl_2$), (2) 278 mM potassium chloride (KCl) and (3) 297 mM potassium nitrate (KNO_3), and generated a final ratio of 1:5 (w/v). The priming solution concentrations created an osmotic potential of Ψ_s= -1.25 MPa. These priming treatments and the respective concentrations were selected because they have been shown to improve the performance of both direct seeded and transplanted rice, under non-saline and saline conditions (Farooq et al., 2007a, Farooq et al., 2007b, Rehman et al., 2011, Afzal et al., 2012, Theerakulpisut et al., 2017). Falcon tubes were placed on the tube rotator (Stuart Scientific CO. LTD; Made in UK) for 36 hours at 25 ^{o}C $\pm$2 ^{o}C. Afterward, soaked seeds were rinsed thrice with autoclaved distilled water, placed in the petri dishes with one layer of filter paper, and re-dried to original weight and moisture level. Drying of seeds was carried out in LEEC plant growth incubator (Models PL2, PL3, and PL33 with JUMO dTRON 316 CONTROL) at 27 ^{o}C $\pm$3 ^{o}C in the dark for 24 hrs. A subset of six centrifuge tubes, each containing the 40 seeds of one of the six rice accessions, was surface sterilized on sowing date and was not primed to serve as control (Ruan et al., 2002, Farooq et al., 2009, Afzal et al., 2012).

3.2.3 Rice Plant Establishment: Hydroponic System Setup, Seed Sowing and Salt Treatment

3.2.3.1 Hydroponic System Setup

Primed rice seeds were germinated and left developing into plants in a supported hydroponic system in a Fitotron plant growth chamber (WEISS GallenKamp), at the School of Agriculture, Policy and Development, University of Reading, United Kingdom. Growth conditions were set up based on rice plants' requirements for grain production; day and night temperatures were 28 ^{o}C and 23 ^{o}C respectively, relative humidity 60%, PAR light intensity 300–600 µmol m^{-2} s^{-1} at plant height and photoperiod 12 h from sowing up to 132 days and 10 h thereafter (Köhl, 2015). Ten dark grey food grade polypropylene tanks (REF: 3-6413-13-CASE GREY RANGE EURO CONTAINER CASE - 26 LITRES (600 X 400 X 155MM).- http://www.plastor.co.uk/) were used as the hydroponic tanks and were aerated with aeration stones, connected to plastic tubes and two air pumps. Rectangular metallic frames of 51x31x12 cm were positioned inside of the ten tanks to support trays with Rockwools Cubes (Grodan Rockwool Cubes 3.8x3.8x3.99 cm - https://www.grodan.com/) with plants (**Figure 3.1**) (Munns and James, 2003, Köhl, 2015, Bado et al., 2016). Nutrient solution was prepared following Yoshida et al. (1976) with modifications made by Gregorio et al. (1997) as described in **Section 2.2.3.1 of Chapter 2**.

3.2.3.2 Seed Sowing

The experiment was set up in a 2x5x6 factorial design (salt treatments vs priming treatments vs rice accessions). Therefore, five tanks corresponding to the five priming treatments were assigned to each salt treatment (control and 80mM NaCl). Primed seeds of the six rice

accessions were directly sown in trays, in which two seeds per Rockwool cube and eight replicates per rice accession were randomly placed (**Figure 3.1**) (Köhl, 2015). The nutrient solution was gradually introduced over three weeks, hence tanks were filled with autoclaved distilled water at sowing and with Yoshida solution ¼ strength, ½ strength and full strength, respectively at leaf stages one, two and three (Munns and James, 2003, Bado et al., 2016).

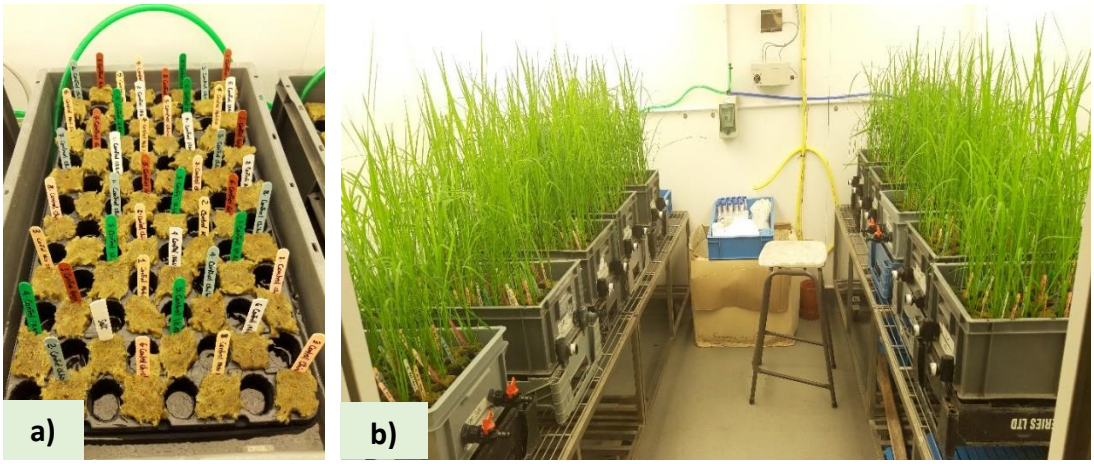


Figure 3.1: Experiment setup: **a)** tank containing nutrient solution, metallic frame with aeration stone at the bottom and Rockwool in trays. In each tank, seeds of the six rice accessions were randomly direct seeded in Rockwool with eight replicates in groups of two seeds per hole (on the left side) and **b)** 25 days old seedlings in different priming treatments (on the right side).

3.2.3.3 Salt Treatment

After seedling establishment, twenty five days after seed sowing, salinity stress was imposed. Dry NaCl was added to the nutrient solution of five tanks. The introduction of salt was carried out over two days with an increment of 40 mM NaCl per day, to reach the final concentration of 80 mM NaCl (Munns and James, 2003, Haq et al., 2014). In this experiment salt concentration was reduced to 80 mM NaCl to allow rice plants to develop up to maturity stage. The concentration of salt was measured with a portable waterproof conductivity meter, Multi-Parameter Testr 35 Series. Autoclaved distilled water was added, in each tank, thrice a week to replace water lost from evaporation and transpiration and keep the original

volume**.** Salinity stress was applied for two months (60 days) and then suspended to allow the plant to recover and produce the grain.

3.2.4 Data Collection

3.2.4.1 Shoot Dry Weight and Salt Tolerance

After 70 days from sowing and 45 days of salt treatment (mid vegetative stage – tillering stage), plants from four replicates per rice accession in all treatments (salt treatment x priming treatment) were collected. These samples were washed with autoclaved distilled water, dried with tissue paper, placed in paper bag and dried to a constant weight in the oven at 80 ^{o}C for 4 d, at which point shoot dry weight was measured. Salt tolerance was calculated as the percentage of shoot dry weight in saline conditions in relation to shoot dry weight in non-saline conditions in all priming treatments. The remaining four replicates were left in the tanks for further studies.

3.2.4.2 Na^+ Exclusion and K^+/Na^+ Discrimination

The above four replicates, collected in all treatments to measure the shoot dry weight, were combined as one sample and ground in a Foss CT 293 Cyclotec Laboratory Mill. From each milled sample, 0.5 g was weighed, placed on MARSXpress digestion tubes, digested, diluted and submitted to ICP analysis for the measurement of tissue Na and K concentrations as detailed on **Section 2.2.4.2**.

3.2.5 Statistical Data Analysis

Three-factor (salt treatments*priming treatments*rice accessions) Analysis of Variance (p-value < 0.05) was used in the statistical package GenStat 19th Edition to assess the differences in the means of shoot dry weight and tissue Na and K concentrations among rice accessions, priming treatments and salt treatments. Salt tolerance was calculated as the percentage of shoot dry weight production under salt stress in relation to the respective control; the ratios of K^+/Na^+ were calculated based on the values of respective tissue Na and K concentrations. The assumptions of ANOVA (Shapiro-Wilk Test for Normality and Test of Homogeneity) were checked and the data were normally distributed.

3.3 Results

3.3.1 Shoot Dry Weight Production

There was a significant difference in mean shoot dry weight (g/plant) between the six rice accessions (p-value < 0.001), two salt treatments (p-value < 0.001), and five priming treatments (p-value < 0.001). There were significant interactions between salt treatments vs priming treatments (p-value < 0.001), salt treatments vs rice accessions (p-value < 0.001), priming treatments vs rice accessions (p-value = 0.019), and salt treatments vs priming treatments vs rice accessions (p-value < 0.001). Therefore, the mean shoot dry weight among the rice accessions are dependent on both salt treatments and priming treatments.

Plants growing in the control (0 mM NaCl) treatment showed the highest mean shoot dry weight (4.11 g/plant) compared to plants growing in the 80 mM NaCl salt treatment (2.36 g/plant). Overall, salt treatment decreased mean shoot dry weight by 43%. Under 80 mM NaCl salt treatment, the higher shoot dry weight was observed in the rice accessions IRGC 7546 (Gaza), IRGC 116793 (IR64), and IRGC 12048 (Moroberekan); whereas, the lower in the rice accessions IRGC 32695 (IR46), IRGC 7547 (Chincherica), and IRGC 7548 (Chibica) (**Appendix: Chapter 3**). However under 80 mM NaCl salt treatment, compared to 0 mM NaCl salt treatment (control), the highest reduction in shoot dry weight was observed on the rice accessions IRGC 12048 (Moroberekan) (61%), followed by IRGC 7547 (Chincherica) (57%), IRGC 7546 (Gaza) (52%) and IRGC 7548 (Chibica) (31%). There was a slight increase in shoot dry weight on the rice accessions IRGC 116793 (IR64) (3%) and IRGC 32695 (IR46) (4%) (**Appendix: Chapter 3**). However, this change was only significant to the rice accession IRGC 12048 (Moroberekan), IRGC 7546 (Gaza), and IRGC 7547 (Chincherica).

Across all rice accessions and salt treatments, priming treatments KCl, KNO_3, H_2O, and Non-primed showed higher mean of shoot dry weight; whereas, priming treatment $CaCl_2$ showed lower mean of shoot dry weight. Overall, priming treatments KCl, KNO_3, and H_2O increased the mean of shoot dry weight by 27%, 24% and 24% respectively; whereas, priming treatment $CaCl_2$ lowered mean of shoot dry weight by 7%, in relation to the non-primed treatment (**Appendix: Chapter 3**).

However, mean shoot dry weight among the rice accessions was dependent on both salt treatment and priming treatments. Under the 80 mM NaCl salt treatment, the highest mean shoot dry weights were observed on the KNO_3 priming treatment for the rice accessions IRGC 12048 (Moroberekan), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), and IRGC 116793 (IR64); on the $CaCl_2$ priming treatment for the rice accession IRGC 7546 (Gaza); and on the KCl priming treatment for the rice accession IRGC 32695 (IR46) (**Table 3.2**). There was an increase in the mean of shoot dry weight of the rice accessions IRGC 12048 (Moroberekan) (47%), IRGC 7548 (Chibica) (26%), IRGC 7547 (Chincherica) (31%), and IRGC 116793 (IR64) (33%) under KNO_3 priming treatment compared to the non-primed treatment, while in IRGC 7546 (Gaza) (18%) a similar effect was observed under $CaCl_2$ priming treatment, and in IRGC 32695 (IR46) (63%) under KCl priming treatment. However, this change was only significant to the rice accession IRGC 32695 (IR46) (**Table 3.2**).

Table 3.2: Effects of salt treatments and priming treatments on mean shoot dry weight (g/plant) of 70-days old seedlings. Six rice accessions (IRGC 12048 (Moroberekan), IRGC 7546 (Gaza), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), IRGC 116793 (IR64) and IRGC 32695 (IR46)) treated with five priming treatments (Non-primed (control), Hydropriming, $CaCl_2$, KCl, and KNO_3) were grown under control (0mM NaCl) and salt treatment (80mM NaCl) in hydroponic system in a Fitotron plant growth chamber. Salt stress was imposed at leaf 3 stage and for 45 days. Data are means (n=4). Means with the same letters are not significantly different within each rice accession column (95% CI)

Salt Treatments	Priming Treatments	Rice Accessions					
		Moroberekan	Gaza	Chibica	Chincherica	IR64	IR46
Control (0mM salt)							
	Non-primed	5.81^{bcd}	5.87^{abc}	1.93^{a}	3.59^{ab}	1.89^{a}	2.06^{ab}
	Hydropriming	6.48^{cd}	9.21^{c}	3.06^{a}	4.52^{bc}	3.19^{ab}	1.82^{ab}
	$CaCl_2$	4.22^{abc}	4.23^{ab}	2.52^{a}	4.30^{bc}	1.62^{a}	1.50^{a}
	KCl	8.43^{d}	7.57^{bc}	3.28^{a}	7.07^{c}	2.01^{a}	2.37^{ab}
	KNO_3	5.63^{bcd}	5.37^{abc}	2.50^{a}	4.25^{abc}	4.75^{b}	2.24^{ab}
80mM salt							
	Non-primed	1.97^{a}	2.92^{ab}	2.19^{a}	1.95^{ab}	2.63^{ab}	1.35^{a}
	Hydropriming	2.64^{ab}	3.19^{ab}	1.60^{a}	1.74^{ab}	2.59^{ab}	2.33^{ab}
	$CaCl_2$	2.24^{a}	3.58^{ab}	0.79^{a}	2.19^{ab}	2.75^{ab}	1.81^{ab}
	KCl	1.32^{a}	2.73^{a}	1.63^{a}	1.41^{a}	1.94^{a}	3.62^{b}
	KNO_3	3.70^{abc}	3.02^{ab}	2.94^{a}	2.83^{ab}	3.91^{ab}	1.27^{a}
s.e.d.= 0.878							

3.3.2 Salt Tolerance

Tolerance to salt of the six rice accessions under each of the five priming treatments was considered as percentage of mean shoot dry weight under salt treatment 80 mM NaCl in relation to the respective 0mM NaCl salt treatment (Control). Rice accessions exhibited different degrees of tolerance to salt within priming treatments. The highest increase in tolerance to salt on the KNO_3 priming treatment were recorded for rice accessions IRGC 12048 (Moroberekan) (32%), followed by IRGC 7547 (Chincherica) and IRGC 7548 (Chibica), with an increase of 12 %, and 5 %, respectively; on the $CaCl_2$ priming treatment increases of 35 % and 31 % were recorded for rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) respectively; an increase of 87% was observed for rice accession IRGC 32695 (IR46) with KCl priming treatment (**Figure 3.2**).

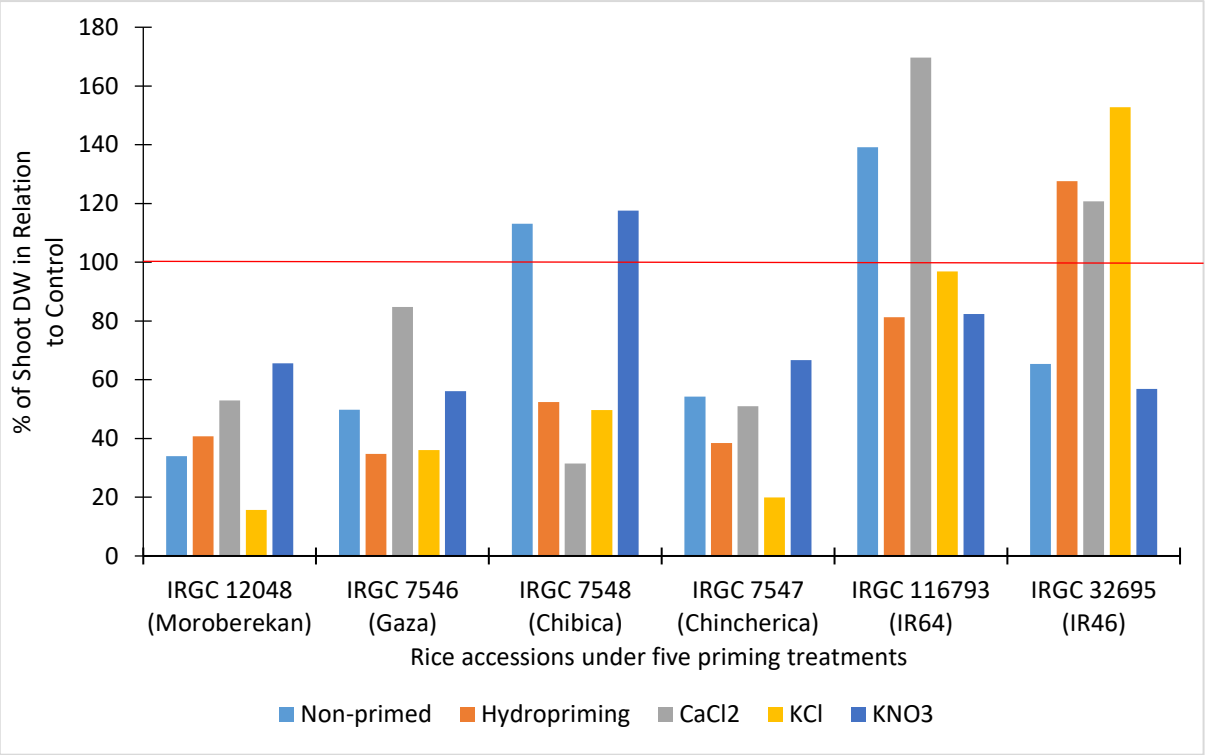


Figure 3.2: Salt tolerance of 70-days old seedlings. Six rice accessions (IRGC 12048 (Moroberekan), IRGC 7546 (Gaza), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), IRGC 116793 (IR64) and IRGC 32695 (IR46)) treated with five priming treatments (Non-primed (control), Hydropriming, $CaCl_2$, KCl, and KNO_3) were grown under control (0mM NaCl) and salt treatment (80mM NaCl) in hydroponic system in a Fitotron plant growth chamber. Salt stress was imposed for 45 days. Data are shown as percentage of the means of shoot dry weight under salt stress in relation to control (n=4). The red line indicate salt tolerance of 100%.

3.3.3 Na^+ Exclusion

As expected, the 0 mM NaCl salt treatment (control) showed lower mean shoot Na concentrations (1.29 mg Na g^{-1} DW) compared to the 80 mM NaCl salt treatment (21.70 mg Na g^{-1} DW). Variation in shoot Na concentration among rice accessions was lower under control, but under 80 mM NaCl salt treatment large variation was observed (**Table 3.3**). Under 80 mM NaCl salt treatment the lowest value of shoot Na concentration were observed on KNO_3 priming treatment for the rice accessions IRGC 12048 (Moroberekan), IRGC 7546 (Gaza), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), and IRGC 116793 (IR64); on KCl

priming treatment the lowest value of shoot Na concentration was observed for rice accession IRGC 32695 (IR46) (**Table 3.3).** There were lower rates of shoot Na concentration accumulation for rice accessions IRGC 12048 (Moroberekan) (92.5%), IRGC 7546 (Gaza) (87.9%), IRGC 7548 (Chibica) (95.5%), IRGC 7547 (Chincherica) (88.7%), and IRGC 116793 (IR64) (76%) under KNO_3 priming treatment compared to the 0 mM NaCl salt treatment (control); and for rice accession IRGC 32695 (IR46) (79.3%) under KCl priming treatment.

3.3.4 K^+/Na^+ Discrimination

Ratios of K^+/Na^+ discrimination were calculated from the values of shoot Na concentration and shoot K concentration under the control and 80 mM NaCl. A an over ten-fold decrease of K^+/Na^+ ratio under salt stress (salt treatment 80 mM NaCl K^+/Na^+ ratio mean = 1.69) compared to control (salt treatment 0 mM NaCl K^+/Na^+ ratio mean = 21.16) was observed. Variation in the K^+/Na^+ ratio among rice accessions was observed in control and 80 mM NaCl salt treatments (**Table 3.3**). Under the 80 mM NaCl salt treatment, the six rice accessions exhibited variation in the reduction of K^+/Na^+ ratio under priming treatments. The highest value of shoot K^+/Na^+ ratios was observed on the KNO_3 priming treatment for rice accessions IRGC 12048 (Moroberekan), IRGC 7546 (Gaza), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), and IRGC 116793 (IR64); on KCl priming treatment for rice accession IRGC 32695 (IR46) (**Table 3.3**).

Table 3.3: Shoot Na concentration (mg Na g^{-1} DW) and K^+/Na^+ ratios of 70-days old seedlings. Six rice accessions were grown under two treatments of salinity control (0mM NaCl) and (80mM NaCl), and five priming treatments, in hydroponic system in a Fitotron plant growth chamber. Salt stress was imposed for 45 days. These values were measured from four replicates of shoot dry weight combined as one sample.

Priming Treatments and Rice Accessions	Na^+ Exclusion		K^+/Na^+ Discrimination	
	Control (0mM NaCl)	80mM NaCl	Control (0mM NaCl)	80mM NaCl
IRGC 12048 (Moroberekan)				
Non-primed	1.37	29.89	13.46	0.87
Hydropriming	1.76	28.64	11.37	1.08
$CaCl_2$	1.41	24.15	16.40	1.18
KCl	1.92	32.24	8.04	0.84
KNO_3	1.37	18.26	13.81	1.35
IRGC 7546 (Gaza)				
Non-primed	1.29	17.86	15.99	1.92
Hydropriming	0.78	17.96	27.55	1.86
CaC_{l2}	1.03	16.08	25.11	2.07
KCl	1.45	12.13	13.69	2.67
KNO_3	1.02	8.45	24.15	3.44
IRGC 7548 (Chibica)				
Non-primed	0.56	25.63	40.43	1.01
Hydropriming	0.56	25.02	48.05	1.20
$CaCl_2$	0.55	48.40	48.20	0.53
KCl	0.70	20.95	30.99	1.55
KNO_3	0.61	13.42	39.74	2.13
IRGC 7547 (Chincherica)				
Non-primed	1.29	24.47	17.09	1.14
Hydropriming	1.16	32.92	21.59	0.91
$CaCl_2$	1.73	24.39	14.70	1.25
KCl	1.81	23.23	10.38	1.31
KNO_3	1.14	10.11	21.45	3.28
IRGC 116793 (IR64)				
Non-primed	1.59	16.59	14.74	1.93
Hydropriming	1.29	16.28	20.17	1.81
$CaCl_2$	1.76	15.46	12.65	1.88
KCl	1.81	19.03	12.65	1.72
KNO_3	1.73	7.22	11.69	3.47
IRGC 32695 (IR46)				
Non-primed	1.84	33.37	14.13	0.88
Hydropriming	1.13	25.89	28.30	1.15
$CaCl_2$	1.19	35.66	24.21	0.79
KCl	1.72	8.30	11.89	3.85
KNO_3	1.20	19.09	22.09	1.59

3.3.5 Relationship of salt tolerance, and the physiological mechanisms: Na^+ concentrations and K^+/Na^+ ratio

There was strong negative and significant correlation between the shoot Na concentrations and salt tolerance (r = - 0.9587, p-value = 0.0100), and strong positive and significant correlation between shoot K^+/Na^+ discrimination and salt tolerance (r = 0.9575, p-value = 0.0105) for the rice accession IRGC 12048 Moroberekan. There was moderate negative correlation between the shoot Na concentrations and salt tolerance and moderate positive correlation between shoot K^+/Na^+ discrimination and salt tolerance for the rice accession IRGC 7548 (Chibica), IRGC 7547 Chincherica and IRGC 32695 (IR46). There was a weak positive correlation between the shoot Na concentrations and salt tolerance and weak negative correlation between shoot K^+/Na^+ discrimination and salt tolerance for the rice accession IRGC 116793 (IR64) and IRGC 7546 Gaza. However, the association between shoot Na concentrations, and K^+/Na^+ ratio with the salt tolerance was only significant for the rice accession IRGC 12048 Moroberekan (**Table 3.4**).

Table 3.4: Association between salt tolerance, shoot Na concentrations, shoot K^+/Na^+ discrimination and shoot K concentrations in the shoots of rice accessions (the correlation coefficient n=5), and the respective p-values.

Shoot Na^+, K^+/Na^+, and K^+	Salt tolerence					
	Rice Acessions					
	Moroberekan	**Gaza**	**Chibica**	**Chincherica**	**IR64**	**IR46**
Na^+	- 0.9587	0.0010	- 0.6575	- 0.5774	0.2880	- 0.3553
K^+/Na^+	0.9575	- 0.0190	0.5684	0.6246	- 0.3854	0.5384
K^+	- 0.2099	- 0.0443	- 0.2115	0.3116	0.2616	0.3070

Shoot Na^+, K^+/Na^+, and K^+	p-value					
	Rice Acessions					
	Moroberekan	**Gaza**	**Chibica**	**Chincherica**	**IR64**	**IR46**
Na^+	0.0100	0.9988	0.2278	0.3080	0.6384	0.5574
K^+/Na^+	0.0105	0.9758	0.3174	0.2600	0.5217	0.3493
K^+	0.7347	0.9437	0.7327	0.6098	0.6707	0.6153

3.4 Discussion

Salt stress reduces growth of crops, including rice (Munns and Tester, 2008, Shahbaz and Ashraf, 2013, Gerona et al., 2019) and seed priming has been reported as alleviating the negative effect of abiotic stress, including soil salinity (Taylor et al., 1998, McDonald, 2000, Farooq et al., 2006a, Iqbal et al., 2006). In this study, under 80 mM NaCl salt treatment we observed an overall reduction of shoot dry weight production by 43%. The negative effect was more prominent in the rice accessions IRGC 12048 (Moroberekan), IRGC 7547 (Chincherica), IRGC 7546 (Gaza) and IRGC 7548 (Chibica). Under 80 mM NaCl salt treatment, KCl priming treatment increased shoot dry weight and salt tolerance of rice accession IRGC 32695 (IR46), $CaCl_2$ priming treatment increased shoot dry weight and salt tolerance of rice accession IRGC 7546 (Gaza) and increased salt tolerance of IRGC 116793 (IR64) (**Table 3.2 & Figure 3.2**). These results are in concordance with Afzal et al. (2012) who noticed that seed priming salt tolerant and salt sensitive fine aromatic rice cultivars with 200mM $CaCl_2$ or 278 mM NaCl KCl salt solutions of Ψ_s= -1.25 MPa osmotic potential, for 36 h at 25 $^oC \pm 2\ ^oC$ room temperature, improved shoot dry weight and salt tolerance, under moderate salinity stress of 40 and 80 mM NaCl; furthermore, seed priming coarse and fine rice with the same inorganic salt solution, for 48 h at 27 ^oC + 3 ^oC , increased the emergence of seedlings and crop establishment of direct seeded rice grain under normal conditions in farmer fields (Farooq et al., 2007a, Rehman et al., 2011). The positive effects of priming were higher under KCl than $CaCl_2$ priming treatments under normal conditions (Farooq et al., 2007a). Conversely, Theerakulpisut et al. (2017), found that priming rice seeds with 200 mM $CaCl_2$ or 200 mM KCl, for 48 hours at room temperature, to alleviate the effect of 150 mM NaCl salinity stress did not affect shoot dry weight. They presumed diverging of their results from those reported from other groups was associated with the genotype and the concentration

of priming solution applied in the different studies. In this study, the effect of priming depended on both salt treatment and rice accession, with the rice accession IRGC 7546 (Gaza), IRGC 116793 (IR64), and IRGC 32695 (IR46) grown at 80mM NaCl salt concentrations responding positively to the $CaCl_2$ and KCl priming treatments. It should be noted that, the KCl priming concentration from Theerakulpisut et al. (2017) study was relatively lower and salt concentration was higher than in the present study. The alleviation of salinity impact is achieved through the improvement of the growth parameters (roots, leaves, stems) and also considerable change in Na-ions and K-ions concentrations, which lead to higher ability for osmotic adjustment (Cayuela et al., 1996, Iqbal and Ashraf, 2007, Afzal et al., 2008). Afzal et al. (2012) noticed that priming with $CaCl_2$ or KCl decreased Na-ions and increased K-ions concentrations in the leaves, which resulted in better salt tolerance of salt tolerant and sensitive fine aromatic rice cultivars. Under the KCl priming treatment, the rice accession IRGC 32695 (IR46) showed the lowest shoot Na-ions accumulation (79.3% shoot Na-ions increase from non-saline to saline) and highest K^+/Na^+ ratio (3.85) (**Table 3.3**). These may have contributed to the increase of salt tolerance of this accession, under KCl priming treatment, though the association between salt tolerance, shoot Na concentration, shoot K concentration and shoot K^+/Na^+ ratio was moderate and not statistically significant (**Table 3.4**). The same change in the shoot Na concentrations and K^+/Na^+ ratio was not observed for the rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) under $CaCl_2$ treatment, although improved salt tolerance in these accession was observed following priming with this salt (**Table 3.3**). Moreover, there was a weak and positive association between salt tolerance and shoot Na concentrations, concomitantly with and a weak negative association with shoot K^+/Na^+ ratios in IRGC 7546 (Gaza) and IRGC 116793 (IR64) (**Table 3.4**). These indicate that Na accumulation in the transpiration stream may favour salt tolerance in these

accessions. It has indeed been reported that a small amount of Na accumulated in the leaves may contribute to osmotic adjustment and ensure survival of plants under salinity stress, as long as this is combined with efficient compartmentation in the vacuoles (Flowers et al., 1986, Shabala and Munns, 2017). Moreover, priming seeds with $CaCl_2$ solution benefit the seeds via the influence of Ca^{2+} on membranes (Shannon and Francois, 1977)**.** Calcium (Ca^{2+}) is reported to have a key role in the processes that preserve the integrity of plant membrane structures and functions, in the stabilization of cell wall structures, in regulation of ion transport and discrimination, control of ion-exchange performance, and the activities of cell wall enzyme (Rengel, 1992, George et al., 2012). However, Ca^{2+} is easily replaceable by other cations in the membranes bindings sites, which may compromise its functions if its availability is substantially reduced (Tuna et al., 2007). For this reason, supplementation of Ca^{2+} in saline solution may contribute in the alleviation of ion toxicities, in particular for plants that are susceptible to sodium injury (Maas, 1993, Grattan and Grieve, 1998). Afzal et al. (2012) observed that $CaCl_2$ priming treatment offer protection to adverse impact of salt stress and enhance crop growth under saline conditions. This could also be the reason for the increase of salt tolerance of rice accession IRGC 7546 (Gaza) and IRGC 116793 (IR64) under $CaCl_2$ priming treatment.

Priming with KNO_3 increased shoot dry weight of the rice accessions IRGC 12048 (Moroberekan), IRGC 7548 (Chibica), IRGC 7547 (Chincherica), and IRGC 116793 (IR64) (**Table 3.2**). There was also an increase of salt tolerance of the rice accessions IRGC 12048 (Moroberekan), IRGC 7548 (Chibica), IRGC 7547 (Chincherica) (**Figure 3.2**). Similarly, Theerakulpisut et al. (2017) observed that priming with 0.25%, 0.50% or 0.75% KNO_3 for 48 hours at room temperature decreased the negative impact of 150 mM NaCl salinity stress in growth of young rice seedlings. They observed that under salt stress there was an increase

of shoot dry weight by about 25 - 31.15% and the ratio Na^+/K^+ were relatively low. In this study, the lowest shoot Na concentrations and highest K^+/Na^+ ratios of the rice accessions IRGC 12048 (Moroberekan), IRGC 7548 (Chibica), and IRGC 7547 (Chincherica) were recorded under the KNO_3 priming treatment (**Table 3.3**). The rice accession IRGC 12048 (Moroberekan) may have benefited by the changes in Na and K concentration, since there was a strong association between salt tolerance, shoot Na concentration, shoot K concentration and shoot K^+/Na^+ ratio. However, there was moderate and not significant association for the rice accession IRGC 7548 (Chibica), and IRGC 7547 (Chincherica), which indicate that for these accessions may have additional mechanisms conferring higher salt tolerance of these accessions under KNO_3 priming treatment (**Table 3.4**). In addition to providing K, priming with KNO_3 may also provide nitric oxide (NO). NO is a gaseous redox-active molecule, which plays an important role in plant growth and development, because it is involved in protection strategies against abiotic stresses (Kim et al., 2014, Fancy et al., 2017, Adamu et al., 2018). It is reported in the literature that NO metabolizes and produces osmolytes in plants, reducing the negative effects of abiotic stresses (Ahmad et al., 2017). Ruan et al. (2004) observed in wheat, that NO activated the biosynthesis and accumulation of proline, improving the maintenance of ion homeostasis.

3.5 Conclusions

80 mM NaCl Salt treatment from leaf 3 stage decreased growth of the rice accessions. Overall, there was a reduction of shoot dry weight by 43%. The reduction was more prominent on the rice accessions IRGC 12048 (Moroberekan), IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 7548 (Chibica). Salt treatment was associated with an increase of shoot Na concentrations, and decrease of K^+/Na^+ ratios in all six rice accessions. Priming treatments had significant impact on the production of shoot dry weight of rice accessions. Some priming treatments were helpful under non-saline and others also ameliorated the impact of 80 mM NaCl salt treatment. KCl priming treatment increased shoot dry weight and salt tolerance, and these effects were accompanied in the rice accession IRGC 32695 (IR46) by a low accumulation of Na-ions in the shoot and increased K^+/Na^+; $CaCl_2$ priming treatment increased shoot dry weight and salt tolerance of the rice accession IRGC 7546 (Gaza), and salt tolerance of IRGC 66970 (IR64), but did not show the lowest shoot Na concentration and highest K^+/Na^+ ratio. This indicates that rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) may have been benefited by the increased concentrations of Na in the shoot or that $CaCl_2$ offered a specific mechanism for plant survival under salt stress. KNO_3 priming treatment increased shoot dry weight, salt tolerance, lowered the shoot Na concentrations and increased K^+/Na^+ of the rice accessions IRGC 12048 (Moroberekan), IRGC 7547 (Chincherica), and IRGC 7548 (Chibica). However, except, for rice accession IRGC 12048 (Moroberekan), there was no strong association between shoot Na concentration, shoot K concentration and shoot K^+/Na^+ ratio and salt tolerance in IRGC 7547 (Chincherica), and IRGC 7548 (Chibica). KNO_3 may have provided additional mechanism for better plant performance under salt stress. Therefore, changes in shoot Na and K concentrations are not the only strategic physiological mechanisms promoted by priming treatments for the survival of

plants under salt stress. The results suggest that there are multiple strategies of seed protection provided by KCl, KNO_3, and $CaCl_2$ priming treatments.

Seed priming treatments (KCl, KNO_3, and $CaCl_2$) showed a potential to increase salt tolerance in rice accessions cultivated in Mozambique under medium-higher salinity stress. KCl seed priming treatment would be recommended for Indica IRRI lines in Mozambique, $CaCl_2$ seed priming treatment for both Indica Mozambique (landraces) and Indica IRRI lines in Mozambique, whereas KNO_3 seed priming treatments for Indica Mozambique (landraces).

CHAPTER 4. Impact of Seed Priming on Germination of Mozambique Rice Accessions under Salty Water Imbibition

4.1 Introduction

In experiments reported in chapter 3 of this thesis, we showed that, $CaCl_2$ and KNO_3 priming treatments increased shoot dry weight and salt tolerance of indica Mozambique (landrace) rice accessions IRGC 7547 (Chincherica) and IRGC 7546 (Gaza), and the IRRI line in Mozambique IRGC 66970 (IR64), when these were grown under 80 mM NaCl salt concentration. Therefore, the aim of this chapter was to quantify the impact of medium high salt stress and different priming treatments on germination, plant growth and development of rice accessions from Mozambique. It was hypothesized that salinity stress decreases mean percent germination, root and shoot lengths of seedlings of rice accessions from Mozambique, while priming treatments reduce these negative impact of salinity stress.

4.2 Materials and Methods

4.2.1 Plant Material and Priming Treatments

The rice accessions and priming treatments for this experiment were selected based on the results of the previous experiment (**Experiment II: 2019, Chapter 3**).

Table 4.1 Rice accessions and priming treatments for the germination test.

Rice accessions	Grown	Priming treatments
IRGC 7546 Gaza	Mozambique (landrace)	$CaCl_2$, KNO_3
IRGC 7547 Chincherica	Mozambique (landrace)	KNO_3, $CaCl_2$
IRGC 116793 IR 64	IRRI line in Mozambique	$CaCl_2$, KNO_3

The order of priming treatments indicate which provided best results on previous study **(Chapter 3)*

4.2.2 Seed Surface Sterilization

A total of 50 seeds per rice accession was separately placed in nine (three rice accessions x three priming treatments) 15 mL falcon tubes, surface sterilized as described in **Section 3.2.2 of Chapter 3**

4.2.3 Seed Priming

Priming treatments, namely: halopriming (200mM calcium chloride ($CaCl_2$) and 297mM potassium nitrate (KNO_3)) and non-priming (control) were applied to the sterilized seeds in the falcon tubes. These priming treatments were prepared as described in **Section 3.2.2 of Chapter 3**.

4.2.4 Seed Germination Setup

The experiment was laid out in a 2x3x3 factorial design with two salt treatments (0 mM NaCl and 80 mM NaCl salt concentrations), three rice accessions (IRGC 7547 (Chincherica), IRGC 7546 (Gaza) and IRGC 66970 (IR64)) and three priming treatments ($CaCl_2$, KNO_3, non-primed) with four replicates of 25 seeds each. This was carried out over four weeks with one replicate per week. An 80 mM NaCl solution and autoclaved distilled water were prepared. Sets of four HOSTESS 230 mm x 310 mm paper towels were arranged, and a line was drawn in the middle of the upper paper. Subsequently, each set of four paper towels was immersed either in a saline solution or in autoclaved distilled water, and wrung to reduce excess moisture. Twelve/thirteen seeds were placed in the middle line on top of the three stacked papers and a fourth paper towel was used to cover the seeds, leaving a space of about 2 cm on the left and basal edges. Afterward, each arrangement of seeds was rolled loosely and placed in plastic bags. The plastic bags were labelled and kept in the LEEC plant growth incubator (Models PL2, PL3, and PL33 with JUMO dTRON 316 CONTROL) at 34/11 °C day/night with 16hrs light/8 hrs dark (Ueno and Miyoshi, 2005). Germinated seed was recorded on eighth day according to the Association of Official Seed Analysis (AOSA), who recommend a germination test duration of 5 to 14 days for rice crop, with a deviation of one to three days allowed (AOSA, 1993)(**Figure 4.1**).

Figure 4.1: Procedure for seed germination: **A)** sets of four papers towels immersed either in a saline (80 mM NaCl) solution or autoclaved distilled water. **B-E)** Seeds sown on moist paper towel, which were loosely rolled, placed in labelled plastic bags and **F)** kept in the incubator at 34/11 °C day/night with 16hrs light/8 hrs dark for eight days.

4.2.5 Data Collection

After eight days in the incubator, seeds were removed and prepared for the measurement of percent germination, root and shoot lengths. A black cardboard of approximate size to the paper towels was prepared, a horizontal line in the middle and a scale in one of the vertical side of the cardboard were drawn. The seeds in each set of four paper towels were transferred to the middle line of the black cardboard, maintaining their position from the paper towels. Individual photos were taken with a mobile phone digital camera and stored as jpg images (**Figure 4.2**).

Figure 4.2: jpg images with seeds of the rice accession IRGC 7546 Gaza, primed with 200mM $CaCl_2$, sown in 80mM NaCl saline solution, germinated in the incubator at 34/11˚C day/night with 16hrs light/8 hrs dark for eight days, and transferred to the black cardboard. The seeds follow the same position as in the paper towels during the germination period.

4.2.5.1 Percent Germination

The percent germination was calculated as the number of the germinated seeds in relation to the total number of sown seeds per replicate. A seed was considered as germinated when root length reached 2 mm (Janmohammadi et al., 2008, Tahjib-Ul-Arif et al., 2018).

4.2.5.2 Seminal Root and Shoot Visualization.

The root and shoot lengths were measured in the software packages SmartRoot, ImageJ 1.53a and RootReader2D v4. The jpg images were converted to greyscale in the SmartRoot software package and stored as “tif” files. The vertical scale in each “tif” file was converted from centimetre to pixel under the software package ImageJ 1.53a. Therefore, from the software ImageJ 1.53a, the tool *straight*, segmented or freehand, or arrows was selected

and a vertical line between two numbers, on the vertical scale from the “tif” image, was drawn. In this study the same numbers, 4 and 5, were used in all “tif” images (**Figure 4.3**). Thereafter, the length of roots and shoots were quantified on RootReader2D v4 software package.

4.2.5.3 RootReader2D v4 Software Setup

The tools Options, Measurement and Modify under the RootReader2D toolbar were configured for this data collection. The Options tool was set for Image Processing and Root Selecting. Therefore, under the Image Processing four options were selected: Bright roots on dark background, Double Adaptive Thresholding, Use Dust Removal Filter (Runs after thresholding), and Use Filling Filter (Runs after thresholding and/or dust removal). While, two options were selected from Root Selecting: Selected roots share a common endpoint (e.g. the seed), and Automatic prediction of furthest endpoint (must select a start endpoint first, e.g. the seed). The Measurement tool was set for the options Set Dust Removal Filter and Set Scale. The Set Dust Removal Filter was 25 pixels, and Set Scale was the respective value converted from centimetre to pixels in ImageJ 1.53a. The Modify tool was adjusted in the option Set Pencil/Eraser Size to 25 pixels.

4.2.5.4 Seminal Root and Shoot Lengths Measurement

Single image saved in grayscale as “tif” file was opened under the RootReader2D v4 Software and the roots and shoots were separately measured. Therefore, to each root or shoot, the tools (Threshold, Region of Interest, Skeletonize, Build Segments and Measure) were applied. Data on the Measuring Log were saved as “csv” file, from which the column

corresponding to the longest root or shoot was selected for the statistical analysis (**Figure 4.3**).

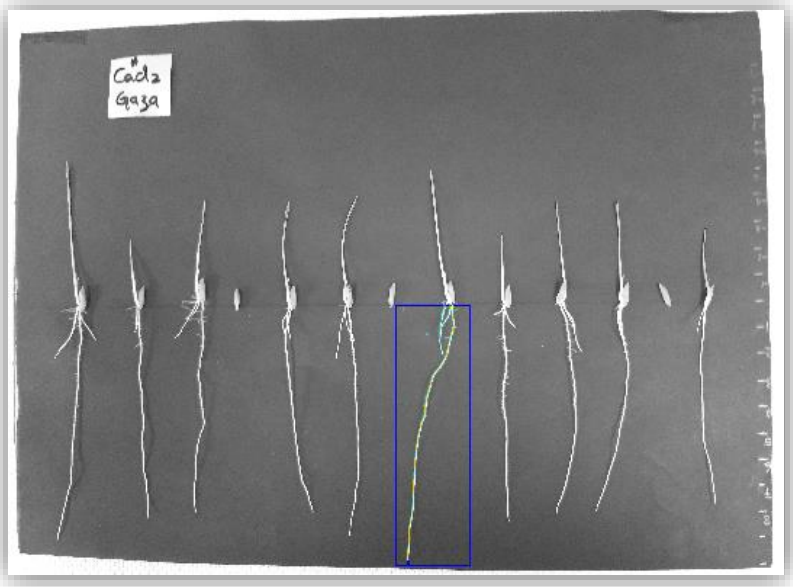


Figure 4.3: jpg images converted to greyscale and stored as "tif" file. From each "tif" image the vertical scale was converted from centimetre to pixel under the software package ImageJ 1.53a. Root of the rice accession IRGC 7546 Gaza, primed with 200mM $CaCl_2$, sown in a saline solution of 80mM NaCl, germinated in the incubator at 34/11◦C day/night with 16hrs light/8 hrs dark for eight days, and ready for the measurement. The root was thresholded, the region of Interest selected, skeletonized, build segments and measured on the RootReader2D v4 Software.

4.2.6 Statistical Analysis

Three-factor (salt treatments*priming treatments*rice accessions) Analysis of Variance (p-value < 0.05) was used in the statistical package GenStat 19th Edition. The difference in the means of percent germination, root and shoot lengths were assessed using Bonferroni test at P<0.05. The assumptions of ANOVA ((Shapiro-Wilk Test for Normality and Test of Homogeneity) were checked and the data were homogenous and normally distributed.

4.3 Results

4.3.1 Percent Germination of Primed and Non-Primed Rice Seedlings Grown under Salt Treatments

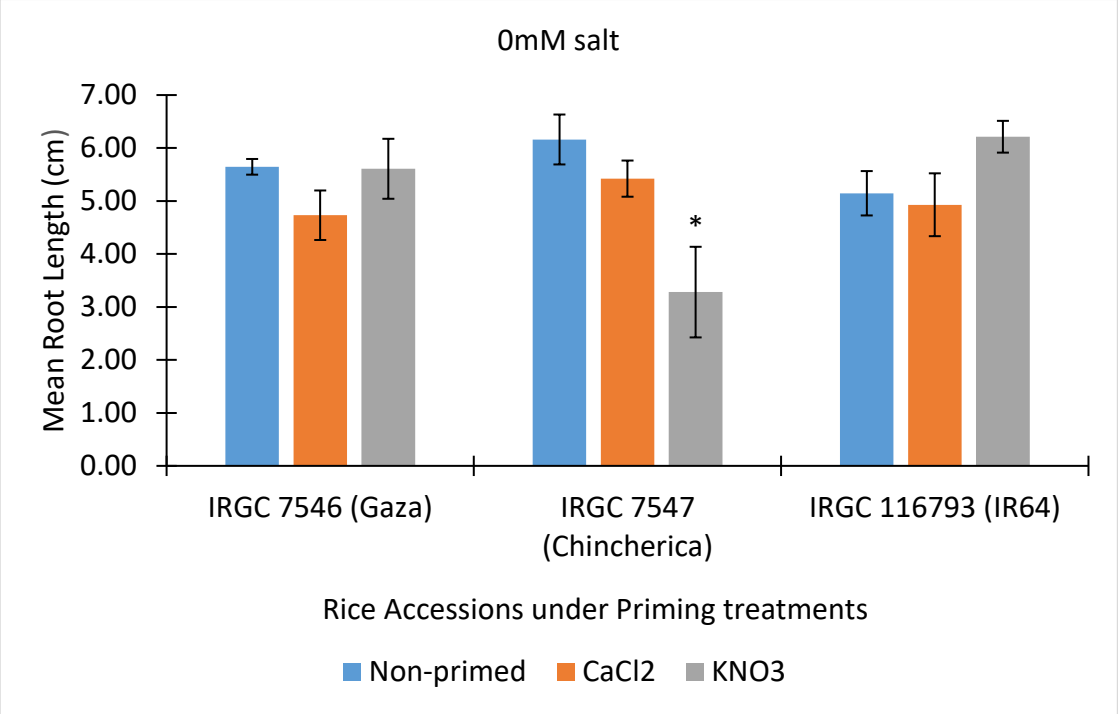
Mean percent germination was not significantly different between the two salt treatments (p-value = 0.947). There were also no significant interactions between salt treatments and priming treatments (p-value = 0.725), salt treatments and rice accessions (p-value = 0.875), priming treatments and rice accessions (p-value = 0.076), and salt treatments, priming treatments and rice accessions (p-value = 0.874). However, there was significant difference in mean percent germination between the three priming treatments (p-value < 0.001) and the three rice accessions (p-value < 0.001) (**Appendix: Chapter 4**). Overall, the KNO_3 priming treatment, had the lowest percent germination of 74.68%; whereas, $CaCl_2$ priming treatment and non-primed treatment had similar mean percent germination of 86.32 % and 94.32 %, respectively. Rice accession IRGC 7546 (Gaza), had the lowest percent germination of 70%. Rice accession IRGC 7547 (Chincherica) and IRGC 116793 (IR64) had similar mean percent germinations of 90.84 % and 94.48 %, respectively.

4.3.2 Root Length of Primed and Non-primed Rice Seedlings Grown under Salt Treatments

Mean root length was not significantly different between three rice accessions (p-value = 0.157), two salt treatments (p-value =0.977), or three priming treatments (p-value =0.210). There were no significant interactions between salt treatments and priming treatments (p-value =0.908), salt treatments and rice accessions (p-value =0.311), and salt treatment, priming treatments and rice accessions (p-value =0.634). However, there was significant

interaction between priming treatments and rice accessions (p-value =0.003) (**Appendix: Chapter 4**).

$CaCl_2$ and KNO_3 priming treatments decreased mean root length of rice accessions IRGC 7546 (Gaza) and IRGC 7547 (Chincherica), but this reduction (about 35% of mean root length) was only significant for rice accession IRGC 7547 (Chincherica) for the KNO_3 priming treatment compared to the non-primed treatment. $CaCl_2$ priming treatment decreased mean root length, while KNO_3 priming treatment increased mean root length, of rice accession IRGC 116793 (IR64). However, these changes were not significant on either of priming treatments (**Figure 4.4**).



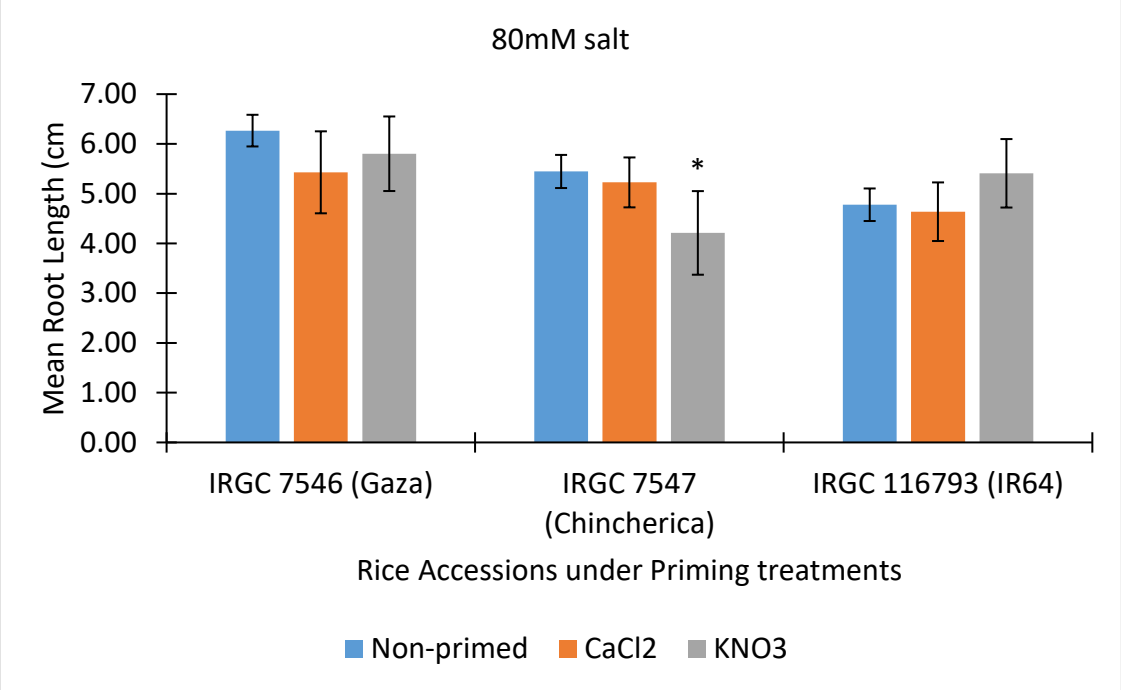
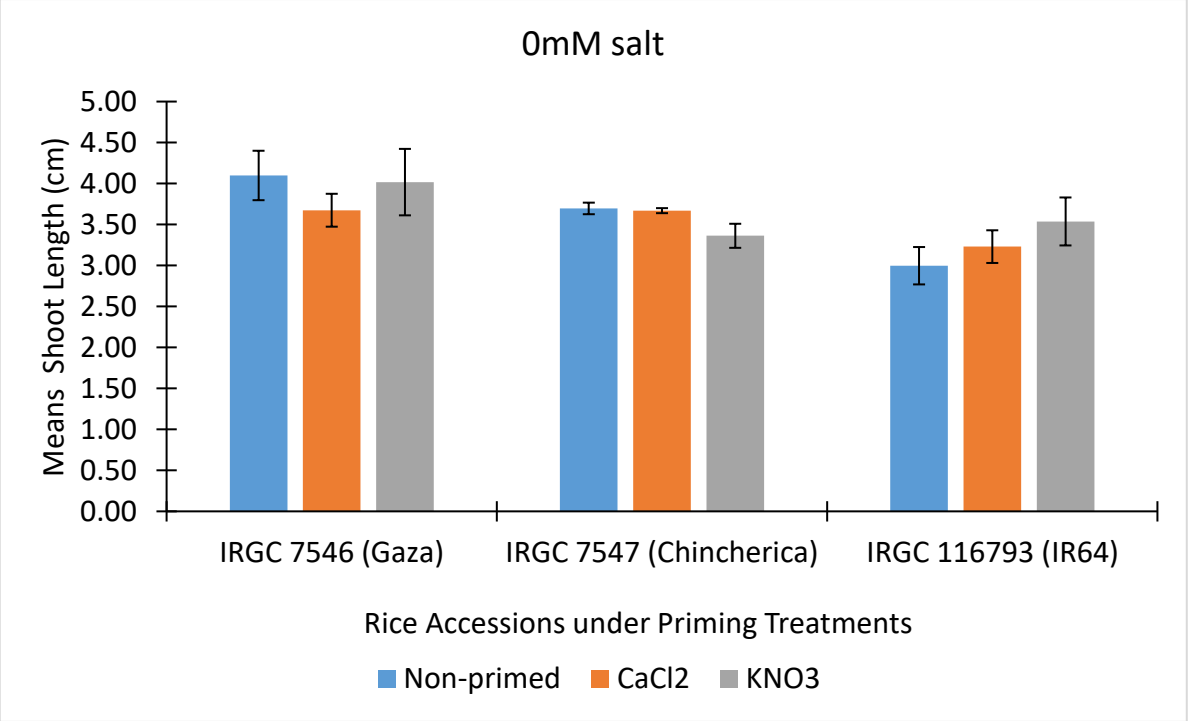


Figure 4.4: Effect of priming treatments on mean root length of 8-days old seedlings. Three rice accessions (IRGC 7546 (Gaza), IRGC 7547 (Chincherica) and IRGC 116793 (IR64) treated with three priming treatments (non-primed,200 mM $CaCl_2$, and 297 mM KNO_3) were grown in an incubator at 34/11°C. day/night with 16hrs light/8 hrs dark. Data are shown as mean value +/- SEM of hundred individual replications (n=100). The *sign means significantly different (p- value < 0.05) within each rice accession. The error bars represent SEM.

4.3.3 Shoot Length of Primed and Non-primed Rice Seedlings Grown under Salt Treatments

Mean shoot length was not significantly different between priming treatments (p-value =0.362). The interactions between salt treatments and priming treatments (p-value =0.612), salt treatment and rice accessions (p-value =0.678), salt treatment, priming treatments and rice accessions (p-value =0.992) were also not significant. However, there was a significant difference in mean shoot length between salt treatments (p-value <0.001) and between rice accessions (p-value <0.001). There was also a significant interaction between priming treatments and rice accessions (p-value =0.028) (**Appendix: Chapter 4**). The 0 mM NaCl treatment had higher mean shoot length (3.587 cm) compared to 80 mM NaCl (2.297 cm). Rice accessions IRGC 66970 (IR64), and IRGC 7547 (Chincherica) had the lowest mean shoot lengths of 2.588 cm and 2.893 cm respectively, while rice accession IRGC 7546 (Gaza) had the highest mean shoot length of 3.345 cm.

$CaCl_2$ and KNO_3 priming treatments slightly decreased mean shoot length of the rice accession Gaza and slightly increased mean shoot length of rice accession IRGC 116793 (IR64); $CaCl_2$ priming treatment increased and KNO_3 priming treatments decreased mean shoot length of rice accession IRGC 7547 (Chincherica). However, within each rice accession there was no significant difference in mean shoot length; and among rice accessions there was only significant difference between rice accessions IRGC 7546 (Gaza) and IRGC 66970 (IR64) under non-primed treatment (p-value =0.0143) (**Figure 4.5**)



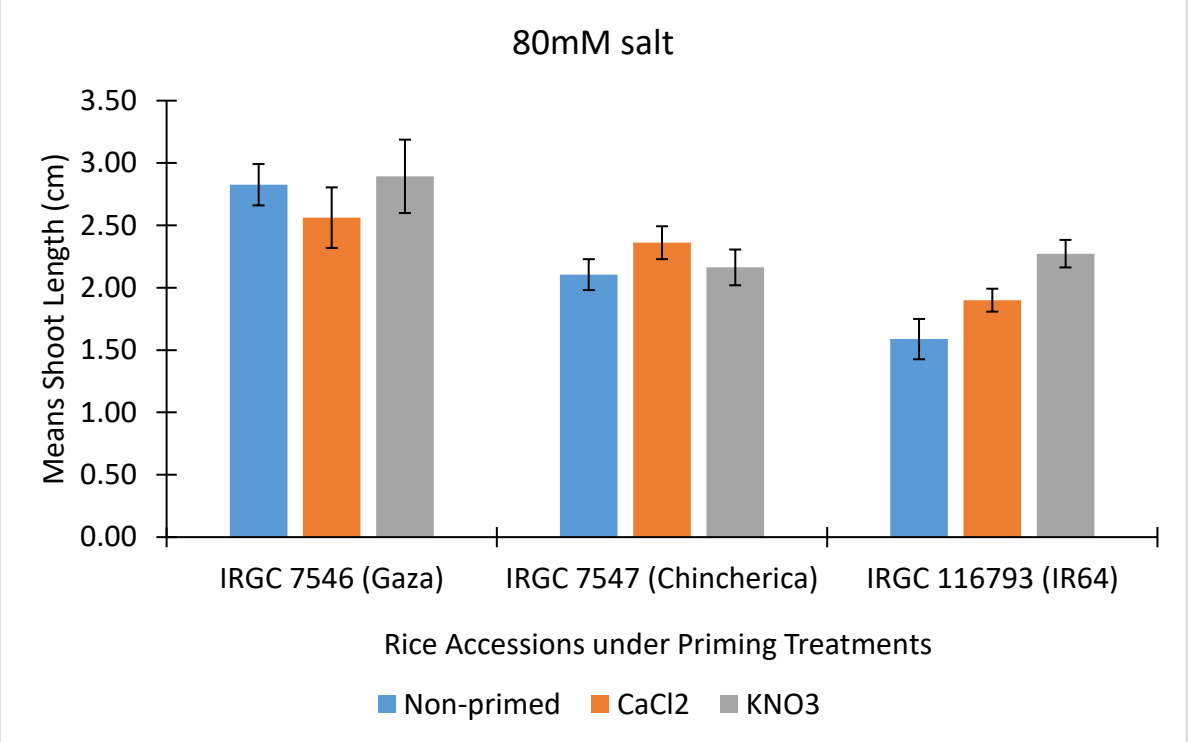


Figure 4.5: Effect priming treatments on mean shoot length of 8-days old seedlings. Three rice accessions (IRGC 7546 (Gaza), IRGC 7547 (Chincherica) and IRGC 116793 (IR64) treated with three priming treatments (non-primed,200 mM $CaCl_2$, and 297 mM KNO_3) were grown in an incubator at 34/11°C. day/night with 16hrs light/8 hrs dark. Data are shown as mean value +/- SEM of hundred individual replications (n=100). The error bars represent SEM.

4.4 Discussion

4.4.1 Percent Germination

Salt treatments did not affect percent germination of the three rice accessions.

On the contrary, both tested priming treatments reduced percent germination, regardless of salt treatments and rice accessions. However, the 8% reduction in germination observed following 200 mM $CaCl_2$ priming treatment, compared to non-primed treatment, was not statistically significant. Afzal et al. (2012) observed that moderate salinity stress of 80 mM NaCl reduced percent germination by 10% in a tolerant rice variety but did not affect the percent germination of the salt-sensitive rice variety compared to control (non salt treatment); 200 mM $CaCl_2$ priming treatment increased by 6.67% the percent germination of fine aromatic rice cultivars sensitive to salinity and by 13.33% the percent germination of salt tolerant cultivars, with only the latter increase, being significant. Therefore, effects of salt stress and priming treatments on germination are more prominent in the tolerant variety compared to a salt sensitive variety. Previous experiments (**Chapter 2**) indicate that rice accessions IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 116793 (IR64) are moderately tolerant to salinity stress with salt tolerance of 61 %, 64 % and 68 % respectively. Possibly, this is the reason why no effect of salt stress was observed and the effect of 200 mM $CaCl_2$ priming treatment was not significant. The 297 mM KNO_3 priming treatment significantly reduced percent germination (19.64% less) compared to non-primed treatment. This is in contrast with Dhillon et al. (2021) who reported that 198 mM KNO_3 priming treatment increased percent germination of rice plants by 3-4%, under non-saline conditions. Ali et al. (2020) observed that 247.5 mM and 495 mM KNO_3, increased percent germination of rice plants by 16% - 48% under mild, moderate and severe drought

conditions; except under 495 mM KNO_3 priming treatment and severe drought condition where 16% decrease in percent germination was observed. This suggests that the concentration of KNO_3 that can be efficiently utilized by germinating seeds varies with the hydration state of the plant. In this study, 297 mM KNO_3 priming treatment is relatively high combined with a relatively high 80 mM NaCl salt concentrations, and this may have negatively affected the germination of rice accessions. The 80 mM NaCl salt concentrations may have created too high osmotic pressure, that reduced the hydration of the seeds, decreasing the efficient use of 297 KNO_3 priming treatment.

4.4.2 Seminal Root Length

Salt stress did not affect seminal root length. However, although not significantly, 200mM $CaCl_2$ priming treatment reduced seminal root length of rice accessions IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 116793 (IR64) by 8%, 15%, and 4% respectively (**Figure 4.4**). Similarly, Theerakulpisut et al. (2017) observed that 200mM $CaCl_2$ priming treatment decreased root length of 10 d old rice seedlings by 20%, under 150 mM NaCl concentrations; while, Afzal et al. (2012) observed that 200mM $CaCl_2$ priming treatment increased root length under non-saline and moderate saline conditions (80 mM NaCl salt concentrations). This increase was more prominent in salt tolerant varieties (25%) than in salt sensitive varieties (8%) at the maximum germination stage (Afzal et al., 2012). In this study, although the results were negative and not significant, the impact was lower in IRGC 116793 (IR64) rice accession, which is relatively tolerant to salt stress compared to IRGC 7547 (Chincherica) and IRGC 7546 (Gaza) rice accessions. However, it should be considered that our observations and scoring were done at day 8^{th} from imbibition and it is possible the

seeds had not reached a developmental stage where the effects of the salt treatment begin to limit growth and the benefits of priming become evident (which would have evidenced the benefits of $CaCl_2$ priming treatment). The 297 mM KNO_3 priming treatment decreased seminal root length of rice accessions IRGC 7547 (Chincherica) and IRGC 7546 (Gaza) by 35% and 4 % respectively, which was significant for IRGC 7547 (Chincherica); though not significant, increase seminal root length by 17% was also reported for IRGC 116793 (IR64) rice accession (**Figure 4.4**). Similarly, Dhillon et al. (2021) observed that 14 d after germination, 198 mM KNO_3 priming treatment increased seminal root by 17% under normal condition. Ali et al. (2020) reported that 247.5 mM and 495 mM KNO_3 priming treatments increased seminal root length by 30-70% on 51 d old seedlings; this positive impact was greater in moderate than in severe drought stress. Theerakulpisut et al. (2017) observed that 49.5 mM KNO_3 priming treatment increased root length of 10 d old rice seedlings by 37%, under 150 mM NaCl concentrations. Therefore, there is a tendency for the effects of priming to vary with experiment duration, growth conditions (normal or stressed), priming concentrations, and the tested rice accessions (degree of tolerance to stress) and this may have contributed to the reduction in root length of IRGC 7547 (Chincherica) and IRGC 7546 (Gaza) rice accessions, which are relatively more salt sensitive compared to IRGC 116793 (IR64).

4.4.3 Shoot Length

Overall, 80 mM NaCl salt treatment decreased shoot length by 36% compared to control (0 mM NaCl). Salinity stress is known to reduce photosynthetic area and the rate of photosynthesis, decreasing the movement of assimilates to the growing tissues, with effects

more evident in leaves compared to roots (Greenway and Munns, 1980, Shabala and Munns, 2017). This could explain why no significant effect of salt stress on root length was observed. The 200mM $CaCl_2$ and 297mM KNO_3 priming treatments affected shoot length, but the response varies with rice accessions (**Figure 4.5**). Afzal et al. (2012) reported that 200mM $CaCl_2$ priming treatment increased shoot length of salt tolerant and salt sensitive fine aromatic rice cultivars, and this increase was more prominent in salt tolerant (20%) than in salt sensitive (6%) cultivars. Theerakulpisut et al. (2017) observed that 200mM $CaCl_2$ priming treatment decreased shoot length by 37%, and therefore did not alleviate the negative impact of 150 mM NaCl salinity stress in two different genotypes. In this study, there was an increase of shoot length in IRGC 7547 (Chincherica) and IRGC 116793 (IR64) rice accessions and decrease in IRGC 7546 (Gaza), and although the changes were not statistically significant they suggest that the effect of priming treatment depends on the rice genotype and its level of tolerance to the stress (**Figure 4.5**). The 297mM KNO_3 priming treatment increased shoot length of IRGC 116793 (IR64) rice accession and decreased that of IRGC 7547 (Chincherica) and IRGC 7546 (Gaza), but this was not significant. Similarly, Dhillon et al. (2021) noted a non-significant increase in shoot length at 14 days after sowing following a 198mM KNO_3 priming treatment. On the other hand, Ali et al. (2020) reported that 247.5 mM and 495 mM KNO3 priming treatments caused a significant increase of shoot length by 30-70% on 51 d old rice plants. Theerakulpisut et al. (2017) observed that 49.5 mM KNO_3 priming treatment alleviated the negative impact of 150 mM NaCl salinity stress on growth of young rice seedlings (10 d old rice seedlings). They observed that under salt stress there was about a 41% increase in shoot length. This suggests that, with prolonged experiment duration, significant results might have been obtained in the IRGC 116793 (IR64) rice accession, had we scored the seedling after a longer interval from sowing, rather than 8 days. This effect

was observed in previous experiment (**Chapter 3**: **Table 3.2**), in which 70 d old plants of IRGC 116793 (IR64) showed 33% increase in mean shoot dry weight when primed with KNO_3 compared to non-primed controls.

4.5 Conclusions

Salt treatment (80 mM NaCl salt concentrations) did not affect percent germination and seminal root length, and decreased mean shoot length of 8-day old seedlings of the three rice accessions.

The 200mM $CaCl_2$ priming treatment did not affect percent germination whilst the 297 mM KNO_3 priming treatment decreased percent germination of the three rice accessions.

The 200mM $CaCl_2$ priming treatment did not affect seminal root lengths, and shoot lengths of the three rice accessions. The 297 mM KNO_3 priming treatment did not affect seminal root lengths and shoot lengths of rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64), while in rice accession IRGC 7547 (Chincherica) decreased seminal root length but did not affect shoot length. Therefore, 200mM NaCl $CaCl_2$ and 297mM NaCl KNO_3 priming treatments did not reduce the negative impact of salinity stress (80 mM NaCl salt concentrations) of seedlings of rice accessions IRGC 7547 (Chincherica), IRGC 7546 (Gaza) and IRGC 116793 (IR64), at least when determined at 8 days from starting of germination inducing conditions.

The present results and comparing with the literature, suggests that there is a trend for priming treatments to increase seedling growth under normal and saline conditions, but this tendency appears to vary with duration of experiment (timing for observations and scoring), growth conditions (normal or stressed), specific priming salt and concentration and rice genotypes.

CHAPTER 5. Effect of Salinity Stress on Growth, Physiological Traits, Grain Yield and Grain Composition (Grain Nutritional Quality) of Primed and Non-primed Rice Accessions from Mozambique

5.1 Introduction

In chapter 3 we have showed that $CaCl_2$ and KNO_3 priming treatments improved shoot dry weight and salt tolerance of indica Mozambique (landrace) rice accessions IRGC 7547 (Chincherica) and IRGC 7546 (Gaza), and indica IRRI line in Mozambique, IRGC 66970 (IR64), under 80 mM NaCl salt concentration. The aim of this chapter was to determine the effect of salinity stress and evaluate the impact of different priming treatments on plant growth (mature plant biomass), development, grain yield and grain composition (grain nutritional quality) of rice accessions from Mozambique. It was hypothesized that salinity stress decreases mean grain yield, grain starch concentration, and grain amylose concentration; and increases mean grain protein concentration, while priming treatments counteract the effect of salt stress on rice accessions from Mozambique.

5.2 Materials and Methods (Experiment III - 2020)

5.2.1 Plant Material

The rice accessions and priming treatments for this study were selected as described in **Section 4.2.1 (Table 4.1) of Chapter 4**.

5.2.2 Seed Surface Sterilization and Priming Treatments

Seeds were surface sterilized as described in **Section 4.2.2 of Chapter 4**; and priming treatments were prepared and applied to rice seeds as described in **Section 4.2.3 of Chapter 4**.

5.2.3 Rice Plant Establishment: Hydroponic System Setup, Seed Sowing and Salt Treatment

5.2.3.1 Hydroponic System Setup

After priming treatments, rice plants were grown in a Fitotron plant growth chamber (WEISS GallenKamp), in a supported hydroponic system. Growth conditions were set up based on rice plants' requirements for grain production. Therefore, the day and night temperatures were 29 °C and 26 °C respectively, the relative humidity was 70%, the PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy, and the photoperiod was 10 h. (**Figure 5.1**) (Yoshida, 1981, Köhl, 2015).

Six dark grey food grade polypropylene tanks (REF: 3-6413-13-CASE GREY RANGE EURO CONTAINER CASE - 26 LITRES (600 X 400 X 155MM) - http://www.plastor.co.uk/), with aeration stones at the bottom of each and linked to two air pumps were arranged in the

growth room. Rectangular metallic meshes of 510x310x120 mm were positioned inside each tank and filled with Rockwool cubes (Grodan Rockwool Cubes Grow Blocks 4" Large Hole X6 Hydroponics - https://www.grodan.com/) (Munns and James, 2003, Köhl, 2015, Bado et al., 2016). The nutrient solution was prepared and replenished following Yoshida et al. (1976) with modifications made by Gregorio et al. (1997) as described in **Section 2.2.3.1 of Chapter 2** (**Figure 5.1**)

5.2.3.2 Seed Sowing

The experiment was set up in a 2x3x3 factorial design (salt treatments vs priming treatments vs rice accessions). Three tanks were assigned to 0 mM NaCl salt concentration and three to 60 mM NaCl salt concentration, in which each of the three priming treatments was allocated. Seeds of the three rice accessions were randomly sown in each tank, with eight replicates (Köhl, 2015). The nutrient solution was gradually introduced over two weeks, thus tanks were filled with autoclaved distilled water at sowing and with Yoshida solution, ½ strength and full strength, respectively at leaf stages one and three (**Figure 5.1**) (Munns and James, 2003, Bado et al., 2016).

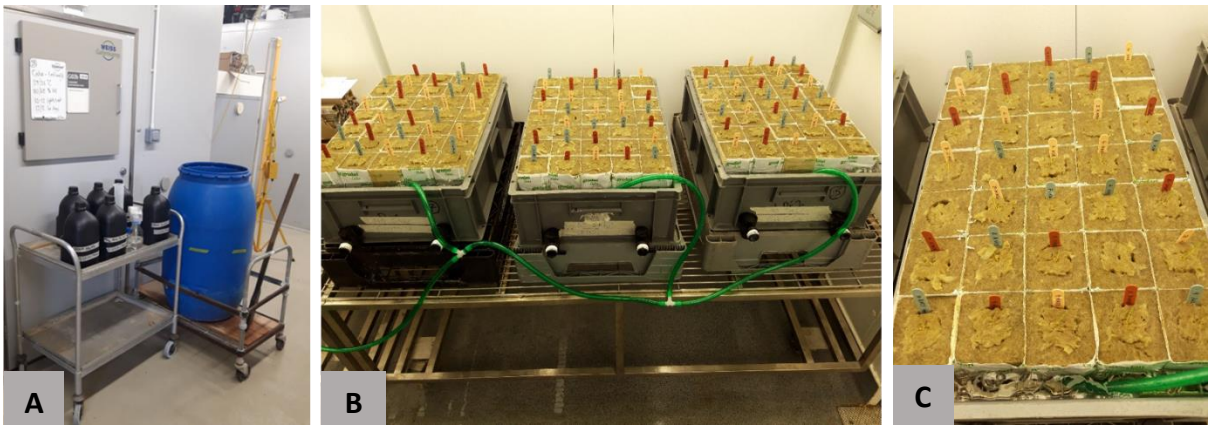


Figure 5.1. A) Fitotron plant growth chamber (WEISS GallenKamp) set up for rice grain production (day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy, photoperiod was 10 h); **B)** supported hydroponic system; and **C)** tank ready for rice direct seeding in Rockwool cubes: each treatment (rice accession, priming treatment and salt treatment) had eight replicates, represented with the same labelling colour, and randomly distributed.

5.2.3.3 Salt Treatment

Thirty-two days after sowing, corresponding to three weeks after emergence (beginning of mid vegetative phase – plants with 3 tillers in average) the salt stress was introduced. Dry NaCl was added to the nutrient solution in the tanks allocated to 60 mM NaCl treatment. The salt was introduced in two applications over three days, with increments of 30 mM NaCl on day 1 and 3, to reach the final concentration of 60 mM NaCl (Munns and James, 2003, Haq et al., 2014). In this experiment, the applied salt concentration was reduced to 60 mM NaCl to allow rice plants to grow and develop to the reproductive and ripening stages The concentration of salt was measured with a portable waterproof conductivity meter, Multi-Parameter Testr 35 Series. Autoclaved distilled water was added in each tank thrice a week to replace water lost from evaporation and transpiration and keep the original volume**.** Salinity stress was applied up to physiological maturity stage (when about 80% of the grain were straw, yellow-colored (**Figure 5.2**).

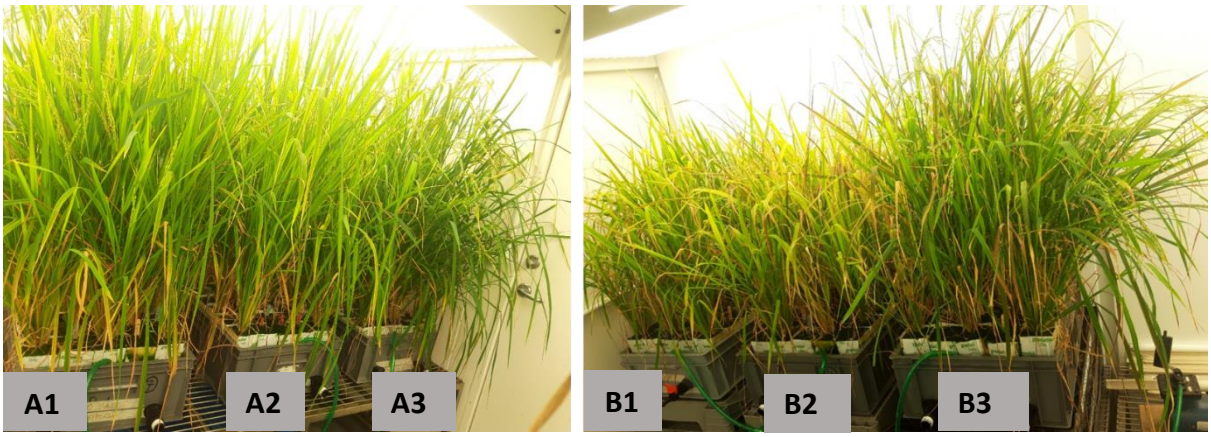


Figure 5.2: Rice plants at maturity stage: **A)** under 0 mM NaCl salt concentration, and the three priming treatments (**A1** - non-primed, **A2** - KNO_3, and **A3** - $CaCl_2$) and **B)** under 60 mM NaCl salt concentration and the three priming treatments (**B1** - KNO_3, **B2** - $CaCl_2$, and **B3** - non-primed,).

5.2.4 Data Collection

5.2.4.1 Shoot Dry Weight and Grain Yield

Plants were grown up to physiological maturity stage (when about 80% - 85% of the grain were straw, yellow-colored), and a week before harvesting, the nutrient solution was removed from the tanks. Plants aerial tissues (i.e. roots system excluded) were collected and separated from the panicles, washed with autoclaved distilled water, dried with tissue paper and placed in paper bags. Plants (the vegetative biomass) were then dried in the oven at 80 °C for four days, to achieve constant weight. Dry weight was measured in grams per plant. The panicles were threshed and the grain yield was measured in grams per plant.

5.2.4.2 Na^+ Exclusion and K^+/Na^+ Discrimination

All eight replicates of dried plants corresponding to specific rice accession / priming treatment / salt treatment were combined (bulked) as one sample and ground in a Foss CT 293 Cyclotec Laboratory Mill. A subsample of 0.5 g per sample was weighed and placed on

MARSXpress digestion tubes in three technical replicates. These samples were digested, diluted and submitted to ICP analysis for the measurement of tissue Na and K concentrations as detailed on **Section 2.2.4.2**.

5.2.4.3 Grain Starch Concentration

Seeds were harvested and moisture measured (10% on wet weight basis). All eight replicates of grain yield per treatment (rice accession / priming treatment / and salt treatment combinations) were bulked and ground on the Laboratory Mill 3303 (Perten Instruments, Warrington, UK), from which the grain starch, amylose, and protein concentrations were measured.

The starch is defined as total available carbohydrate minus water soluble carbohydrate. Total available carbohydrate is defined as the hot water soluble material that is broken down to reducing sugars by incubation with amyloglucosidase followed by mild acid hydrolysis (Smith et al., 1964). Water soluble carbohydrate is defined as the cold water soluble material that is broken down to reducing sugars by mild acid hydrolysis. The starch concentration was measured using SKALAR METHODS ANALYSIS: TOTAL REDUCING SUGARS (after inversion) RANGE: 50 - 1000 mg $C_6H_{12}O_6$/liter SAMPLE: Plant Extraction of Total Available Carbohydrates from Grass as described below:

A. <u>Total Available Carbohydrate</u>

About 0.3 g of ground sample (in duplicate) was weighed into a 50 mL screw top tube, 25 mL of distilled water was added, and the cap were screwed and mixed thoroughly. The tubes were placed in boiling water bath for 2 h. The tube contents were mixed by inversion every

15 min. The tube contents were allowed to cool below 70 ^{o}C, and 15 mL amyloglucosidase solution (~100U/mL in pH 4.5 acetate buffer) was added to the tubes and mixed. The tubes were placed in a water bath at 55 ^{o}C for 90 min, and mixed by inversion every 15 min. The tubes were then cooled and the contents filtered through Whatman filter paper 9.0 cm. From the filtered samples, a 2 mL subsample was placed in 15 mL screw top tube, and 6 mL 0.133M sulphuric acid was added and the cap replaced and the tubes inverted to mix. The tubes were placed in water bath at 70 ^{o}C for 30 min. After cooling, the hydrolysate was analysed for reducing sugar concentration using a continuous flow auto-analyser (**San^{++}** Automated Wet Chemistry Analyzer – Continuous Flow Analyzer (CFA) (Skalar Analytical B.V., The Netherlands). Recommended Operational Settings: 1. System sample time: 70 sec., wash time: 70 sec., air time: 3 sec. 2. Module sample time: 70 sec., wash time: 70 sec., air time: 3 sec. 3. Calibration type: 1st order ISO 8466-1., standardised with 50:50 glucose: fructose solution in distilled water.

B. <u>Water Soluble Carbohydrate</u>

About 0.5 g of ground sample (in duplicate) was weighed into a 50 mL Erlenmeyer flask, 25 mL distilled water was added and sample swirled gently to mix. The Flasks were placed on Stuart Orbital Shaker SSL1 set at approximately 160 rpm and left for 2 h. The extract was filtered through Whatman filter paper 9.0 cm. From the filtered samples, a 2 mL subsample was placed in 15 mL screw top tube, and 6 mL 0.133M sulphuric acid was added and the cap replaced and inverted to mix. The tubes were then placed in water bath at 70 ^{o}C for 30 min. After cooling, the hydrolysate was analysed for reducing sugar concentration using a continuous flow auto-analyser, standardised with 50:50 glucose:fructose solution in distilled water.

C. Grain Starch Concentration Calculation

Total available carbohydrate and water soluble carbohydrate were derived from the equation (1) and the starch concentration from the equation (2) below:

$$(1)\ CHO = \frac{C \times D \times 100}{W \times 1000}$$

Where: CHO is the total carbohydrate or the water soluble carbohydrate content (% by weight)

D is the dilution factor, i.e. the extract volume in mL prior to hydrolysis

C is the concentration of reducing sugar in the hydrolysate in mg/L

W is the weight of sample taken in mg

$$(2)\ Starch = \frac{0.9 \times (TC - WC) \times 100}{SR}$$

Where: Starch is the starch concentration (% by weight)

a factor of 0.9 is applied to correct for water gained on hydrolysis

TC is the total carbohydrate concentration as obtained from (1)

WC is the water soluble carbohydrate concentration as obtained from (1)

SR is the starch recovery (%)

5.2.4.4 Grain Amylose Concentration

The amylose concentration was measured following the Megazyme (www.megazyme.com) protocol; therefore, the measurement of the amylose was carried out through sections A, B, and C below:

A. Starch Pre-treatment

Flour sample of 25 mg was added into screw capped Kimax sample tube and 1 mL of Dimethyl sulphoxide (DMSO) was added to the tube, with gentle stirring at low speed on a vortex mixer (IKA VORTEX GENIUS 3). The tubes were capped and the tube contents were heated, for approximately one minute, in a boiling water bath (on PIERCE Reacti-Therm Heating Stirring Module, Pierce Chemical Company, Rockford) until the flour sample was completely dispersed, ensuring that there was no gelatinous lumps of starch remaining. The tube was vigorously mixed at high speed on a vortex mixer, and returned to the boiling water bath and heated for a further 15 min, with intermittent high speed stirring on a vortex mixer. The tubes were stored at room temperature for approximately five minutes and then 2mL of 95 % (v/v) ethanol was added with continuous stirring on a vortex mixer, and a further 4 mL of 95 % (v/v) ethanol was added. The tubes were capped and inverted to mix, and the starch precipitate was formed. The tubes were allowed to stand for 15 minutes and centrifuged (on Eppendorf Centrifuge 5810 R, Germany) at 2 000 g for 5 min. The supernatant was discarded and the tubes were drained for 10 minutes on a tissue paper, ensuring that all of the ethanol has drained. To the starch pellet, 2 mL of DMSO was added with gentle vortex mixing and the tubes were placed in boiling water for 15 minutes and mixed occasionally, ensuring that there were no gelatinous lumps. The tubes were removed

from boiling water bath and immediately 4 mL of Con A solvent (working concentration) was added while vigorously mixing the tube, and quantitatively the tube contents was transferred to a 25 mL volumetric flask and diluted to volume with Con A solvent (working concentration). This solution was filtered through Whatman filter paper 9.0 cm and named solution A.

B. <u>Con A Precipitation of Amylopectin and Determination of Amylose</u>

From solution A above, 1.0 mL was transferred to a 2.0 mL Eppendorf microfuge tube; 0.50 mL of Con A solution (Bottle 1) was added. The tubes were capped and gently mixed by repeated inversion, avoiding frothing of the sample. The tubes were then allowed to stand for 1 h at room temperature, and centrifuged (on Eppendorf Centrifuge 5424) at 14,000 g for 10 min, at room temperature. A 1 mL subsample of the supernatant was transferred to a 15 mL centrifuge tube, and 3 mL of 100 mM sodium acetate buffer, pH 4.5 was added and mixed before being heated in a boiling water bath for 5 min to denature the Con A. The tubes were placed in water bath (SUB Aqua 12 Plus, Grant Instruments, Cambridge) at 40 ^{o}C and allowed to equilibrate for 5 min, and 0.1 mL of amyloglucosidase/ α-amylase enzyme mixture (page 3; solution 2 (Bottle 2)) was added, and incubated (on Stuart incubator S160D) at 40^{o}C for 30 min. The tubes were centrifuged (on Eppendorf Centrifuge 5810 R, Germany) at 2,000 g for 5 min and 1 mL aliquots of the supernatant were transferred to new 15 mL centrifuge tubes. To which 4 mL of GOPOD Reagent (Reagent B) (Bottle 3 + 4) was added, and incubated at 40 ^{o}C for 20 min, concurrently with Reagent Blank (1 mL of 100 mM sodium acetate buffer, pH 4.5 and 4 mL of GOPOD Reagent (Reagent B) (Bottle 3 + 4)) and two D-

Glucose Controls (0.1 mL of D-glucose standard solution, 0.9 mL of 100 mM sodium acetate buffer, pH 4.5 and 4 mL of GOPOD Reagent (Reagent B) (Bottle 3 + 4)).

C. Determination of Total Starch

From solution A prepared above, in 15 mL centrifuge tubes, 0.5 mL was mixed with 4 mL of 100 mM sodium acetate buffer, pH 4.5, and 0.1 mL of amyloglucosidase/ α-amylase solution (page 3; solution 2 (Bottle 2)) was added and the mixture was incubated at 40°C for 10 min. From this solution, an aliquot of 1.0 mL was transferred in duplicate to new 15 mL centrifuge tubes, and 4 mL of GOPOD Reagent (Reagent B) (Bottle 3 + 4) was added, mixed well, and incubate at 40°C for 20 min. This last incubation was performed concurrently with the samples, Reagent Blank and the D-Glucose Controls detailed in section B. The absorbance of each sample, and the D-Glucose Controls were read at 510 nm against the reagent blank on SpectraMax i3x (Molecular Devices). The percentage of amylose was calculated as the ratio of absorbance of Con A supernatant (section B) and the absorbance of total starch aliquot (section C) multiplied by 66.8 dilution factor.

5.2.4.5 Grain Protein Concentration

Elemental nitrogen (N) was determined though the Dumas method (or combustion method) in the Leco CHN 628 Carbon/Hydrogen/Nitrogen analyser (LECO Corporation, United States of America) and protein derived using conversion factor. Therefore, samples were combusted, reduced, separated, and N detected, as described here. Three replicates of well homogenized flour samples of about 0.2 g were heated for rapid combustion in a high-temperature oven at over 1000 °C in the presence of pure oxygen and produced water (water vapour), carbon

dioxide and nitrogen in the form of diverse oxides. This mixture of gases went through the reduction chamber containing copper heated to around 650 °C to convert the nitrogen oxides into elemental N and collects the oxygen in excess. Total N concentration was measured by a thermal conductivity detector and the protein concentration was calculated using a N to protein conversion factor of 5.7.

5.2.4.6 Total Protein Extraction

About 50 mg of homogenous and fine flour per treatment (rice accession, priming treatment, and salt treatment) was placed in 2 mL Eppendorf tube and 100 mM Tris-HCl (pH 6.8) extraction buffer containing 1% w/v dithiothreitol (DTT) and 1% sodium dodecyl sulfate (SDS) was added to sample (20 µL extraction buffer per mg of flour, hence approximately 1000 µL was added to each sample). Samples were vortexed briefly on IKA VORTEX GENIUS 3 and then placed on an Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1,000 rpm speed followed by centrifugation for 20 min at 10,000 g (rpm) on Centrifuge 5424. The supernatant was transferred to a new tube, the Bolt 4X LDS Sample Buffer (Novex, Life Technologies was added (150 µL supernatant and 50 µL of Buffer) and stored at -20 ^{o}C until the one-dimensional SDS-PAGE analysis.

5.2.4.7 Sequential Extraction of Rice Protein

Rice protein of the accessions IRGC 7546 (Gaza), and IRGC 116793 (IR64) was extracted in series as follows: Albumins – Globulins - Cysteine Poor Prolamins (CPP) - Cysteine Rich Prolamins (CRP) - Glutelins. Therefore, about 25 mg of homogenous and fine flour per treatment (rice accession, priming treatment, and salt treatment) were placed in 2 mL

Eppendorf tubes and 250 µl of autoclaved distilled water was added. Tubes were vortexed briefly on IKA VORTEX GENIUS 3 and then placed on an Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1000 rpm and centrifuged at 10,000 g (rpm) for 20 min on Centrifuge 5424. The supernatant, corresponding to an albumin-reach extract, was removed to clean 2 mL Eppendorf tubes and named Albumin (A). To the pellet, 250 µl 0.5 M NaCl, 10 mM Tris –HCl, pH 7.5 was added. Tubes were vortexed briefly on IKA VORTEX GENIUS 3 and then placed on Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1,000 rpm and centrifuged at 10,000 g for 20 min on Centrifuge 5424. The supernatant corresponding to a globulin-reach extract with residual albumins, was removed to clean 2 mL Eppendorf tubes and named Albumins/Globulins (A/G). The pellet was resuspended with 250 µl autoclaved distilled water, and the tubes were vortexed briefly on IKA VORTEX GENIUS 3 and then placed on Eppendorf Thermo Mixer for 10 min at 50 ^{o}C and 1000 rpm and centrifuged at 10 000 g for 20 min on Centrifuge 5424. The supernatant (possibly containing residual A/G) was transferred to clean 2 mL Eppendorf tubes and named Water. The pellet was resuspended in 250 µl of 60 % (v/v) 1-propanol solution, and the tubes vortexed briefly on IKA VORTEX GENIUS 3 and then placed on an Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1,000 rpm and centrifuged at 10,000 g for 20 min on Centrifuge 5424. The supernatant was removed to clean 2 mL Eppendorf tubes and named Cysteine Poor Prolamins (CPP). The pellet was resuspended in 250 µl of 60 % (v/v) n-propanol + 1% DTT was added, the tubes were vortexed briefly on IKA VORTEX GENIUS 3 and then placed on an Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1,000 rpm and centrifuged at 10,000 g for 20 min on Centrifuge 5424. The supernatant was removed to clean 2 mL Eppendorf tubes and named Cysteine Rich Prolamins (CRP). Finally, the pellet was resuspended in 250 µl of SDS buffer (50 mM ris –HCl, pH 6.8, 4 % SDS, 5 % 2-mercaptoethanol + 1.5 % DTT), tubes were vortexed briefly on IKA

VORTEX GENIUS 3, and then placed on an Eppendorf Thermo Mixer for 15 min at 50 ^{o}C and 1,000 rpm after which were centrifuged at 10,000 g for 20 min on Centrifuge 5424. The supernatant was removed to clean 2 mL Eppendorf tubes and named Glutelins. All extracted samples were stored at -20 ^{o}C.

5.2.4.8 One-Dimensional SDS-PAGE Analysis of the Total and Sequential Protein Extract

Samples of total extraction and sequential extraction were run in the gel with the same procedure. Therefore, samples were thawed at room temperature, briefly heated for 8 min at 65 °C on a preheated Eppendorf Thermo Mixer and centrifuged for 2 min at 10,000 g on Centrifuge 5424. An Invitrogen by Thermo Fisher Scientific NuPAGE 10% Bis- Tris precast gel (1.0 mm x 15 Well) was placed in a Bolt Mini Gel Tank, then the NuPAGE MES SDS Running Buffer (20X) was added. Each well was loaded with 10 µL per sample from individual rice accession, priming treatment, and salt treatment combinations and 3.5 µL of PageRuler Prestained Protein Ladder. The gel was run at 200 V for about 60 min. The gel was fixed with 12% trichloroacetic acid for 15 min, washed twice with 250 mL autoclaved distilled water on a gyro-rocker (SSL3, Stuart, UK) for 15 min, stained with 50 mL of PageBlue Protein Staining Solution for 2 h, and then de-stained overnight with autoclaved distilled water. For densitometric analysis of total protein extracts, samples on gels were arranged based on the three rice accessions ((IRGC 7546 (Gaza), IRGC 116793 (IR64), and IRGC 7547 (Chincherica)), three priming treatments, and two salt treatments. For densitometric analysis of sequential protein extraction samples were arranged based on protein type (Albumins - Albumins/Globulins (A/G) - Water - Cysteine Poor Prolamins (CPP) - Cysteine Rich Prolamins

(CRP) – Glutelins), two salt treatments within each of the two rice accession ((IRGC 7546 (Gaza) and IRGC 116793 (IR64)). All the precast gels were run in duplicate.

5.2.4.9 Protein Identification and Densitometric Analysis

Individual images of precast gels from total and sequential extractions were taken on the gel doc system at the molecular genetics laboratory. A LED Pad (Light Pad Drawing A4 Tracing Light Table NXENTC LED) was used for a better visualization of the precast gel and the bands. A square was drawn in the middle of the Led Pad for uniformity in the measurement, each precast gel was placed on the drawn square, inserted in the gel doc system, and photos were taken and stored as Jpeg images. The Jpeg images were analyzed in the Gel Analyzer 19.1 Package. From the Jpeg images from total extractions, the single bands in the SDS-PAGE grain protein profile of the rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) were thoroughly analyzed to check if there was any difference between the lanes. From sequential protein extraction, the different protein types (Albumins - Albumins/Globulins (A/G) - Water - Cysteine Poor Prolamins (CPP) - Cysteine Rich Prolamins (CRP) – Glutelins (Gt)) were identified and quantified on GelAnalyzer 19.1 Package.

In the Gel Analyzer 19.1 Package, the Jpeg images of the sequential extractions of rice accession IRGC 7546 (Gaza) under KNO_3 priming treatment and the two salt treatments (0mM NaCl and 60 mM NaCl salt treatments) were analyzed following the settings: File – New analyzer; Dark on Light; Select a Crop Region; Add a New Lane; Band Mode; Add Band Manually; Rf Calibration Code; Add Rf Curve; MW Calibration Mode; and Insert Ladder MW (molecular weight).

5.2.5 Statistical Data Analysis

Three-factor (salt treatments*priming treatments*rice accessions) Analysis of Variance (p-value < 0.05) was used in the statistical package GenStat 19th Edition, to assess the difference in the means of shoot dry Weight, shoot Na concentration, shoot K^+/Na^+ discrimination, grain yield, grain starch concentration, amylose concentration, and protein concentration. The assumptions of ANOVA (Shapiro-Wilk Test for Normality and Test of Homogeneity) were checked and the data were normally distributed.

5.3 Results

5.3.1 Shoot Dry Weight Production

There was no significant difference in mean shoot dry weight between the three rice accessions (p-value= 0.577) and three priming treatments (p-value = 0.535). There were also no significant interactions between salt treatments and priming treatments (p-value = 0.545), priming treatments and rice accessions (p-value = 0.314), and salt treatments, priming treatments and rice accessions (p-value = 0.877). However, there was a significant difference in mean shoot dry weight between the two salt treatments (p-value = 0.003) and a significant interaction between salt treatments and rice accessions (p-value <0.001) (**Appendix: Chapter 5**). Across the three rice accessions and three priming treatments, 0 mM NaCl salt treatment (control) showed higher mean of shoot dry weight (19.68 g/plant) than 60 mM NaCl salt treatment (12.2 g/plant). Overall, 60 mM NaCl salt treatment reduced mean shoot dry weight by 38% compared to control. Under 60 mM NaCl salt treatment, the highest shoot dry weight was observed in rice accession IRGC 116793 (IR64) (18.09 g/plant), followed by IRGC 7546 (Gaza) (10.95 g/plant); whereas, the lowest shoot dry weight was in rice accession IRGC 7547 (Chincherica) (4.72 g/plant) (**Table 5.1**). Compared to 0 mM NaCl salt treatment (control), under 60 mM NaCl salt treatment the rice accession IRGC 7547 (Chincherica) showed the highest and most significant reduction in shoot dry weight (85%) (p-value < 0.001); the rice accession IRGC 116793 (IR64) showed an increase in shoot dry weight (37%), but this was not significant (p-value = 1.000) (**Table 5.1**).

Across the three rice accessions and two salt treatments, KNO_3 priming treatment showed the highest mean shoot dry weight of 17.75 g/plant, followed by Non-primed (17.49 g/plant), whereas $CaCl_2$ priming treatment showed the lowest mean shoot dry weight of

14.04 g/plant. Overall, KNO_3 priming treatment increased mean shoot dry weight by 1.5%, whereas $CaCl_2$ priming treatment lowered mean shoot dry weight by 18%, in relation to the non-primed treatment. Under 60 mM NaCl salt treatment, compared to Non-primed treatment (control), $CaCl_2$ and KNO_3 priming treatments decreased the mean shoot dry weight of the rice accessions IRGC 7546 (Gaza) (35% and 27% respectively) and IRGC 116793 (IR64) (43% and 25% respectively); and increased the mean shoot dry weight of rice accession IRGC 7547 (Chincherica) (21% and 33% respectively). However, there was no significant difference in mean shoot dry weight within all three rice accessions and priming treatments (**Table 5.1**).

Table 5.1: Effect of salt treatments and priming treatments on mean shoot dry weight (g/plant). Three rice accessions IRGC 7546 (Gaza), IRGC 7547 (Chincherica) and IRGC 116793 (IR64) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Salt stress was imposed from 32 d after sowing up to maturity. Data are shown as mean value +/- SEM of eight individual replications (n = 8).

Salt Treatments	Priming Treatments	Shoot Dry Weight Production (g/plant)	
		Mean	Std.Err.
IRGC 7546 (Gaza)			
0mM salt	Non-primed	18.053	2.359
0mM salt	$CaCl_2$	12.970	1.608
0mM salt	KNO_3	24.503	4.108
60mM salt	Non-primed	13.840	1.806
60mM salt	$CaCl_2$	8.980	2.671
60mM salt	KNO_3	10.082	1.153
IRGC 7547 (Chincherica)			
0mM salt	Non-primed	23.820	13.426
0mM salt	$CaCl_2$	35.722	10.823
0mM salt	KNO_3	35.422	12.845
60mM salt	Non-primed	4.027	0.503
60mM salt	$CaCl_2$	4.856	1.928
60mM salt	KNO_3	5.343	1.941
IRGC 116793 (IR64)			
0mM salt	Non-primed	17.665	1.721
0mM salt	$CaCl_2$	9.607	1.383
0mM salt	KNO_3	12.684	1.008
60mM salt	Non-primed	23.462	3.917
60mM salt	$CaCl_2$	13.468	2.566
60mM salt	KNO_3	17.670	0.983

5.3.2 Na^+ Exclusion

Significant differences in shoot Na concentration between the three rice accessions (p-value < 0.001), two salt treatments (p-value <0.001), and three priming treatments (p-value < 0.001) were observed. The interactions between salt treatments and priming treatments, salt treatments and rice accessions, priming treatments and rice accessions, and salt treatments, priming treatments and rice accessions were all significant (p-value < 0.001) (**Appendix: Chapter 5**). Therefore, mean shoot Na concentration among the rice accessions are dependent on both salt treatments and priming treatments.

Across the three rice accessions and three priming treatments, plants grown under 0 mM NaCl salt treatment (control) had a lower mean shoot Na concentration (2.442 mg Na g^{-1} DW) than those grown under 60 mM NaCl salt treatment (18.675 mg Na g^{-1} DW). The highest increase in mean shoot Na concentration from control to 60mM NaCl, was observed in rice accessions IRGC 7547 (Chincherica) (89%)(p-value < 0.001), followed by IRGC 7546 (Gaza) (87%)(p-value < 0.001); whereas, the lowest increase mean shoot Na concentration was observed in rice accession IRGC 116793 (IR64) (83%)(p-value < 0.001) (**Appendix: Chapter 5**). Variation in shoot Na concentration among rice accessions was lower under 0 mM NaCl salt treatment (control), but under 60 mM NaCl salt treatment large variation was observed. Under 60 mM NaCl salt treatment, the lowest mean shoot Na concentration was observed in plants whose seeds had been treated with KNO_3 priming treatment for all three rice accessions IRGC 7546 (Gaza), IRGC 7547 (Chincherica), and IRGC 116793 (IR64) (**Table 5.2**). The lowest rates of shoot Na concentration accumulation, in relation to 0 mM NaCl salt treatment (control), were observed in plants whose seeds had been treated with KNO_3 priming treatment for rice accessions IRGC 7546 (Gaza) (84%) and IRGC 7547 (Chincherica)

(84%); and in Non-primed treatment (75%) followed by KNO_3 priming treatment (76%) for rice accession IRGC 116793 (IR64) (**Table 5.2**).

5.3.3 K^+/Na^+ Discrimination

Significant differences in shoot K^+/Na^+ discrimination between the three rice accessions (p-value < 0.001), two salt treatments (p-value < 0.001), and three priming treatments (p-value < 0.001) were observed. Significant interactions between salt treatments and priming treatments, salt treatments and rice accessions, priming treatments and rice accessions, and salt treatments, priming treatments and rice accessions (p-value < 0.001) were also observed (**Appendix: Chapter 5**). Therefore, the means K^+/Na^+ ratio among the rice accessions are dependent on both salt treatments and priming treatments.

Across the three rice accessions and three priming treatments, there was a decrease in shoot K^+/Na^+ discrimination under salt stress (salt treatment 60 mM NaCl K^+/Na^+ ratio mean = 2.551) compared to control (salt treatment 0 mM NaCl K^+/Na^+ ratio mean = 10.502). The highest reduction in mean shoot K^+/Na^+ discrimination was observed in rice accessions IRGC 7547 (Chincherica) (86% less)(p-value < 0.001), followed by IRGC 116793 (IR64) (69% less)(p-value < 0.001); whereas the lowest reduction was observed in rice accession IRGC 7546 (Gaza) (68% less)(p-value < 0.001) (**Appendix: Chapter 5**). Variation of shoot K^+/Na^+ ratio across the three rice accessions and priming treatments was observed in both 0 mM NaCl (control) and 60 mM NaCl salt treatments (**Table 5.2**).

Under 60 mM NaCl salt treatment, KNO_3 and $CaCl_2$ priming treatments increased the shoot K^+/Na^+ discrimination of the rice accessions IRGC 7546 (Gaza) and IRGC 7547 (Chincherica); whereas KNO_3 priming treatment increased and $CaCl_2$ priming treatment decreased the

shoot K^+/Na^+ discrimination of the rice accession IRGC 116793 (IR64). The lowest reduction of shoot K^+/Na^+ discrimination under 60 mM NaCl salt treatment, in relation to control (0 mM NaCl salt treatment), were observed on KNO_3 priming treatment for all three rice accessions: IRGC 7546 (Gaza) (65% less) and IRGC 7547 (Chincherica) (77% less), and IRGC 116793 (IR64) (60% less) (**Table 5.2**).

Table 5.2: Effects of salt treatments and priming treatments on shoot Na concentration and shoot K^+/Na^+ discrimination on three rice accessions. Three rice accessions IRGC 7546 (Gaza), IRGC 7547 (Chincherica) and IRGC 116793 (IR64) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Salt stress was imposed from 32 d after sowing up to maturity. Data are shown as mean value +/- SEM of three individual replications (n = 3). Treatments sharing the same letter are not significantly different within each column (95% CI).

Salt Treatments	Priming Treatments	Shoot Na concentration (mg Na g^{-1} DW)	K^+/Na^+
IRGC 7546 (Gaza)			
0 mM salt	Non-primed	3.83 ^{e}	5.07 ^{e}
0 mM salt	CaCl2	3.11 ^{de}	6.31 ^{f}
0 mM salt	KNO3	3.03 ^{cde}	7.09 ^{g}
60 mM salt	Non-primed	32.29 ^{n}	1.15 ^{a}
60 mM salt	CaCl2	23.07 ^{l}	2.24 ^{c}
60 mM salt	KNO3	19.30 ^{j}	2.46 ^{c}
IRGC 7547 (Chincherica)			
0 mM salt	Non-primed	1.79 ^{ab}	18.98 ^{l}
0 mM salt	CaCl2	2.47 ^{bcd}	9.62 ^{i}
0 mM salt	KNO3	2.60 ^{bcd}	9.91 ^{i}
60 mM salt	Non-primed	24.83 ^{m}	1.25 ^{a}
60 mM salt	CaCl2	22.13 ^{k}	1.80 ^{b}
60 mM salt	KNO3	16.75 ^{i}	2.32 ^{c}
IRGC 116793 (IR64)			
0 mM salt	Non-primed	2.16 ^{abc}	8.97 ^{h}
0 mM salt	CaCl2	1.72 ^{ab}	12.27 ^{j}
0 mM salt	KNO3	1.27 ^{a}	16.29 ^{k}
60 mM salt	Non-primed	8.67 ^{g}	3.06 ^{d}
60 mM salt	CaCl2	15.68 ^{h}	2.16 ^{bc}
60 mM salt	KNO3	5.35 ^{f}	6.54 ^{f}
		s.e.d.= 0.2223	**s.e.d.= 0.0981**

5.3.4 Grain Yield

The rice accession IRGC 7547 (Chincherica) did not produce yield under salt stress, as the plants started drying at the mid vegetative stage (tillering stage), before the reproductive (panicle initiation, booting, heading and flowering) and ripening stages; thus it was excluded in the following grain analysis.

There was significant difference in mean grain yield between the two salt treatments (p-value < 0.001), and the three priming treatments (p-value < 0.001); and no significant difference between the two rice accessions which produced seeds (p-value = 0.520). There was no significant interactions between salt treatments and priming treatments (p-value = 0.758) and salt treatments, priming treatments and rice accessions (p-value = 0.502). However, there was significant interactions between salt treatments and rice accessions (p-value <0.001) and priming treatments and rice accessions (p-value = 0.019). Therefore, the means of grain yield of the rice accessions are separately influenced by salt treatments or priming treatments (**Appendix: Chapter 5**).

0 mM NaCl salt treatment (control) showed higher mean grain yield (12.325 g/plant) than 60 mM NaCl salt treatment (2.588 g/plant). Overall, 60 mM NaCl salt treatment significantly impacted grain yield in all two rice accessions (minus 79% mean grain yield). Salt treatment resulted in 95% (p-value < 0.001) and 55% (p-value = 0.0002) yield reductions of rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64), respectively (**Table 5.3**).

Non-primed treatment showed higher mean grain yield of 10.165 g/plant compared to $CaCl_2$ priming treatment (5.290 g/plant) and KNO_3 priming treatment (6.915 g/plant). Overall, compared to the non-primed treatment, $CaCl_2$ and KNO_3 priming treatments significantly decreased mean grain yield by 48% (p-value = 0.0009) and 32% (p-value = 0.0001)

respectively. However, mean grain yield varied with rice accessions. $CaCl_2$ and KNO_3 priming treatments did not significantly affect mean grain yield of rice accessions IRGC 7546 (Gaza) and decreased the mean grain yield of rice accessions IRGC 116793 (IR64) by 64 % (p-value = 0.00008) and 53 % (p-value = 0.0005) respectively (**Table 5.3**).

Table 5.3: Effect of salt treatments and priming treatments in mean grain yield (g/plant). Two rice accessions (IRGC 7546 (Gaza) and IRGC 116793 (IR64)) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Salt stress was imposed from 32 d after sowing up to maturity. Data are shown as mean value +/- SEM of eight individual replications (n = 8).

Salt Treatments	Rice Accessions	Yield (g/plant)	
		Mean	Std.Err.
0 mM NaCl (Control)	IRGC 7546 (Gaza)	14.771	1.055
	IRGC 116793 (IR64)	9.879	0.856
60 mM NaCl	IRGC 7546 (Gaza)	0.760	0.985
	IRGC 116793 (IR64)	4.417	0.907

Priming Treatments	Rice Accessions	Yield (g/plant)	
		Mean	Std.Err.
Non-primed	IRGC 7546 (Gaza)	8.580	1.168
	IRGC 116793 (IR64)	11.749	1.020
$CaCl_2$	IRGC 7546 (Gaza)	6.384	1.129
	IRGC 116793 (IR64)	4.196	1.093
KNO_3	IRGC 7546 (Gaza)	8.331	1.431
	IRGC 116793 (IR64)	5.499	1.126

5.3.5 Grain Starch Concentration

Significant differences in mean grain starch concentration (%) between the two rice accessions (p-value = 0.0013), two salt treatments (p-value <0.001), and three priming treatments (p-value < 0.001) were observed. Significant interactions between salt treatments and priming treatments (p-value < 0.001), salt treatments and rice accessions (p-value < 0.001), priming treatments and rice accessions (p-value < 0.001), and salt

treatments, priming treatments and rice accessions (p-value = 0.009) were observed (**Appendix: Chapter 5**). Therefore, mean grain starch concentration (%) among the rice accessions are dependent on both salt treatments and priming treatments.

Grain from rice plants under 0 mM NaCl (control) showed higher mean grain starch concentration (60.394%) than grain from rice plants under 60 mM NaCl (58.078%). Overall, compared to control, 60 mM NaCl salt treatment significantly decreased mean grain starch concentration by, on average, 2% (p-value < 0.001). However, this reduction varied with rice accessions. The rice accession IRGC 7546 (Gaza) showed a 7% reduction (p-value < 0.001) compared to a reduction of 3 % (p-value < 0.0495) for the rice accession IRGC 116793 (IR64) (**Table 5.4**).

Grain from plants subjected to the $CaCl_2$ priming treatment showed highest mean grain starch concentration (60.342 %); whereas, grain from plants subjected to the KNO_3 priming treatment showed lowest mean grain starch concentration (57.604 %). Overall, compared to non-primed treatment (control), $CaCl_2$ priming treatment increased the mean grain starch concentration by 0.6% (p-value = 0.051) but this was not significant; whereas, KNO_3 priming treatment significantly lowered mean grain starch concentration by 2% (p-value = 0.0003). Under 60 mM NaCl salt treatment, compared to non-primed, $CaCl_2$ and KNO_3 priming treatments did not significantly affect the grain starch concentration (%) of both rice accessions IRGC 7546 Gaza and IRGC 116793 (IR64) (**Table 5.4**). However, the highest means grain starch concentrations under 60 mM NaCl and the lowest reductions of mean grain starch concentration (%) under 60 mM NaCl, compared to the respective 0mM NaCl (control), were observed with KNO_3 priming treatment (3.7%) and $CaCl_2$ priming treatment (5.05%) for the rice accession IRGC 7546 (Gaza); and with $CaCl_2$ priming treatment (0.38%)

for IRGC 116793 (IR64). Under KNO_3 priming treatment and IRGC 116793 (IR64), 60 mM NaCl salt treatment was associated with an increase of 5.68% (p-value = 0.0006) in mean grain starch concentration (%) (**Table 5.4**).

Table 5.4: Effects of salt treatments and priming treatments in grain starch concentration (%) on two rice accessions. Two rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Data are shown as mean value +/- SEM of two individual replications (n = 2).

Salt Treatments	Priming Treatments	Starch Concentration (%)	
		Mean	Std.Err.
IRGC 7546 (Gaza)			
0mM salt	Non-primed	63.833	0.485
0mM salt	$CaCl_2$	62.347	0.485
0mM salt	KNO_3	61.500	0.485
60mM salt	Non-primed	56.659	0.485
60mM salt	$CaCl_2$	57.300	0.686
60mM salt	KNO_3	57.800	0.686
IRGC 116793 (IR64)			
0mM salt	Non-primed	60.913	0.485
0mM salt	$CaCl_2$	61.053	0.485
0mM salt	KNO_3	52.715	0,485
60mM salt	Non-primed	57.636	0.485
60mM salt	$CaCl_2$	60.669	0.485
60mM salt	KNO_3	58.402	0.485

5.3.6 Grain Amylose Concentration

The two rice accessions exhibited intermediate (20-25%) grain amylose concentration with overall mean of 24.456 % and 21.478 % for IRGC 7546 Gaza and IRGC 116793 (IR64), respectively.

There was no significant difference in mean grain amylose concentration (%) between the two rice accessions (p-value = 0.116), three priming treatments (p-value = 0.519), and two

salt treatments (p-value = 0.381). There were also no significant interactions between salt treatments and priming treatments (p-value = 0.801), salt treatments and rice accessions (p-value = 0.625), priming treatments and rice accessions (p-value = 0.907), and salt treatments, priming treatments and rice accessions (p-value = 0.464) (**Appendix: Chapter 5**).

Overall, 60 mM NaCl salt treatment slightly decreased grain amylose concentrations (%), while priming treatments slightly increased grain amylose concentrations (%). Under 60 mM NaCl salt treatment, $CaCl_2$ and KNO_3 priming treatments increased grain amylose concentration (%) of rice accessions IRGC 7546 Gaza and IRGC 116793 (IR64), and particularly increased grain amylose concentrations (%) from low (10 - 19%) to intermediate (20-25%) in rice accession IRGC 116793 (IR64) (**Table 5.5**). However, the effect of salt treatments and priming treatments were not significant.

Table 5.5: Effects of salt treatments and priming treatments in grain amylose concentration (%) on two rice accessions. Two rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Data are shown as mean value +/- SEM of two individual replications (n = 2).

Salt Treatments	Priming Treatments	Amylose Concentrations (%)	
		Mean	Std.Err.
IRGC 7546 (Gaza)			
0mM salt	Non-primed	24.266	2.481
0mM salt	$CaCl_2$	27.061	2.481
0mM salt	KNO_3	25.673	2.481
60mM salt	Non-primed	21.946	2.026
60mM salt	$CaCl_2$	22.506	3.509
60mM salt	KNO_3	25.283	3.509
IRGC 116793 (IR64)			
0mM salt	Non-primed	22.037	3.509
0mM salt	$CaCl_2$	21.035	3.509
0mM salt	KNO_3	22.421	3.509
60mM salt	Non-primed	18.183	2.481
60mM salt	$CaCl_2$	24.509	2.481
60mM salt	KNO_3	20.682	2.481

5.3.7 Grain Protein Concentration

A significant difference in grain protein concentration between the two rice accessions (p-value < 0.001), two salt treatments (p-value < 0.001) and three priming treatments (p-value <0.001) were observed. There were also significant interactions between salt treatments and priming treatments (p-value < 0.001), salt treatments and rice accessions (p-value < 0.001); priming treatments and rice accessions (p-value < 0.001); and salt treatments, priming treatments and rice accessions (p-value < 0.001). Therefore, the mean grain protein concentration in the rice accessions are dependent on both salt treatments and priming treatments (**Appendix: Chapter 5**).

0 mM NaCl salt treatment (control) showed lower mean grain protein concentration (9.20%) than 60 mM NaCl salt treatment (12.45%). Overall, 60 mM NaCl salt treatment significantly increased mean grain protein concentration by 3%. However, this increase varied with rice accessions. The rice accession IRGC 7546 (Gaza) showed higher increase of 4.414% (p-value < 0.001), than the rice accession IRGC 116793 (IR64), which showed an increase of 2.536% (p-value < 0.001) (**Table 5.6**).

Across the two rice accessions and two salt treatments, grain from plants subjected to the $CaCl_2$ priming treatment showed highest mean grain protein concentration (11.203%), followed by grain from plants subjected to the KNO_3 priming treatment (10.879%) and the non-primed treatment (10.392%). However, compared to non-primed treatment, both $CaCl_2$ (p-value = 0.321) and KNO_3 (p-value = 1.000) were not significantly higher. Under 60 mM NaCl salt treatment, compared to non-primed, $CaCl_2$ (p-value < 0.001) and KNO_3 (p-value = 0.0012) priming treatments significantly increased grain protein concentration (%) of rice accession IRGC 7546 Gaza by 1.65% and 1% respectively; $CaCl_2$ priming treatment (p-value =

1.000) did not have significant effect and KNO_3 priming treatment (p-value < 0.001) significantly decreased (0.94% less) grain protein concentration (%) of rice accession IRGC 116793 (IR64) (**Table 5.6**).

Table 5.6: Effects of salt treatments and priming treatments in grain protein concentration (%) on two rice accessions. Two rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) treated with three priming treatments (non-primed, $CaCl_2$, and KNO_3) were grown under control (0mM NaCl) and salt treatment (60mM NaCl) in a Fitotron plant growth chamber (WEISS GallenKamp): day and night temperatures 29 °C and 26 °C respectively, relative humidity 70%, PAR light intensity was 300–600 µmol m^{-2} s^{-1} at crop canopy. Data are shown as mean value +/- SEM of three individual replications (n = 3).

Salt Treatments	Priming Treatments	Grain Protein Concentrations (%)	
		Mean	Std.Err.
IRGC 7546 (Gaza)			
0mM salt	Non-primed	7.571	0.087
0mM salt	$CaCl_2$	8.382	0.087
0mM salt	KNO_3	8.658	0.087
60mM salt	Non-primed	11.985	0.087
60mM salt	$CaCl_2$	13.639	0.151
60mM salt	KNO_3	12.995	0.151
IRGC 116793 (IR64)			
0mM salt	Non-primed	9.737	0.087
0mM salt	$CaCl_2$	10.310	0.087
0mM salt	KNO_3	10.526	0.087
60mM salt	Non-primed	12.274	0.087
60mM salt	$CaCl_2$	12.482	0.151
60mM salt	KNO_3	11.338	0.087

5.3.8 Grain Protein Profile

Comparison of the SDS-PAGE grain protein profile of rice accessions IRGC 7546 (Gaza) and IRGC 116793 (IR64) under the three priming (non-primed, $CaCl_2$, and KNO_3) and two salt treatments (0 mM NaCl and 60 mM NaCl) (**Figure 5.3**) showed some differences between the profile of the rice accession IRGC 7546 Gaza under KNO3 priming treatment and 60 mM NaCl salt concentration (**G2 in Figure 5.3**) compared to all other treatments for that same rice accession (**G1, G3, G4, G5 and G6**). In G2 there was an increase in density in some major bands corresponding to protein of apparent molecular size in the range 12 kDa -38 kDa (green rectangle) and a decrease in some bands corresponding to the 50 kDa-115 kDa range (red rectangle) (**Figure 5.3)**. No differences in protein expression were observed among the different treatment for IRGC 116793 (IR64).

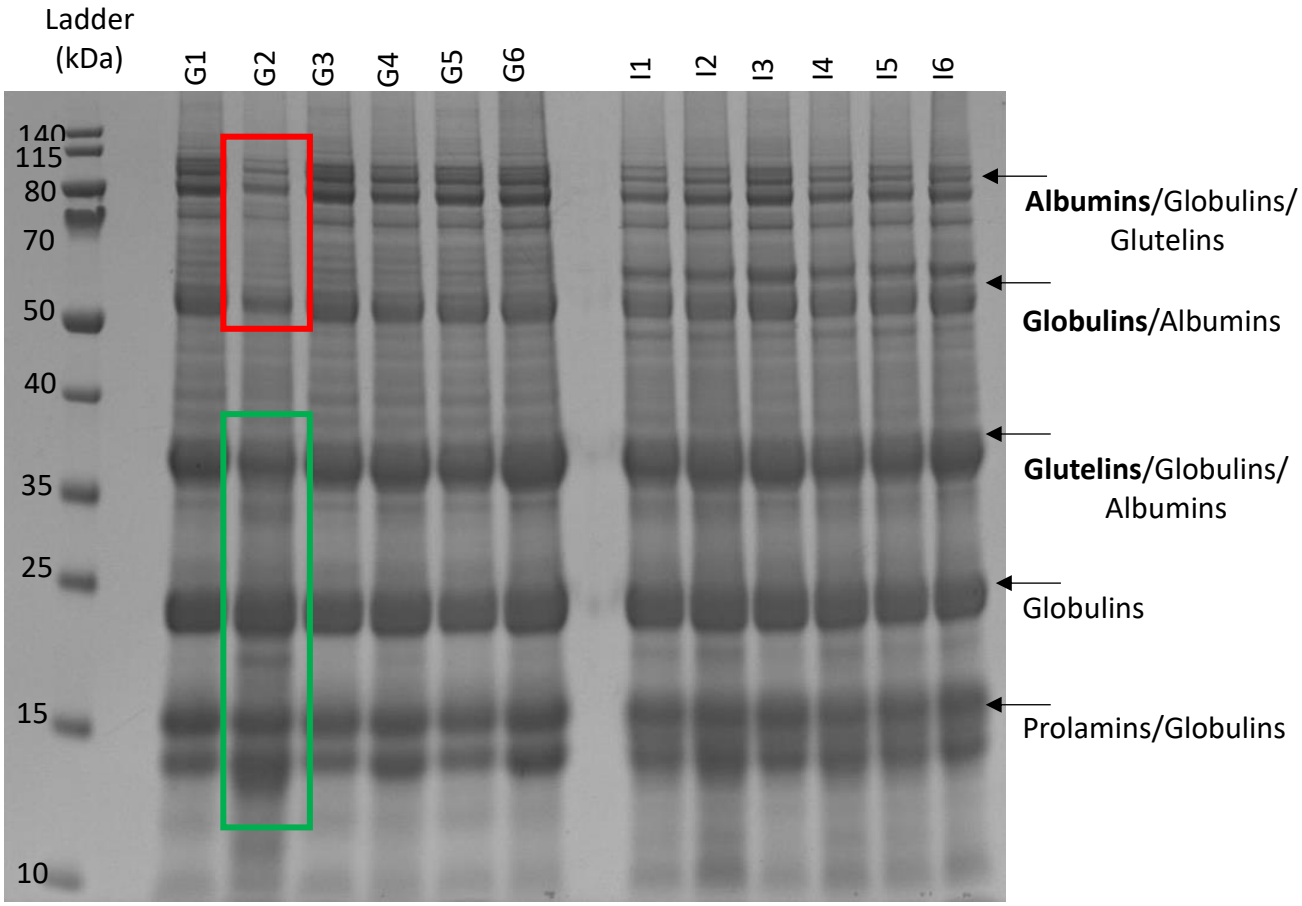


Figure 5.3: SDS- PAGE grain protein profile of the rice accessions IRGC 7546 Gaza and IRGC 116793 (IR64) under two salt treatments (0mM NaCl and 60mM NaCl) and three priming treatments (non-primed, $CaCl_2$, and KNO_3). **G1**: Gaza KNO_3 0mM NaCl; **G2**: Gaza KNO_3 60mM NaCl; **G3**: Gaza non-primed 0mM NaCl; **G4**: Gaza non-primed 60mM NaCl; **G5**: Gaza $CaCl_2$ 0mM NaCl; and **G6**: Gaza $CaCl_2$ 60mM NaCl. **I1**: IR64 KNO_3 0mM NaCl; **I2**: IR64 KNO_3 60mM NaCl; **I3**: IR64 $CaCl_2$ 0mM NaCl; **I4**: IR64 $CaCl_2$ 60mM NaCl; **I5**: IR64 non-primed 0mM NaCl; **I6**: IR64 non-primed 60mM NaCl. Assignment of specific protein bands to protein solubility classes (on the right of the gel) was done on the basis of results from **Figure 5.4**.

Flour from **G2** (Gaza KNO_3 60 mM NaCl) was selected on the basis of its distinctive protein profile and subjected, together with the respective control flour **G1** (Gaza KNO_3 0 mM NaCl), to sequential extraction and SDS-PAGE densitometry analysis, in order to identify and quantify grain storage proteins types differentially accumulated in the salt two treatments (**Figure 5.4**).

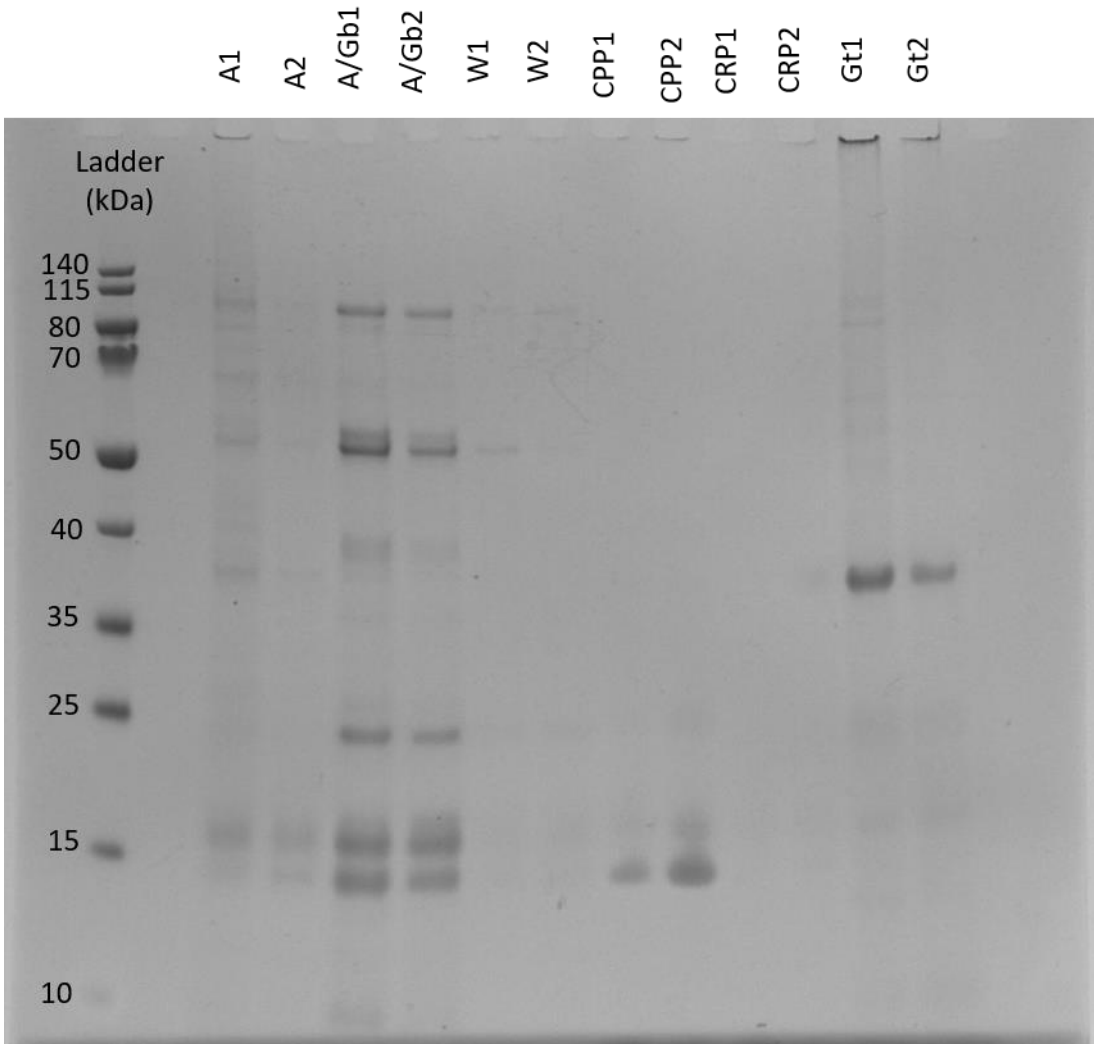


Figure 5.4: Sequential Protein Extract of the rice accessions IRGC 7546 Gaza under two treatments of salinity (0mM NaCl and 60mM NaCl) and KNO_3 priming treatment. Gaza KNO_3 0mM NaCl Albumins (**A1**); Gaza KNO_3 60mM NaCl Albumins (**A2**); Gaza KNO_3 0mM NaCl Albumins/Globulins (A/G) (**A/Gb1**); Gaza KNO_3 60mM NaCl Albumins/Globulins (A/G) (**A/Gb2**); Gaza KNO_3 0mM NaCl Water (**W1**); Gaza KNO_3 60mM NaCl Water (**W2**); Gaza KNO_3 0mM NaCl Cysteine Poor Prolamins (CPP) (**CPP1**); Gaza KNO_3 60mM NaCl Cysteine Poor Prolamins (CPP) (**CPP2**); Gaza KNO_3 0mM NaCl Cysteine Rich Prolamins (CRP) (**CRP1**); Gaza KNO_3 60mM NaCl Cysteine Rich Prolamins (CRP) (**CRP2**); Gaza KNO_3 0mM NaCl Glutelins (**Gt1**); Gaza KNO_3 60mM NaCl Glutelins (**Gt2**).

The bands in the lanes were selected and the apparent molecular weight (MW) and raw volume (refers to density of each band) were detected and associated with the protein type in the sequential extraction (**Figure 5.5**).

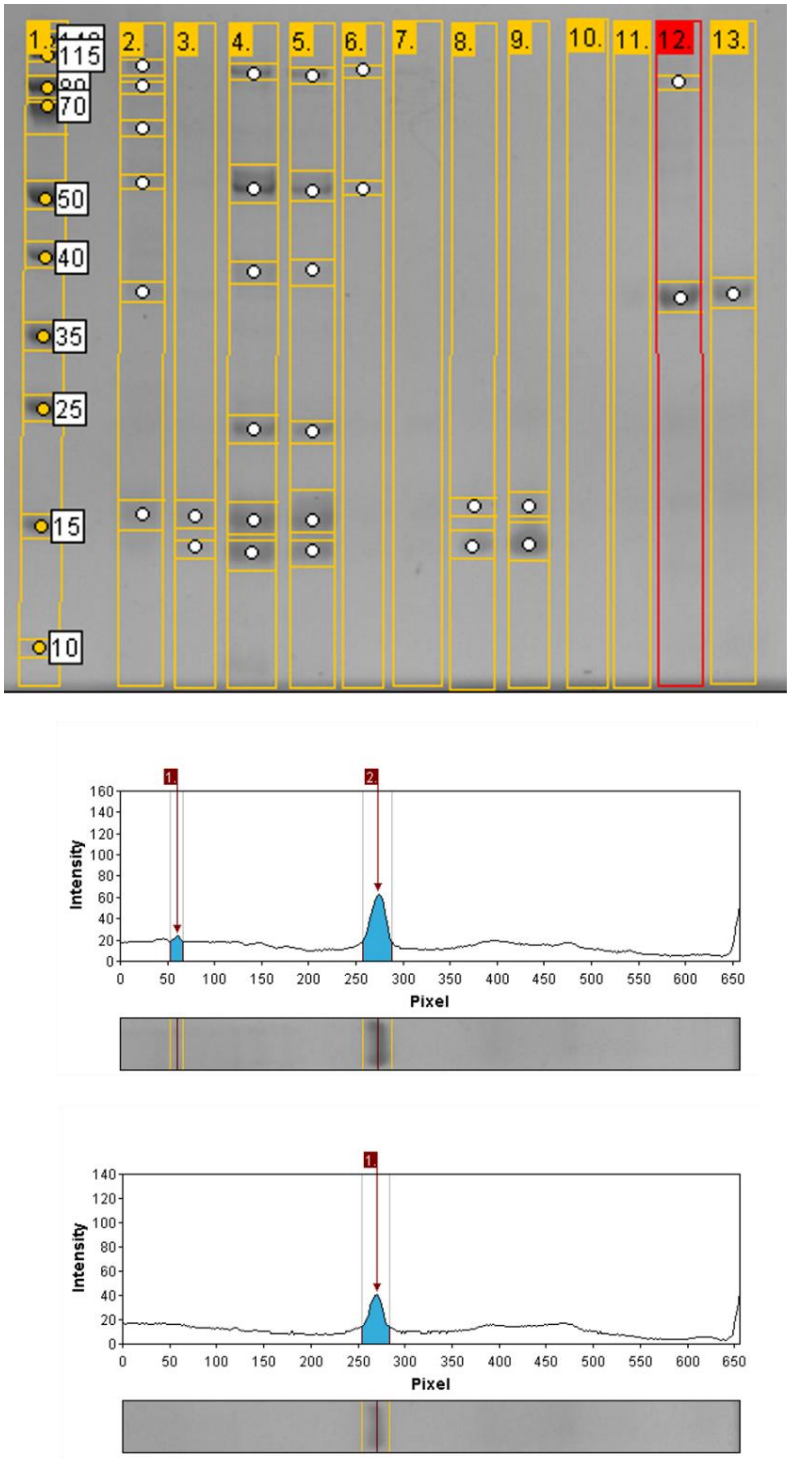


Figure 5.5. **A** Densitometry analysis of a Jpeg image of SDS-PAGE gel separating protein fractions obtained from sequential extraction of flour from two treatments (Gaza KNO_3 0 mM NaCl – **G1,** and Gaza KNO_3 60 mM NaCl – **G2**). Across different lanes bands were selected (framed) and quantified Lane 2 and 3 correspond to water extracts (Albumins - **A**); lane 4 and 5 correspond to salt extracts (Albumins/Globulins - **A/G**), lane 6 and 7 correspond to water extracts (Water - **W**), lane 8 and 9 correspond to alcohol extracts (Cysteine Poor Prolamins - **CPP**), lane 10 and 11 correspond to alcohol extracts (Cysteine Rich Prolamins - **CRP**), lane 12 and 13 correspond to SDS-extracted residue (Glutelins - **Gt**) all from **G1** and **G2**, respectively. **B) & C)** indicating the selection and quantification of SDS extracted protein in the lanes 12 and 13 in **A)**.

The analysis of variance of the densitometry analysis data from the sequential extractions of rice accession IRGC 7546 (Gaza) under KNO_3 priming treatment and the two salt treatments (0mM NaCl and 60 mM NaCl salt treatments): **G1** and **G2** showed that there were significant differences in mean protein raw volume (band density) between two salt treatments (p-value = 0.012), five protein types (p-value < 0.001) and between the interaction protein types and salt treatments (p-value = 0.0021) **(Appendix: Chapter 5)**. Overall, 60 mM NaCl salt treatment decreased mean grain protein raw volume (band density) by 26%. Across the two salt treatments under the KNO_3 priming treatment, the salt soluble fraction of protein (Albumins/Globulins (**A/G**) - mainly globulins) showed the highest raw volume (mean = 4473.125) followed by the water soluble fraction type (mainly albumins, mean = 2506.250); the alcohol soluble prolamins (mean = 1095.500) and the SDS-extracted residue, consisting of glutelins (mean = 1048.250) showed the lower raw volume (band density). The band density of globulin fraction (A/G) was significantly higher than the albumin (p-value < 0.001), prolamins (p-value < 0.001) and glutelins (p-value < 0.001) enriched fractions.

Therefore, salt treatment applied to KNO_3-primed rice resulted in lower relative band density of albumins, globulins, and glutelins, and in higher band density of prolamins, but this change was only significant for albumins (p-value = 0.0079), which showed 59.13% of mean protein raw volume reduction (**Figure 5.6**).

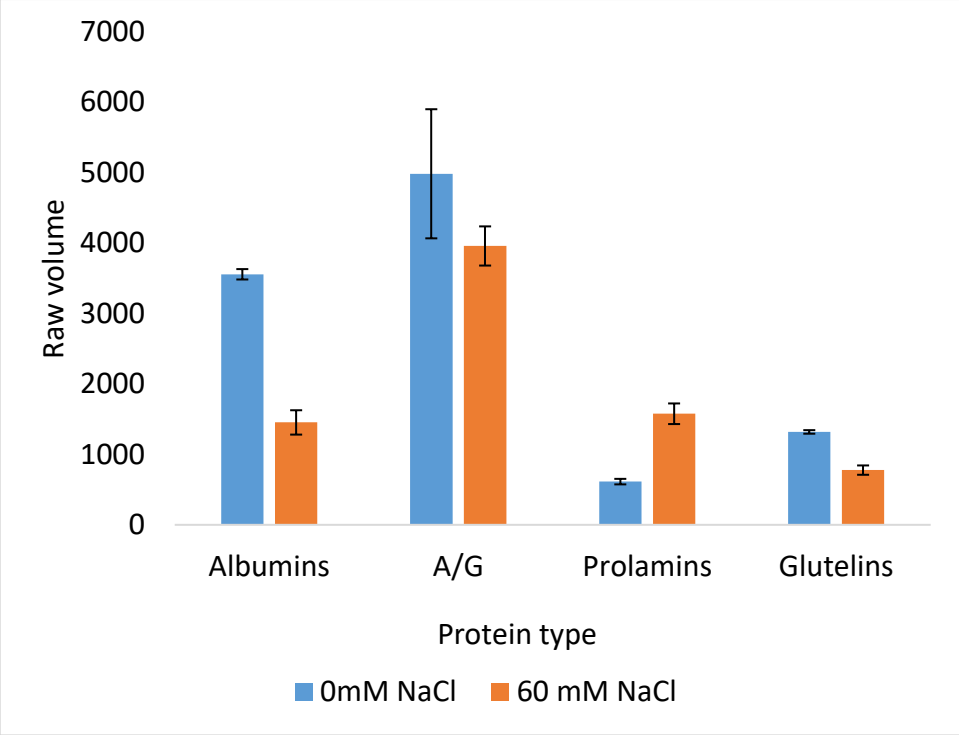


Figure 5.6: Densitometry graphic from sequential protein extract of the rice accession IRGC 7546 Gaza under two treatments of salinity (0mM NaCl and 60mM NaCl) and KNO_3 priming treatment, indicating the relative band density of storage protein (Albumins, Albumins/Globulins (A/G), Prolamins, and Glutelins). Data are shown as mean value +/- SEM of two individual replications (n=2). The bars with * are significantly different (p- value < 0.05). The error bars represent SEM.

5.3.9 Correlations between Grain Yield and Shoot Dry Weight, Shoot Na Concentration, Shoot K^+/Na^+ Discrimination, Starch Concentration, Amylose Concentration and Protein Concentration on Two Rice Accessions (IRGC 7546 (Gaza) and IRGC 116793 (IR64))

Under the two salt treatments and the three priming treatments, for the rice accession IRGC 7546 Gaza there was a significant and positive correlation between grain yield and shoot dry weight (r = 0.8245), grain yield and shoot K^+/Na^+ discrimination (r = 0.9248), grain yield and grain starch concentration (%) (r = 0.9344); not significant and positive correlation between grain yield and grain amylose concentration (r=0.5919); and significant and negative correlation between grain yield and shoot Na concentration (r = -0.9023), and grain yield and grain protein concentration (%) (r=-0.9692) (**Table 5.7**). For the rice accession IRGC

116793 (IR64), there was no significant correlation between grain yield and all other variables: shoot dry weight (r = 0.2804), shoot Na concentration (r = -0.6432), K^+/Na^+ discrimination (r = 0.3733), grain starch concentration (%) (r=-0.0493), grain amylose concentration (%) (r=-0.3953); grain protein concentration (%) (r=-0.6508) (**Table 5.7**).

Table 5.7 Correlation (r) between shoot dry weight (g/plant), shoot Na concentration (mg Na g^{-1} DW), shoot K^+/Na^+ discrimination, starch concentration (%), amylose concentration (%) and protein concentration (%) and grain yield (g/plant) variables of the two rice accessions (IRGC 7546 (Gaza) and IRGC 116793 (IR64)), under two salt treatments (0mM NaCl and 60mM NaCl), and three priming treatments (non-primed, $CaCl_2$, and KNO_3), ($p < 0.05$, n=6).

Variable	SDW (g/plant)	Na^+	K^+/Na^+	Starch (%)	Amylose (%)	Protein (%)
IRGC 7546 (Gaza)						
Yield (g/plant)	0.8245*	-0.9023*	0.9248*	0.9344*	0.5919	-0.9692*
IRGC 116793 (IR64)						
Yield (g/plant)	0.2804	-0.6432	0.3733	-0.0493	-0.3953	-0.6508

** Significant*

5.3.10 Correlations between Grain Starch Concentration (%) and Grain Protein Concentration (%) on Two Rice Accessions (IRGC 7546 (Gaza) and IRGC 116793 (IR64))

Under the two salt treatments and the three priming treatments, the rice accession IRGC 7546 Gaza showed a significant and negative correlation between grain starch concentrations (%) and grain protein concentrations (%) (r = - 0.951), and the rice accession IRGC 116793 (IR64) did not show a significant correlation between grain starch concentrations (%) and grain protein concentrations (%) (r = - 0.002). Therefore, the increase of grain protein concentrations is associated with the decreased of grain starch concentrations only for the rice accession IRGC 7546 Gaza (**Table 5.8**).

Table 5.8: Correlation (r) between grain starch concentrations (%) and grain protein concentrations (%) variables of the two rice accessions (IRGC 7546 (Gaza) and IRGC 116793 (IR64)), under two salt treatments (0mM NaCl and 60mM NaCl), and three priming treatments (non-primed, $CaCl_2$, and KNO_3), ($p < 0.05$, n=6).

Salt Treatments	Priming Treatments	Grain Starch Concentrations (%)	Grain Protein Concentrations (%)
IRGC 7546 (Gaza)			
0 mM NaCl	Non-primed	63.833	7.571
60 mM NaCl	Non-primed	56.659	11.985
0 mM NaCl	$CaCl_2$	62.347	8.382
60 mM NaCl	$CaCl_2$	57.300	13.639
0 mM NaCl	KNO_3	61.500	8.658
60 mM NaCl	KNO_3	57.800	12.995
Means		**59.907**	**10.538**
Std.Dev.		**3.023**	**2.636**
r ($p < 0.05$ n=6)	**- 0.951***		
IRGC 116793 (IR64)			
0 mM NaCl	Non-primed	60.913	9.737
60 mM NaCl	Non-primed	57.636	12.274
0 mM NaCl	$CaCl_2$	61.053	10.310
60 mM NaCl	$CaCl_2$	60.669	12.482
0 mM NaCl	KNO_3	52.715	10.526
60 mM NaCl	KNO_3	58.402	11.338
Means		**58.565**	**11.111**
Std.Dev.		**3.201**	**1.109**
r ($p < 0.05$ n=6)	**-0.002**		

** significant*

5.4 Discussion

5.4.1 Salt Stress on Shoot Dry Weight, Shoot Na Concentration, Shoot K^+/Na^+ Discrimination, and Grain Yield

As hypothesized, overall salt treatment reduced shoot dry weight production and this was more prominent on relatively sensitive rice accession IRGC 7547 Chincherica (**Table 5.1**). This is similar to what reported by Gerona et al. (2019), who observed that under salt stress there was an overall reduction of shoot dry weight, and that the magnitude of salt stress was higher on sensitive genotypes. In our first salt stress screening study, with a shorter experiment duration and higher salt concentrations (100 mM NaCl), rice accession IRGC 7547 Chincherica showed lower salt tolerance (salt tolerance = 61%) than rice accessions IRGC 7546 Gaza (salt tolerance = 64%) and IRGC 116793 (IR64) (salt tolerance = 68%) (**Chapter 2: Figure 2.4**). These differences in salt tolerance may not appear to be large, but at lower salt concentrations (60 mM NaCl) and in a prolonged experiment, the difference in salt sensitivity may have increased.

In this study as expected, the addition of 60 mM NaCl salt concentration to the hydroponic solution, overall increased shoot Na concentrations, and decreased shoot K^+/Na^+ ratios. This increase of shoot Na concentrations and decrease of shoot K^+/Na^+ ratios was more prominent in the relatively sensitive rice accession IRGC 7547 Chincherica, followed by the rice accession IRGC 7546 Gaza; and lower in relatively tolerant rice accession IRGC 116793 (IR64) **(Appendix: Chapter 5)**. This is in concordance with literature which reports that tolerant varieties maintain lower shoot Na concentrations and higher shoot K^+/Na^+ ratios than sensitive varieties (Flowers and Yeo, 1981, Haq et al., 2009, Haq et al., 2014).

In this experiment, under 60mM NaCl salt treatment, the rice accession IRGC 7547 Chincherica did not reach the reproductive stage. Furthermore, the stress of 60mM NaCl salt concentrations dramatically reduced the grain yield of the two rice accessions IRGC 7546 Gaza and IRGC 116793 (IR64), with yield losses of 95% and 55% respectively **(Table 5.3)**. Salt concentrations of about 60 mM NaCl were reported in literature to reduce the grain yield of rice by 50 % (Maas and Hoffman, 1977), but lower salt concentrations of 20 mM NaCl or 40 mM NaCl can already cause yield reductions of 33 % and 50 %, respectively (Lutts et al., 1995, Abdullah et al., 2001, Grattan et al., 2002, Baxter et al., 2011). The yield reductions we observed in our study are therefore higher than the expected under 60 mM NaCl from (Maas and Hoffman, 1977). This difference from Maas and Hoffman (1977) and our study may be explained by the fact that the 50% grain yield reduction value was predicted based on a traditional model that simulates crops yield response under saline conditions, which was created based on the literature. This model is intended to provide a general guide for crop management under salt stress and it may not be always accurate.

The response to salinity varies based on stage of growth and development, severity, and duration of stress (Zeng et al., 2001, Ismail and Horie, 2017). In this study, salinity was applied from 32 d after sowing, up to physiological maturity (from mid vegetative phase, through grain filling, to harvest ready), plants had therefore prolonged exposure to salinity stress. It is probable that an earlier or a shorter duration of the application of salinity stress would have had a lower impact on yield. Moreover, the sensitivity to salinity varies among genotypes (Lee et al., 2003, Kurotani et al., 2015). From previous study (**Chapter 2: Figure 2.4)** these three rice accessions IRGC 7547 Chincherica, IRGC 7546 Gaza, and IRGC 116793 (IR64) are among the sensitive genotypes and within them different degree of salt sensitivity was displayed. In this study, these accessions also showed different degrees of tolerance to

60 mM NaCl salt stress, and it was lower in IRGC 7547 Chincherica, followed by IRGC 7546 Gaza and IRGC 116793 (IR64) **(Table 5.3).**

5.4.2 The Influence of Priming Treatments on Shoot Dry Weight, Shoot Na Concentration, Shoot K^+/Na^+ Discrimination, and Grain Yield

Our hypothesis that priming treatments ($CaCl_2$, and KNO_3) would ameliorate the negative effects of salt stress (60 mM NaCl salt concentrations) did not proved right: priming treatment with 200mM $CaCl_2$ decreased shoot dry weight of rice accessions IRGC 7546 Gaza, and IRGC 116793 (IR64), both on 0 mM NaCl and 60 mM NaCl salt stress, therefore displaying lower salt tolerance with respect to non-primed samples, and although increased shoot dry weight of rice accession IRGC 7547 Chincherica, these changes were not significant (**Table 5.1**). Findings from our study are in agreement with Theerakulpisut et al. (2017): although an higher level of salinity stress (150 mM NaCl) was applied in that study compared to ours, they noticed that 200 mM $CaCl_2$ priming treatment did not have significant effect on shoot dry weight. They underlined that the insignificant effect of $CaCl_2$ priming treatment, which was different from previous studies, was possibly due to the rice genotype and priming concentration applied in that study.

In this study, priming treatment with 297 mM KNO_3 decreased shoot dry weight of the rice accessions IRGC 7546 Gaza and IRGC 116793 (IR64), while increased shoot dry weight of the rice accession IRGC 7547 Chincherica, but these changes were not significant (**Table 5.1**). In contrast, Theerakulpisut et al. (2017), noticed that under 150 mM NaCl salinity stress, KNO_3 priming treatment significantly increased shoot dry weight by 25% to 31.15%. (Theerakulpisut et al., 2017). It has been reported that seed priming with inorganic salts

ameliorates the negative effect of salinity through improvement of growth parameters (roots, leaves, stems) as a result of induction and *de novo* synthesis of hydrolases (amylases, lipases, proteases), antioxidants (catalases, superoxide dismutase, and peroxidases) and osmolytes (proline content) (Farooq et al., 2006a, Farooq et al., 2009, Guo et al., 2022). However, also due to the significant changes of shoot Na concentrations and shoot K^+/Na^+ ratios, which enable better osmotic adjustment (Cayuela et al., 1996, Iqbal and Ashraf, 2007, Afzal et al., 2008). As hypothesized, 200 mM $CaCl_2$ and 297 mM KNO_3 priming treatments decreased mean shoot Na-concentrations and increased mean shoot K^+/Na^+ ratios of rice accessions IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 66970 (IR64). Under 60 mM NaCl salt treatment, KNO_3 followed by $CaCl_2$ priming treatments showed lowest shoot Na concentration and highest shoot K^+/Na^+ discrimination (**Table 5.2**). Similarly, Afzal et al. (2012) reported that under moderate salinity stress of 40 and 80 mM NaCl, halopriming with 200 mM $CaCl_2$ salt solutions of Ψ_s= -1.25 MPa osmotic potential decreased Na-ions and increased K-ions concentrations in the leaves, resulting in improved tolerance of both salt tolerant and sensitive fine (i.e. long or medium grain – indica rice) aromatic rice cultivars. Moreover, Theerakulpisut et al. (2017) observed that under 150 mM NaCl salt treatment, KNO_3 priming treatment alleviated the negative impact of salinity on growth of young rice seedlings and the ratios Na^+/K^+ were relatively low compared to the non-primed control. In this study, the 200 mM $CaCl_2$ and 297 mM KNO_3 priming treatments may have been crucial in lowering mean shoot Na-concentrations and in increasing mean shoot K^+/Na^+ ratios, and this may have slightly benefited the sensitive rice accession IRGC 7547 Chincherica, which showed an increase of shoot dry weight under both priming treatments (**Table 5.2**).

Different to what initially hypothesized in this study, independent to salt treatment, priming treatment with 200 mM $CaCl_2$ and 297 mM KNO_3 decreased mean grain yield of the rice

accessions IRGC 7546 Gaza and IRGC 116793 (IR64). These changes were significant for the rice accession IRGC 116793 (IR64), hence priming with 200 mM $CaCl_2$ and 297 mM KNO_3 negatively affected the growth and development of this accession independent of the concentrations of salt treatment (no effect of salinity stress was observed) (**Table 5.3**). This findings are different from that reported by Farooq et al. (2006a),Farooq et al. (2006e) and Rehman et al. (2011), who observed increased grain yield of direct seeded rice under normal conditions in farmer fields following the application of 200 mM $CaCl_2$ salt solutions of Ψ_s= -1.25 MPa osmotic potential. The increased of grain yield is reported with osmopriming followed by re-drying, or osmohardening (soaking the seeds for 24 h in 200 mM $CaCl_2$ salt solutions of Ψ_s= -1.25 MPa osmotic potential, re-drying to initial moisture content, and repeating the cycle once), while our study evaluated the effectiveness of 200 mM $CaCl_2$ salt solutions for 36 h, re-drying to initial moisture content followed by sowing. Dhillon et al. (2021) observed that the application of KNO_3 priming treatment increased grain yield in about 8 to 10% of direct seeded rice under normal conditions in a field experiment. However, unlike our study, this experiment was carried out with lower priming concentration (198 mM KNO_3), shorter priming duration (12 h and 14 h) and it was in a field experiment.

5.4.3 The Effect of Salt Stress on Starch, Amylose, and Protein Concentrations

Starch. As by our hypothesis, compared to control (0mM NaCl salt treatment), 60 mM NaCl salt treatment decreased grain starch concentration of the two rice accessions IRGC 7546 (Gaza) and IRGC 66970 (IR64). However, the specific effect varied with rice accession. The rice accession IRGC 7546 Gaza showed a higher reduction in grain starch concentration

under 60 mM NaCl compared to control plants (**Table 5.4**). This partly is in concordance with Siscar-Lee et al. (1990) who reported that under EC = 50-60 mM NaCl, compared to the control (normal soil), there was a reduction of starch concentration in four rice varieties with different degrees of salt tolerance, however, this reduction was not associated to the degree of salt tolerance. Rao et al. (2013), on the other hand observed that under EC = 40 and 80 mM NaCl salinity stress, compared to the control, there was a reduction in grain starch concentration, in nineteen genotypes categorized as tolerant, semi-tolerant and sensitive, and this was more prominent in the salt sensitive varieties.

Amylose. In this study, on the other hand, the 60mM NaCl salt treatment did not significantly affect the amylose concentration, compared to control (0mM NaCl salt treatment). Grain of the two rice accessions remained within the intermediate class of amylose concentration (20 - 25%) (Kumar and Khush, 1986), also in the case of grain produced under 60mM NaCl salt treatment (**Table 5.5**). Therefore, they would be expected to have gelatinization temperature of 70 ^{o}C to 74 ^{o}C, soft gel consistency (length of gel > 61 mm), cook moist and tender and not harden when cool, which represent preferred cooking and eating quality parameters (**Chapter 1: Table 1.2**). Similarly, Thitisaksakul et al. (2015) observed that when rice plants were grown, in greenhouse conditions, in pots with soil mixture flooded with salt solution of EC = 40 mM NaCl and EC = 20 mM NaCl, applied respectively from seedling stage (four weeks after transplant) or after the anther appearance up to physiological maturity, compared to the control, did not result in significant change in amylose concentration in the grain flour as well as in single grain of the rice cultivar Nipponbare. On the other hand, Rao et al. (2013) observed that when rice plants of nineteen genotypes categorized as tolerant, semi-tolerant and sensitive were grown up to physiological maturity, in the lysimeters filled with saline soils of EC = 40 mM NaCl and EC=80 mM NaCl, compared to the control (normal

soil), there was a reduction in grain amylose concentration. The difference in rice genotypes, salt concentration is in the same range, may explain the different effect of amylose concentration from our study.

Protein. As hypothesized, in this study, under 60mM NaCl salt treatment, compared to control (0mM NaCl salt treatment), there was an overall increase in grain protein concentration. This increase was higher in the rice accession IRGC 7546 Gaza than in accession IRGC 116793 (IR64) (**Table 5.6**). Moreover, the increased grain protein concentration (%) is associated with the decrease of grain starch concentration (%) for the rice accession IRGC 7546 Gaza and not for the rice accession IRGC 116793 (IR64) (**Table 5.8**). Similarly, Siscar-Lee et al. (1990) noticed that under EC = 50-60 mM NaCl, compared to the control (normal soil), there was an increase of brown rice protein concentration in four rice varieties with different degrees of salt tolerance. Thitisaksakul et al. (2015) observed that under EC = 40 mM NaCl salinity stress imposed at anthesis stage, compared to the control in greenhouse conditions, the grain protein concentration increased by 20.1 %, which was a result of the increase of the glutelin followed by prolamin.

5.4.4 The Influence of Priming Treatments on Starch, Amylose, and Protein Concentrations

In this study, under 60 mM NaCl salt treatment, priming with 200mM $CaCl_2$ and 297mM KNO_3 did not significantly affect grain starch **(Table 5.4)** and grain amylose concentrations **(Table 5.5)** but significantly increased grain protein concentration **(Table 5.6**) of the rice accession IRGC 7546 Gaza. It is documented that the grain of rice consists of four types of protein which are water soluble albumins, salt soluble globulins, alkaline soluble glutelins,

and alcohol soluble prolamins (Chen et al., 2012). The alkaline soluble glutelins (80%) and alcohol soluble prolamins (20%) are the most predominant (Yamagata et al., 1982, Chen et al., 2012). Densitometry analysis of the grain protein profiles (**Figure 5.6**) suggests that salt treatment under KNO_3 priming treatment lowered the band density of albumins, globulins, and glutelins, and increased the prolamins. Therefore, the increase in grain protein concentration in the rice accession IRGC 7546 Gaza (**Table 5.6 & Figure 5.6**) is associated with a higher content of prolamins. However, the exact concentrations of the protein type's fractions that would support this assumption were not measured. Priming with 200mM $CaCl_2$ and 297mM KNO_3 did not significantly affect grain starch **(Table 5.4)** and grain amylose **(Table 5.5)** concentrations, priming with 200mM $CaCl_2$ did not affect grain protein concentration, and priming with 297mM KNO_3 decreased protein concentration **(Table 5.6**) of the rice accession IRGC 116793 (IR64). Farooq et al. (2006a) observed that priming rice seed with 200mM $CaCl_2$ salt solutions of Ψ_s= -1.25 MPa osmotic potential decreased the amylose concentration and increased the percentage of crude protein of direct seeded rice grain under non-saline conditions. Moreover, Rehman et al. (2011) also noticed that priming rice seed with 200mM $CaCl_2$ salt solution of Ψ_s= -1.25 MPa osmotic potential increased grain translucence of direct seeded rice, under normal conditions in farmer fields. It is reported that increased translucency of rice grain is associated with increased amount of amylose (Rani and Bhattacharya, 1989, Lisle et al., 2000, Singh et al., 2003).

Limited information is available on the impact of KNO_3 seed priming on rice grain quality under direct seeded conditions; and to our knowledge our study is the first one to evaluate the change on rice grain composition under salt conditions following KNO_3 priming treatment.

For the rice accessions IRGC 7546 (Gaza) there was strong positive association between shoot dry weight, shoot K^+/Na^+ discrimination, starch concentration, amylose concentration and grain yield; and strong negative association between shoot Na concentration, protein concentration and grain yield (**Table 5.7**). Although priming treatments had the reverse effect of salinity stress in shoot Na concentration and shoot K^+/Na^+ discrimination, they did not affect the shoot dry weight and starch concentration. Priming treatments increased grain protein concentrations. However, grain protein concentrations showed a negative correlation with grain starch concentrations for the rice accession IRGC 7546 (Gaza) (**Table 5.8**). Therefore, these may be the reasons why there was not significant effect of priming treatments in the grain yield of the rice accessions IRGC 7546 (Gaza).

For the rice accession IRGC 116793 (IR64), there was no significant association between the measured variables (shoot dry weight, shoot Na concentration, shoot K^+/Na^+ discrimination, starch concentration, amylose concentration, protein concentration) and the grain yield, neither between grain starch concentrations and grain protein concentrations (**Table 5.7 & 5.8**). However, despite of being the least affected by salinity stress, this accession was negatively affected by priming treatments. This suggests that perhaps priming treatments may have negatively influenced other physiological variables not included in this study (e.g. hydrolases, antioxidants, and osmolytes), which may have negatively affected the growth and development of the rice accession IRGC 116793 (IR64), resulting in a significant decrease in yield.

5.5 Conclusions

Salt treatment (60 mM NaCl) decreased shoot dry weight, increased shoot Na-concentrations, and decreased shoot K^+/Na^+ ratios of rice accessions IRGC 7547 (Chincherica), IRGC 7546 (Gaza), and IRGC 66970 (IR64). These changes were more prominent in the rice accession IRGC 7547 (Chincherica), followed by the rice accession IRGC 7546 (Gaza).

Salt treatment (60 mM NaCl) resulted in 100% sterility in rice accession IRGC 7547 (Chincherica) and decreased grain yield of rice accessions IRGC 7546 (Gaza) and IRGC 66970 (IR64). These reduction was higher in the rice accession IRGC 7546 (Gaza) than in IRGC 66970 (IR64).

Salt treatment decreased grain starch concentration, did not affect grain amylose concentration and increased grain protein concentration of the rice accessions IRGC 7546 (Gaza), and IRGC 66970 (IR64). These changes were more prominent in the rice accession IRGC 7546 (Gaza) than in IRGC 66970 (IR64).

Under 60 mM NaCl salt treatment, $CaCl_2$ and KNO_3 priming treatments did not benefit shoot dry weight or grain yield of the three rice accessions from this study. Moreover, priming had a negative effect on grain yield of the rice accession IRGC 116793 (IR64). Except for IRGC 66970 (IR64) under $CaCl_2$ priming treatment, $CaCl_2$ and KNO_3 priming treatments decreased shoot Na concentration, and increased shoot K^+/Na^+ discrimination in all three rice accessions, and this was more prominent in the sensitive rice accession IRGC 7547 (Chincherica), followed by the rice accession IRGC 7546 (Gaza). $CaCl_2$ and KNO_3 priming treatments did not impact grain starch concentration or the amylose concentration of the rice accessions IRGC 7546 (Gaza) and IRGC 66970 (IR64). However, $CaCl_2$ and KNO_3 priming

treatments increased grain protein concentration in IRGC 7546 (Gaza) and decreased in KNO_3 primed IRGC 116793 (IR64).

CHAPTER 6. General Discussion

This research aimed to determine salt tolerance of rice accessions grown in Mozambique; to quantify the impact of salt stress on development, grain yield and grain composition; to establish the mechanisms that could be at the basis of a greater tolerance to salt; to evaluate which seed priming approaches could be effective in increasing salt tolerance of rice accessions from Mozambique and improve their yield and grain composition.

6.1 Salinity Tolerance of Rice Accessions from Mozambique (Chapter 2)

The first specific objective of this research was to determine the salinity tolerance of rice accessions representative to those cultivated in Mozambique. The twelve rice accessions showed large variation in the response to salt stress. This study showed that indica Mozambique landraces rice accessions are moderately tolerant, while the indica IRRI lines rice accessions are tolerant to high salinity stress during the seedling stage. Therefore, this finding suggests adoption of indica IRRI lines rice accessions for a better rice production in saline soils of Mozambique. However, from an agronomical point of view, it should be noted that, while indica IRRI lines, which are genetically improved accessions, may better cope with the stress of salinity, in general, compared to the indica Mozambique landraces, they are less adapted to the Mozambican environment or climate and more demanding in terms of agricultural inputs (machinery, irrigation system and water, good seeds, fertilizers, pesticides, etc.). Furthermore, they are currently the least preferred by the farmers and rice consumers in Mozambique, in terms of grain quality (storage, milling and marketing properties, physical appearance of the caryopsis, cooking and eating quality, and nutritional value). Consequently, studies that aim to improve the productivity of indica Mozambique

landraces, with the purpose of providing farmers desired alternatives of rice accessions with better performance under salt stress, are relevant.

6.2 Salt Tolerance of Rice Accessions and Seed Priming (Chapter 3)

Seed priming treatments were evaluated in terms of their ability to alleviate the negative effects of salt stress in rice accessions from Mozambique, and ion analysis was carried out to establish if differences in response were correlated with different accumulation of Na and K ions, which have been reported to promote osmotic adjustments in the cells, hence conferring higher salt tolerance.

Priming treatments showed that there is scope for better rice production in salt affected soils in Mozambique. However, the positive effects are observable with an accurate combination of rice accessions and the inorganic priming salt used for seed hydration or seed preparation before sowing. Therefore, before moving towards the adoption of priming treatments, pilot studies should be carried out to identify the combination of rice accessions and the inorganic priming salt that provides best results. $CaCl_2$ and KNO_3 seed priming treatment would be recommended for Indica Mozambique (landraces).

This study also showed that the increase of salt tolerance conferred by the priming treatments is not exclusively based in the physiological changes caused by Na and K concentrations in the shoots, there are additional physiological mechanisms provided by each inorganic salt applied for priming. For instance, $CaCl_2$ priming treatment appears to have benefited rice plants by providing Ca^{2+} on the membranes, which would promote integrity of plant membrane structures and functions (Shannon and Francois, 1977, Rengel, 1992, George et al., 2012), while KNO_3 priming treatment benefited rice plants by providing

nitric oxide (NO), which is known to be involved in defense strategies against abiotic stresses, such as the production of osmolytes (e.g. proline and glycine betaine) in plants (Fancy et al., 2017, Adamu et al., 2018, Ahmad et al., 2017). Therefore, future related studies may also consider including parameters such as the measurement of Ca^{2+} on the membranes for $CaCl_2$ priming treatment, and the concentrations of nitric oxide (NO) or the content of osmolytes (e.g. proline and glycine betaine) for KNO_3 priming treatment, for better understanding of how each inorganic salt protects plants from salt stress. These complementary data may facilitate the establishment or identification of mechanisms that confer higher salt tolerance of rice accessions in Mozambique.

6.3 Salt Stress and Seed Priming on Germination of Rice Accessions (Chapter 4)

The impact of salt stress and different priming treatments on germination, plant growth and development was quantified. 80 mM NaCl salt treatment did not affect percent germination and seminal root length, and decreased shoot growth of the three rice accessions. Salt treatment did not affect rice seed vigor, but the reduction of shoot length may lead to low leaf area and poor crop establishment. Poor crop establishment may prevent rice plants from competing with weeds for solar radiation, soil water and nutrients, decreasing the rate of photosynthesis, causing poor plant performance and ultimately a low yield.

Our results suggest positive $CaCl_2$ priming effects on shoot length of rice accessions IRGC 7547 (Chincherica) and IRGC 116793 (IR64) **(Figure 4.5)**, and positive KNO_3 priming effects on root and shoot lengths of rice accession IRGC 116793 (IR64) **(Figure 4.4 & 4.5)**, and that these effects may have become significant if the measurement was done after a longer time of incubation in germination conditions (12-14 days, rather than 8 days), lower salt

concentrations, and, possibly, lower priming salt concentrations (Theerakulpisut et al., 2017, Ali et al., 2020, Dhillon et al., 2021). Therefore, our results suggest that the adoption of seed priming, whilst not impacting on germination per se, may still alleviate the negative effects of salinity in the early stages of rice crop development. However, further studies are required to disclose the best priming salt concentrations and duration that provide satisfactory results at each growth conditions (salt concentrations) and rice accession.

6.4 Salt Stress and Seed Priming on Growth, Physiological Traits, Grain Yield and Grain Composition of Rice Accessions (Chapter 5)

The beneficial effects of priming treatments ($CaCl_2$ and KNO_3) observed in the earlier vegetative stages, in the mitigation of the effect of salinity stress on rice accessions from Mozambique, were evaluated to determine whether these also benefit rice accessions in terms of mature plant biomass, grain yield and grain composition (grain nutritional quality). Priming treatments ($CaCl_2$ and KNO_3), under salinity stress, did not affect shoot dry weight; decreased mean grain yield of tested rice accessions, being significant for IRGC 66970 (IR64); did not affect grain starch concentration and grain amylose concentration; and increased grain protein concentration. Therefore, priming treatments ($CaCl_2$ and KNO_3), did not benefit the tested rice accessions, under salinity stress. These results reinforce that there is a need for further studies to optimize the combination of rice accessions and inorganic priming salt treatment (concentrations and duration) that could be effective at mitigating different levels of salinity stress. Additionally, our results indicate that priming treatments possibly have negatively interfered in other physiological parameters such as the content of hydrolases, antioxidants, and osmolytes of rice accession IRGC 66970 (IR64). Therefore, future studies

should also include these parameters. Thereafter, favorable priming approaches may be disseminated to smallholder farmers to increase rice grain yield and quality, thereby improve the food and nutritional security in Mozambique.

CHAPTER 7. Appendices

Chapter 2

Analysis of Variance

Variate: **Shoot Dry Weight** (g/plant)

Effects of salt stress on shoot dry weight of twelve rice accessions

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Salt.treatments	27.2	1	27.2	118	<0.001
Rice accessions	71.06	11	6.46	118	<0.001
Salt.treatments.Rice Accessions	32.37	11	2.94	118	0.002

Shoot Dry Weight means differences (*Bonferroni* test at P<0.05)

Cell No.	Bonferroni test; variable **Shoot Dry Weight** Probabilities for Post Hoc Tests Error: Between MS = .04361, df = 120.00													
	!Salinity level	! Rice accessions	{1} - .4028	{2} .011 66	{3} - .2111	{4} - .5355	{5} - .3005	{6} - .4234	{7} - .3518	{8} - .3010	{9} - .2719	{10} - .5290	{11} - .3812	{12} - .5005
1	Salt	Gaza		0.22 2838	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
2	Salt	Chincherica	0.22 2838		1.00 0000	0.00 3741	1.00 0000	0.12 4327	0.86 8085	1.00 0000	1.00 0000	0.00 4652	0.40 2764	0.01 1830
3	Salt	Chibica	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
4	Salt	IRGC 12048 (Moroberekan)	1.00 0000	0.00 3741	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
5	Salt	IRGC 32695 (IR46)	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
6	Salt	CO39	1.00 0000	0.12 4327	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
7	Salt	IRGC 53435 (IR54)	1.00 0000	0.86 8085	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000
8	Salt	IRGC 55969 (IR54)	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000	1.00 0000
9	Salt	IRGC 66970 (IR64)	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000	1.00 0000
10	Salt	IRGC 101363 (Moroberekan)	1.00 0000	0.00 4652	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000	1.00 0000
11	Salt	IRGC 116793 (IR64)	1.00 0000	0.40 2764	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000		1.00 0000
12	Salt	IRGC 126 505 (IR52)	1.00 0000	0.01 1830	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	1.00 0000	

13	Control	Gaza	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
14	Control	Chincherica	0.000196	1.000000	0.109434	0.000001	0.006871	0.000092	0.001213	0.006748	0.017287	0.000002	0.000429	0.000005
15	Control	Chibica	0.027561	1.000000	1.000000	0.000316	0.522382	0.014416	0.126972	0.514830	1.000000	0.000399	0.053415	0.001096
16	Control	IRGC 12048 (Moroberekan)	0.073588	1.000000	1.000000	0.000998	1.000000	0.039610	0.314614	1.000000	1.000000	0.001253	0.138260	0.003326
17	Control	IRGC 32695 (IR46)	1.000000	0.582159	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
18	Control	CO39	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
19	Control	IRGC 53435 (IR54)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
20	Control	IRGC 55969 (IR54)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
21	Control	IRGC 66970 (IR64)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
22	Control	IRGC 101363 (Moroberekan)	0.363182	1.000000	1.000000	0.006755	1.000000	0.206140	1.000000	1.000000	1.000000	0.008362	0.644388	0.020832
23	Control	IRGC 116793 (IR64)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
24	Control	IRGC 126 505 (IR52)	1.000000	0.118714	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000

Analysis of Variance

Variate: **Shoot Na Concentrations** (mg Na g^{-1} DW)

Effects of salt stress on Shoot Na Concentrations of twelve rice accessions

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Salt treatments	1721.89	1	1721.89	46	<0.001
Rice accessions	46.62	11	4.24	46	<0.001
Salt treatments. Rice accessions	43.58	11	3.96	46	<0.001

Shoot Na Concentrations means differences (*Bonferroni* test at P<0.05)

Cell No.	Bonferroni test; variable **Shoot Na Concentrations** Probabilities for Post Hoc Tests Error: Between MS = .03464, df = 48.000												
	!Salinity level	! Rice accessions	{1} 1.5482	{2} 1.4572	{3} 1.5025	{4} 1.8318	{5} 1.4417	{6} 1.6533	{7} .86820	{8} 1.4229	{9} 1.1808	{10} 1.8076	{11} 1.3978	{12} 1.6419
2	Salt	Chincherica	1.000000		1.000000	1.000000	1.000000	1.000000	0.088928	1.000000	1.000000	1.000000	1.000000	1.000000
3	Salt	Chibica	1.000000	1.000000		1.000000	1.000000	1.000000	0.034515	1.000000	1.000000	1.000000	1.000000	1.000000

4	Salt	IRGC 12048 (Moroberekan)	1.000000	1.000000	1.000000		1.000000	1.000000	0.000021	1.000000	0.024227	1.000000	1.000000	1.000000
5	Salt	IRGC 32695 (IR 46)	1.000000	1.000000	1.000000	1.000000		1.000000	0.122037	1.000000	1.000000	1.000000	1.000000	1.000000
6	Salt	CO 39	1.000000	1.000000	1.000000	1.000000	1.000000		0.001261	1.000000	0.870470	1.000000	1.000000	1.000000
7	Salt	IRGC 53435 (IR 54)	0.012975	0.088928	0.034515	0.000021	0.122037	0.001261		0.178257	1.000000	0.000037	0.292859	0.001628
8	Salt	IRGC 55969 (IR 54)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.178257		1.000000	1.000000	1.000000	1.000000
9	Salt	IRGC 66970 (IR 64)	1.000000	1.000000	1.000000	0.024227	1.000000	0.870470	1.000000	1.000000		0.040528	1.000000	1.000000
10	Salt	IRGC 101363 (Moroberekan)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.000037	1.000000	0.040528		1.000000	1.000000
11	Salt	IRGC 116793 (IR 64)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.292859	1.000000	1.000000	1.000000		1.000000
12	Salt	IRGC 126505 (IR 52)	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.001628	1.000000	1.000000	1.000000	1.000000	
13	Control	Gaza	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
14	Control	Chincherica	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
15	Control	Chibica	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
16	Control	IRGC 12048 (Moroberekan)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
17	Control	IRGC 32695 (IR 46)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000007	0.000000	0.000000	0.000000	0.000000	0.000000
18	Control	CO 39	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000012	0.000000	0.000000	0.000000	0.000000	0.000000
19	Control	IRGC 53435 (IR 54)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000001	0.000000	0.000000	0.000000	0.000000	0.000000
20	Control	IRGC 55969 (IR 54)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
21	Control	IRGC 66970 (IR 64)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
22	Control	IRGC 101363 (Moroberekan)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
23	Control	IRGC 116793 (IR 64)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000002	0.000000	0.000000	0.000000	0.000000	0.000000
24	Control	IRGC 126505 (IR 52)	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000

Analysis of Variance

Variate: **Shoot K Concentrations** (mg K g^{-1} DW)

Effects of salt stress on Shoot K Concentrations of twelve rice accessions

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Salt.treatments	338.62	1	338.62	46	<0.001
Rice accessions	92.14	11	8.38	46	<0.001
Salt treatments. Rice accessions	59.98	11	5.45	46	<0.001

Shoot K Concentrations means differences (*Bonferroni* test at P<0.05)

Cell No.	Bonferroni test; variable **Shoot K Concentrations** Probabilities for Post Hoc Tests Error: Between MS = .00147, df = 48.000													
	!Salinity level	! Rice accessions	{1} 1.6501	{2} 1.6158	{3} 1.5594	{4} 1.4407	{5} 1.5774	{6} 1.6677	{7} 1.6928	{8} 1.6423	{9} 1.5686	{10} 1.4551	{11} 1.5294	{12} 1.5916
1	Salt	Gaza		1.000000	1.000000	0.000006	1.000000	1.000000	1.000000	1.000000	1.000000	0.000030	0.093058	1.000000
2	Salt	Chincherica	1.000000		1.000000	0.000283	1.000000	1.000000	1.000000	1.000000	1.000000	0.001383	1.000000	1.000000
3	Salt	Chibica	1.000000	1.000000		0.113761	1.000000	0.313458	0.025659	1.000000	1.000000	0.452549	1.000000	1.000000
4	Salt	IRGC 12048 (Moroberekan)	0.000006	0.000283	0.113761		0.018158	0.000001	0.000000	0.000014	0.045277	1.000000	1.000000	0.004045
5	Salt	IRGC 32695 (IR 46)	1.000000	1.000000	1.000000	0.018158		1.000000	0.157686	1.000000	1.000000	0.079129	1.000000	1.000000
6	Salt	CO 39	1.000000	1.000000	0.313458	0.000001	1.000000		1.000000	1.000000	0.734019	0.000004	0.015349	1.000000
7	Salt	IRGC 53435 (IR 54)	1.000000	1.000000	0.025659	0.000000	0.157686	1.000000		1.000000	0.065530	0.000000	0.001022	0.602011
8	Salt	IRGC 55969 (IR 54)	1.000000	1.000000	1.000000	0.000014	1.000000	1.000000	1.000000		1.000000	0.000072	0.200928	1.000000
9	Salt	IRGC 66970 (IR 64)	1.000000	1.000000	1.000000	0.045277	1.000000	0.734019	0.065530	1.000000		0.189272	1.000000	1.000000
10	Salt	IRGC 101363 (Moroberekan)	0.000030	0.001383	0.452549	1.000000	0.079129	0.000004	0.000000	0.000072	0.189272		1.000000	0.018608
11	Salt	IRGC 116793 (IR 64)	0.093058	1.000000	1.000000	1.000000	1.000000	0.015349	0.001022	0.200928	1.000000	1.000000		1.000000
12	Salt	IRGC 126505 (IR 52)	1.000000	1.000000	1.000000	0.004045	1.000000	1.000000	0.602011	1.000000	1.000000	0.018608	1.000000	
13	Control	Gaza	0.053238	0.001393	0.000003	0.000000	0.000019	0.300600	1.000000	0.023803	0.000007	0.000000	0.000000	0.000094
14	Control	Chincherica	0.651117	0.022259	0.000047	0.000000	0.000351	1.000000	1.000000	0.313848	0.000131	0.000000	0.000002	0.001669
15	Control	Chibica	1.000000	0.429490	0.001300	0.000000	0.009104	1.000000	1.000000	1.000000	0.003529	0.000000	0.000046	0.039791
16	Control	IRGC 12048 (Moroberekan)	0.259423	0.007899	0.000015	0.000000	0.000117	1.000000	1.000000	0.121104	0.000043	0.000000	0.000001	0.000565

17	Control	IRGC 32695 (IR 46)	0.74 1268	0.02 5808	0.00 0055	0.00 0000	0.00 0411	1.00 0000	1.00 0000	0.35 9093	0.00 0153	0.00 0000	0.00 0002	0.00 1950
18	Control	CO 39	0.07 4076	0.00 1992	0.00 0004	0.00 0000	0.00 0028	0.40 9255	1.00 0000	0.03 3382	0.00 0010	0.00 0000	0.00 0000	0.00 0136
19	Control	IRGC 53435 (IR 54)	0.36 2872	0.01 1495	0.00 0023	0.00 0000	0.00 0174	1.00 0000	1.00 0000	0.17 1272	0.00 0065	0.00 0000	0.00 0001	0.00 0836
20	Control	IRGC 55969 (IR 54)	0.16 5609	0.00 4804	0.00 0009	0.00 0000	0.00 0069	0.86 2829	1.00 0000	0.07 6262	0.00 0026	0.00 0000	0.00 0000	0.00 0338
21	Control	IRGC 66970 (IR 64)	1.00 0000	0.73 3830	0.00 2455	0.00 0000	0.01 6818	1.00 0000	1.00 0000	1.00 0000	0.00 6595	0.00 0000	0.00 0088	0.07 1812
22	Control	IRGC 101363 (Moroberekan)	1.00 0000	0.06 8920	0.00 0161	0.00 0000	0.00 1189	1.00 0000	1.00 0000	0.87 0004	0.00 0448	0.00 0000	0.00 0005	0.00 5516
23	Control	IRGC 116793 (IR 64)	1.00 0000	0.31 4184	0.00 0903	0.00 0000	0.00 6390	1.00 0000	1.00 0000	1.00 0000	0.00 2462	0.00 0000	0.00 0031	0.02 8271
24	Control	IRGC 126505 (IR 52)	1.00 0000	0.07 7943	0.00 0185	0.00 0000	0.00 1360	1.00 0000	1.00 0000	0.97 0815	0.00 0513	0.00 0000	0.00 0006	0.00 6289

CHAPTER 3

Analysis of Variance

Variate: **Shoot Dry Weight** (g/plant)

Effects of salt stress and priming treatments on shoot dry weight of six rice accessions

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1		183.861	183.861	119.32	<.001
Priming_treatments	4		39.342	9.835	6.38	<.001
Rice_accessions	5		242.74	48.548	31.51	<.001
Salt_treatments.Priming_treatments	4		39.445	9.861	6.4	<.001
Salt_treatments.Rice_accessions	5		150.18	30.036	19.49	<.001
Priming_treatments.Rice_accessions	20		57.036	2.852	1.85	0.019
Salt_treatments.Priming_treatments.Rice_accessions	20		76.998	3.85	2.5	<.001
Residual	177	-3	272.737	1.541		
Total	236	-3	1056.783			

Means of shoot dry weight on six rice accessions under two salt treatments

Salt Treatments	Rice Accessions					
	Chibica	Chincherica	Gaza	IR46	IRGC 116793 (IR64)	IRGC 12048 (Moroberekan)
80mM salt	1.83	2.02	3.09	2.07	2.77	2.37
Control (0mM salt)	2.66	4.75	6.45	2.00	2.69	6.11
s.e.d.= 0.393						

Overall means of shoot dry weight under five priming treatments

Priming Treatments					
	$CaCl_2$	KCl	KNO_3	Non-primed	Water
	2.65	3.61	3.54	2.85	3.53
s.e.d.= 0.253					

❖ **Germination Test**

Analysis of variance

Variate: **Seedling Percent Germination** (%)

Effects of salt stress and priming treatments on seedling percent germination of three rice accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1	0.06	0.06	0	0.947
Priming_treatments	2	293.44	146.72	11.72	<.001
Rice_accessions	2	523.86	261.93	20.92	<.001
Salt_treatments.Priming_treatments	2	8.11	4.06	0.32	0.725
Salt_treatments.Rice_accessions	2	3.36	1.68	0.13	0.875
Priming_treatments.Rice_accessions	4	112.39	28.1	2.24	0.076
Salt_treatments.Priming_treatments.Rice_accessions	4	15.22	3.81	0.3	0.874
Residual	54	676	12.52		
Total	71	1632.44			

❖ **Root Length**

Analysis of variance

Variate: **Root Length** (cm)

Effects of salt stress and priming treatments on Root Length of three rice accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1	0.001	0.001	0	0.977
Priming_treatments	2	3.987	1.994	1.61	0.21
Rice_accessions	2	4.762	2.381	1.92	0.157
Salt_treatments.Priming_treatments	2	0.24	0.12	0.1	0.908
Salt_treatments.Rice_accessions	2	2.96	1.48	1.19	0.311
Priming_treatments.Rice_accessions	4	22.646	5.662	4.56	0.003
Salt_treatments.Priming_treatments.Rice_accessions	4	3.193	0.798	0.64	0.634
Residual	54	67.016	1.241		
Total	71	104.806			

❖ **Shoot Length**

Analysis of variance

Variate: **Shoot Length** (cm)

Effects of salt stress and priming treatments on Shoot Length of three rice accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1	29.9493	29.9493	174.23	<.001
Priming_treatments	2	0.3559	0.1779	1.04	0.362
Rice_accessions	2	6.9708	3.4854	20.28	<.001
Salt_treatments.Priming_treatments	2	0.1704	0.0852	0.5	0.612
Salt_treatments.Rice_accessions	2	0.1346	0.0673	0.39	0.678
Priming_treatments.Rice_accessions	4	2.0239	0.506	2.94	0.028
Salt_treatments.Priming_treatments.Rice_accessions	4	0.046	0.0115	0.07	0.992
Residual	54	9.2825	0.1719		
Total	71	48.9334			

- **Shoot Dry Weight**

Analysis of Variance

Variate: Shoot Dry Weight **(g/plant)**

Effects of salt stress and priming treatments on shoot dry weight of three rice accessions

Effect	Univariate Tests of Significance for DW/plant Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	25163.29	1	25163.29	157.3749	0.000000
! Salt treatments	2320.20	1	2320.20	14.5109	0.000258
! Priming treatments	201.52	2	100.76	0.6302	0.534889
! Rice accessions	176.96	2	88.48	0.5534	0.576995
! Salt treatments*! Priming treatments	195.53	2	97.76	0.6114	0.544867
! Salt treatments*! Rice accessions	4047.51	2	2023.76	12.6569	0.000015
! Priming treatments*! Rice accessions	770.95	4	192.74	1.2054	0.314260
! Salt treatments*! Priming treatments*! Rice accessions	191.67	4	47.92	0.2997	0.877425
Error	14070.67	88	159.89		

❖ **Na^+ Exclusion:**

Analysis of variance

Variate: **Shoot Na Concentrations** (mg Na g^{-1} DW)

Effects of salt stress and priming treatments on Shoot Na Concentrations of three rice accessions

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1		3557.639	3557.639	47999.29	<.001
Priming_treatments	2		176.9698	88.48492	1193.83	<.001
Rice_accessions	2		658.4464	329.2232	4441.85	<.001
Salt_treatments.Priming_treatments	2		156.3086	78.1543	1054.45	<.001
Salt_treatments.Rice_accessions	2		452.6233	226.3117	3053.37	<.001
Priming_treatments.Rice_accessions	4		105.7443	26.43608	356.67	<.001
Salt_treatments.Priming_treatments.Rice_accessions	4		101.1458	25.28644	341.16	<.001
Residual	34	-2	2.52003	0.07412		
Total	51	-2	4788.229			

❖ **Rice Accessions * Salt Treatments**

Salt Treatments	Rice Accessions		
	Chincherica	Gaza	IRGC 116793 (IR64)
0mM salt	2.285	3.323	1.717
60mM salt	21.239	24.886	9.901

s.e.d.= 0.1283

❖ **K^+/Na^+ Discrimination**

Analysis of variance

Variate**: Shoot K^+/Na^+ Ratios**

Effects of salt stress and priming treatments on Shoot K^+/Na^+ Ratios of three rice accessions

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Salt_treatments	1		853.5642	853.5642	59085.29	<.001
Priming_treatments	2		26.33834	13.16917	911.59	<.001
Rice_accessions	2		172.4269	86.21344	5967.85	<.001
Salt_treatments.Priming_treatments	2		10.39106	5.19553	359.64	<.001
Salt_treatments.Rice_accessions	2		107.9402	53.97009	3735.91	<.001
Priming_treatments.Rice_accessions	4		150.0547	37.51368	2596.77	<.001
Salt_treatments.Priming_treatments.Rice_accessions	4		106.9393	26.73483	1850.63	<.001
Residual	34	-2	0.49117	0.01445		
Total	51	-2	1370.269			

❖ **Rice Accessions * Salt Treatments**

Salt Treatments	Rice Accessions		
	Chincherica	Gaza	IRGC 116793 (IR64)
0 mM salt	12.837	6.16	12.51
60 mM salt	1.788	1.947	3.917

s.e.d.= 0.0567

❖ **Grain Yield**

Analysis of variance

Variate**: Grain Yield** (g/plant)

Effects of salt stress and priming treatments on Grain Yield of three rice accessions

Effect	Univariate Tests of Significance for Yield/plant Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	4004.002	1	4004.002	244.4882	0.000000
! Salt treatments	1706.591	1	1706.591	104.2061	0.000000
! Priming treatments	329.770	2	164.885	10.0680	0.000153
! Rice accessions	6.856	1	6.856	0.4187	0.519851
! Salt treatments*! Priming treatments	9.105	2	4.553	0.2780	0.758192
! Salt treatments*! Rice accessions	329.022	1	329.022	20.0904	0.000030
! Priming treatments*! Rice accessions	137.163	2	68.582	4.1877	0.019400
! Salt treatments*! Priming treatments*! Rice accessions	22.804	2	11.402	0.6962	0.502096
Error	1080.887	66	16.377		

❖ **Grain Starch Concentration**

Analysis of variance

Variate: Grain Starch Concentration **(%)**

Effects of salt stress and priming treatments on Grain Starch Concentration of three rice accessions

Effect	Univariate Tests of Significance for Starch Concentration (%) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	72182.24	1	72182.24	153347.5	0.000000
!Rice accessions	9.26	1	9.26	19.7	0.001264
!Priming treatments	27.04	2	13.52	28.7	0.000072
!Salt Treatments	27.58	1	27.58	58.6	0.000017
!Rice accessions*!Priming treatments	21.43	2	10.71	22.8	0.000190
!Rice accessions*!Salt Treatments	46.00	1	46.00	97.7	0.000002
!Priming treatments*!Salt Treatments	34.45	2	17.22	36.6	0.000025
!Rice accessions*!Priming treatments*!Salt Treatments	7.47	2	3.74	7.9	0.008621
Error	4.71	10	0.47		

❖ **Grain Amylose Concentration**

Analysis of variance

Variate: Grain Amylose Concentration **(%)**

Effects of salt stress and priming treatments on Grain Amylose Concentration of three rice accessions

Effect	Univariate Tests of Significance for Amylose Concentration (%) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	9114.731	1	9114.731	740.4373	0.000000
!Rice accessions	38.314	1	38.314	3.1125	0.115701
!Priming treatments	17.525	2	8.762	0.7118	0.519383
!Salt Treatments	10.568	1	10.568	0.8585	0.381253
!Rice accessions*!Priming treatments	2.445	2	1.223	0.0993	0.906549
!Rice accessions*!Salt Treatments	3.177	1	3.177	0.2581	0.625121
!Priming treatments*!Salt Treatments	5.629	2	2.815	0.2287	0.800629
!Rice accessions*!Priming treatments*!Salt Treatments	20.853	2	10.427	0.8470	0.463817
Error	98.479	8	12.310		

❖ **Grain Protein Concentration**

Analysis of variance

Variate: Grain Protein Concentration **(%)**

Effects of salt stress and priming treatments on Grain Protein Concentration of three rice accessions

Effect	Univariate Tests of Significance for Protein (%) Whole Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	2812.206	1	2812.206	122756.5	0.000000
!Rice accession	1.968	1	1.968	85.9	0.000000
!Priming treatments	2.894	2	1.447	63.2	0.000000
!Salt Level	63.567	1	63.567	2774.8	0.000000
!Rice accession*!Priming treatments	1.689	2	0.845	36.9	0.000000
!Rice accession*!Salt Level	12.008	1	12.008	524.2	0.000000
!Priming treatments*!Salt Level	1.388	2	0.694	30.3	0.000002
!Rice accession*!Priming treatments*!Salt Level	0.901	2	0.451	19.7	0.000030
Error	0.412	18	0.023		

❖ **Grain Protein Profile**

Analysis of variance

Variate: **Grain Protein Raw Volume** (%)

Effects of salt stress on Grain Protein Raw Volume on rice accession IRGC 7546 (Gaza)

Effect	Univariate Tests of Significance for Raw Volume Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	166462820	1	166462820	339.1817	0.000000
!Protein Type	62249858	3	20749953	42.2797	0.000000
!Salt Treatment	3665955	1	3665955	7.4697	0.011591
!Protein Type*!Salt Treatment	9737157	3	3245719	6.6134	0.002052
Error	11778666	24	490778		

CHAPTER 8. References

ABDULLAH, Z., KHAN, M. A. & FLOWERS, T. J. 2001. Causes of Sterility in Seed Set of Rice under Salinity Stress. *Journal of Agronomy and Crop Science,* 187**,** 25-32.

ADAMU, T. A., MUN, B.-G., LEE, S.-U., HUSSAIN, A. & YUN, B.-W. 2018. Exogenously applied nitric oxide enhances salt tolerance in rice (Oryza sativa L.) at seedling stage. *Agronomy,* 8**,** 1-15.

AFZAL, I., BUTT, A., UR REHMAN, H., AHMAD BASRA, A. B. & AFZAL, A. 2012. Alleviation of salt stress in fine aromatic rice by seed priming. *Australian Journal of Crop Science,* 6**,** 1401 - 1407.

AFZAL, I., NOOR, M., BAKHTAVAR, M., AHMAD, A. & HAQ, Z. 2015. Improvement of spring maize performance through physical and physiological seed enhancements. *Seed Science and Technology,* 43**,** 1-12.

AFZAL, I., RAUF, S., BASRA, S. M. A. & MURTAZA, G. 2008. Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress. *Plant, Soil and Environment,* 54**,** 382-388.

AFZAL, I., REHMAN, H. U., NAVEED, M. & BASRA, S. M. A. 2016. Recent Advances in Seed Enhancements. *New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology.* IntechOpen.

AHMAD, P., AHANGER, M. A., ALYEMENI, M. N., WIJAYA, L. & ALAM, P. 2017. Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. *Protoplasma,* 255**,** 79-93.

ALI, L. G., NULIT, R., IBRAHIM, M. H. & YIEN, C. Y. S. 2020. Enhancement of germination and early seedling growth of rice (Oryza sativa) var. FARO44 by seed priming under normal and drought stressed conditions. *Journal of Plant Nutrition,* 43**,** 1579-1593.

AOSA 1993. Association of Official Seed Analysis: Rules for Testing Seeds. *Journal of Seed Technology* 16**,** 1-113.

ASCH, F., DINGKUHN, M., DÖRFFLING, K. & MIEZAN, K. 2000. Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. *Euphytica,* 113**,** 109 - 118.

ASHRAF, M. & FOOLAD, M. R. 2005. Pre-Sowing Seed Treatment—A Shotgun Approach to Improve Germination, Plant Growth, and Crop Yield Under Saline and Non-Saline Conditions. 88**,** 223-271.

ASHRAF, M. & HARRIS, P. J. C. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science,* 166**,** 3-16.

AWAN, T. H., AHMADIZADEH, M., JABRAN, K., HASHIM, S. & CHAUHAN, B. S. 2017. Domestication and development of rice cultivars. *Rice Production Worldwide.* Springer.

BADO, S., FORSTER, B. P., GHANIM, A. M., JANKOWICZ-CIESLAK, J., BERTHOLD, G. & LUXIANG, L. 2016. Protocol for Screening for Salt Tolerance in Rice. *Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley.* Springer.

BAKHTAVAR, M. A., AFZAL, I., BASRA, S. M., AHMAD, A. U. & NOOR, M. A. 2015. Physiological Strategies to Improve the Performance of Spring Maize (Zea mays L.) Planted under Early and Optimum Sowing Conditions. *PLoS One,* 10**,** 1-15.

BAXTER, G., ZHAO, J. & BLANCHARD, C. 2011. Salinity alters the protein composition of rice endosperm and the physicochemical properties of rice flour. *Journal of the Science of Food and Agriculture,* 91**,** 2292-2297.

BECKLES, D. M. & THITISAKSAKUL, M. 2014. How environmental stress affects starch composition and functionality in cereal endosperm. *Starch - Stärke,* 66**,** 58-71.

BELDER, P., SPIERTZ, J. H. J., BOUMAN, B. A. M., LU, G. & TUONG, T. P. 2005. Nitrogen economy and water productivity of lowland rice under water-saving irrigation. *Field crops research,* 93**,** 169-185.

BEWLEY, J. D. 1997. Seed Germination and Dormancy. *The Plant Cell,* 9**,** 1055-1066.

BEWLEY, J. D. & BLACK, M. 1994. *Seeds: Physiology of Development and Germination.*, Springer Science & Business Media.

BOONCHUAY, P., CAKMAK, I., RERKASEM, B. & PROM-U-THAI, C. 2013. Effect of different foliar zinc application at different growth stages on seed zinc concentration and its impact on seedling vigor in rice. *Soil science and plant nutrition (Tokyo),* 59**,** 180-188.

BRAY, C. 1995. Biochemical processes during the osmopriming of seeds. *Seed Development and Germination***,** 767-789.

CALINGACION, M., LABORTE, A., NELSON, A., RESURRECCION, A., CONCEPCION, J. C., DAYGON, V. D., MUMM, R., REINKE, R., DIPTI, S., BASSINELLO, P. Z., MANFUL, J., SOPHANY, S., LARA, K. C., BAO, J., XIE, L., LOAIZA, K., EL-HISSEWY, A., GAYIN, J., SHARMA, N., RAJESWARI, S., MANONMANI, S., RANI, N. S., KOTA, S., INDRASARI, S. D., HABIBI, F., HOSSEINI, M., TAVASOLI, F., SUZUKI, K., UMEMOTO, T., BOUALAPHANH, C., LEE, H. H., HUNG, Y. P., RAMLI, A., AUNG, P. P., AHMAD, R., WATTOO, J. I., BANDONILL, E., ROMERO, M., BRITES, C. M., HAFEEL, R., LUR, H. S., CHEAUPUN, K., JONGDEE, S., BLANCO, P., BRYANT, R., THI LANG, N., HALL, R. D. & FITZGERALD, M. 2014. Diversity of global rice markets and the science required for consumer-targeted rice breeding. *PLoS One,* 9**,** 1-12.

CAYUELA, E., PÉREZ-ALFOCEA, F., CARO, M. & BOLARIN, M. 1996. Priming of seeds with NaCl induces physiological changes in tomato plants grown under salt stress. *Physiologia Plantarum,* 96**,** 231-236.

CHAMPAGNE, E. T., WOOD, D. F., JULIANO, B. O. & BECHTEL, D. B. 2004. The rice grain and its gross composition. *Rice chemistry and technology,* 3**,** 77-107.

CHANG, H. 2002. Effect of seed priming with mixed-salt solution on germination and physiological characteristics of seedling in rice (Oryza sativa L.) under stress conditions. *Journal of Zhejiang Agricultural University,* 28**,** 175 -178.

CHANG, J., CHEONG, B. E., NATERA, S. & ROESSNER, U. 2019. Morphological and metabolic responses to salt stress of rice (Oryza sativa L.) cultivars which differ in salinity tolerance. *Plant physiology and biochemistry,* 144**,** 427-435.

CHEN, Y., WANG, M. & OUWERKERK, P. B. F. 2012. Molecular and environmental factors determining grain quality in rice. *Food and Energy Security,* 1**,** 111-132.

CRUZ, N. D. & KHUSH, G. 2000. Rice grain quality evaluation procedures. *Aromatic rices,* 3**,** 15-28.

DELAUNEY, A. J. & VERMA, D. P. S. 1993. Proline biosynthesis and osmoregulation in plants. *The plant journal,* 4**,** 215-223.

DHILLON, B. S., KUMAR, V., SAGWAL, P., KAUR, N., SINGH MANGAT, G. & SINGH, S. 2021. Seed Priming with Potassium Nitrate and Gibberellic Acid Enhances the Performance of Dry Direct Seeded Rice (Oryza sativa L.) in North-Western India. *Agronomy,* 11**,** 1-20.

DU, B., LUO, H., HE, L., ZHANG, L., LIU, Y., MO, Z., PAN, S., TIAN, H., DUAN, M. & TANG, X. 2019. Rice seed priming with sodium selenate: Effects on germination, seedling growth, and biochemical attributes. *Scientific reports,* 9**,** 1-9.

DUTTA, P. 2018. Seed Priming: New Vistas and Contemporary Perspectives. *Advances in Seed Priming.* Singapore.

EDA 2005. Development Strategy for the Rice Sector in Mozambique. Final Report. 1-59.

ELLIS, R. H., HONG, T. & ROBERTS, E. H. 1985. The Conduct of Seed Germination Tests. *Handbook of seed technology for genebanks. Volume I. Principles and methodology* International Board for Plant Genetic Resources. Handbooks for Genebanks, No. 2.

FANCY, N. N., BAHLMANN, A. K. & LOAKE, G. J. 2017. Nitric oxide function in plant abiotic stress. *Plant, cell and environment,* 40**,** 462-472.

FAROOQ, M., BARSA, S. M. A. & WAHID, A. 2006a. Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regulation,* 49**,** 285-294.

FAROOQ, M. & BASRA, S. 2006. Seed priming enhances emergence, yield, and quality of direct-seeded rice. *International Rice Research Notes,* 31**,** 42-46.

FAROOQ, M., BASRA, S., AFZAL, I. & KHALIQ, A. 2006b. Optimization of hydropriming techniques for rice seed invigoration. *Seed Science and Technology,* 34**,** 507-512.

FAROOQ, M., BASRA, S., CHEEMA, M. & AFZAL, I. 2006c. Integration of pre-sowing soaking, chilling and heating treatments for vigour enhancement in rice (Oryza sativa L.). *Seed science and technology,* 34**,** 499-506.

FAROOQ, M., BASRA, S., WAHID, A., KHALIQ, A. & KOBAYASHI, N. 2009. Rice seed invigoration: a review. *Organic farming, pest control and remediation of soil pollutants.* Springer.

FAROOQ, M., BASRA, S. M. A. & AHMAD, N. 2007a. Improving the performance of transplanted rice by seed priming. *Plant Growth Regulation,* 51**,** 129-137.

FAROOQ, M., BASRA, S. M. A., KHALID, M., TABASSUM, R. & MAHMOOD, T. 2006d. Nutrient homeostasis, metabolism of reserves, and seedling vigor as affected by seed priming in coarse rice. *Botany,* 84**,** 1196-1202.

FAROOQ, M., BASRA, S. M. A. & KHAN, M. B. 2007b. Seed priming improves growth of nursery seedlings and yield of transplanted rice. *Archives of Agronomy and Soil Science,* 53**,** 315-326.

FAROOQ, M., BASRA, S. M. A., TABASSUM, R. & AFZAL, I. 2006e. Enhancing the Performance of Direct Seeded Fine Rice by Seed Priming. *Plant Production Science,* 9**,** 446-456.

FITZGERALD, M. A., MCCOUCH, S. R. & HALL, R. D. 2009. Not just a grain of rice: the quest for quality. *Trends in Plant Science,* 14**,** 133-139.

FLOWERS, T., HAJIBAGHERI, M. & CLIPSON, N. 1986. Halophytes. *The quarterly review of biology,* 61**,** 313-337.

FLOWERS, T. & YEO, A. 1981. Variability in the resistance of sodium chloride salinity within rice (Oryza sativa L.) varieties. *New Phytologist,* 88**,** 363-373.

GEORGE, E., HORST, W. J. & NEUMANN, E. 2012. Adaptation of Plants to Adverse Chemical Soil Conditions. *Marschner's Mineral Nutrition of Higher Plants.* Academic press.

GERONA, M. E. B., DEOCAMPO, M. P., EGDANE, J. A., ISMAIL, A. M. & DIONISIO-SESE, M. L. 2019. Physiological Responses of Contrasting Rice Genotypes to Salt Stress at Reproductive Stage. *Rice Science,* 26**,** 207-219.

GHOSH, B., MD, N. A. & GANTAIT, S. 2016. Response of rice under salinity stress: a review update. *Rice Research: Open Access,* 4**,** 1-8.

GRAHAM, R., SENADHIRA, D., BEEBE, S., IGLESIAS, C. & MONASTERIO, I. 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field crops research,* 60**,** 57-80.

GRATTAN, S. & GRIEVE, C. 1998. Salinity–mineral nutrient relations in horticultural crops. *Scientia horticulturae,* 78**,** 127-157.

GRATTAN, S. R., ZENG, L., SHANNON, M. C. & ROBERTS, S. R. 2002. Rice is more sensitive to salinity than previously thought. *California Agriculture,* 56**,** 189-198.

GREENWAY, H. & MUNNS, R. 1980. Mechanisms of Salt Tolerance in Nonhalophytes. *Annual Review of Plant Physiology,* 31**,** 149-190.

GREGORIO, G. B., SENADHIRA, D. & MENDOZA, R. D. 1997. Screening rice for salinity tolerance. *IRRI Discussion Paper Series.*

GUO, X., ZHI, W., FENG, Y., ZHOU, G. & ZHU, G. 2022. Seed priming improved salt-stressed sorghum growth by enhancing antioxidative defense. *Plos one,* 17**,** 1-13.

HAQ, T., AKHTAR, J., NAWAZ, S. & AHMAD, R. 2009. Morpho-physiological response of rice (Oryza sativa L.) varieties to salinity stress. *Pakistan Journal of Botany* 41**,** 2943-2956.

HAQ, T. U., AKHTAR, J., STEELE, K. A., MUNNS, R. & GORHAM, J. 2014. Reliability of ion accumulation and growth components for selecting salt tolerant lines in large populations of rice. *Functional Plant Biology,* 41**,** 379–390.

HEINEMANN, R. J. B., FAGUNDES, P. L., PINTO, E. A., PENTEADO, M. V. C. & LANFER-MARQUEZ, U. M. 2005. Comparative study of nutrient composition of commercial brown, parboiled and milled rice from Brazil. *Journal of Food Composition and Analysis,* 18**,** 287-296.

HOANG, T. M. L., TRAN, T. N., NGUYEN, T. K. T., WILLIAMS, B., WURM, P., BELLAIRS, S. & MUNDREE, S. 2016. Improvement of salinity stress tolerance in rice: Challenges and opportunities. *Agronomy (Basel),* 6**,** 1-23.

HUSSAIN, M., FAROOQ, M. & LEE, D. J. 2017. Evaluating the role of seed priming in improving drought tolerance of pigmented and non-pigmented rice. *Journal of Agronomy and Crop Science,* 203**,** 269-276.

INDEXMUNDI 2021a. https://www.indexmundi.com/agriculture/?commodity=milled-rice&graph=production

INDEXMUNDI 2021b. https://www.indexmundi.com/agriculture/?country=mz&commodity.

IQBAL, M. & ASHRAF, M. 2007. Seed Preconditioning Modulates Growth, Ionic Relations, and Photosynthetic Capacity in Adult Plants of Hexaploid Wheat under Salt Stress. *Journal of Plant Nutrition,* 30**,** 381-396.

IQBAL, M., ASHRAF, M., JAMIL, A. & UR-REHMAN, S. 2006. Does Seed Priming Induce Changes in the Levels of Some Endogenous Plant Hormones in Hexaploid Wheat Plants Under Salt Stress? *Journal of Integrative Plant Biology,* 48**,** 181-189.

ISMAIL, A. & TUONG, T. 2009. Brackish water coastal zones of the monsoon tropics: challenges and opportunities. *Natural resource management for poverty reduction and environmental sustainability in rice-based systems***,** 113-121.

ISMAIL, A. M., HEUER, S., THOMSON, M. J. & WISSUWA, M. 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Molecular Biology,* 65**,** 547-70.

ISMAIL, A. M. & HORIE, T. 2017. Genomics, Physiology, and Molecular Breeding Approaches for Improving Salt Tolerance. *Annual Review Plant Biology* 68**,** 405-434.

JANMOHAMMADI, M., DEZFULI, P. M. & SHARIFZADEH, F. 2008. Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *Genetic Applied Plant Physiology,* 34**,** 215-226.

JISHA, K. C., VIJAYAKUMARI, K. & PUTHUR, J. T. 2013. Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum,* 35**,** 1381-1396.

JULIANO, B. 1972. The rice caryopsis and its composition. *In:* HOUSTON, D. F. (ed.) *Rice Chemistry and Techonology.* American Association of Cereal Chemists.

JULIANO, B. 1979. The chemical basis of rice grain quality. *Chemical Aspects of Rice Grain Quality.* International Rice Research Institute.

JULIANO, B., SANTIAGO, R., CRUZ, L. J. & GB, C. 1964a. Some physicochemical properties of rice in Southeast Asia. *Cereal Chemistry,* 41**,** 275-286.

JULIANO, B. O., BAUTISTA, G. M., LUGAY, J. C. & REYES, A. C. 1964b. Rice Quality, Studies on Physicochemical Properties of Rice. *Journal of Agricultural and Food Chemistry,* 12**,** 131-138.

KHAN, A. A. 1992. Preplant physiological seed conditioning. *Horticultural reviews,* 13**,** 131-181.

KHUSH, G. S. 2005. What it will take to Feed 5.0 Billion Rice consumers in 2030. *Plant Molecular Biology,* 59**,** 1-6.

KHUSH, G. S., PAULE, C. & DE LA CRUZ, N. M. Rice grain quality evaluation and improvement at IRRI. Proceedings of the workshop on chemical aspects of rice grain quality, 1978. Los Banos, Philippines, International Rice Research Institute, 1979.

KIM, S.-M., SUH, J.-P., LEE, C.-K., LEE, J.-H., KIM, Y.-G. & JENA, K. K. 2014. QTL mapping and development of candidate gene-derived DNA markers associated with seedling cold

tolerance in rice (Oryza sativa L.). *Molecular genetics and genomics : MGG,* 289**,** 333-343.

KÖHL, K. 2015. Growing rice in controlled environments. *Annals of Applied Biology,* 167**,** 157-177.

KUMAR, I. & KHUSH, G. 1986. Gene dosage effects of amylose content in rice endosperm. *The Japanese Journal of Genetics,* 61**,** 559-568.

KUROTANI, K.-I., YAMANAKA, K., TODA, Y., OGAWA, D., TANAKA, M., KOZAWA, H., NAKAMURA, H., HAKATA, M., ICHIKAWA, H., HATTORI, T. & TAKEDA, S. 2015. Stress tolerance profiling of a collection of extant salt-tolerant rice varieties and transgenic plants overexpressing abiotic stress tolerance genes. *Plant and Cell Physiology,* 56**,** 1867-1876.

LAL, S. K., KUMAR, S., SHERI, V., MEHTA, S., VARAKUMAR, P., RAM, B., BORPHUKAN, B., JAMES, D., FARTYAL, D. & REDDY, M. K. 2018. Seed priming: An emerging technology to impart abiotic stress tolerance in crop plants. *Advances in Seed Priming.* Springer.

LEE, I. S., LEE, J. O. & GE, L. 2009. Comparison of Terrestrial Laser Scanner with Digital Aerial Photogrammetry for Extracting Ridges in the Rice Paddies. *Survey Review,* 41**,** 253-267.

LEE, K.-S., CHOI, W.-Y., KO, J.-C., KIM, T.-S. & GREGORIO, G. B. 2003. Salinity tolerance of japonica and indica rice (Oryza sativa L.) at the seedling stage. *Planta,* 216**,** 1043-1046.

LEESAWATWONG, M., JAMJOD, RERKASEM, B. Nitrogen S., KUO, J., DELL, B. & fertilizer alters milling quality and protein distribution in head rice. 4th International Crop Science Congress, Brisbane, Australia, 2004.

LEKKLAR, C., SURIYA-ARUNROJ, D., PONGPANICH, M., COMAI, L., KOSITSUP, B., CHADCHAWAN, S. & BUABOOCHA, T. 2019. Comparative Genomic Analysis of Rice with Contrasting Photosynthesis and Grain Production under Salt Stress. *Genes,* 10**,** 1-20.

LINARES, O. F. 2002. African Rice (Oryza glaberrima): History and Future Potential. *Proceedings of the National Academy of Sciences - PNAS,* 99**,** 16360-16365.

LISLE, A. J., MARTIN, M. & FITZGERALD, M. A. 2000. Chalky and Translucent Rice Grains Differ in Starch Composition and Structure and Cooking Properties. *Cereal Chemistry,* 77**,** 627-632.

LIU, Q., ZHOU, X. & SUN, Z. 2017. Application of silicon fertilizer affects nutritional quality of rice. *Chilean Journal of Agricultural Research,* 77**,** 163-170.

LUTTS, S., KINET, J. M. & BOUHARMONT, J. 1995. Changes in plant response to NaCl during development of rice (Oryza sativa L.) varieties differing in salinity resistance. *Journal of Experimental Botany,* 46**,** 1843-1852.

MAAS, E. 1993. Salinity and citriculture. *Tree physiology,* 12**,** 195-216.

MAAS, E. V. & HOFFMAN, G. J. 1977. Crop salt tolerance–current assessment. *Journal of the Irrigation and Drainage Division,* 103**,** 115-134.

MAHAJAN, G., SARLACH, R. S., JAPINDER, S. & GILL, M. S. 2011. Seed Priming Effects on Germination, Growth and Yield of Dry Direct-Seeded Rice. *Journal of Crop Improvement,* 25**,** 409-417.

MCDONALD, M. B. 2000. Seed priming. *Seed Technology and its Biological Basis.* Sheffield: Sheffield Academic Press.

MUNNS, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment,* 25**,** 239-250.

MUNNS, R. 2005. Genes and salt tolerance: bringing them together. *New Phytologist* 167**,** 645-63.

MUNNS, R. & JAMES, R. A. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil,* 253**,** 201-218.

MUNNS, R., JAMES, R. A. & LÄUCHLI, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany,* 57**,** 1025-1043.

MUNNS, R. & TESTER, M. 2008. Mechanisms of salinity tolerance. *Annual Review Plant Biology,* 59**,** 651-81.

MURAMATSU, Y., TAGAWA, A., SAKAGUCHI, E. & KASAI, T. 2006. Water Absorption Characteristics and Volume Changes of Milled and Brown Rice During Soaking. *Cereal Chemistry,* 83**,** 624-631.

NRDS 2009. National Rice Development Strategy. Minister of Agriculture, Mozambique.

PANDITA, V., ANAND, A. & NAGARAJAN, S. 2007. Enhancement of seed germination in hot pepper following presowing treatments. *Seed Science and Technology,* 35**,** 282-290.

PAPARELLA, S., ARAÚJO, S. S., ROSSI, G., WIJAYASINGHE, M., CARBONERA, D. & BALESTRAZZI, A. 2015. Seed priming: state of the art and new perspectives. *Plant Cell Reports,* 34**,** 1281-1293.

PATTANAGUL, W. & THITISAKSAKUL, M. 2008. Effect of salinity stress on growth and carbohydrate metabolism in three Rice (Oryza sativa L.) cultivars differing in salinity tolerance. *Indian Journal of Experimental Biology,* 46**,** 736-742.

PEIRIS, B. D., SIEGEL, S. M. & SENADHIRA, D. 1988. Chemical characteristics of grains of rice (Oryza sativa L.) cultivated in saline media of varying ionic composition. *Journal of Experimental Botany,* 39**,** 623-631.

PLATTEN, J. D., EGDANE, J. A. & ISMAIL, A. M. 2013. Salinity tolerance, Na+ exclusion and allele mining of HKT1;5 in Oryza sativa and O. glaberrima: Many sources, many genes, one mechanism? *BMC Plant Biology,* 13**,** 1-16.

QADIR, M., QUILLÉROU, E., NANGIA, V., MURTAZA, G., SINGH, M., THOMAS, R. J., DRECHSEL, P. & NOBLE, A. D. 2014. Economics of salt-induced land degradation and restoration. *Natural Resources Forum,* 38**,** 282-295.

QIN, H., LI, Y. & HUANG, R. 2020. Advances and challenges in the breeding of salt-tolerant rice. *International journal of molecular sciences,* 21**,** 1-15.

RANI, M. R. S. & BHATTACHARYA, K. R. 1989. Slurry Viscosity as a Possible Indicator of Rice Quality. *Journal of Texture Studies,* 20**,** 139-149.

RAO, B. S., MURTHY, A. V. & SUBRAHMANYA, R. The amylose and the amylopectin contents of rice and their influence on the cooking quality of the cereal. Proceedings of the Indian Academy of Sciences-Section B, 1952. Springer India, 70-80.

RAO, P. S., MISHRA, B. & GUPTA, S. R. 2013. Effects of Soil Salinity and Alkalinity on Grain Quality of Tolerant, Semi-Tolerant and Sensitive Rice Genotypes. *Rice Science,* 20**,** 284-291.

RAZZAQ, A., ALI, A., SAFDAR, L. B., ZAFAR, M. M., RUI, Y., SHAKEEL, A., SHAUKAT, A., ASHRAF, M., GONG, W. & YUAN, Y. 2020. Salt stress induces physiochemical alterations in rice grain composition and quality. *Journal of Food Science,* 85**,** 14-20.

REHMAN, H., BASRA, S. M. A., FAROOQ, M., AHMED, N. & AFZAL, I. 2011. Seed Priming with CaCl2 Improves the Stand Establishment, Yield and Quality Attributes in Direct

Seeded Rice (Oryza sativa). *International Journal of Agriculture and Biology,* 13**,** 786-790.

RENGASAMY, P. 2006. World salinization with emphasis on Australia. *Journal of experimental botany,* 57**,** 1017-1023.

RENGEL, Z. 1992. The role of calcium in salt toxicity. *Plant, Cell & Environment,* 15**,** 625-632.

RICHARDS, L. A. 1969. *Diagnosis and improvement of saline and alkali soils*, United States Department Of Agriculture; Washington.

ROESSNER, U. & BECKLES, D. M. 2012. Metabolomics for salinity research. *Methods Molecular Biology,* 913**,** 203-215.

RUAN, H.-H., SHEN, W.-B. & XU, L.-L. 2004. Nitric oxide involved in the abscisic acid induced proline accumulation in wheat seedling leaves under salt stress. *Acta Botanica Sinica - English Edition,* 46**,** 1307-1315.

RUAN, S., XUE, Q. & TYLKOWSKA, K. 2002. The influence of priming on germination of rice (Oryza sativa L.) seeds and seedling emergence and performance in flooded soil. *Seed Science and Technology,* 30**,** 61-67.

SALEETHONG, P., SANITCHON, J., KONG-NGERN, K. & THEERAKULPISUT, P. 2013. Effects of exogenous spermidine (spd) on yield, yield-related parameters and mineral composition of rice ('oryza sativa'L. ssp.'indica') grains under salt stress. *Australian Journal of Crop Science,* 7**,** 1293-1301.

SHABALA, S. & CUIN, T. A. 2008. Potassium transport and plant salt tolerance. *Physiologia Plantarum,* 133**,** 651-669.

SHABALA, S. & MUNNS, R. 2017. Salinity stress: physiological constraints and adaptive mechanisms. *Plant stress physiology, 2nd edn. CABI, Wallingford***,** 24-63.

SHABALA, S. & SHABALA, L. 2011. Ion transport and osmotic adjustment in plants and bacteria. *Biomolecular concepts,* 2**,** 407-419.

SHAHBAZ, M. & ASHRAF, M. 2013. Improving Salinity Tolerance in Cereals. *Critical Reviews in Plant Sciences,* 32**,** 237-249.

SHAN, Z. 2006. Mineral elements and human health. *Studies of Trace Elements and Health,* 23**,** 66-67.

SHANNON, M. C. & FRANCOIS, L. E. 1977. Influence of Seed Pretreatments on Salt Tolerance of Cotton during Germination. *Agronomy Journal,* 69**,** 619-622.

SHAVRUKOV, Y. 2013. Salt stress or salt shock: which genes are we studying? *Journal of Experimental Botany,* 64**,** 119-127.

SINGH, N., SODHI, N. S., KAUR, M. & SAXENA, S. K. 2003. Physico-chemical, morphological, thermal, cooking and textural properties of chalky and translucent rice kernels. *Food Chemistry,* 82**,** 433-439.

SISCAR-LEE, J. J. H., JULIANO, B. O., QURESHI, R. H. & AKBAR, M. 1990. Effect of saline soil on grain quality of rices differing in salinity tolerance. *Plant Foods for Human Nutrition,* 40**,** 31-36.

SMAJGL, A., TOAN, T. Q., NHAN, D. K., WARD, J., TRUNG, N. H., TRI, L. Q., TRI, V. P. D. & VU, P. T. 2015. Responding to rising sea levels in the Mekong Delta. *Nature Climate Change,* 5**,** 167-174.

SMITH, D., PAULSEN, G. M. & RAGUSE, C. A. 1964. Extraction of Total Available Carbohydrates from Grass and Legume Tissue. *Plant physiology (Bethesda),* 39**,** 960-962.

SUN, M. M., ABDULA, S. E., LEE, H. J., CHO, Y. C., HAN, L. Z., KOH, H. J. & CHO, Y. G. 2011. Molecular aspect of good eating quality formation in Japonica rice. *PLoS One,* 6**,** 1-12.

TAHJIB-UL-ARIF, M., SAYED, M. A., ISLAM, M. M., SIDDIQUI, M. N., BEGUM, S. N. & HOSSAIN, M. A. 2018. Screening of rice landraces (Oryza sativa L.) for seedling stage salinity tolerance using morpho-physiological and molecular markers. *Acta Physiologiae Plantarum,* 40**,** 1-12.

TAYLOR, A. G., ALLEN, P. S., BENNETT, M. A., BRADFORD, K. J., BURRIS, J. S. & MISRA, M. K. 1998. Seed enhancements. *Seed Science Research,* 8**,** 245-256.

TESTER, M. & DAVENPORT, R. 2003. Na^+ Tolerance and Na^+ Transport in Higher Plants. *Annals of Botany,* 91**,** 503-527.

THAKUR, M., SHARMA, P. & ANAND, A. 2019. Seed priming-induced early vigor in crops: an alternate strategy for abiotic stress tolerance. *Priming and Pretreatment of Seeds and Seedlings.* Springer.

THEERAKULPISUT, P., KANAWAPEE, N. & PANWONG, B. 2017. Seed priming alleviated salt stress effects on rice seedlings by improving Na+/K+ and maintaining membrane integrity. *International Journal of Plant Biology,* 7**,** 53-58.

THITISAKSAKUL, M., JIMÉNEZ, R. C., ARIAS, M. C. & BECKLES, D. M. 2012. Effects of environmental factors on cereal starch biosynthesis and composition. *Journal of Cereal Science,* 56**,** 67-80.

THITISAKSAKUL, M., TANANUWONG, K., SHOEMAKER, C. F., CHUN, A., TANADUL, O. U., LABAVITCH, J. M. & BECKLES, D. M. 2015. Effects of timing and severity of salinity stress on rice (Oryza sativa L.) yield, grain composition, and starch functionality. *Journal of Agricultural and Food Chemistry,* 63**,** 2296-2304.

THORNTON, J. M. & POWELL, A. A. 1992. Short-term aerated hydration for the improvement of seed quality in Brassica oleracea L. *Seed Science Research,* 2**,** 41-49.

TSAI, Y.-C., CHEN, K.-C., CHENG, T.-S., LEE, C., LIN, S.-H. & TUNG, C.-W. 2019. Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency. *BMC plant biology,* 19**,** 1-17.

TUNA, A. L., KAYA, C., ASHRAF, M., ALTUNLU, H., YOKAS, I. & YAGMUR, B. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environmental and Experimental Botany,* 59**,** 173-178.

UENO, K. & MIYOSHI, K. 2005. Difference of optimum germination temperature of seeds of intact and dehusked japonica rice during seed development. *Euphytica,* 143**,** 271-275.

UMEDA, M., OHTSUBO, H. & OHTSUBO, E. 1991. Diversification of the rice Waxy gene by insertion of mobile DNA elements into introns. *The Japanese Journal of Genetics,* 66**,** 569-586.

VAUGHAN, D. A., LU, B.-R. & TOMOOKA, N. 2008. The evolving story of rice evolution. *Plant Science (Limerick),* 174**,** 394-408.

VERMA, T. S. & NEUE, H. U. 1984. Effect of soil salinity level and zinc application on growth, yield, and nutrient composition of rice. *Plant and soil,* 82**,** 3-14.

VILLAREAL, R. M. & JULIANO, B. O. 1978. Properties of glutelin from mature and developing rice grain. *Phytochemistry,* 17**,** 177-182.

VIRMANI, S. S. & ILYAS-AHMED, M. 2007. Rice breeding for sustainable production. *Breeding major food staples.*

YAMAGATA, H., SUGIMOTO, T., TANAKA, K. & KASAI, Z. 1982. Biosynthesis of Storage Proteins in Developing Rice Seeds. *Plant Physiology,* 70**,** 1094-1100.

YAMAKAWA, H., HIROSE, T., KURODA, M. & YAMAGUCHI, T. 2007. Comprehensive Expression Profiling of Rice Grain Filling-Related Genes under High Temperature Using DNA Microarray. *Plant Physiology,* 144**,** 258-277.

YANG, L., WANG, Y., DONG, G., GU, H., HUANG, J., ZHU, J., YANG, H., LIU, G. & HAN, Y. 2007. The impact of free-air CO2 enrichment (FACE) and nitrogen supply on grain quality of rice. *Field Crops Research,* 102**,** 128-140.

YANG, Y., GUO, M., SUN, S., ZOU, Y., YIN, S., LIU, Y., TANG, S., GU, M., YANG, Z. & YAN, C. 2019. Natural variation of OsGluA2 is involved in grain protein content regulation in rice. *Nature communications,* 10**,** 1949-1949.

YOSHIDA, S. 1981. *Fundamentals of rice crop science*, International Rice Research Institute.

YOSHIDA, S., FORNO, D. A. & COCK, J. 1976. *Laboratory manual for physiological studies of rice*, International Rice Research Institute.

ZENG, L., SHANNON, M. C. & LESCH, S. M. 2001. Timing of salinity stress affects rice growth and yield components. *Agricultural Water Management,* 48**,** 191-206.

ZHENG, M., TAO, Y., HUSSAIN, S., JIANG, Q., PENG, S., HUANG, J., CUI, K. & NIE, L. 2016. Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. *Plant growth regulation,* 78**,** 167-178.