RNA-seq based Transcriptomic Variations and Expression Analysis of SKC1 gene involved in Salt Tolerance in Green Super Rice

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CERTIFICATE

The Thesis submitted by **Muhammad Ammar Amanat** to National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Center (NARC), Islamabad, Pakistan, is accepted in its current form. This thesis fulfills all the requirements for facilitating him with a Degree of Master of Philosophy in **Plant Genomics and Biotechnology.**

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DECLARATION

I would like to declare that the data presented in this thesis "**RNA-seq based transcriptomic variations and expression analysis of SKC1 gene involved in salt tolerance in Green Super Rice**" is generated by myself from original research work, under the supervision of **Dr. Muhammad Ramzan Khan** at Department of Plant Genomics and Biotechnology (PGB), PARC Institute of Advanced Studies in Agriculture (PIASA), National Agriculture Research Centre (NARC), Quaid-I-Azam University, Islamabad, Pakistan. The results and material used in this thesis never presented anywhere else earlier.

_____

Muhammad Ammar Amanat

Dated: ……..

DEDICATION

I sincerely dedicate this lifetime achievement to my loving and very caring parents whose prays and support made my path smooth and comfortable to my goals. They are my mentor and I am proud of them.

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Muhammad Ammar Amanat

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LIST OF ABBREVIATIONS

Word	Abbreviation
GSR	Green Super Rice
NIGAB	National Institute for Genomics and Advanced Biotechnology
NARC	National Agricultural Research Center
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
ANOVA	Analysis of Variance
RCBD	Randomized Complete Block Design
CV	Co-efficient of Variation
SSI	Stress Susceptibility Index
PCA	Principle Component Analysis
BLUP	Basic Linear Unbiased Prediction
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Real Time PCR
cDNA	Complementary deoxyribonucleic acid
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleic Acid
PH	Plant Height
NT	Number of Tillers
GY	Grain Yield
SY	Straw Yield
HI	Harvest Index
GW	Grain Weight
SL	Seed Length
SL	Shoot Length

RL	Root Length
SFW	Shoot Fresh Weight
RFW	Root Fresh Weight
SDW	Shoot Dry Weight
RDW	Root Dry Weight
SNC	Shoot Sodium Concentration
SKC	Shoot Potassium Concentration
RNC	Root Sodium Concentration
RKC	Root Potassium Concentration
Vg	Genetic Variance
Ve	Environmental Variance
Vp	Phenotypic Variance
GCV	Genetic co-efficient of variance
PCV	Genetic co-efficient of variance
Mha	million hactares, ,
MMT	million metric tons of milled rice
MT ha^{-1}	metric tons of milled rice per hectare
mg	Milligram
ml	Milliliter
ul	Microliter
mM	millimoloar
pm	Pico mole
pH	Power of Hydrogen Ion
rpm	Revolution Per Minute
gm	gram

ABSTRACT

Rice (*Oryza sativa*) is the important staple food crop worldwide, especially in Southeast Asia. About one third of rice cultivation area is under saline soil. Salinity stress is among the major abiotic stresses globally, that inhibits the rice growth and affects crop productivity. Plants have adopted the multiple tolerance mechanisms to tackle salinity stress. This study was carried out to identify such mechanism of salt tolerance at normal field, saline field, and hydroponic condition. The present study investigates the saline tolerance mechanism among the 22 GSR lines along with two local checks. In this study, we screened the experimental rice genotypes with the help of morpho-physiological, biochemical and genetic regulators under normal and salinity stress conditions. Field trial conducted during June, 2020 according to triplicated randomized complete block design (RCBD) with two treatments i.e. normal and salinity condition at NIGAB and Pindibhattian respectively. Morphology based attributes i.e., plant height (PH), number of tillers (NT), grain yield per plant (GY), straw yield per plant (SY), harvest index (HI), salt susceptibility index (SSI), 1000-grain weight (GW), and grain length (GL) were measured at maturity stage.

Hydroponic experiment was also conducted at seedling stage to screen the saline tolerant lines. Morpho-physiological attributes include; shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) were taken under control, 140mM and 200mM salt stress. Based on the salinity tolerance ranking, three saline tolerant (NGSR S13, NGSR S19, NGSR S20) and three saline susceptible lines (NGSR S3, NGSR S12, and NGSR S16) were selected for further molecular evaluation experiment. Using online transcriptome data, five differential expressed genes and two already reported genes (SKC1 and qSE3) were selected for expression evaluation in salt tolerant and susceptible lines. RT-PCR was performed to understand the genetic mechanism underlying salinity tolerance among the salt tolerant and susceptible lines. In future, functional validation of the salinity tolerant genes in rice and other crops will help us to understand the genetic mechanism underlying salinity tolerance. Salinity tolerant lines are useful genetic resource for salinity breeding programs.

1 INTRODUCTION

1.1 Origin and Taxonomy of Rice

Rice is amongst the most important crops of the world and it is a staple food for more than half of the world's population. Rice (*Oryza sativa*) is grown in the tropical and subtropical humid parts of all the continents in the world (Asia, Africa, Europe, Australia, and Americas) except Antarctica which is covered with snow, making it inappropriate for farming. (Chang, 1985).

The cultivated rice belongs to the genus *Oryza* and the family Poaceae (Gramineae) meaning "true grass". The genus *Oryza* is further divided into four species complexes (i) *sativa,* (ii) *officinalis*, (iii) *meyerina, and* (iv) *ridley* (G. Khush, 2005). Only sativa complex amongst all the complexes has the two species that have been domesticated and are cultivated. *O. sativa* is cultivated all over the world and *O. glabberinna* is grown in a limited area in Africa. The other species amongst the genus *Oryza* are wild species that could not be domesticated due to lodging and shattering (Callaway, 2014). Crop domestication involves the selection and screening of desired traits amongst the wild species to make them suitable for human use (Kovach, Sweeney, & McCouch et al., 2007). As this screening process is intense, the domestication process makes the crops unfit for survival in wild (He et al., 2011). Domestication involves the screening of desired traits, so many genes are lost from the gene pool ultimately decreasing g genetic diversity (Wright & Gaut, 2005).

There are about 21 wild relatives of Rice (Sweeney & McCouch, 2007). These wild relatives of *O. sativa* and *O.glabberinna* in the genus *Oryza* can be manipulated for the crop improvement because of the genetic variation. Rice domestication began long time ago around 9000 years ago (Molina et al., 2011). *Oryza sativa* has been domesticated from the wild species *O. nivara* and *O. rufipogon* (G. S. Khush, 1997; Kovach et al., 2007; Sweeney & McCouch, 2007).

O. sativa has two major subspecies (i) *Japonica* and (ii) *Indica.* Both of these species are more closely related to their distinct wild relatives than they are to themselves. They are so named because of their origin of domestication: *japonica* from China and *indica* from India. They have same non shatter mutation in gene sh4. According to (Sang & Ge, 2013), mutation arose in *japonica* prior to *indica.* It is thought that the

domestication of rice in China may have started in the Pearl River Valley (Huang et al., 2012) or lower Yangtze River Valley (Fuller et al., 2009; Vaughan, Lu, & Tomooka et al., 2008). It is evident that the domestication of rice began in the Asia in Assam-Meghalaya area in India and in Southeast China covering river valley regions (Fuller, 2011; Swaminathan, 1984). Japonicas are characterized as short, erect, stiff, having round grains and are found in temperate regions, whereas Indicas have weak stem, hanging leaves, tall stature, long grain size, high number of tillers and show poor response to high nutrient input (Kang, 2010).

Rice are semi aquatic plants that usually grow submerged in water. Rice has 3 to 6 months of growth cycle that varies with the variety. It is an annual crop but may produce tillers after it has been harvested. Mature plant of *O. sativa* consists of a main stem and several tillers. These tillers will ultimately bear panicle and terminal flowering head once the plant has passed through the reproductive phase. In its life, the plant of *O. Sativa* passes through two phases, vegetative and reproductive phase. During vegetative phase, the plant passes through germination, seedling stage, tillering, and stem elongation, while in the reproductive phase, the plant goes through booting, panicle initiation and grain filling (Maclean, Dawe, & Hettel et al., 2002). Rice is considered to be a natural inbred crop that makes it very valuable for the plant breeders (Blair, Hedetale, & McCouch et al., 2002). Rice having simple genome serves as a valuable model specie for cereals as well as monocots i.e. Wheat, Maize, Sorghum and Barley.

O. sativa has a relatively compact genome size and it consists of 12 pairs of chromosomes. It has a genome size of about 443 Mb that is the smallest genome of any domesticated cereal crop. Rice genome has been completely sequenced and because of its small genome size it was the first crop plant whose genome was sequenced (Project Intl Rice 2005). Its two subspecies, *Indica* and *Japonica* are mostly diploid (2n=2x=24). Both of these species has the genome AA (Li, Pinson, Paterson, Park, & Stansel et al., 1997; Smith & Dilday, 2002). On the other hand, wild relatives of *Oryza sativa* in the genus *Oryza* contains diploid (2n=2x=24) as well as tetraploid (2n=4x=48) forms. These different genome forms make up about 10 different types of genomes; AA, BB, CC, EE, FF, GG, BBCC, CCDD, HHJJ, and HHKK (Vaughan, Morishima, & Kadowaki et al., 2003). The genetic diversity within the genus *Oryza* amongst the wild relatives of the *Oryza sativa* can be manipulated in the rice improvement for

introgression of desired traits in Rice. This strategy can be very helpful especially in improving Rice's response against biotic and abiotic stresses (Ratnayaka, 1999).

1.2 Rice Status worldwide

Though, the world has seen an increase in the rice yield over the period of time but the rate of growth of rice has been declining. This is evident from the findings of Adjao et al. that during the year 1962 – 1979, the compound growth rate of rice was 2.5 % per annum (pa) but during the years 1980 – 2011 the growth rate declined to 1.4 % per annum (pa) (Adjao & Staatz, 2015). According to the Wales and Cheves et al. by 2015 – 2016 rice-harvested area is most likely to be 160 million hectares (M ha), and might not change by the year 2020 – 2021 (Wailes & Chavez, 2012).

Table 1. 1 Predictions of area, production, consumption, and yield of rice

Item	2010/11	2015/16	2020/21
Area (M ha)	157.3	160.2	160.5
Production (MMT)	451.4	481.5	502.7
Consumption (MMT)	446.2	478.5	501.5
Average Yield (MT ha^{-1})	2.87	3.01	3.13

Source: (Wailes & Chavez, 2012), Mha million hactares, MMT million metric tons of milled rice, MT ha^{-1} metric tons of milled rice per hectare

Almost 80 % of the world's rice is cultivated in the eight countries of the Asia i.e. China, Bangladesh, India, Indonesia, Thailand, Philippines, Vietnam and Myanmar. These countries also hold about the world's 46.6 % of the population. Asia alone contributes to the world's 90% cultivable area of Rice (Wassmann et al., 2009). On the other hand, Africa's contribution towards Rice cultivable area is 5 % (8 M ha) (Zeigler, 2006), Caribbean and Latin America contributes about 5.5 M ha area (Pulver, Jaramillo, Moreira, & Zorilla, 2010) and Brazil contributes about 2.8 M ha (Lafranco, 2010). Comparatively, Africa has seen an increase in their rice growth rate as compared to Asia. During the years 1980 – 2011, rice area growth rate in Africa has been recorded to be 3.1 % which is significantly higher in comparison to Asia that was 0.4 % (Adjao & Staatz, 2015). If we view the total rice production, China was the largest contributor contributing about 30.1 % in the World's total rice production in 2010. By the passage of time, China's contribution in the World's total rice production may slightly decrease, while India's contribution might increase (Wailes & Chavez, 2012). Asia, the world's

largest rice producing region might also lose some of its share to Africa in total rice production per annum (Wailes & Chavez, 2012).

1.3 Importance of Rice

Rice is thought to be the most consumed cereal grain of the globe as it is the staple food for more than half of the world's population (Krishnan, Ramakrishnan, Reddy, & Reddy, 2011; Y. Yu, Huang, & Zhang, 2012). Rice is also said to be the first crop to be domesticated that could be used by human to fulfill their nutrition requirement. Rice is cultivated on the world's 9% fertile land. Rice is ranked third highest in the worldwide food production after maize and wheat (FAO STAT 2010). Rice is the most important cereal crop as it provides 15 % per capita protein and 21 % per capita energy, if we talk in terms of calorific intake and human consumption. This percentage is even higher in the developing countries being 20 % for per capita protein and 27 % for per capita energy (G. Khush & Virk, 2000). Rice cultivation around the globe has been a significant source of livelihood for the millions of people. Rice cultivation is the major source for foreign exchange and revenue for numerous Asian counties.

Global warming and changes in climate proves to be disastrous for the crops and poses tremendous threat to the world's food production, ultimately affecting economic development of the countries. Population of the world is expected to increase by 50 % in the next 50 years, along with an increase in insufficiency of land and water. According to IRRI, 800 million tons of rice are required to meet the consumption demand of rice in 2025 (Purevdorj & Kubo, 2005). While land and water resources are depleting, there are other major challenges posed by abiotic stresses such as the salinity, drought, cold, and heat and biotic stresses such as diseases, insects and pests.

1.4 Status of Rice in Pakistan

Rice, in Pakistan, is not only the second most consumed food after wheat, but also an important economical product with major export importance second only to cotton (Chandio & Yuansheng, 2018; GOP 2021). In terms of GDP, rice contributes 0.7% and to the value addition, the contribution is around 3.5% in Pakistan's economy (GOP 2021). Punjab and Sindh are, generally, the major cultivation sites for rice in Pakistan. Here, both surface and subsoil irrigation systems are utilized. The commonly grown

varieties of rice in both the provinces include Basmati, D-98, IRRI-9, IRRI-6 and Begghi (Chandio & Yuansheng, 2018; Ghulam et al. 2012).

IRRI and Basmati are the two mainly cultivated varieties in around 11% of total agricultural area in Pakistan (Ahmed & Schmitz, 2011; S. Ali et al., 2017). Punjab and Sindh are the main producers (about 90%) of superior quality of rice to provide for the domestic use and fulfill export needs (Chandio & Yuansheng, 2018; GOP 2018). Rice meets sixty percent of the food needs of Pakistan, which is also a major food source for animals worldwide in winter (Drake, Nader, & Forero et al., 2002; Nguyen et al., 2008). It is a staple food for local population and Pakistan exports it to other countries including Afghanistan, Iran, Saudi Arabia and United Arab Emirates (UAE) to earn foreign exchange (Chandio et al. 2017). The estimated area used for rice cultivation in 2010-2011 was about 2365.3 (000 ha), Punjab accounts for 1766.8, Sindh for 361.2, Khyber Pakhtunkhwa (KPK) for 46.1 and Baluchistan for 191.2 (000 ha). In 2010-2011, estimated rice production in Pakistan was 4823.3 (000 t). Punjab contributed 3384.0, Sindh around 1230.3, Khyber Pakhtunkhwa (KPK) around 78.4 and Baluchistan contributed 130.6 (000 t) of the total rice production (see Table 1) (GOP 2011).

Since the last decade, trends of rice productivity depict the decline in rice cultivation. The reason may be weather changes, which may result in abiotic stresses (Joyo et al. 2018) and/or land degradation (Magsi & Sheikh, 2017). Many researchers (Hanif, Syed, Ahmad, Malik, & Nasir et al. , 2010; Rasul, Mahmood, Sadiq, & Khan et al., 2012; Zhu, Ringler, Iqbal, Sulser, & Goheer et al., 2013), in Pakistan, have studied the impact of climate change on Agriculture. In addition, several researches have tried to increase the rice yield by introducing the resistance in rice towards abiotic stresses (Abid, Ngaruiya, & Zulfiqar et al., 2017; Abid, Scheffran, & Elahi et al., 2019; A. Ali & Erenstein, 2017; Arshad, Amjath-Babu, & Müller et al., 2016; Arshad et al., 2017; Gorst, Dehlavi, & Groom, 2018).

1.5 Green Super Rice

By 2050, the world population will grow beyond nine billion, which necessitates a 70% growth in food production (Desa, 2015; FAO 2013). This growth is vital for securing the living standards and world food security. Traditionally, crop improvement involved

crossing plants with agronomically desirable phenotypes. These techniques produce semi-dwarf varieties providing heterosis and lodging resistance, leading to increased rice yields over past fifty years. These methods are not only expensive but also have adverse effects on environment (G. S. Khush, 2001; Valdés, Hazell, & Pomareda et al., 1986; Yorobe Jr et al., 2016).

Green Super Rice (GSR) is a rice cultivar capable of producing stable and high yield at low input (Zhang, 2007). Green Super Rice (GSR) was introduced a decade ago (Zhang, 2007) for increasing the yield while lowering the associated ecological footprint and production cost. The recent findings in bioinformatics and genomics research have revealed that multiple techniques such as back breeding, marker-assisted selection, whole genome selection breeding, pedigree breeding and combining ability breeding have been utilized for producing generation containing various elite genes and GSR varieties with desirable traits. These methods were exploited to transfer the genes linked to rice quality, disease and insect resistance, yield, drought tolerance and increasing nutrient use (S. Yu, Ali, Li, & Zhang et al., 2020). GSR varieties have decreased requirements for water, pesticides and fertilizer. They also can withstand salt and alkali stress, have high yields, are more nutritious and palatable, can grow on marginal lands and produce low amount of greenhouse gases(Wing, Purugganan, & Zhang et al., 2018).

1.6 Salinity

Salinity, as abiotic stress in soil and water, poses a worldwide problem both to irrigated and non-irrigated crops. Sodium, with concentration more than 4dSm-1 or 40 mM, is primarily the leading cause of soil and water salinity (Munns & Tester, 2008; Szabolcs, 1994). However, sodium concentrations of over 150mM- 200mM have already been investigated (Kawasaki et al., 2001; Rabbani et al., 2003; Ren et al., 2005). According to the Food and Agriculture Organization of the United Nations (FAO), there are about 397 M ha of land affected by salinity worldwide. Twenty percent of the 230 M ha of land used for crop production is affected by salinity with a staggering cost of USD 11 billion per year (Thomas and Morini, 2005).

Salinity is caused by the natural climatic factors. Seawater pollutes the rivers and aquifers. Many soluble salts are released when basalt rocks withers in arid and semi-arid areas. Poor drainage system and irrigation utilizing salt-rich water causes

secondary salinization(Yadav, Irfan, & Hayat et al., 2011). Heavy concentration of sodium ions in soil lowers the capacity of plants to take up water and minerals leading to retardation of plant growth and yield (Flowers, 2004; Munns & Tester, 2008). Soil salinity affects 20 % of the cultivated lands in the world leading to alkalinity and water logging(Hakim et al., 2014). Rice is the most sensitive economic cereal to salinity stress (Munns & Tester, 2008). Salt level of 10dSm-1 causes the death of seedlings (Munns, James, & Läuchli, 2006) and at reproductive stage, high yield loss of up to 90% is caused by salt stress of 3.5 dSm-1 (Asch, Dingkuhn, & Miezan et al., 2000). In Pakistan, out of 79.6 M ha of area, 22 M ha is cultivated (GOP, 1999) 6 M ha is damaged through salinity(Mahmood et al., 2003). Among salt affected soil, 2 M ha is damaged by salinity and 3 M ha by sodicity (Qureshi, Aslam, & Rafiq et al, 1993). Irrigated land in Sindh and Punjab are 40 % and 25 % salt-affected respectively, which has caused a major damage to the livelihood of 10-20 million people (Barrett-Lennard & Hollington, 2006).

1.7 Mechanisms of Salt Stress Tolerance in Rice

Many rice varieties at their seedling and reproductive stage are sensitive to the toxicity of sodium ion accumulation caused by salinity. Few rice varieties using sophisticated physiological mechanism including sodium compartmentalization into apoplast, sequestration into older tissues, and upregulation of antioxidants, stomatal responsiveness and sodium exclusions have developed tolerance against salinity (Deng et al., 2015; Prasad, Bagali, & Shashidhar et al., 2000; Ren et al., 2005; Thomson et al., 2010).

Salinity causes ionic and osmotic stress in plants. In osmotic stress, due to higher amount of salts in soil or water near roots, the availability of nutrients and water is restricted (Munns & Tester, 2008; Roy, Negrão, & Tester, 2014). To combat osmotic stress, plants close their stomata for water conservation and limit transpiration, which is involved in the movement of sodium ions from roots to shoots of plants (Flowers & Flowers, 2005).

Compounds of low molecular weights and water use aquaporins, membrane proteins, to enter plants cells. During osmotic stress, the genes for aquaporins show down regulation and over expression, which shows the important role these proteins have in homeostasis. Furthermore, there is an accumulation of solutes like mannitol, sucrose,

betaine, proline and glycerin for adjustment of osmotic environment and prevention of dehydration through restoration of water uptake (Horie, Karahara, & Katsuhara, 2012).

The survival of plants decreases with the buildup of sodium ions under salinity stress (Yeo, Yeo, Flowers, & Flowers, 1990). In plants, there is not any sodium selective membrane recognized yet. They are taken up into cells through nonselective cation channels or K+ transporters (Demidchik & Maathuis, 2007; Flowers & Flowers, 2005). Transporters are involved in salinity tolerance by reducing the buildup of Na+ ions through ion exclusion (Sonia Negrão et al., 2011). One of the best-known transporters involved in tolerance against salinity include high-affinity K+ transporters or HKT. HKT 1;1 has shown selective permeability towards sodium ions, at high concentration of Na+ ions in Arabidopsis, and assisted in the removal and recirculation to roots from leaves (Horie et al., 2005). Another scientist, (Ren et al., 2005) testified the function of OsHKT1;5 gene in rice for Na+/K+ homeostasis.

Compartmentalization of sodium ions in vacuoles is one of the salinity tolerance mechanism accepted as tissue tolerance. Na+/H+ transporters (NHX) present in tonoplast perform the Selective sequestration of sodium ions in vacuoles (Sonia Negrão et al., 2011). In association with this process, there is an accumulation of potassium ions and other solutes in the cytosol to stabilize the osmotic pressure of in vacuoles (Munns & Tester, 2008).

In addition, there was an observation of Na+ ion secretion in halophytes' leaves by specialized modified cells (Flowers & Flowers, 2005). Oryza coarctata, a wild relative of rice with KKLL genome, due to its distinctive trichomes was found extremely tolerant to salinity stress (Bal & Dutt, 1986).

1.8 Genetics and QTL Mapping of Salt Tolerance in Rice

Salt tolerance in rice accessions is present in wide varieties. Rice landraces including Nona Bokra and Pokkali can endure certain levels of salt stress (Yeo & Flowers, 1986). Salt tolerance in rice is considered a complex trait both physiologically and genetically (Bonilla, Dvorak, Mackell, Deal, & Gregorio, 2002; Gregorio & Senadhira, 1993; Walia et al., 2005). Salinity tolerance shows complexity due to variety of plant's responses to salinity stress. Under salt stress, the phenotypic characterization in rice of mapping populations for physiological and morphological traits showed presence of

transgressive segregants, continuous distribution and substantial interaction of genotype with environment. These are the signs of a quantitative trait and shows salinity tolerance as of polygenic nature (Flowers, 2004; Gregorio & Senadhira, 1993; Koyama, Levesley, Koebner, Flowers, & Yeo, 2001). Furthermore, traits with salinity tolerance when mapped using QTL, showed existence of some QTLs with large-effect and many QTLs with small-effect. Mapping for osmotic related QTL by Robin et al., was carried out using backcross population of IR62266-426-2 × IR60080-46A (Robin et al., 2003). 14 QTL were identified on 8 chromosomes. Using the rice F2 population of Cheriviruppu × Pusa Basmati 1, at seedling and reproductive stage, Hossain et al. studied agronomic traits under salinity which lead to identification of 16 QTL for tolerance against salt (Hossain, Rahman, Alam, & Singh, 2015). Ghomi et al., using the 148 F2 population obtained from Gharib (indica) × Sepidroud (indica) found 41 QTL having an effect on 12 physiological traits related to salt tolerance (Ghomi, Rabiei, Sabouri, & Sabouri, 2013). 14 QTL in rice effecting salt tolerance related traits were mapped through F2 population of salt sensitive cv. Khazar and Tarommahali (Sabouri et al., 2009).

Cloning of QTL genes for rice ST in addition to genetic analysis of ST through QTL mapping have been performed. One of the genes include SKC1 encoding for a HKT-type transporter, which, under salt stress, maintains homeostasis of potassium ions in ST variety (Ren et al., 2005). Another gene OsCCCl^- (cation-Cl− cotransporter) is considered to be involved in transport of ions, k^+ and Cl^-, and plays important role in homeostasis of these ions and in rice development (Kong et al., 2011). OsEATB is another cloned gene, linked to panicle branching and tillering, obtained from rice 9311 variety and was down-regulated under salinity stress (Qi et al., 2011). Some other ST cloned genes include OsMAPK33 which plays negative role in ST by increasing sodium ion uptake through over expression under salinity (Lee et al., 2011), and OsNHX1, an antiporter of Na+/H+ in rice (Fukuda et al., 2004) which under higher concentration of NaCl and KCl, increases expression in roots and shoots.

Many studies to identify QTLs were carried out using small number of parents of bi-parental segregating population, which fails to show the complete genetic variation in salt tolerance in rice germoplasms (Xu 2012 Abbr). The use of natural populations over the bi-parental populations have shown some advantage in identifying QTL/QTN to find out complex traits (Agrama, Eizenga, & Yan, 2007; Zhao et al., 2011). Rice

landraces used by Emon et al. contained 220 multiple markers linked to ST, 8 of which were markers with sequence-tagged-site established for genes Sa1T, DST and SKC1 (Emon, Islam, Halder, & Fan, 2015). Kumar et al. found 20 SNPs, in 220 rice accessions, highly associated with Na+/K+ ratio explaining phenotypic variance of 5–18% (Kumar 2015). Negrão et al. used 392 rice lines and found interaction of P140A and OsHKT1;5 with transmembrane domain in salt tolerance (Sónia Negrão et al., 2013). Platten et al. utilized plant shoots at seedling stage of 103 rice lines to mine alleles at OsHKT1;5 locus and found out 7 major and 3 minor alleles associated with Na+ (Platten, Egdane, & Ismail, 2013). A study to find the association of haplotype distribution and natural genetic variation in rice for a candidate gene of salt responsiveness conducted by Mishra et al. revealed twenty two salt responsive genes in indian germplasm with multiple ST haplotypes and phenotypes (Mishra, Singh, Misra, Rai, & Singh, 2016).

1.9 Transcriptome Analysis

RNA plays a vital role as intermediary between genome and proteome. This discovery in molecular biology has provided information regarding identification of transcripts and in gene expression quantification. The dual feature of discovery and quantification in sequencing RNA can be utilized to obtain a single high-throughput sequencing assay known as RNA-sequencing (RNA-seq) (Conesa et al., 2016). There has been a shift towards Next-Generation Sequencing (NGS) based RNA sequencing method due to improvement in high–throughput technologies. This method is best in identifying the novel transcripts, a feature useful in stress experiments (Chandran et al., 2019). RNA-Seq based salt analyses of transcriptome have been performed using root samples (Chandran et al., 2019; Mizuno et al., 2010; Zhou et al., 2016).

1.10 Aims and Objectives

- Phenomic evaluation of 22 GSR lines along with two checks under control and salinity stress.
- Selection of salinity tolerant and susceptible lines among the studied genotypes.
- Identification of differentially expressed genes based on transcriptome analysis.

- Molecular characterization of selected salinity related genes among salinity tolerant and susceptible genotypes.

2 MATERIALS AND METHODS

2.1 Selection of *Oryza sativa* Lines/ Varieties

22 different best performing GSR lines NGSR-S1 to NGSR-S22 were selected from 552 GSR lines for experiment, based on their phenotypic characters along with two checks IRRI 6 and Kissan Basmati. Seeds of these GSR lines were acquired from NIGAB (National Institute for Genomics and Advanced Biotechnology), NARC (National Agriculture Research Centre), Islamabad. Three independent experiment were run for these 24 lines to check the response of these GSR lines against salt stress and to screen out the salt tolerant and salt susceptible lines,

2.2 Field Trial

The healthy seeds of 22 GSR Accessions and two checks were sown in plastic trays having 128 wells. Each line was grown in the separate tray. Two seeds were sown in each well of the tray in the month of June. The seeds were sown in mixture of soil, peet moss and sand in a ratio 2: 2: 1. In the month of July, at three leaf seedling stage about three weeks after sowing, half of the rice seedlings were transported for transplantation to the field of Soil Salinity Research Institute, Tehsil Pindi Bhattian, District Hafizabad, while half of the rice seedlings were left to grow as a control in National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Centre (NARC), Islamabad. The experiment design was randomized complete block design (RCBD) with three replicates for each field.

2.3 Evaluation of Morphological Traits for Field

Before harvesting of the rice plants, the following morphological data was recorded for each GSR accession; plant height, and number of tillers. After harvesting grain yield per plant, straw yield per plant, seed length, and 1000 grain weight were recorded. The data was recorded randomly from each replication to minimize the error. Plant height was recorded upto the grains, and only fertile tillers were counted. Grain yield was computed by weighing the total grains of a plant on a weighing balance in grams, whereas straw yield was calculated by weighing grain less straw on weighing balance. Seed length was measured in millimetres by digital Vernier caliper.

2.4 Glasshouse Trial no. 1

Seeds of 22 GSR lines along with two checks were surface sterilized with 30% hydrogen peroxide solution for 10 minutes followed by thoroughly washing with autoclaved distilled water. After sterilization, seeds were placed in petri dishes to germinate for 72 hours in growth chamber at 24-28°C with 14 hours light period and 10 hours dark period. Three day old uniform seedlings were selected and transferred to polyethylene lined plastic trays containing 128 wells. The three day old seedlings were transplanted in trays containing mixture of soil, peat moss and sand in the ratio 2: 2: 1. These trays were than immersed in water, on a tray filled with water ensuring continuous supply of water to the rice seedlings. The polyethylene lined plastic trays were kept in glass house under controlled conditions having light period of 14 hours followed by 10 hours dark period, humidity 55% to 65% and temperature 26 - 28 °C. The design of the experiment was randomized complete block design (RCBD) with three replications. Eight seedlings were transplanted for each replicate for two treatments; one as a control and the other one as a salt treatment.

Rice seedlings were left to grow until 7th day. At 7th day after transplantation, seedlings in one batch were given salt treatment of 140 mM NaCl. They were left to grow for further 7 days with one batch as a control and the other batch as with salt stress. The salt solution was changed at the 4th day after applying salt stress.

2.5 Glasshouse Trial no. 2

Seeds of same 22 GSR lines along with two checks were grown in a same manner as for glasshouse trial no. 01 described above. The only difference was the plants were given salt stress with a higher salt concentration and lower time duration for stress.

Rice seedlings were left to grow for 14 days. At 14th day after transplantation, seedlings in one batch were given salt treatment of 200 mM NaCl while the other batch was left to grow as a control. They were left to grow for further 7 days. The salt solution was changed at the 4th day after applying salt stress.

2.6 Evaluation of Morphological Traits for Glasshouse Trials

The 24 rice cultivars were evaluated in three experimental rounds for measuring trait values. Post to salt treatment, three rice seedlings per line in each replication were randomly selected for measuring shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW). The mean phenotypic value of the cultivars collected out of three experimental rounds were normalized by BLUP analysis using R software. The BLUP analysis was employed to eradicate the difference amongst the root data readings because some of the roots were lost in washing.

2.7 Evaluation of Biochemical Traits for Glasshouse Trials

Two replicates from plants in 200 mM were selected to analyse for Na+ and K+ concentration. The roots and shoots were rinsed with distilled water several times after they had been harvested at the 7^{th} day of salinization with 200 mM salt. The samples were kept in oven to make them dry for 72 hours at 60°C. After the samples had dried, the sodium and potassium concentration was measured according to the method proposed by (Kushizaki, 1968) using flame photometer. Ratio of sodium concentrations of shoot and root to that of potassium concentrations of shoot and root was also calculated.

2.8 Statistical Analysis

ANOVA was employed for the field trial to compute significance amongst the ttraits and genotypes. Best linear unbiased prediction (BLUP) value for the traits recorded in glasshouse was recorded using R software. R code “ggcorrplot2” was used for finding correlation amongst the traits using the R software. XLSTAT was used for Principal Component Analysis (PCA).

2.9 Identification of Salinity Related Genes

Published literature was used to identify key genes controlling salt tolerance in rice. Though, there are many QTLs that have been identified to contribute to salt tolerance mechanisms. SKC1 and qSE2 are the two genes that have been characterized and have

proven to exhibit a role in salt stress tolerance in rice (Ren et al., 2005; He, Y et al., 2019). Uncharacterized genes that were also selected that originated from the different transcriptome data available online. CDS sequence of these gene were retrieved from the Rice Genome Annotation Project (http://rice.uga.edu/) browser.

2.10 Screening for Salt tolerant and Salt Susceptible lines

Rice seedlings for two glasshouses were evaluated according to (SES, IRRI). The scoring for the salt toxicity symptoms was recoded. Morphological data from the two glass house trials (shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight) and field trial (plant height, number of tillers, grain yield, straw yield, harvest index and stress susceptibility index) was used to evaluate the behavior of 22 GSR lines and two checks towards salt stressed conditions. The stressed data for the lines was compared with that of control and ranked according to their performance. Tolerant and susceptible GSR lines were then selected according to their scores. A high score indicates susceptible whereas a low score indicates tolerant line.

2.11 RNA Extraction

2.11.1 Sample Preparation

The roots of the seedlings were washed thoroughly to dispose of the excessive soil and peat moss on the roots. After washing, the root tissue from all the lines were immediately stored in liquid nitrogen prior to storage in – 80 °C freezer. The root tissue were also collected in three replicates.

2.11.2 Extraction of RNA

Total RNA from the roots of the most tolerant and most susceptible GSR lines were extracted. The total RNA was extracted using the TRIzol reagent and the method devised by (Sah, Kaur, & Kaur, 2014). Chomczynki and Sacchi developed TRIzol reagent (Chomczynski & Sacchi, 1987). 50 - 100mg of root tissue preserved at -80 °C was ground with the help of sterile mortar and pestle. Powdered ground samples were then transferred to 2 ml sterile eppendorf tube. 1 ml TRIzol is added in the Eppendorf tube containing the sample. The Eppendorf tube was then vortexed and incubated at room temperature for 5 minutes. After incubation, 200 µl chloroform was added. Again,

samples were left to incubate at room temperature for 5 minutes. Samples were then centrifuged for 15 minutes at 12,000 rpm at 4 °C. Upper aqueous phase was carefully picked and transferred to another sterile Eppendorf tube; not letting any debris while transferring. 500 µl isopropyl alcohol was added in the tube containing supernatant. Isopropyl alcohol helps to precipitate RNA. The samples were mixed and left to incubate at room temperature. Samples were then centrifuged at 12,000 rpm for 10 minutes at 4 °C. The precipitated RNA will form a pellet most likely to be on the side of the tube or at the bottom of the micro centrifuge tube. Supernatant above the precipitated RNA was discarded. 75 % chilled ethanol was added and tubes were vortexed. Samples were centrifuged at 12,000 rpm for 10 minutes at 4 °C. This step has to be repeated twice. Supernatant was discarded and pellet was left to air dry for about 2 – 3 minutes. RNA was dissolved in 60 µl of RNase free water. For short time storage, eluted RNA was stored at - 20 °C but after that RNA was preserved at – 80 °C.

2.11.3 Gel Electrophoresis

The Agarose Gel Electrophoresis technique was employed to analyse RNAs extracted from the root tissue of *Oryza sativa.* 1 % agarose gel was used to analyse different RNAs obtained from rice. 1 % agarose gel was prepared in a flask using 1X TAE buffer (prepared from 50 X Tae buffer). The mixture of TAE buffer and agarose powder was allowed to heat in the microwave oven until the agrose was completely dissolved in the mixture and the solution became transparent. The mixture was then allowed to cool for few minutes until flask gets warm. 4 µl ethidium bromide (intercalating dye) was then added in the solution. The mixture was then poured onto the gel tray which had already been assembled. After a while, gel became solid and was ready to be loaded with the samples. Comb was removed very carefully making sure that wells do not break. 5 µl of RNA saample was mixed with 3 µl of 6X loading dye (Thermofisher). 1 kb ladder was also run with the rice RNA samples in the first well of the gel. The gel was then run with the voltage of 80 volts for forty minutes in the caster that had 1X TAE buffer. After forty minutes, to confirm the presence of bands gel was viewed and analysed under gel documentation system.

2.11.4 DNase treatment

Extracting RNA with TRIzol reagent can sometimes result in the accumulation of the unwanted products i.e. DNA and proteins in the extracted RNA. The unwanted DNA was detected in some samples by the Agarose gel electrophoresis. To remove the genomic DNA, samples were subjected to DNase treatment.

Equal amount of 5M NaCl to that of eluted RNA was added in the RNA samples. Samples were vortexed for 10 to 15 seconds and incubated at -20 °C freezer for 60 minutes. Samples were centrifuged at 12,000 rpm for 30 minutes. Supernatant was discarded. For washing, 0.5 ml of 70 % ethanol was added in each of the sample. Samples were centrifuged at 4°C at 12,000 rpm for 10 minutes. We discarded the supernatant. Remaining ethanol was sucked out with the pipette. RNA pellet was allowed to air dry for several seconds. Later, RNA pellet was dissolved in 40 ul RNase free water.RNA quality was again checked at the agarose gel to confirm that all the genomic DNA has been eradicated and integrity of the RNA is still in shape.

2.12 Quantification of RNA

Synthesis of cDNA needs pure RNA to avoid any contamination. To determine the integrity, quality, and quantity of RNA prior to cDNA synthesis, we used Biospec™ (Biospec nano spectrophotometer for life sciences). The OD for the RNA was measured at the wavelength 260/280nm for all purified samples. The samples that were low in quantity and showed poor quality were extracted again to maintain and get uniform expression of genes.

2.13 Synthesis of cDNA

The total RNA extracted from the rice samples was taken as a template for synthesising cDNA. Thermo Scientific Revert Aid reverse transcriptase-III, First Strand cDNA Synthesis Kit was used to synthesize cDNA from the RNA extracted from the rice samples. Amount of RNA used for the RNA synthesis was equivalent to 1μg. To make all the concentrations of RNA equal to 1 μg, the RNA values obtained from nanodrop were normalised. The concentration of RNA was kept as denominator and 1000 was divided by the denominator. In such manner the values of RNA concentration were

normalised. Total reaction mixture for one sample was 20 µl. 20 µl reaction mixture contained 4 µl RT buffer, 2 µl dNTPs, 1 µl oligodT 1 µl, RT enzyme and rest 0f the 12 µl contained water and RNA sample. So subsequently, amount of water to be used was also calculated using the value obtained after normalising the RNA concentrations.

12 µl water and RNA along with 1 µl of oligodT were added in the PCR tube. The mixture was then run on PCR (Applied Biosystem-96 wells thermo cycler by Thermo Scientific). Mixture was allowed to incubate at 65 °C for 5 minutes in the PCR. After incubation 7 µl of the mixture (4 µl RT buffer, 2 µl dNTPs, and 1 µl, RT enzyme) were added in the PCR tube. The reaction mixture was then run at 42°C for 1 hour and the final extension step for 72°C for 10 minutes. Synthesised cDNA was then stored at 4°C refrigerator.

2.14 Transcriptomic Analysis

The transcriptome data for two studies (Chandran et al., 2019; Shankar et al., 2016) was downloaded from NCBI and EMBL-EBI Array Express. From these transcriptome data, the data only related to roots (control and under salt stress) was downloaded. The downloaded data was analysed in LINUX based system.

The data was downloaded in the SRA format from the GEO NCBI. Reference genome for the *Oryza sativa* was downloaded from the www.plants.ensembl.org. Hisat2 version 2.1.0 https://daehwankimlab.github.io/hisat2/ was used for the reference genome indexing of *Oryza sativa.* Split command in the LINUX system was used to split the paired end library of transcriptome data into FastQ files. By employing a particular command line in the LINUX based computer, alignment of the reads was completed. Files in .sam were converted into .bam files. Files were then sorted and merged for use in future. To calculate the differential gene expression of the DEGs (differentially expressed genes) we brought two tools into our use. Ballgown was downloaded from https://bioconductor.org/packages/release/bioc/html/ballgown.html and another tool employed was featurecount.

2.15 Differentially Expressed Genes (DEGs)

Log 2 values obtained for the DEGs were used to construct heat map. Cluster heat map for both the studies were constructed using R code “pheatmap” in R software. 5 DEGs

with the higher log 2 values were selected to check for the expression in the selected GSR varieties.

2.16 Primers Designing for Gene Expression

Amplifix software was used for designing the primers of *Oryza sativa* to examine the expression of genes conferring salt resistance to rice plants. Those primers were then checked with the help of two different softwares Multiple Primer Analyzers (Thermofisher Scientific) and primer stat (Stothard 2000). The specificity of the designed primers was tested by UCS PCR at UCSC-In Silico PCR genome browser (https://genome.ucsc.edu/) (Rhead et al. 2009). The designed primers for yield related gene expression analysis are given in (Table 2.1)

Table 2. 1 Designed Primers for the genes

Sr.no	Primer's name	Primer's Sequence
1	LOC_Os08g34540_F	AATCACGCCATGAAGGGAGACTAC
2	LOC_Os08g34540_R	TCTTTTGCCTGGGAGAACCACTTG
3	LOC_Os10g31120_F	GAACCCAAGTGGCATGCTGTTT
4	LOC_Os10g31120_R	TCATCCTTGCCTTTGCCTTTGC
5	LOC_Os05g33260_F	CATCGTGACTGACAGATGGCAGAA
6	LOC_Os05g33260_R	ACTCCCAACCGTAACATCAACTCG
7	LOC_Os01g46890_F	TGTTGGGCGTCTACGTCTTCAC
8	LOC_Os01g46890_R	GCAGAATCATGCAGCCATGGAAGT
9	LOC_Os01g50430_F	ATGCAGTTTGCCAGGTCAGA
10	LOC_Os01g50430_R	AGAACCTCGCTGACGACATGAT
11	LOC_Os03g37930_F	CATGCCGAGGATATGCTTACACCA
12	LOC_Os03g37930_R	GAAGCGATGAGTGAGGCCTTACAA
13	LOC_Os01g20160_R	AACTACAGCGTCCTCAACATCGTC
14	LOC_Os01g20160_F	TGAGAGTGAGCTTCCCTTGTTTGC

2.17 qRT PCR expression analysis

1000 ng or 1 µg of cDNA in the qRT-PCR will create a lot of noise and ultimately the results will not be reliable. To overcome this, 20 µl of cDNA in the PCR tube was diluted to 100 µl.

Table 2. 2 RNA concentration used for qRT PCR

Sample Name	Nucleic Acid Concentration	OD 260/ 280 nm	RNA used for cDNA synthesis(µl)	Water used for cDNA synthesis(µl)
S3-C	724	2.00	1.38	10.62
S12-C	510	2.00	1.96	10.04
S16-C	506	1.97	1.98	10.02
S13-C	380	1.98	2.63	9.37
S19-C	367	1.99	2.72	9.28
S20-C	550	2.00	1.82	10.18
S3-T	466	1.97	2.15	9.85
S12-T	826	1.98	1.21	10.79
S16-T	453	1.97	2.21	9.79
S13-T	710	1.98	1.41	10.59
S19-T	870	1.97	1.15	10.12
S20-T	531	1.99	1.88	9.02

The expression of salinity related genes in the Green Super Rice (GSR) was analysed using the comparative ΔCT method in the Applied Biosystems Step-One-Plus real-time PCR. Primers used were Gene specific. SYBR-GREEN master mix prepared already by the Thermofisher was used. Actin was used as an endogenous control for the 13 genes that were used in this study. Total reaction volume for one reaction was 10µl. 5µl SYBR-GREEN master mix, 1µl cDNA, 0.2µl forward primer, 0.2µl reverse primer and 3.6µl water was used in each reaction mixture.

The profile set for the qRT-PCR was as follows; first stage was denaturation at 94ºC for 10 minutes, second stage consisted of forty cycles with 95ºC for forty seconds, 58ºC for 32 seconds, and 72º C for 32 seconds, For melt curve step and hold type PCR was used. For melt curve stage we set the profile of PCR at 95ºC for 15 seconds and 60ºC for 1 min. Melt curve and amplification plot were analysed. ΔCT values for the actin and genes of the study were further used for calculating the expression.

3 RESULTS

3.1 ANOVA

The combined analysis of variance (ANOVA) displayed highly significant differences amongst all the genotypes and for most of the yield related traits (Table) 3.1. This suggests the sufficient amount of variability of among different rice genotypes in this study for the traits. Coefficient of variation is simply a measure of dispersion of variability. In this study, variation amongst the traits ranged from 3.17 % to 47.72 %. The minimum CV was obtained was for the grain length 3.17 % and maximum CV obtained was for the stress susceptibility index 47.72 %.

Table 3. 1 Descriptive statistics and analysis of variance for field trial

Traits	Mean	Max.	Min.	Mean Sq. Value	C.V. (%)	Most promising lines
Plant Height	88.33	117.53	81.400	158.27***	4.94	NGSR-S3 NGSR-S1 NGSR-S13
No. of Tillers	14.33	22.00	9.8000	18.92**	15.89	NGSR-S9 NGSR-S19 NGSR-S13
Grain Yield	23.92	51.47	13.600	125.79**	18.76	NGSR-S8 NGSR-S9 NGSR-S22
Straw Yield	43.60	125.60	36.400	907.39*	24.31	NGSR-S1 NGSR-S10 NGSR-S5
Harvest Index (HI)	0.16	0.41	0.0000	0.0093***	16.92	NGSR-S16 NGSR-S13 NGSR-S21
Stress Susceptibility Index (SSI)	0.8592	2.0940	-0.482	0.0228	47.72	NGSR-S6 NGSR-S19 NGSR-S13
1000 Grain Weight	17.43	30.63	14.810	24.94***	7.01	NGSR-S3 NGSR-S10 NGSR-S9
Grain Length	7.76	12.25	7.7330	2.174***	3.17	NGSR-S3 NGSR-S16 NGSR-S9

3.2 Evaluation of Morphological Traits for Field Trial

3.2.1 Plant Height

The mean square value of 22 GSR lines and 2 checks (IRRI 6 and Kissan Basmati) for plant height exhibited highly significant variation at $p < 0.001$ (Table 3.1). The CV (co-

efficient of variation) for the plant height was 4.94 %. Mean values for the plant height among the 24 studied rice genotypes ranged from 81.40 cm to 133.20 cm. The minimum (88.33 cm) for the plant height was recorded for the NGSR S18 and the maximum (117.53 cm) for the plant height was recorded for the NGSR S3. Amongst the checks, 113.80 cm was recorded for the IRRI 6 and 114.00 cm was recorded for the Kissan Basmati. Only NGSR S3 had higher plant height as compared to the two checks under salt stressed conditions. Plant height of each genotype under control condition compared with salt stress condition (Figure 3.1). These results suggest that the

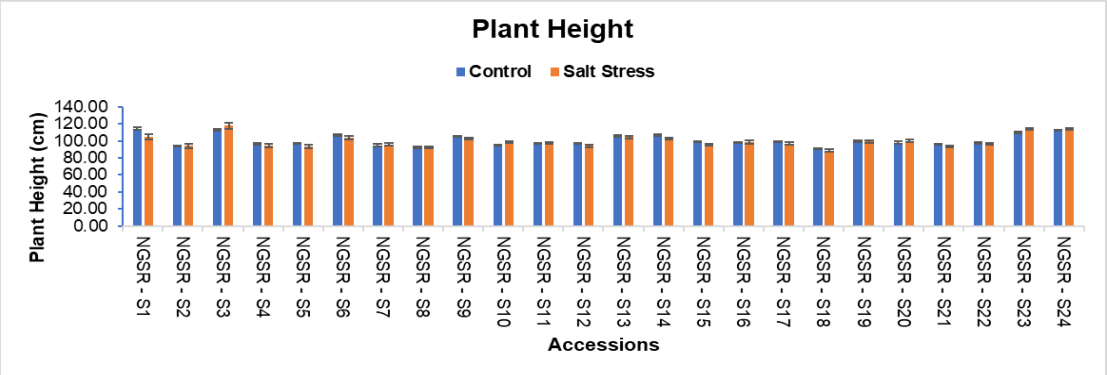


Figure 3. 1 Plant height (cm) for the 24 rice genotypes grown in control and saline conditions

genotypes plant height under study were not greatly affected by salt stressed conditions.

3.2.2 Number of Tillers

The mean square value for all the 22 GSR genotypes and checks (IRRI 6 and Kissan Basmati) for the number of tillers displayed very significant variation $p < 0.01$ (Table 3.1). The CV for the number of tillers was 15.89 %. Mean values for the number of tillers for the 22 rice genotypes ranged from the 14.33 to 22.00, whereas mean values for the number of tillers of the checks was 16.27 for the IRRI 6 and 21.80 for the Kissan basmati. The minimum (14.33) number of tillers were counted for the NGSR S4 and the maximum number of tillers (22.00) were counted for the NGSR S9. NGSR S19 also had the same number of tillers (22.00) as that of NGSR S9, whereas NGSR S16 had 21.00 and NGSR S13 had 21.33 number of tillers.

All the 22 genotypes had comparatively less number of tillers as compared to their control, which suggests that this trait was affected by salinity in the soil.

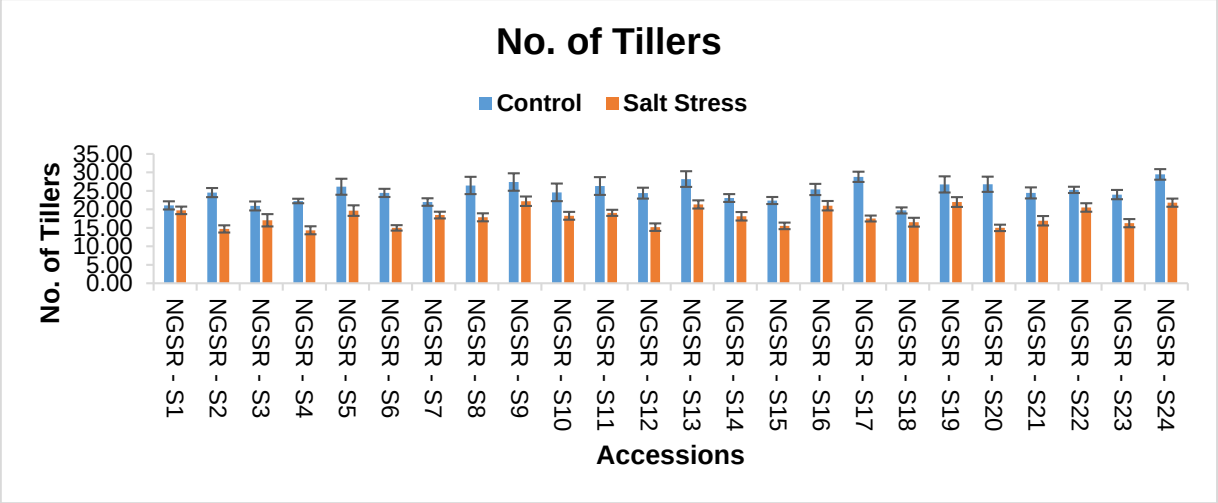


Figure 3. 2 Number of Tillers for the 24 rice genotypes grown in control and saline conditions

3.2.3 Grain Yield

The mean square value for all the 22 rice genotypes and the two checks under salt stressed environment showed remarkably significant results p <0.01 (Table 3.1). The CV for the grain yield was 18.76 %. The grain yield per plant for all the 22 rice genotypes ranged from the 23.93 grams to 51.97 grams. The minimum grain yield per plant (23.93 grams) was recorded for the NGSR S1 while the maximum grain yield per plant (51.97 grams) was recorded for the NGSR S9. The grain yield per plant recorded for the two checks were 27.20 grams for kissan basmati and 30.80 grams for IRRI 6.

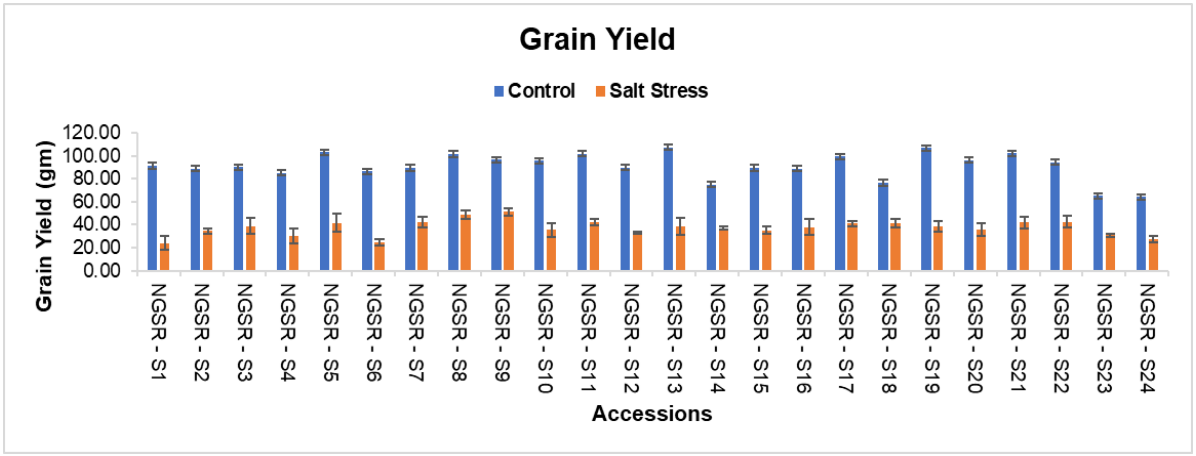


Figure 3. 3 Grain yield (gm) for the 24 rice genotypes grown in control and saline conditions

Most of the 22 rice genotypes except NGSR S1, NGSR S6, and NGSR S4 had higher

grain yield per plant as compared to the two checks grown. NGSR S11, NGSR S22 and NGSR S8 had 42.27, 42.67 and 48.80 grams grain yield per plant respectively.

The grain yield for all of the 22 rice accessions and two checks (IRRI 6 and kashmir basmati) halved under the saline environment. This shows that the lines did behave comparatively well against the checks but the actual yield decreased with the increasing salinity in the soil.

3.2.4 Straw Yield

The mean square value of straw yield for all the 22 rice genotypes along with the two checks under saline conditions exhibited significant differences $p < 0.05$. (Table 3.1) The variation for the straw yield was found to be 24.31 %. The mean straw yield per plant for the 22 rice genotypes showed that all the accessions had more weight than 64.27 grams with an average 88.453grams. The minimum straw yield per plant 64.27 grams was attained from the NGSR S16, while the maximum grain yield per plant was attained from the NGSR S1. However, straw yield per plant for the checks was recorded to be 43.60 grams for the IRRI 6 and 97.07 grams for the kissan basmati. Apart from the NGSR S1, all the decreasing trend for the straw yield per plant was observed in all the rice accessions, which suggests the decrease in the total biomass for the rice under salinity stress.

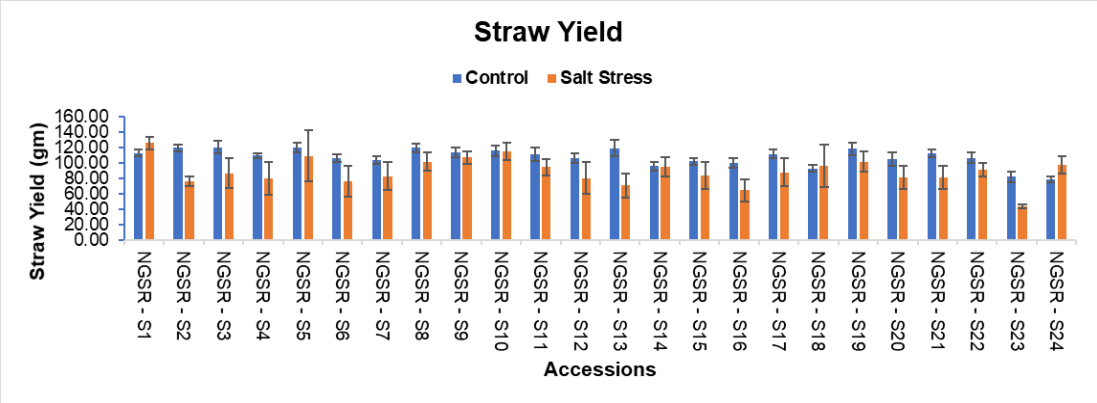


Figure 3. 4 Straw Yield (gm) for 24 rice genotypes grown in control and saline conditions

3.2.5 Harvest Index

The highly significant variation was observed from the mean square value of the harvest index for all the 22 rice lines with the 2 checks in the present study under the salinity stressed environment $p < 0.001$(Table 3.1), and the variation was found to be 16.92 %

for the harvest index. The mean harvest index for the 22 rice genotypes ranged from the 0.16 to 0.38. The minimum harvest index (0.16) among the 22 rice genotypes was obtained from the NGSR S1 and the maximum (0.38) was obtained from the NGSR S16. The mean harvest index for NGSR S16 was almost same as for the other genotypes NGSR S7 (0.35), NGSR S21 (0.35), and NGSR S13 (0.36). The harvest index for the

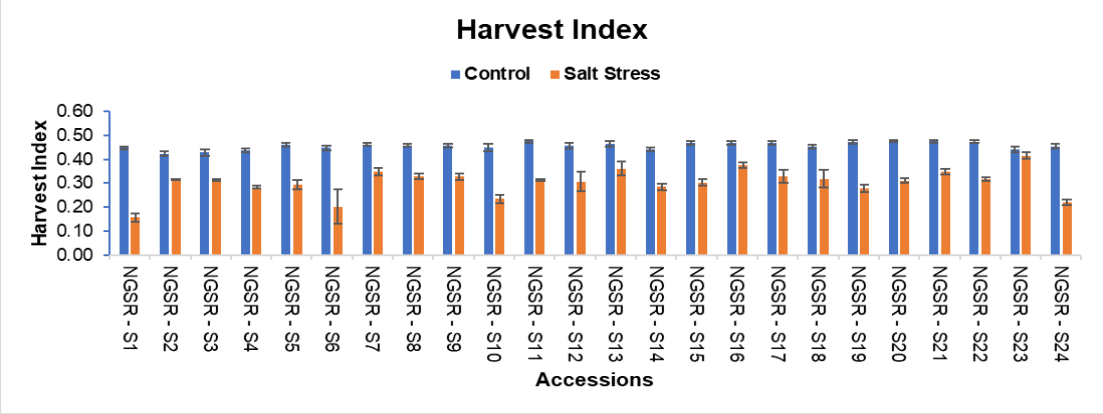


Figure 3. 5 Harvest Index for the 24 rice genotypes.

2 checks was 0.22 for the Kashmir basmati and 0.40 for the IRRI 6. Every line (22 NGSR lines and 2 checks) had less harvest index as compared to that of their control. This depicts that yield of all the lines is affected by the salts present in the soil.

3.2.6 Seed Length

The mean square value for the seed length for all the 22 rice lines under study and the two checks showed highly significant variations $p < 0.001$ (Table 3.1)under salt stressed conditions. The CV for the seed length was 3.17 %. The mean values for the seed length of the 22 rice genotypes ranged from the 7.76 cm to 11.24 cm. The minimum seed length (7.76 cm) recorded was for the NGSR S21 and the maximum seed length (11.24 cm) was recorded for the NGSR S3. The mean of the two checks was recorded to be 9.60 cm for IRRI 6 and 12.25 cm for Kashmir basmati. All the accessions had less seed length as compared to that of the check; kissan basmati (long grain).

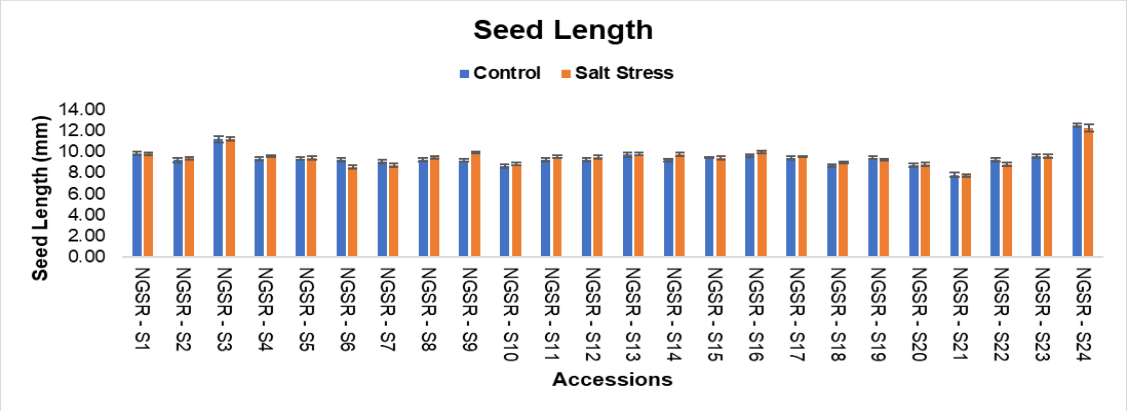


Figure 3. 6 Seed length for the 24 rice genotypes grown in control and saline conditions

3.2.7 Grain Weight

The mean square values of the 22 rice genotypes and the two checks displayed remarkably significant variations $p < 0.001$(Table 3.1)under saline environment. The CV for the grain weight is 7.01 %. The mean values for the 1000 grain weight for the 22 rice genotypes ranged from the 17.43 grams to 24.12 grams. The minimum value (17.43 grams) for the 1000 grain weight was recorded for the NGSR S6 and the maximum value (24.12 grams) for the 1000 grain weight was obtained from the NGSR S3. The mean value for the 1000 grain weight for the two checks was recorded to be 24.63 grams for IRRI 6 and 30.63 cm for kissan basmati. Both the checks had the higher 1000 grains weight compared to those of the 22 rice genotypes. All the varieties also had the less 1000 grain weight as compared to that of control.

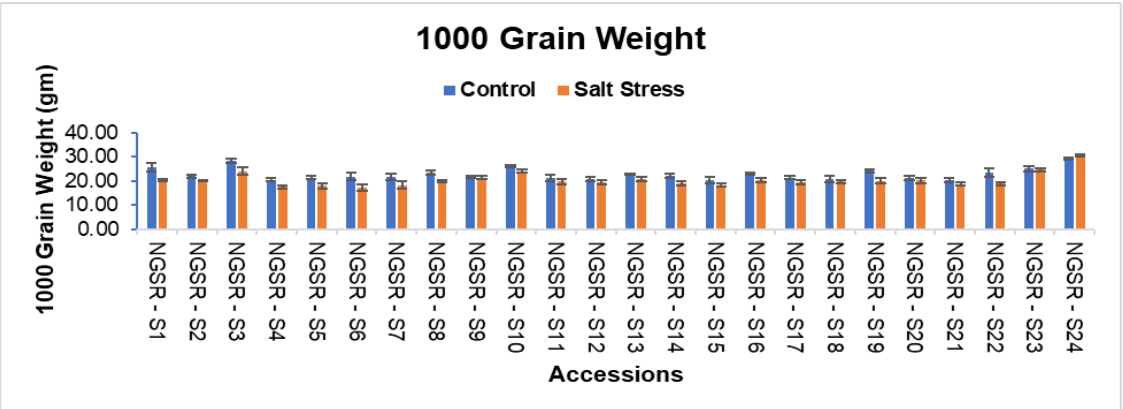


Figure 3. 7 1000 grain weight (gm) for 24 rice genotypes grown in control and saline conditions

3.2.8 Stress Susceptibility Index

The stress susceptibility index for all the 22 rice genotypes and the two checks had no significant differences (1.000) (Table 3.1) but recorded 47.2 % of the total variation.

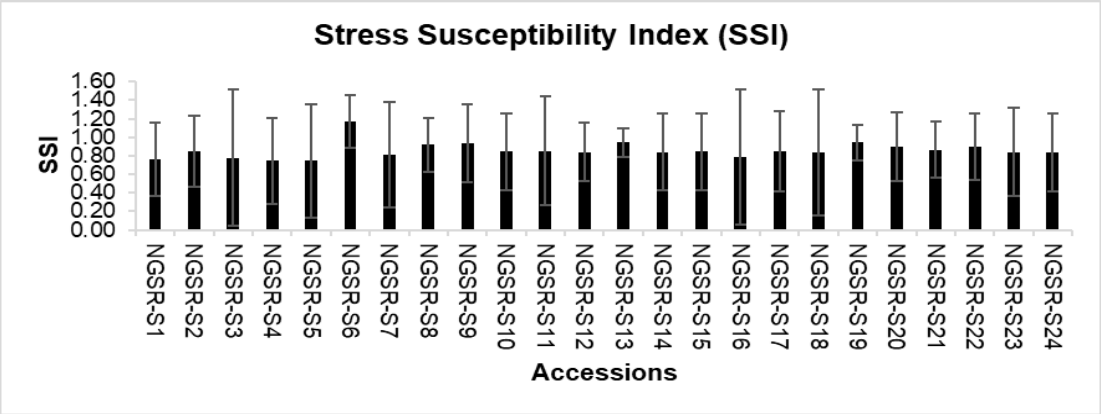


Figure 3. 8 Stress Susceptibility Index (SSI) for 24 rice genotypes

Highest stress susceptibility index was recorded in NGSR S6. Owing to the non-significance of this trait, it would not be useful for further screening of varieties under salt stressed environment.

3.3 Vg, Ve, Vp, GCV, PCV and Heritability

The estimate values for the genetic variance (Vg), environmental variance (Ve), phenotypic variance (Ve), genetic co-efficient of variation (GCV), phenotypic co-efficient of variance (PCV) and heritability for different traits evaluated in this study are presented in the table (Table 3.2). All the traits amongst all the rice varieties exhibited significant variations for the Vg and Ve except for harvest index and number of tillers. Vg with the highest magnitude was observed for the straw yield (148.33) and the lowest value was observed for the stress susceptibility index (-0.05). Vp with the highest magnitude value was observed for the straw yield (302.46), and the lowest value was recorded for the harvest index (0.00) (Table 3.2). Wide variability existed in plant height, grain yield, and 1000 grain weight and grain length.

All the traits evaluated in this study had low to moderate genotypic coefficient of variance (GCV) (Table 3.2). The GCV span between 6.71 % and 13.77 %. The highest GCV was observed for the straw yield 13.77 % and the lowest was observed for the plant height 6.71 %. In comparison to GCV, PCV for all the traits had higher value. The lowest PCV was observed for the plant height 7.29 % and the highest PCV was

observed for the straw yield 19.66 %. The amount of difference between GCV and PCV illustrates the level of environmental influence on any trait. Minor differences between the GCV and PCV depicts the lesser influence of environment against the more genetic effect on the expression of any trait. Plant height, number of tillers, harvest index, 1000 grain weight, and grain length are presumed to have insignificant influence from the environment, as there exists minimum differences between GCV and PCV.

Moderate to high heritability was observed for all the traits except stress susceptibility index. Heritability of the traits ranged from 49.0 to 95.8. Lowest heritability was for the straw yield while 1000 grain weight had the highest heritability. High heritability >60 was observed for plant height, grain yield, harvest index, grain yield and 1000 grain weight, whereas moderate heritability was observed for the number of tillers and straw yield.

Table 3. 2 Vg, Ve, Vp, GCV, PCV, and Heritability of all the traits under study

Traits	Genetic Variance (Vg)	Environmental Variance (Ve)	Phenotypic Variance (Vp)	Genetic co-efficient of Variation (GCV)	Phenotypic co-efficient of Variation (PCV)	Heritability
Plant Height	44.68	8.08	52.76	6.711	7.29	84.7
No. of Tillers	3.55	2.75	6.31	10.414	13.88	56.3
Grain Yield	25.53	16.40	41.93	13.514	17.32	60.9
Straw Yield	148.33	154.13	302.46	13.769	19.66	49.0
Harvest Index (HI)	0.00	0.00	0.00	0.000	0.00	71.9
Stress Susceptibility Index (SSI)	-0.05	0.06	0.01	NA	11.64	-637.3
1000 Grain Weight	7.63	0.69	8.31	13.4895	14.08	91.7
Grain Length	0.69	0.03	0.72	8.740135	8.93	95.8

3.4 Correlation Analysis

Correlation amongst seven agronomic traits was studied. Correlation analysis revealed the association amongst the traits by showing the relatedness of one trait with another (Fig 3.9). Significant positive and negative correlations were observed in the correlation studied traits in saline and control conditions. Grain yield had significantly high positive correlation with straw yield (0.87), harvest index (0.50) and number of tillers (0.32).

But it negatively correlated with plant height (-0.38), 1000 grain weight (-0.31) and seed length (-0.42). Plant height on the other hand correlated positively and significantly high with grain weight (0.63), and seed length (0.66), whereas only moderately positively correlated with number of tillers (0.03). Number of tillers positively correlated with all the traits; HI (0.45), followed by GY (0.32), SL (0.20), GW (0.09), SY (0.07), and PH (0.03). Straw yield highly positively correlated with GY (0.87) followed by NT (0.07), HI (0.01) whereas it negatively correlated with other traits, PH (-0.27), SL (-0.32) and GW (-0.18). Harvest index had a negative correlation with the plant height PH (-0.32), GW (-0.35), SL (-0.30) but had a significantly positive correlation with GY (0.50) followed by NT (0.45) and SY (0.01). Grain weight had remarkable positive correlation with SL (0.74), PH (0.63), and NT (0.09) nut had a negative correlation with HI (-0.35), GY (-0.31) and SY (-0.18). Positive correlation was observed between seed length and GW (0.74), PH (0.66), NT (0.20) whereas the negative correlation for the seed length was observed with GY (-0.42), SY (-0.32) and HI (-0.30). Highest positive correlation was observed amongst grain yield and straw

yield (0.87). Strong negative correlation was observed for grain yield and seed length (- 0.42). Minimum correlation was recorded for HI and SY (0.01).

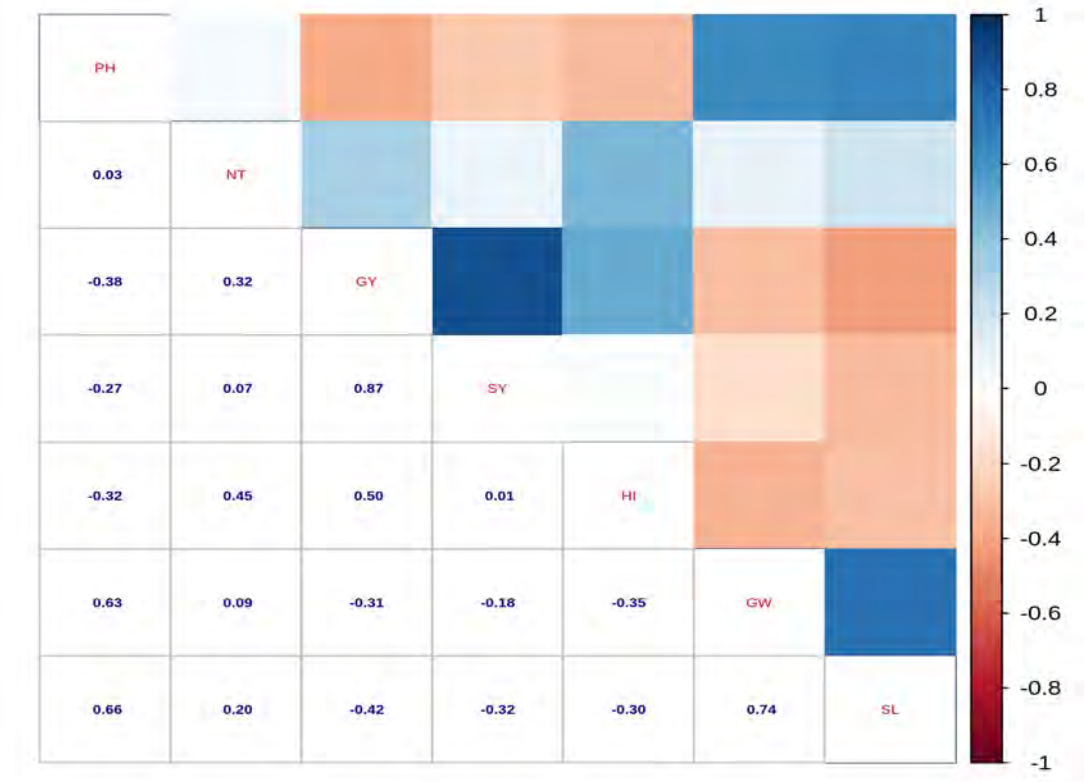


Figure 3. 9 Correlation plot amongst the trials under control and saline environment

3.5 Principle Component Analysis

Genetic diversity amongst different traits was studied by constructing genotype trait (G-T) biplot using PCA. PCA (G-T) biplot was constructed for the both normal as well as salt stressed conditions. >1 eigenvalue was designated as a threshold value for selecting principle components (PC) or factors (F) for further analysis. In the control environment, first 3 PCs had eigenvalue >1 (Table). These 3 PCs contributed 85.28 % of the total variability. The first two PCs (PC1 and PC2) were employed to construct G-T biplot; PC1 and PC2 accounted for 45.05 % and 22.74 % of the total variability (Table 3.3). Comparatively, in the salt stressed environment, the first three PCs had eigenvalue >1. The first three PCs accounted for 86.10 % of the total variability. The individual variability for the PC1 and PC2 was 38.83 % and 24.49 % respectively.

Table 3. 3 Eigenvalue, variability and cumulative variability exhibited by different principle components (PC) in control and saline environment

	Control			Salt		
PCs	**Eigenvalue**	**Variability**	**Cumulative %**	**Eigenvalue**	**Variability**	**Cumulative %**
PC1	3.15	45.05	45.05	2.72	38.83	38.83
PC2	1.59	22.74	67.80	1.71	24.49	63.32
PC3	1.22	17.49	85.28	1.60	22.78	86.10
PC4	0.45	6.41	91.69	0.41	5.86	91.96
PC5	0.36	5.07	96.77	0.29	4.09	96.05
PC6	0.22	3.19	99.96	0.24	3.42	99.47
PC7	0.00	0.04	100.00	0.04	0.53	100.00

For the control experiment, GW (21%), GY (20%), PH (19%), and SL (18%) were responsible for high variability in the principle component 1 (PC1), while no. of tillers (0.63) contributed least in the overall variability of PC1 (Table 3.4). No. of tillers correlated significantly high (41%) followed by GY (15%) and SL (12%) with the PC2, while PH (6%), and GY (6%) contributed less amongst all the traits. Highest contributing values for the PC1 under salt stressed conditions were observed for GW (27%), SL (27%), PH (26%), and lowest for SY (1%). SY (52%) and HI (33%) displayed high contributing variability for the PC2 while the minimum value was observed for GY (0.1%) and SL (0.8%) (Table 3.4).

Table 3. 4 Contribution of principle components (PC) under control and salt conditions

	Control			Salt		
Traits	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
PH	18.67	6.06	1.56	26.01	6.74	0.12
No. Till	0.63	40.82	12.13	6.28	5.83	32.43
GY	20.20	14.68	8.72	6.92	0.16	45.19
SY	11.64	6.31	43.10	1.04	51.74	1.70
HI	10.21	9.10	29.96	5.69	32.33	17.09
GW	17.99	11.15	4.48	27.13	2.34	1.63
SL	20.67	11.90	0.05	26.94	0.80	1.83

3.5.1 Variable analysis based on principle components, variability and correlation

The variability illustrated by the PC1 (45%) and PC2 (23%) was used to construct the biplot for the control environment. Variables were imposed as vector and the length of the vector showed the combined variability in PCs (Yan and Tinker 2005). Trends of correlation among the variables and principle components is illustrated by the positive and negative factor loadings. Four quadrants of PCA biplot are assigned values based on the factor loading values. Vectors away from the origin represents high variability for the trait i.e. number of tillers (No. till), grain weight (GW) and grain yield (GY) under control environment. Correlation amongst the traits is represented by the angles between the two vectors. Angle less than 90° represents higher correlation amongst the traits (Yan and Kang, 2003). The traits that are perpendicular to each other in the variable biplot represents independent behaviour or no correlation amongst them.

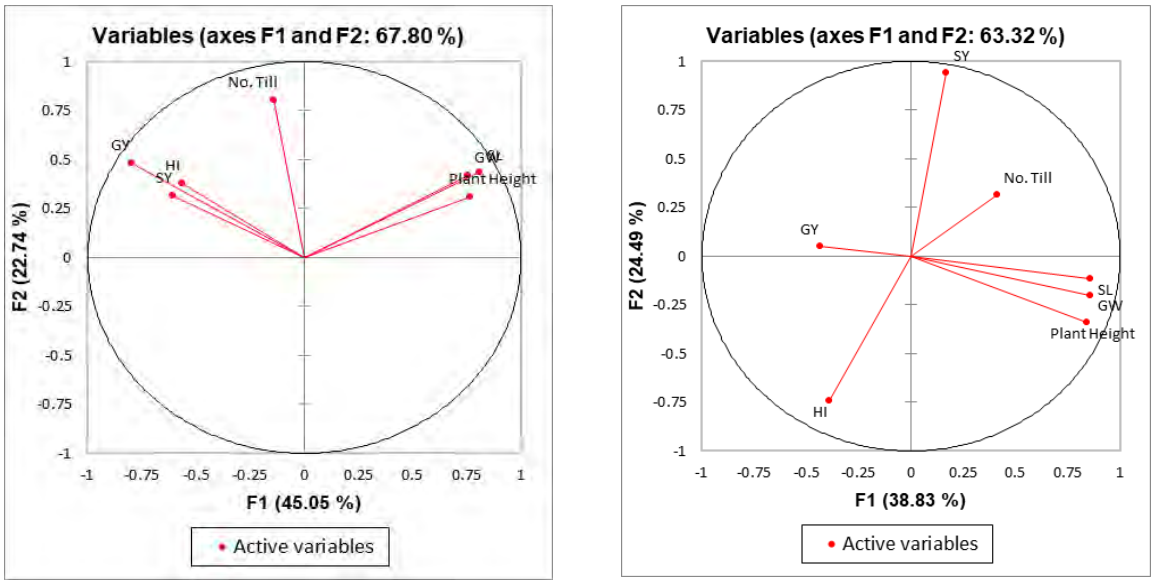
Under saline environment, PC1 and PC2 showed variability as 38.83% and 22.49 % respectively. The two principle components (PC1 and PC2) were used to construct variable biplot. Vectors that are close to the origin represents less variability as represented by number of tillers (no. till) and grain yield (GY). The variability biplot showed that number of tillers (no. till) and straw yield (SY) belong in the first quadrant that has positive values for both factors. Thus, two traits in the first quadrant has positive correlation amongst them. Under control conditions, grain weight (GW), seed length (SL) and plant height (PH) belong to the first quadrant (+, +) showing positive correlation.

3.5.2 Biplot Analysis

Principle components (PC1 and PC2), distributed all the 24 rice genotypes along the 2D plane for representing graphical structure of the genotypes. Biplot with the help of vectors displays the association of different genotypes with vectors of different traits and among themselves.

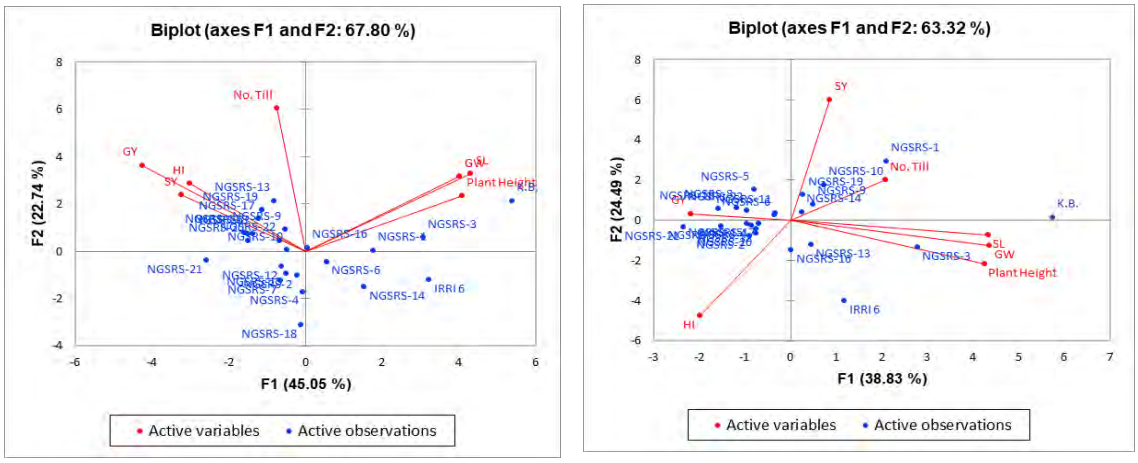
Under control conditions, PC 1 was mainly represented by PH, GY, SY, SL, and GW, whereas PC 2 was mainly represented by NT, GY, SL, and SL. In saline environment, PC 2 was mainly represented by PH, GW and SL, while PC 2 was mainly represented

by SY and HI. 24 rice genotypes spanned along four quadrants of the biplot with the help of the variability of PC1 and PC2 for both environments (control and salt). The rice varieties present in the positive quadrant are more useful, informative and have high probability of being linked. For the plants grown as control, 4 genotypes were found to be in the first quadrant, whereas for saline conditions there were 6 genotypes. The rice accessions that are near to negative axis and lie away from the origin are less informative. Kashmir basmati was found in the group 1 (first quadrant) of both environments.



(a) **(b)**

Figure 3. 10 Variable biplots for the traits studied for 24 rice genotypes (a) control (b) Salt



(a) **(b)**

Figure 3. 11 PCA biplots for the genotypes and traits (a) control (b) salt

3.6 Evaluation of Morphological Traits in Glasshouse Trials

3.6.1 Shoot Length

The mean shoot length for 24 rice genotypes and 3 replicates were evaluated based on the BLUP value. Significant decrease was observed in the shoot length for most of the rice genotypes used in both experiments (140 mM salt stress and 200mM salt stress). The low salt stress experiment (140 mM) significantly decreased the shoot length for all the genotypes under study and the minimum shoot length was observed for NGSR S7 (20.45 cm), followed by NGSR S16 (20.96 cm) comparative to their control NGSR S7 (25.6 cm) and NGSR S16 (26.0 cm). The highest percentage decrease in the shoot length was also observed in the NGSR S3 (5.2 %) and NGSR S16 (5.1 %). The tallest shoots were observed for the NGSR S13 (25.47 cm) under stress conditions. NGSR S13 had low percentage decrease (10.4 %) in its shoot length in comparison to its control. The shoot length for NGSR S13 was even better than the checks K.B. (25.07 cm) and IRRI 6 (21.13 cm).

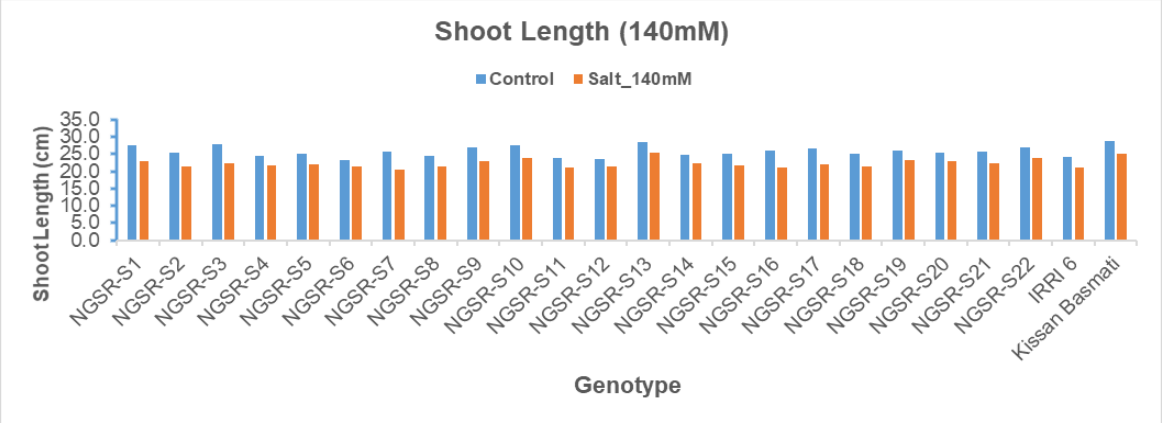


Figure 3. 12 Shoot length for 24 rice genotypes under 140 mM under salt stress

Under high salt stress shortening of shoot length was observed for most of the genotypes but not for all relative to the control. NGSR S1 and NGSR S13 had taller shoots under stress than in control. However, the shortest shoot was observed for the NGSR S3 (21.3 cm). The highest percentage decrease was also observed in the shoot length for NGSR S3 (24.3 %) followed by NGSR S11 (7.2%), illustrating the impact of salt stress on NGSR S3. On the other hand, tallest shoot was recorded for the NGSR S13 (28.9 cm) followed by NGSR S1 (27.8 cm).

The shoot length for the NGSR S3 was most affected under high salt concentrations but was less affected under low salt concentrations, whereas NGSR S13 performed better amongst other genotypes relative to its control and checks IRRI 6 and kissan basmati under low as well as high saline environment.

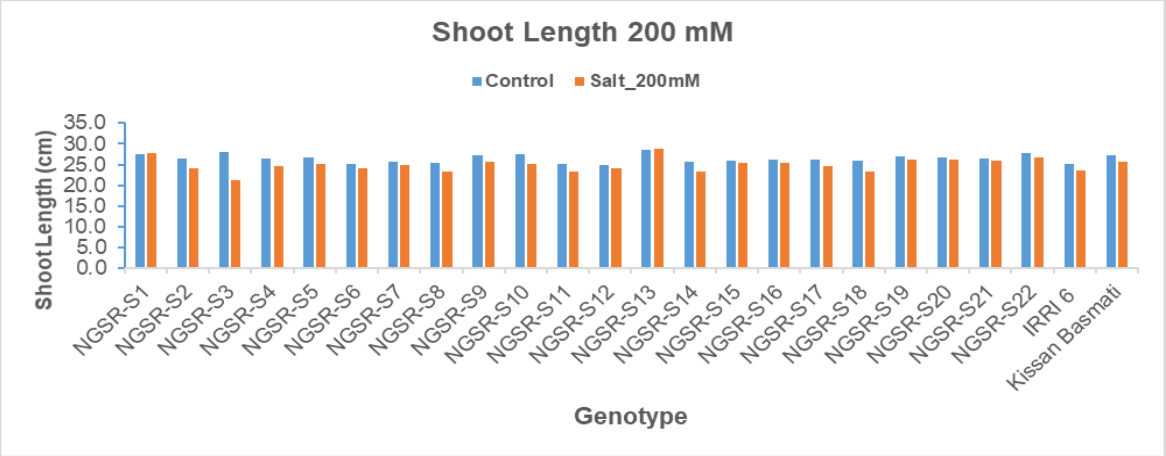


Figure 3. 41 Shoot length for 24 rice genotypes under 200 mM salt stress

3.6.2 Root Length

The mean root length for 24 rice accession and their 3 replicates were analysed based on the value obtained by BLUP. Decrease in root length was observed for most of the rice genotypes evaluated at 140 mM salt concentration and 200mM salt concentration. Significant decrease in the root length of all the rice genotypes were recorded in the low salt concentration (140 mM) environment. The minimum root length was recorded for NGSR S17 genotype (14.22 cm). The highest percentage decrease in the root length was observer in NGSR S14 (30.0 %) followed by NGSR S16 (29.9 %) and NGSR S3 (28.8%) respectively. Though the longest roots were observed for NGSR S15 (15.78 cm) and NGSR S5 (15.62 cm) but NGSR S15 had the high percentage decrease in the

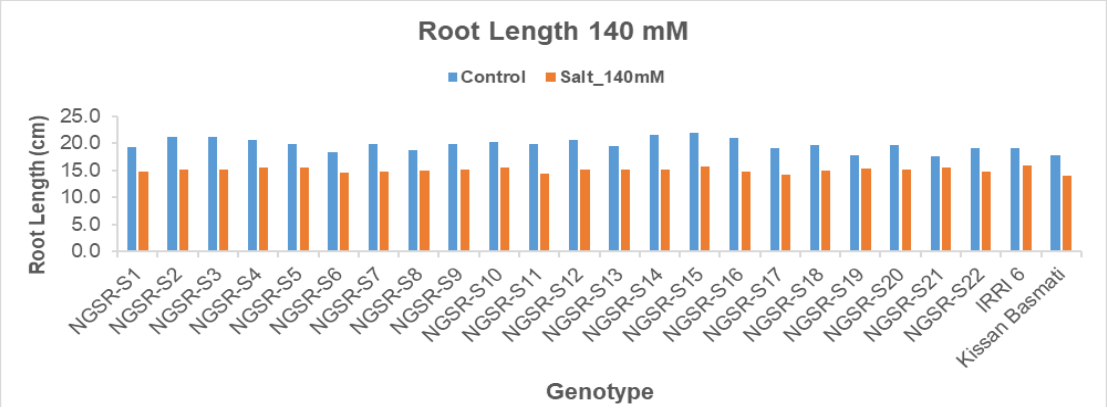


Figure 3. 42 Root length for 24 rice genotypes under 140 mM salt stress

root length (28.1 %). In comparison to the NGSR genotypes, checks IRRI 6 had the longest root (15.91cm) and kissan basmati had the minimum root length (13.94 cm).

Under more saline environment (200 mM), lengths of root did not decrease for all the genotypes relative to their control; NGSR S1 and kissan basmati had longer root under stress than that of their control. Though kissan basmati had longer root (17.4 cm) compared to its control but the length of root was only longer than NGSR S11 (17.0 cm) which is the minimum root length recorded in the experiment (200 mM salt experiment). Despite the minimum root length for the NGSR S11, the highest percentage decrease in the root length was observed in the NGSR S3 (21.4 %) followed by NGSR S11 (20.3%). The maximum root length was recorded for the NGSR S20 (19.6 cm). NGSR S20 performed better than both the checks IRRI 6 (19.2 cm) and kissan basmati (17.4 cm).

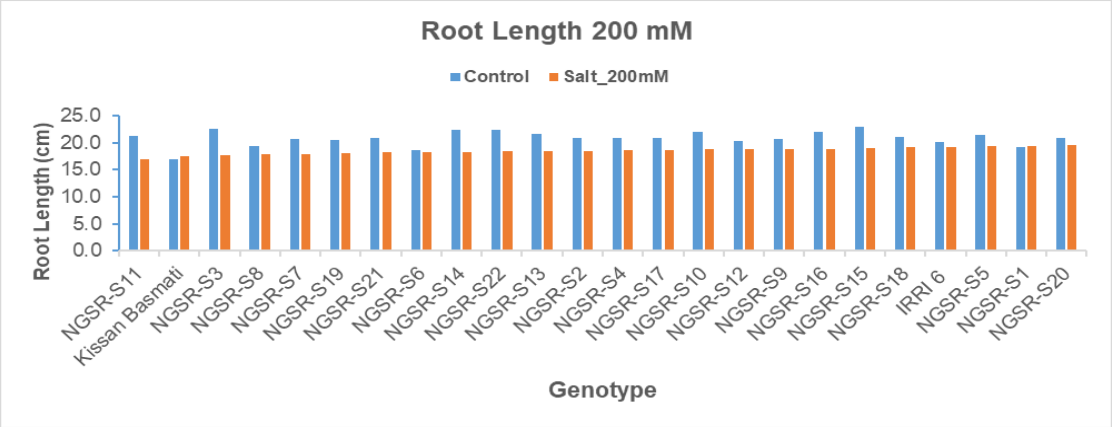


Figure 3. 43 Root length for 24 rice genotypes under 200 mM salt stress

3.6.3 Shoot Fresh Weight

BLUP value was obtained by using R software to evaluate mean shoot fresh weight (SFW) for 24 rice genotypes and their 3 replicates. Shoot fresh weight saw a major decrease in the salt stress environment relative to the control conditions. Under less saline experiment (140 mM), the least fresh weight for the shoot was exhibited by NGSR S11 shoots (0.090 gm) followed by NGSR S12 (0.091gm). The highest decrease in the percentage of the SFW was recorded by NGSR S3 (55.9 %) followed by NGSR S16 (55.8 %). The more shoot fresh weight amongst all the 22 NGSR genotypes was recorded for NGSR S19 (0.126 gm). The performance of NGSR S19 had been on the

better side in comparison to check kissan basmati (0.090 gm) but IRRI 6 (0.130 gm) as the other check performed better than the NGSR genotypes.

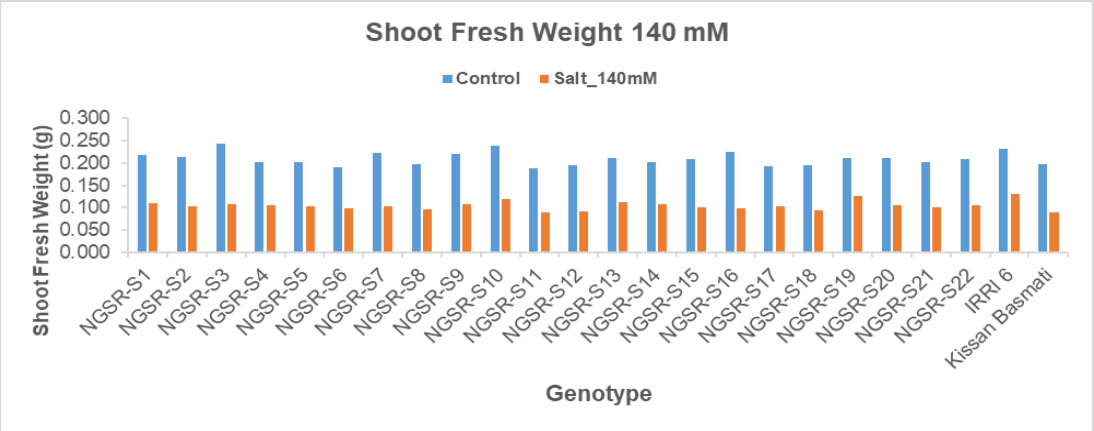


Figure 3. 44 Shoot Fresh Weight for 24 rice genotypes under 140 mM salt stress

The trend of decreasing shoot fresh weight under salt stress environment in high saline environment (200 mM) was same as that of under low salt stress conditions as all the had less shoot fresh weight in comparison to their control. The minimum SFW was recorded for the NGSR S3 (0.110 gm). The highest percentage was also recorded for the NGSR S3 (46.2 %), followed by NGSR S22 (42.2 %). NGSR S13 had the higher SFW (0.133 gm) amongst all the genotypes. Two checks IRRI 6 and kissan basmati had relatively low SFW (0.127 gm) and (0.111 gm) respectively. Though kissan basmati had lesser shoot fresh weight but the percentage decrease was observed more for IRRI 6 (41.4 %) than kissan basmati (32.2 %). The percentage decrease in the shoot fresh weight observed for NGSR S13 was (34.7%).

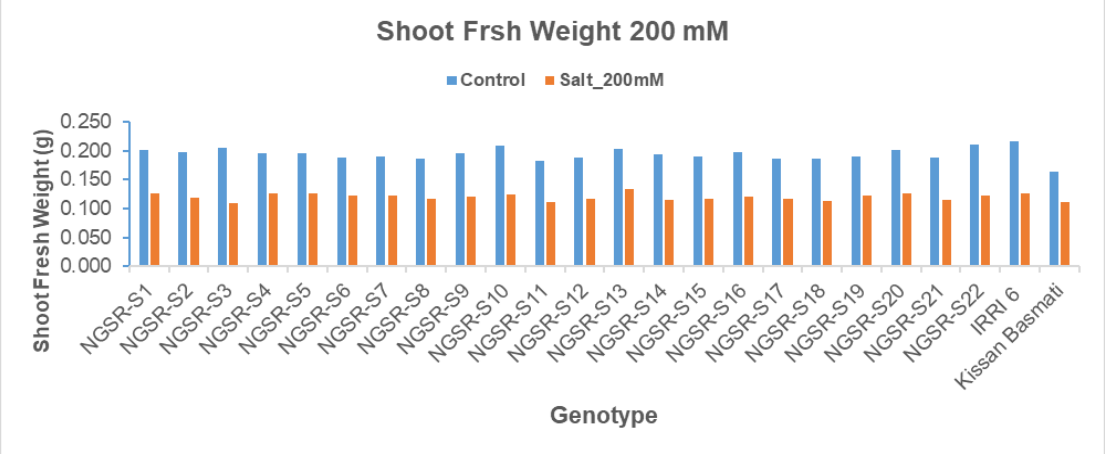


Figure 3. 45 Shoot Fresh Weight for 24 rice genotypes under 200 mM salt stress

3.6.4 Root Fresh Weight

R software was employed to generate BLUP value to analyse root fresh weight (RFW) for 24 different rice lines and their 3 replicates. Significant decrease in the fresh weight of the roots for all the genotypes was observed in both the experiments (140 mM and 200mM). At low salt concentrations in the solution, the least root fresh weight (RFW) was observed for NGSR S22 (0.080 gm) and NGSR S11 (0.080 gm). But the highest percentage decrease in RFW relative to control was recorded in the NGSR S7 (57.9 %) and NGSR S9 (57.9 %), followed by NGSR S17 (57.7 %). The heaviest root was obtained for the genotype NGSR S3 (0.100 gm) and NGSR S14 (0.095 gm), but the percentage decrease in RFW was on the higher end for both the genotypes respectively; (53.5 %) for NGSR S3 and (52.8 %) for NGSR S14. NGSR S3 and NGSR S14 had higher RFW compared to the check IRRI 6 (0.093 gm) and kissan basmati (0.078 gm), which has the least RFW amongst the other genotypes.

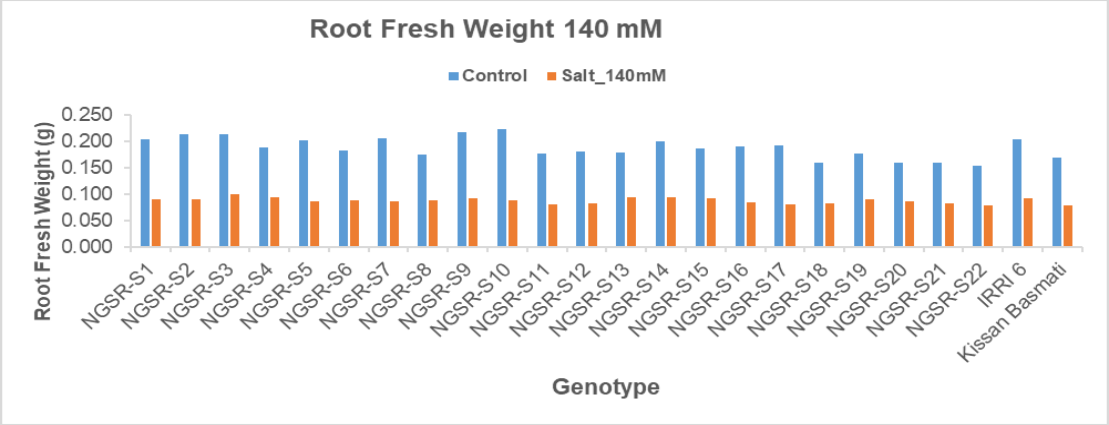


Figure 3. 46 Root Fresh Weight for 24 rice genotypes under 140 mM salt stress

At high salt concentrations (200 mM), same trend for the decrease in RFW relative to control was observed because of the week and less dense root growth. Minimum RFW was observed for the NGSR S12 (0.076 gm) followed by NGSR S3 (0.077 gm). The highest percentage decrease in the RFW was recorded in the NGSR S3 (55.8 %) followed by NGSR S9 (50.6 %), which is much lower than NGSR S3. Maximum RFW amongst 22 NGSR genotypes was recorded for the NGSR S1 (0.109 gm), second to NGSR S1 the maximum RFW was recorded for NGSR S3 (0.104 gm). Check (IRRI 6) performed better amongst 24 rice accessions and had the maximum RFW (0.110 gm).

RFW for the kissan basmati was (0.081 gm). The least percentage decrease was observed for the genotype NGSR S1 (34.8 %).

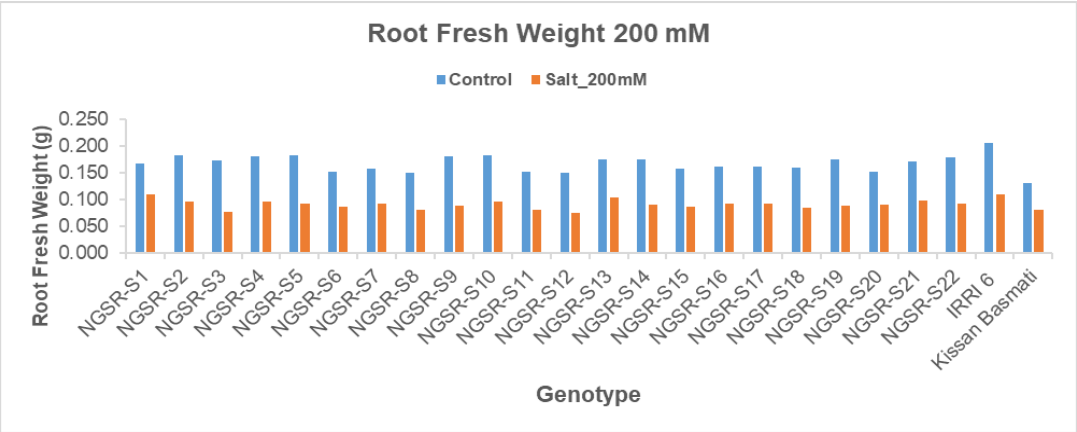


Figure 3. 62 Root Fresh Weight for 24 rice genotypes under 200 mM salt stress

3.6.5 Shoot Dry Weight (SDW)

The mean shoot dry weight (SDW) value for 24 different rice varieties and their 3 replicates were interpreted based on the value obtained by the BLUP. The decrease in the SDW was observed as witnessed in the SFW in all the rice accessions under salt stress relative to their control. At less saline environment (140 mM), the minimum value for the SDW was recorded for the NGSR S11 (0.025 gm) followed by NGSR S6 (0.028 gm). The maximum SDW amongst the 22 rice genotypes was recorded for NGSR S19 (0.040 gm) and NGSR S10 (0.035 gm). Though maximum SDW was observed for NGSR S19 but it had the highest percentage decrease in SDW (72.3%). IRRI 6 had the highest SDW amongst the 24 rice accessions (0.042 gm) while kissan basmati recorded (0.027 gm) SDW.

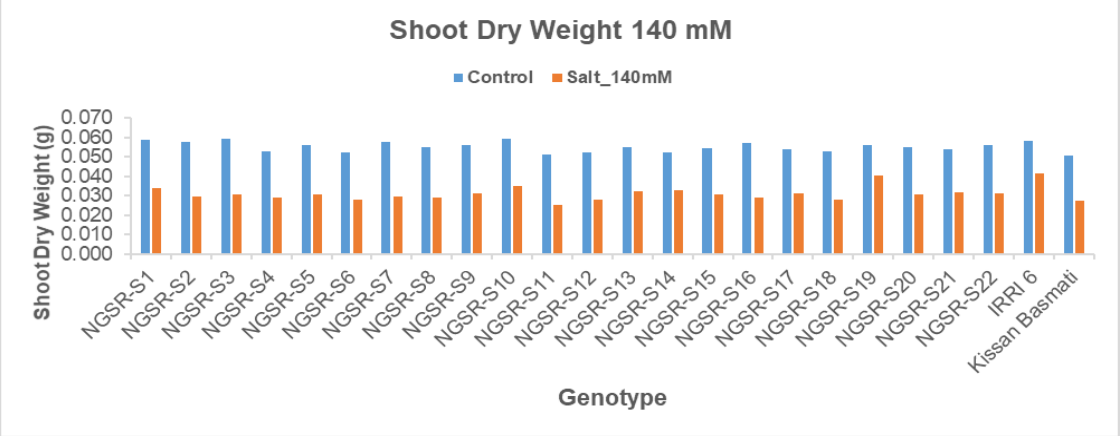


Figure 3. 63 Shoot Dry Weight for 24 rice genotypes under 140 mM salt stress

At high salt concentration (200 mM), NGSR S3 had least SDW (0.030 gm), followed by NGSR S12 (0.031 gm). The highest percentage decrease was also recorded for the NGSR S3 (48.9 %) followed by NGSR S12 (39.8%). There exists a large gap for percentage decrease between NGSR S3 and NGSR S12. The maximum SDW was recorded for NGSR S13 (0.045 gm), it also had the lesser percentage decrease in SDW. Two checks (IRRI 6 and kissan basmati) had (0.044 gm) and (0.034 gm) respectively. NGSR S3 performed better than two checks IRRI 6 and kissan basmati.

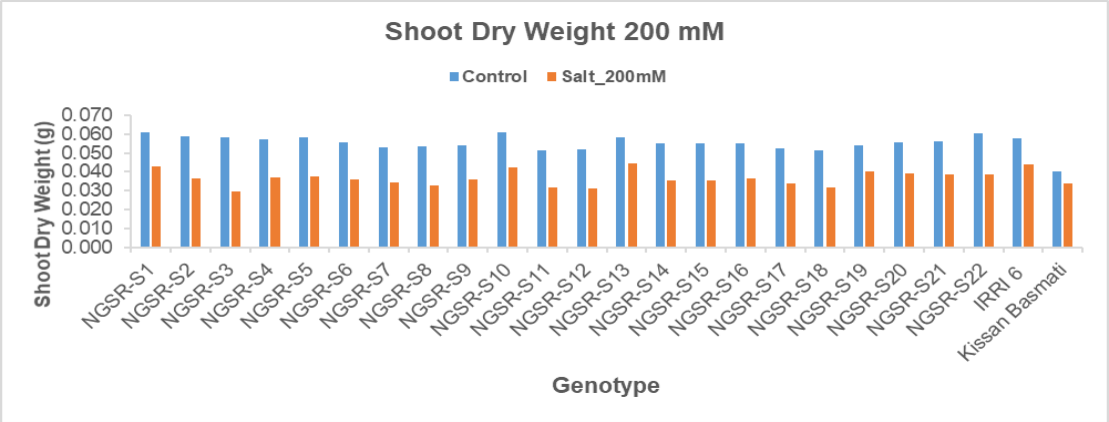


Figure 3. 64 Shoot Dry Weight for 24 rice genotypes under 200 mM salt stress

3.6.6 Root Dry Weight

BLUP value was obtained by using R to estimate the mean root dry weight values of 24 rice genotypes and their 3 replicates. Significant decrease in RDW was observed under salt stressed environment relative to control under both saline concentrations (140 mM and 200 mM). Under less saline conditions (140 mM), the minimum value for SDW was recorded by NGSR S2 (0.012 gm), NGSR S6 (0.012 gm), NGSR S16 (0.012

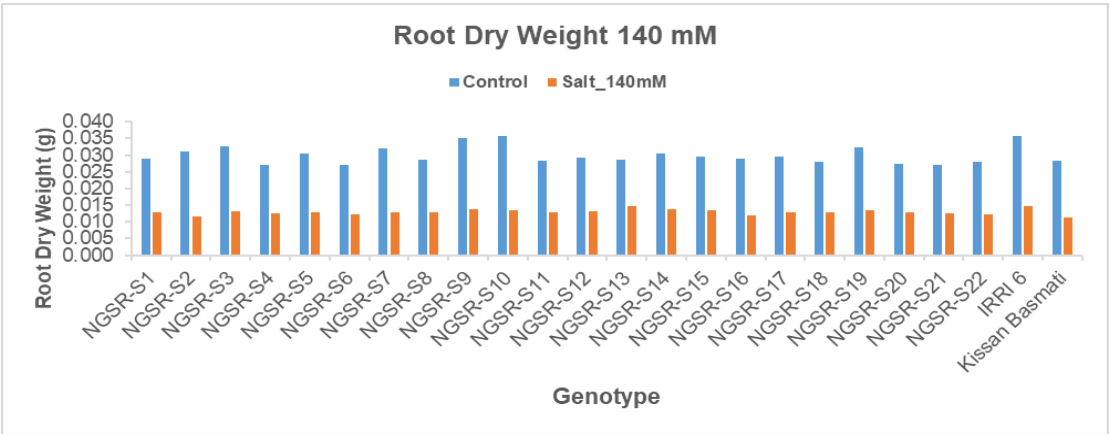


Figure 3. 65 Root Dry Weight for 24 rice genotypes under 140 mM salt stress

gm) and NGSR S22 (0.012 gm). However the highest percentage decrease in the RDW

was observed for NGSR S10 (62.12 %), followed by NGSR S2 (62.09 %). The maximum RDW was recorded for NGSR S 13 (0.015 gm). IRRI 6 also had the highest RDW (0.015 gm) equivalent to NGSR S13, whereas kissan basmati had (0.011 gm).

Under more saline environment (200 mM), the minimum RDW among the 22 NGSR rice genotypes was recorded for NGSR S12 (0.0155 gm), whereas the highest percentage decrease in RDW was recorded for NGSR S15 (46.9 %). The maximum RDW was recorded for NGSR S13 (0.0278 gm). The check IRRI 6 had the highest RDW amongst all 24 rice accessions (0.0185 gm), while kissan basmati RDW was recorded to be (0.0160 gm) respectively.

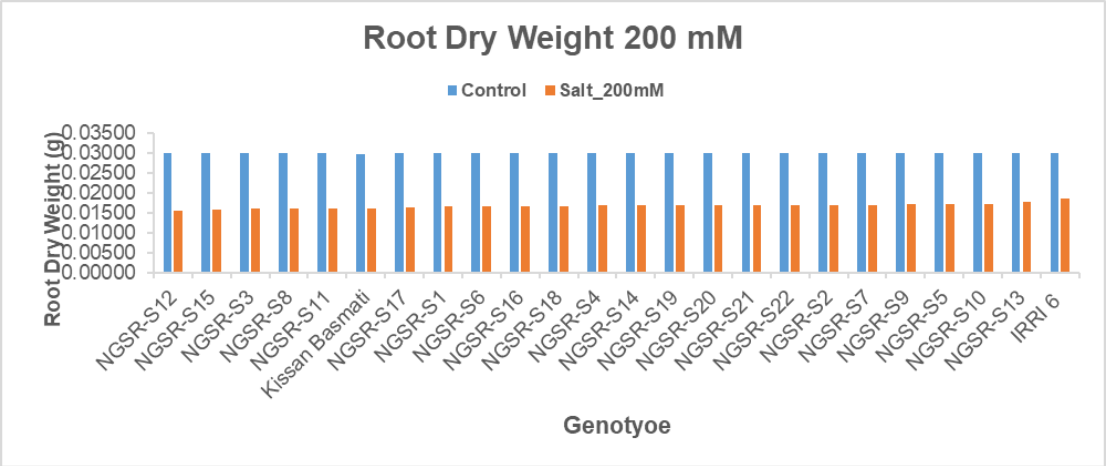


Figure 3. 66 Root Dry Weight for 24 rice genotypes under 200 mM salt stress

3.7 Evaluation of Biochemical Traits

3.7.1 Sodium (Na^+) and Potassium (K^+) ratio

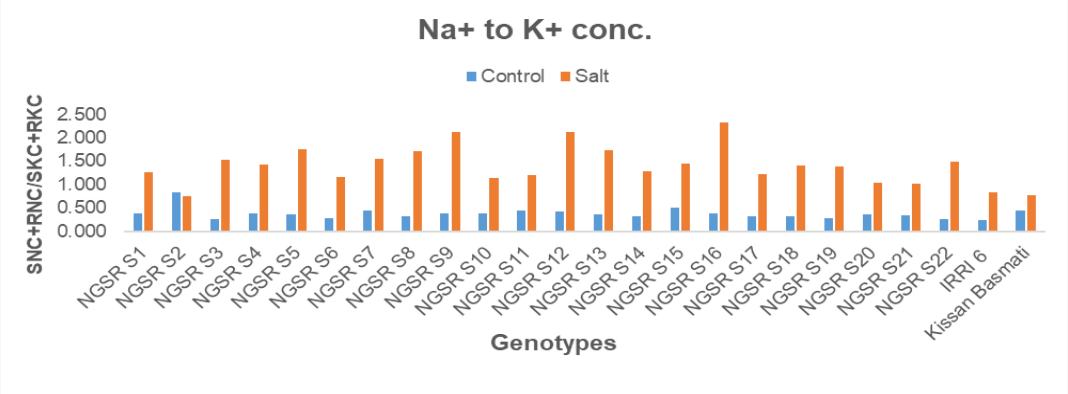


Figure 3. 67 Ratio of Na^+ concentration in shoots and K^+ concentration in shoots and roots under 200 mM salt stress.

Ratio of sodium concentration in shoots (SNC) and roots (SKC) to potassium concentration in shoots (SKC) and roots (RKC) was calculated. Higher values for the ratio were obtained for the plants grown under salt stressed conditions. Least value for the sodium concentration in roots and shoots to potassium concentration was observed in NGSR S2. NGSR S16 exhibited higher concentration of sodium concentration in roots and shoots to potassium concentration in shoots and roots. Sodium concentration to potassium concentration in checks (kissan basmati and IRRI 6) was only higher than NGSR S2 respectively.

3.8 Correlation Analysis for Glasshouse Trials

Six physiological traits for the glasshouse trial no. 01 were evaluated under correlation analysis. The salt concentration for the glasshouse trial no. 01 was 140 mM. The association amongst the traits was revealed by correlation analysis. The correlation for control and saline environment revealed significant positive and negative correlations amongst the traits. Shoot length correlated positively with all the traits except RL where it showed negative correlation (-0.10). The positive correlations for SL was recorded as SFW (0.24), SDW (0.23), RDW (0.12), and RFW (0.02). Significantly high positive correlation was observed for RL with SFW (0.57), RFW (0.57), RDW (0.57) and SDW (0.54). RL negatively correlated with SL (-0.10). SFW correlated positively with all the other traits; remarkably high correlation of this trait was recorded with SDW (0.95), followed by RDW (0.63), RFW (0.60), RL (0.57), and SL (0.24). A positive correlation was observed for RFW was observed with the other traits SFW (0.60), RL (0.57), RDW (0.56), SDW (0.45), and SL (0.02). SDW also showed positive correlation with SFW (0.95), RDW (0.61), RL (0.54), RFW (0.45) and SL (0.23). Positive correlation was also recorded for RDW with SFW (0.63), SDW (0.61), RL (0.57), RFW (0.56), and SL (0.12). Maximum positive correlation amongst the traits was recorded for SFW with

Figure 3. 68 Correlation ratios for the morphological traits studied under 140 mM salt stress

SDW (0.95). Minimum ratio of correlation was between SL and RFW (0.02). The only negative correlation was recorded for SL with RL (-0.10).

Six morphological traits for glasshouse trial no. 02 were studied. The salt concentration for the glasshouse trial no.02 was 200 mM. Correlation analysis revealed the association amongst the traits. A positive correlation of SL for all the traits was recorded i.e. with SDW (0.70), SFW (0.62), RFW (0.54), RL (0.34) and RDW (0.32). Same trend of positive correlation was observed for RL with all the traits as well. The observed value of correlation factor for RL with SFW is (0.56), RFW (0.50), SDW (0.47), RDW (0.38) and SL (0.38). A strong positive correlation for SFW with SDW (0.82), RFW (0.73), RDW (0.71), was observed, followed by SL (0.63) and RL (0.56) respectively. RFW correlated highly positively with SDW (0.87), RDW (0.80) and SFW (0.73). A positive correlation for RFW with SL (0.54) and RL (0.50) was also observed

respectively. Observed values of correlation for SDW other traits are as follows; RFW (0.87), SFW (0.82), SDW (0.78), SL (0.70) and RL (0.47). SDW also correlated positively with all the other traits with the correlation factor value RFW (0.80), SDW (0.78), SFW (0.71), RL (0.38) and SL (0.32). Maximum positive correlation factor value in the plot was recorded between RFW and SDW (0.87). Minimum correlation was observed for SL and RDW (0.32). However, no negative correlation was observed for any trait.

Figure 3. 84 Correlation ratios for the morphological traits studied under 200 mM salt stress

3.9 Screening for Salt Tolerant and Salt Susceptible Lines

Most tolerant and most susceptible lines are selected based upon their ranking for morphological traits and scoring for salt toxicity symptoms. NGSR S3 performance was the lowest amongst all (Table). Score for NGSR S3 was high among all the other genotypes that is an indication of low performance. NGSR S12 and NGSR S16 were the other genotypes that scored high (38 and 37 respectively) and hence proved

susceptible to salt stress. NGSR S13 was most tolerant line as compared to the other rice genotypes in the study. NGSR S13 had the least overall score (18) ranking and salt toxicity symptoms. NGSR S19 and NGSR S21 were the other two lines that had the low scores. So it can be inferred from the scores that NGSR S19 and NGSR S21 were tolerant to salt stress.

Genotype	Ranking			STS		Score	
	140mM	200 mM	Field	140 mM	200 mM		
NGSR-S1	6	2	5	5	9	27	
NGSR-S2	8	5	8	5	7	33	
NGSR-S3	9	10	3	9	9	40	Most Susceptible
NGSR-S4	6	6	7	5	7	31	
NGSR-S5	6	3	6	7	9	31	
NGSR-S6	4	3	9	7	9	32	
NGSR-S7	9	6	4	5	7	31	
NGSR-S8	6	7	6	7	7	33	
NGSR-S9	8	6	2	5	7	28	
NGSR-S10	8	5	4	5	7	29	
NGSR-S11	6	8	6	7	9	36	
NGSR-S12	5	9	6	9	9	38	Susceptible
NGSR-S13	1	1	4	5	7	18	Most tolerant
NGSR-S14	5	7	4	5	7	28	
NGSR-S15	6	5	7	5	7	30	
NGSR-S16	9	6	4	9	9	37	Susceptible
NGSR-S17	6	6	7	7	7	33	
NGSR-S18	4	7	1	7	7	26	
NGSR-S19	1	4	6	3	5	19	Tolerant
NGSR-S20	1	2	4	7	7	21	Tolerant
NGSR-S21	3	6	6	7	9	31	
NGSR-S22	6	7	8	5	7	33	
NGSR-S23	2	6	7	5	5	25	
NGSR-S24	7	6	6	9	9	37	

Table 3. 5 Ranking for the genotypes based on their phenotypic performance and Score for salt toxicity damage

3.10 Cluster Heatmap Analysis

Transcriptome data from two different studies (Chandran et al., 2019; Shankar et al., 2016) was acquired. The raw reads for the two studies were downloaded from GEO and Arrayexpress. The raw reads were processed to acquire gene locus IDs. Locus IDs were used to construct heatmap for the transcriptome data. Heat map and clustering for different transcripts in control environment as well as under salt stress conditions was obtained from the transcriptome data for two studies and is presented in the figure. Heat maps (Fig 3.27) show the expression of different genes based on their FPKM values and log2foldchange values. It can be seen from the figure that group 1 exhibits IR64 and Pokkali in control environment while group2 represents Pokkali 64 and IR 64 under salt stress. The control group is mostly displaying the color that has the vibrant blue shade, which means that less expression is observed in this group. Bright blue represents the least value, light yellow to pale yellow represents lesser value while light red to dark red color shows high level of expression for the genes. Expression of more locus IDs for the (Shankar et al., 2016) study was found to be higher in the group 2 that displays genes under salt stress. More light blue to pale yellow to red color is observed in the second cluster showing the high expression of different genes under salt stress. Heat map for the second study was also constructed using R software. Four different clusters are used to represent heat map based on different treatments; cluster 1 (salinity 1 and salinity 2), cluster 2 (control 2 and control 4), cluster 3 (salinity 3 and salinity 4) and cluster 4 (control 1 and control 3). Dark blue color to yellow to red color shows less to high expression of different genes in the heat map. Same pattern among the treatments is observed as for the study (Chandran et al., 2019). More dark blue color was observed for the genes under control indicating less expression. In saline conditions, (group 1 and group 3), more light blue and yellow color was observed indicating relatively high expression of genes under saline conditions than in control.

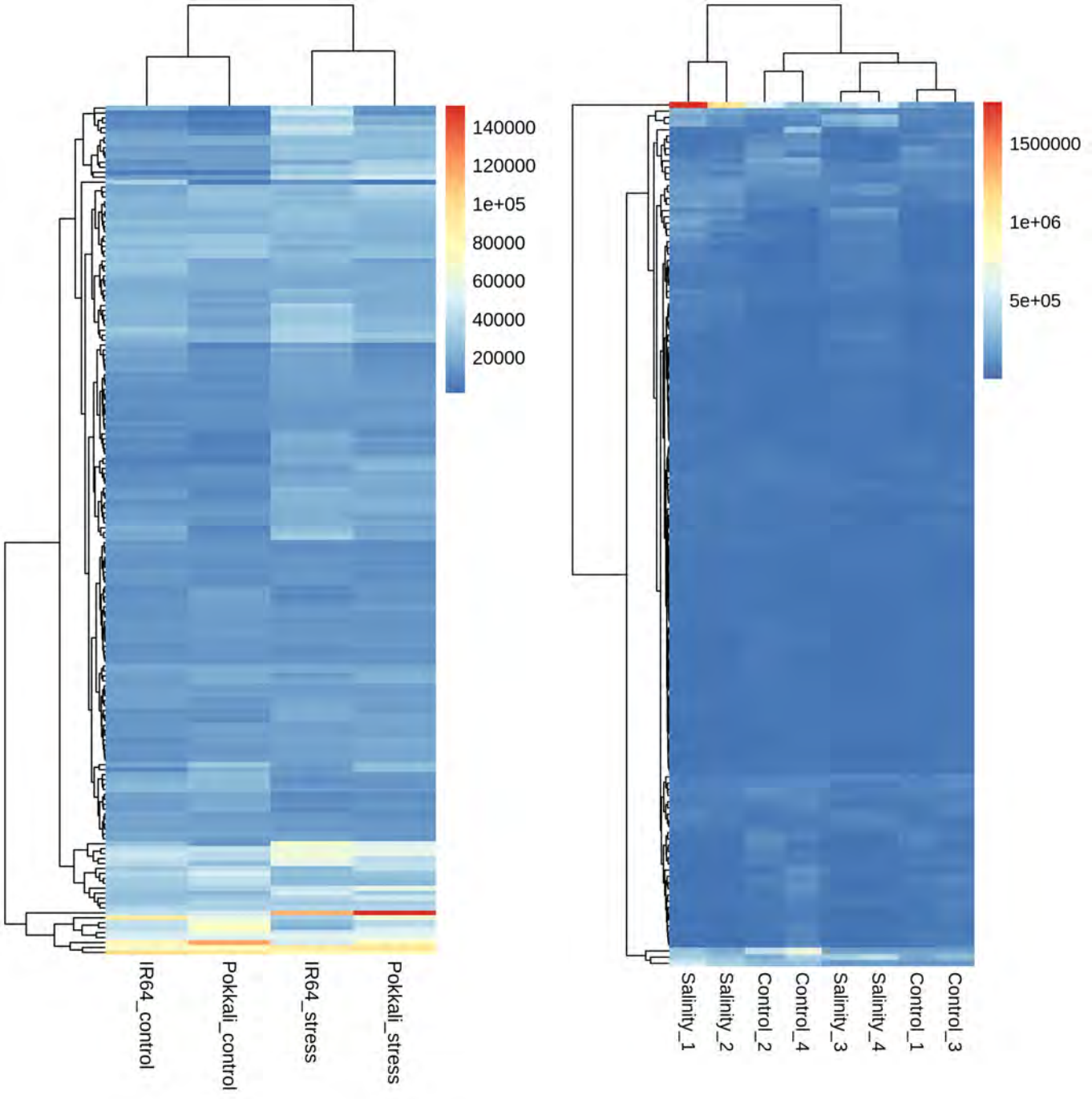


Figure 3. 100 Heat map showing the expression of genes under control and salinity stress. Scale represents lower to higher value, whereas blue color represents lower expression and red color express high expression

3.11 Expression Analysis

To validate transcriptome data acquired by two other studies, genes with the higher FPKM values were selected from the transcriptome data publicly available along with two already known genes responsible for imposing salt stress to rice. The expression of these genes was checked in the roots of six different rice genotypes; three being identified as salt susceptible and three being identified as salt tolerant. Loc_Os8g34540 expression was very high in salt susceptible lines NGSR S3, NGSR S12, and NGSR S16 respectively compared to that of in salt tolerant varieties (NGSR S13, NGSR S19, and NGSR S20) (Fig 3.28a). Same pattern was observed for the gene Loc_Os10g31120 (Fig 3.28b). Significantly high expression of the gene was observed in salt susceptible lines NGSR S16 and NGSR S3, whereas its expression was relatively constant in salt tolerant lines. Loc_Os05g33260 showed high expression in all the lines relative to control (Fig 3.28c). But under salt stress, expression was even higher in identified salt tolerant lines NGSR S13, NGSR S19, and NGSR S20. Expression for the gene Loc_Os01g46890 was induced in all the genotypes relative to control (Fig 3.28d). But the expression data of this gene was not significant as irregular expression was observed. The expression data for the salt tolerant and salt susceptible lines did not have any resemblance to themselves. Expression of Loc_Os1g50430 was also variable as there is a difference of expression in the control as well (Fig 3.28e). qSE 3 expression was induced in salt affected plants as it is evident from the (Fig 3.28f). Similar expression for qSE 3 was observed in NGSR 13 as in salt susceptible lines. But qSE 3 expression was induced more in NGSR 19 and NGSR 20 rice lines as compared to other GSR lines. SKC1 (OsHKT 1; 5) showed way high expression in salt tolerant varieties and relatively low in susceptible lines (Fig 3.28g). Its expression was induced in NGSR S13, NGSR S19, and NGSR S20. Based on the expression analysis data, NGSR S19 and NGSR S20 appeared to be more tolerant to salt amongst all the other genotypes.

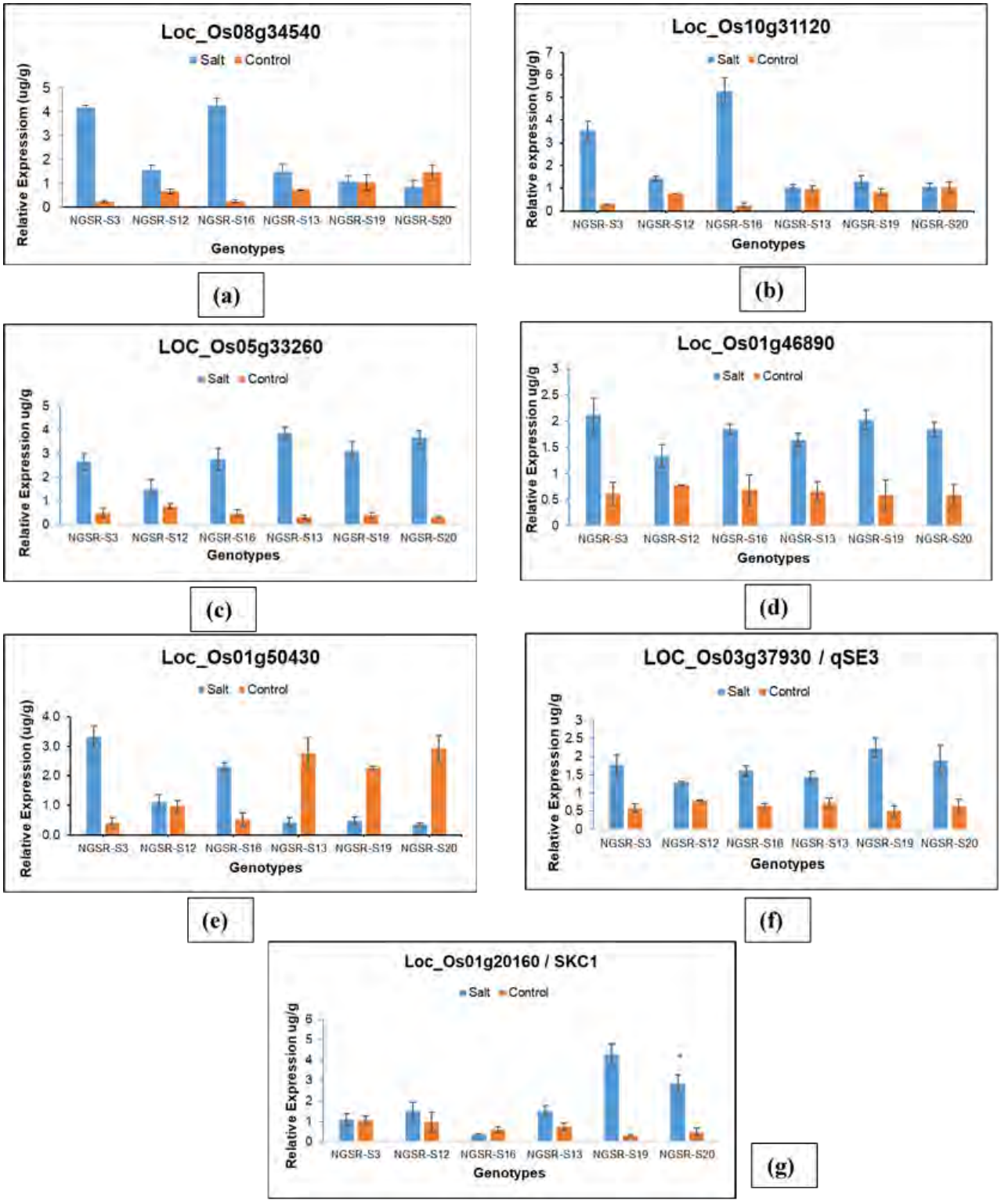


Figure 3. 116 Expression Analysis of different genes evaluated under this study

DISCUSSION

Salinity is a major problem for the reduction in the yield of worldwide rice. Coastal areas and areas that are subjected to drought experience the constant increase in salt content (Tester & Davenport, 2003; Zhu, 2001) (FAO 2016). Due to flooding or irrigation with brackish water, soil in the deltas is rich in minerals and salts (especially NaCl), which hamper the rice production. Rice yield is significantly affected by the salt when soil electrical conductivity reaches 6 us/cm (Hanin, Ebel, Ngom, Laplaze, & Masmoudi, 2016; Munns & Tester, 2008). Rice is very sensitive to salt stress, however, sensitivity of rice to salt is subject to cultivar. There is a great amount of genetic diversity amongst the different crops for salt tolerance (M. Y. Ashraf & Sarwar, 2002; Flowers et al., 2000). Under saline environment, reduction in plant growth is the common phenomenon observed in plants. It is usually visible in the form of stunted shoots. Tolerance to salt stress is a complex phenomenon observed in plants. Understanding of this phenomenon is necessary for developing cultivars tolerant to salt stress (Chinnusamy, Jagendorf, & Zhu, 2005). Plants have multiple level of tolerance mechanisms at morphological, physiological, biochemical and molecular level, that had been studied in the past years (Hanin et al., 2016; Roy, Negrão, & Tester, 2014).

GSR have green super traits which helps rice plant to tolerate against biotic and abiotic stresses and perform better in term of yield under lower nutrient supply. As soil salinization is becoming increasingly challenging for agriculture, selection and breeding of novel lines prove to be a valuable solution to produce the cultivars that grow well in harsh environments and have a better yield. By this way, we would be able to combat global food security to meet the increasing food demand for the growing population. The response to salt stress of the rice accessions under study has not been established. Therefore, scope of this study primarily focuses on to select the salt tolerant varieties and to compare them against the common cultivars. Different transcriptomic studies have been carried out to reveal the differentially expressed genes under salt stress. The raw reads from two trascriptomic studies were processed to identify differentially expressed genes and then identify the expression of those genes with the higher FPKM values in salt tolerant and susceptible lines in this study. Expression

profile of the genes (SKC1 and qSE3) that are known to impart salt stress in rice was also analysed to check the expression in our lines.

The mean values were obtained for all the 22 GSR lines alongwith the 2 checks (IRRI 6 and kissan basmati) were grown in saline field for different morphological traits. These mean values were subjected to analyse for diversity among different traits as well as genotypes. Significant variation for the traits among all the genotypes was observed. Some of the genotypes exhibited high genetic potential for the yield as well as yield related attributes. Thus, genetic diversity exhibited by the lines under study can be exploited to opt for the promising lines. With the help of genomics and breeding, these lines can be utilised to improve rice yield under saline stressed conditions. Two cultivars grown as checks (IRRI 6 & kissan basmati) with the other 22 rice genotypes also displayed high genetic diversity for parameters under study. ANOVA also showed high genetic diversity for all the studied traits and genotypes. The difference of significance between the accessions is more likely attributed to the diversified panel of varieties. The significance for the yield and yield related attributes in this study correlates with other studies who have also reported significant variations in yield and yield related attributes (Chaudhary & Motiramani, 2003; Singh, Singhara, Parray, & Bhat, 2006; Yadav, Suresh, Pandey, & Kumar, 2010).

Plant height decreased in the saline environment as compared to control. Same phenomenon was observed for shoot length of the seedlings that were grown in glasshouse. The percentage decrease in the and shoot length was higher in salt susceptible genotypes. The trend of decrease in shoot length increase with the increase in salt concentration. These results are consistent with the findings of (Islam et al., 2011) and (Miah, Panaullah, Rahman, & Ishaque, 1992). Number of tillers is another important yield related trait as it bears the grains in determining the effect of salt stress on rice. Number of tillers have shown to decrease under saline environment. Highest percentage decrease for the number of tillers was recorded for NGSR S17 (40%) followed by NGSR S6 (39%). In saline conditions, significant reduction in number of tillers on a plant have also been reported by(Zeng, Shannon, & Lesch, 2001). The toxicity effect imposed by salt might reduce the number of tillers in the rice plant. Plants might produce more tillers to dilute salts in order to combat salinity stress (Aslam, Qureshi, Ahmad, & Muhammad, 1989).

Morpho physiological traits i.e. shoot fresh weight, shoot dry weight, root fresh weight and root dry weight complement each other at early stages of growth, so all of these traits can be employed as an indicator for salt tolerance (M. Ashraf, Waheed, Bhatti, Baig, & Aslam, 1999; Noreen & Ashraf, 2008). Plants under salt stress show a decrease in their biomass i.e. SFW, SDW, RFW and RDW (Koca, Bor, Özdemir, & Türkan, 2007; Tuna, Kaya, Dikilitas, & Higgs, 2008). Same trend for the decrease in SFW, SDW, RFW, and RDW was observed in this study. NGSR S3 exhibited highest decrease in shoot fresh weight 55.9 % for 140mM and 46.2 % for 200mM. NGSR S3 proved to be susceptible to salt stress. It is also indicated by rankings and salt toxicity symptoms data. NGSR S3 also recorded 48.9 % decrease in SDW in 200 mM salt stress. Most heavy root was recorded for NGSR S19 in 140mM salt concentration, NGSR S13 on the other hand recorded the least percentage decrease for SDW in 200mM, indicating the tolerance to salt. The genotypes tolerant to salt showed less percentage of decrease in their biomass compared to susceptible genotypes. These results are consistent with the findings of (Grieve & Fujiyama, 1987). Significant reduction in seedling growth under salinity has also been reported by (Shereen, Mumtaz, Raza, Khan, & Solangi, 2005).

The decrease in SDW and SFW under salinity stress is due to less water potential and growth inhibition related to osmotic effects (Munns, Schachtman, & Condon, 1995). Salinity stress can also lead to decrease in cell turgor pressure, and stomatal closure that may ultimately result in less photosynthesis (Gale & Zeroni, 1984).

Many transcriptomic studies have been carried out to address the impact of salinity on rice. All of those studies differ from each other i.e. with the different medium being used for growth, different plant stage and different stress exposure time. Two transcriptome studies were used to identify DEGs present in those data. The DEGs with higher log 2 foldchange values were selected and further processed for expression analysis. These genes were novel genes that were acquired from the transcriptome data. Heat map constructed on the basis of DEGs obtained from the transcriptome data shows the expression of these genes under control and saline environment. The expression profile of these DEGs validated the expression of DEGs in transcriptome data.

The most important adaptation for plant to combat salinity is to block the transport and accumulation of Na^+ ions in the leaves (Munns & Tester, 2008). Expression of OsHKT1;5 is correlated to the salt tolerance of a rice genotype. SKC1 (OsHKT1;5) is present in plants roots, where it helps the retrieval of Na^+ ions into xylem parenchyma cells from xylem prior to its transport in shoots (Munns & Tester, 2008; Ren et al., 2005) According to (Walia et al., 2007) expression for the OsHKT1;5 was less in IR29 (salt susceptible cultivar) than Pokkali (salt tolerant line). Our results are also consistent with Walia et al. as increased expression is observed for the salt tolerant lines.

Conclusion

22 NGSR rice genotypes alongwith two checks (IRRI 6 and kissan basmati) were evaluated in this study and further screened for their tolerance against salt. The large amount of variability amongst all the 22 green super rice genotypes could be utilised for crop improvement. Based on different morpho physiological parameters and complemented by the expression of genes, we selected three salt tolerant rice genotypes (NGSR S13, NGSR S19, and NGSR S20) and three salt susceptible lines (NGSR S3, NGSR S12, and NGSR S16). Consequently, the genotypes performing well under salt stress environment can also be utilised for further breeding programs for variety improvement and development. Such genotypes would be very helpful to breeders in selecting high yielding salt tolerant varieties. Furthermore, DEGs were also selected from the transcriptomic data from two studies. Expression profile of 5 DEGs were evaluated. However, to validate these DEGs, functional validation of such genes in rice and other crops will better help us to understand the mechanism involved in salt tolerance.

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APPENDICES

Appendix-I

10X TAE Buffer Composition

Reagent	Quantity
Tris-Base	242g
Glacial Acetic Acid	57.1g
0.5 EDTA	100ml

1X TAE Buffer

Reagents	Quantity
50X TAE	20ml
Distilled Water	980ml

Appendix-II

Synthesis of first strand cDNA

Total RNA extracted was used as a template for synthesis of cDNA according to the manufacturer instructions using the Reverse Transcriptase III Kit (Fermentas).

PCR mixture performed in 25µl reaction volume. Following reagents were used for the synthesis of first strand cDNA:

4µl 10X Reverse Transcriptase Buffer

3µl 10nM dNTPs mixture

1µl gene specific primer

4µl RNA

1µl Reverse Transcriptase

12µl double nuclease free water

PCR conditions were used as follows:

65ºC for 5 minutes

Chilled sample for 1 minute

55ºC for 60 minutes

72ºC for 10 minutes

Final hold 4 ºC for ∞

Appendix-III

Quantitative real time –PCR

To determine the expression of yield related genes in *Allium sativum* different tissues, Real Time PCR was used using SYBER green master mix.

For 25µl reaction PCR reagents detail are as follow:

SYBER Green Master mix	12
Forward primer	0.8
Reverse primer	0.8
DNA template	3
Double distilled water	up to 25

For amplification of yield related genes with gene specific primer PCR profile is as follow:

Initial denaturation at 94ºC for 10 minutes

Denaturation at 95ºC for 40 seconds

Annealing at 58ºC for 32 seconds 37 cycles

Extension at 72ºC for 32 seconds

Final extension at 95ºC for 15 seconds

Melt curve analysis at 52ºC to 95ºC

Final hold 4 ºC for ∞

Appendix-IV

Ranking rice genotypes

	Field Data							
	Salt/Control							
	PH	No. of T	GY	SY	HI	SL	GW	Ranking
NGSR - S1	0.921	0.937	0.262	1.119	0.349	0.997	0.789	5
NGSR - S10	1.037	0.743	0.368	0.996	0.520	1.024	0.920	4
NGSR - S11	1.008	0.724	0.415	0.845	0.657	1.032	0.929	6
NGSR - S12	0.976	0.623	0.363	0.757	0.669	1.026	0.934	6
NGSR - S13	0.988	0.757	0.359	0.596	0.777	1.005	0.907	4
NGSR - S14	0.962	0.786	0.494	0.995	0.644	1.062	0.859	4
NGSR - S15	0.961	0.693	0.389	0.821	0.645	1.000	0.900	7
NGSR - S16	1.003	0.827	0.428	0.645	0.804	1.037	0.888	4
NGSR - S17	0.978	0.609	0.413	0.787	0.701	1.012	0.914	7
NGSR - S18	0.971	0.838	0.539	1.039	0.701	1.034	0.952	1
NGSR - S19	0.996	0.823	0.359	0.857	0.588	0.979	0.828	6
NGSR - S2	1.004	0.601	0.391	0.633	0.748	1.018	0.913	8
NGSR - S20	1.020	0.560	0.372	0.769	0.652	1.013	0.960	4
NGSR - S21	0.978	0.692	0.409	0.722	0.730	0.992	0.916	6
NGSR - S22	0.986	0.813	0.452	0.858	0.671	0.956	0.807	8
NGSR - S23	1.038	0.678	0.472	0.533	0.939	1.003	0.978	7
NGSR - S24	1.013	0.740	0.422	1.244	0.487	0.979	1.049	6
NGSR - S3	1.043	0.815	0.430	0.717	0.735	1.004	0.854	3
NGSR - S4	0.983	0.644	0.352	0.725	0.649	1.029	0.863	7
NGSR - S5	0.964	0.753	0.404	0.909	0.637	1.006	0.839	6
NGSR - S6	0.965	0.613	0.287	0.714	0.451	0.926	0.800	9
NGSR - S7	1.011	0.839	0.470	0.793	0.751	0.961	0.846	4
NGSR - S8	0.999	0.675	0.480	0.850	0.720	1.025	0.851	6
NGSR - S9	0.974	0.810	0.531	0.937	0.717	1.080	0.982	2

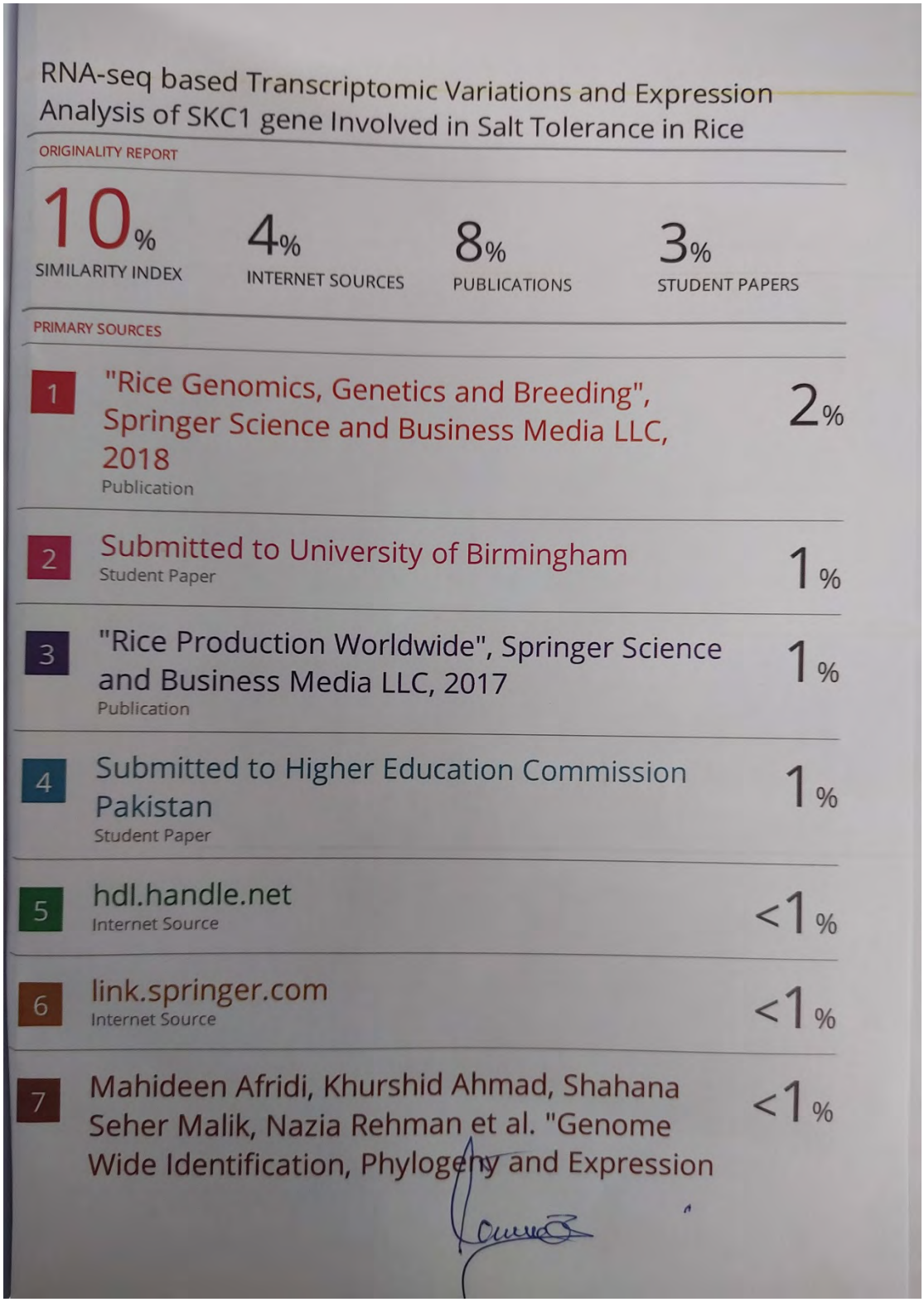
Figure 1 Ranking of rice genotypes grown in field according to their performance

Salt/Control_140mM							
Genotype	SL	RL	SFW	RFW	SDW	RDW	Ranking
NGSR-S1	0.8336	0.7640	0.5092	0.4454	0.5714	0.4468	6
NGSR-S10	0.8731	0.7654	0.4964	0.3986	0.5886	0.3788	8
NGSR-S11	0.8806	0.7250	0.4761	0.4496	0.4926	0.4579	6
NGSR-S12	0.9105	0.7354	0.4713	0.4537	0.5294	0.4475	5
NGSR-S13	0.8958	0.7844	0.5342	0.5255	0.5880	0.5113	1
NGSR-S14	0.9018	0.7000	0.5330	0.4724	0.6351	0.4510	5
NGSR-S15	0.8654	0.7187	0.4853	0.4939	0.5633	0.4610	6
NGSR-S16	0.8053	0.7010	0.4418	0.4406	0.5093	0.4122	9
NGSR-S17	0.8288	0.7454	0.5287	0.4246	0.5815	0.4355	6
NGSR-S18	0.8577	0.7655	0.4792	0.5222	0.5256	0.4617	4
NGSR-S19	0.8921	0.8679	0.5957	0.5136	0.7228	0.4210	1
NGSR-S2	0.8433	0.7145	0.4836	0.4236	0.5087	0.3791	8
NGSR-S20	0.9031	0.7675	0.5006	0.5416	0.5594	0.4735	1
NGSR-S21	0.8694	0.8755	0.4981	0.5207	0.5837	0.4677	3
NGSR-S22	0.8798	0.7784	0.4978	0.5149	0.5599	0.4348	6
NGSR-S23	0.8721	0.8289	0.5609	0.4561	0.7164	0.4078	2
NGSR-S24	0.8662	0.7817	0.4556	0.4632	0.5407	0.3958	7
NGSR-S3	0.8085	0.7121	0.4411	0.4646	0.5202	0.4025	9
NGSR-S4	0.8844	0.7522	0.5238	0.4986	0.5555	0.4637	6
NGSR-S5	0.8769	0.7836	0.5052	0.4270	0.5478	0.4180	6
NGSR-S6	0.9187	0.7988	0.5131	0.4852	0.5294	0.4584	4
NGSR-S7	0.7981	0.7442	0.4638	0.4215	0.5087	0.3967	9
NGSR-S8	0.8727	0.7977	0.4913	0.5008	0.5226	0.4453	6
NGSR-S9	0.8603	0.7630	0.4876	0.4210	0.5562	0.3945	8

Figure 2 Ranking of rice genotypes grown in 140 mM salt stress according to their performance

Salt/Control_200mM							
Genotype	SL	RL	SFW	RFW	SDW	RDW	Ranking
NGSR-S1	1.0052	1.0807	0.6316	0.7290	0.7007	0.5550	2
NGSR-S10	0.9158	0.8796	0.5941	0.5414	0.6910	0.5750	5
NGSR-S11	0.9284	0.6946	0.6112	0.4831	0.6158	0.5357	8
NGSR-S12	0.9628	0.9505	0.6207	0.4259	0.6023	0.5185	9
NGSR-S13	1.0105	0.8503	0.6531	0.6447	0.7651	0.5933	1
NGSR-S14	0.9125	0.8082	0.5956	0.5180	0.6476	0.5595	7
NGSR-S15	0.9851	0.8559	0.6136	0.5323	0.6476	0.5310	5
NGSR-S16	0.9668	0.8806	0.6094	0.5804	0.6654	0.5545	6
NGSR-S17	0.9357	0.8997	0.6248	0.5892	0.6444	0.5474	6
NGSR-S18	0.9062	0.9504	0.6093	0.4934	0.6252	0.5553	7
NGSR-S19	0.9796	0.8612	0.6453	0.5025	0.7403	0.5597	4
NGSR-S2	0.9121	0.8891	0.6071	0.5512	0.6208	0.5667	5
NGSR-S20	0.9750	1.0085	0.6288	0.6079	0.7061	0.5656	2
NGSR-S21	0.9837	0.8558	0.6106	0.5999	0.6903	0.5643	6
NGSR-S22	0.9629	0.8183	0.5783	0.5192	0.6413	0.5637	7
NGSR-S23	0.9387	0.9956	0.5886	0.6018	0.7552	0.6136	6
NGSR-S24	0.9383	0.9150	0.6781	0.5315	0.8390	0.5372	6
NGSR-S3	0.7567	0.7333	0.5377	0.3790	0.5108	0.5336	10
NGSR-S4	0.9319	0.8930	0.6388	0.5491	0.6428	0.5599	6
NGSR-S5	0.9452	0.9474	0.6475	0.5149	0.6451	0.5723	3
NGSR-S6	0.9569	0.9665	0.6511	0.5591	0.6444	0.5555	3
NGSR-S7	0.9672	0.8270	0.6453	0.5979	0.6427	0.5675	6
NGSR-S8	0.9173	0.8713	0.6281	0.5000	0.6127	0.5349	7
NGSR-S9	0.9459	0.9341	0.6145	0.4861	0.6592	0.5705	6

Figure 3 Ranking of rice genotypes grown in 200 mM salt stress according to their performance



RNA-seq based Transcriptomic Variations and Expression Analysis of SKC1 gene Involved in Salt Tolerance in Rice

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