Doctoral Dissertation

Improvement of Salinity Tolerance in Rice (*Oryza sativa* L.) by N-methyl-N-nitrosourea (MNU) Treatment and Exogenous Application

CAN THU HUONG

Graduate School for International Development and Cooperation
Hiroshima University

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CAN THU HUONG

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Committee on Final Examination:

TRAN Dang Xuan, Associate Professor
Chairperson

LEE Han Soo, Associate Professor

HOSAKA Tetsuro, Associate Professor

TSUDZUKI Masaoki, Professor

MORIMOTO Masanori, Professor
Kindai University

Date: July 26th 2021

Approved:

ICHIHASHI Masaru, Professor
Dean

Date: Sep. 3, 2021

Graduate School for International Development and Cooperation
Hiroshima University

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ABSTRACT

Background

More than half of the world's population consumes rice (*Oryza sativa*) as a staple food and considers it as an important agricultural crop with various benefits. In the current context of the rapid increase of world population and impacts of climate change, the rice demand has increased in both productivity and quality. Therefore, the target of rice breeders is breeding and developing new cultivars which have not only high yield and good quality but also possess wide adaptability to severe environmental conditions.

Among climate change impacts, salinity is one of the most problematic hazards to rice cultivation, which adversely affects rice yield and quality. By various damages of ion toxification, nutritional disorders, osmotic imbalance, oxidative stresses, membrane disorganization, reduction of cell division and expansion, and genotoxicity, salinity intrusion significantly reduces rice growth as well as partial sterility, which ultimately results in the reduction of rice yield. Besides, different levels of salt stress can lead to the changes of quality in rice, such as amylose content, protein content, amino acid, and micro and macro mineral nutrients. Although slight salinity induces the increase of some minerals, salt conditions almost have a negative effect to the rice production. Therefore, the breeding of salt-tolerant rice cultivars is an important task to ensure food security and sustainable agriculture.

Tremendous strategies have been conducted to enhance salinity tolerance in rice. Mutation breeding is a potential technique that creates a new rice and selects the elite line through many generations. N-Methyl-N-nitrosourea (MNU) mutation is a chemical induced mutation and widely used for breeding cereal crops. MNU frequently leads to the point mutation. It is possible to alter the DNA structure of plants and cause the biological effects.

MNU-induced mutants are considered as the high frequency mutation which is a valuable material for plant breeding.

In a breeding, molecular marker or DNA marker is used to identify the genotype of plant population. The molecular marker is a prominent innovation of biotechnology that helps breeders to select the target plants quickly and simply by detecting a particular gene or trait. DNA marker has been discovered for genetic analysis since 1980s and still applied for rice breeding. Among developed DNA markers, simple sequence repeat marker (SSR) is one of the powerful techniques to identify the mutant genotypes. This marker is co-dominance and useful for the detection of genetic inheritance in progeny. By the advantage compared to other markers, SSR has been achieved some achievements in mutation breeding including classification of important mutant genotypes.

Earlier studies substantiated the important functions of mineral elements in human, animal, and especially plant nutrition. Plants use minerals as structural components in carbohydrates, proteins, and organic molecules. Magnesium (Mg) is a macronutrient participated in various metabolic processes as photosynthesis, enzyme activators, and osmotic balance of plants. In the last decades, several studies reflected a correlation between magnesium and plant growth in a number of higher plants. However, the role of magnesium in reducing salt-induced damages in rice has not been comprehensively studied.

Objectives

Considering all above mentions, to develop the tolerant rice and improve its resistance to salinity stress, this study is conducted to (1) identify the salinity tolerance of rice mutants by phenotypic measurements and SSR markers, (2) determine the inheritance pattern of

salinity tolerance and beneficial phytochemicals of rice mutants, (3) shorten breeding time by the possible maternal inheritance, and (4) improve salinity tolerance of rice by application of magnesium.

Structure of dissertation

Chapter 1: General introduction

Chapter 2: Identification of salinity tolerance in rice mutants by phenotypic and simple sequence repeat analyses

Chapter 3: Maternal inheritance of salinity tolerance and beneficial phytochemicals in rice

Chapter 4: Improvement of salinity tolerance in rice by exogenous magnesium application

Chapter 5: General discussion

Materials, methods, and results

In this study, rice mutants were created by soaking rice seeds in 150 mM MNU for 3 hours, drying, and keeping in the dark for 3 months in a hermetic condition before being stored at 4 °C. The rice mutants and parents were then cultivated in Higashi-Hiroshima from 2016 to 2019 to assess their phenotypes. After that, salinity tolerance of rice samples was screened. A mutant population with prominent characteristics (TBR1/KD18) also was selected to detect their salt tolerance. From the screening, the strong tolerant and susceptible rice were chosen for experiment with exogenous magnesium.

In chapter 2, salinity tolerance of ten rice cultivars/mutant lines was identified by the combination of phenotypic measurement and genetic analysis. The phenotypic assessments were conducted followed the protocol from International Rice Research Institute. In genetic analysis, forty-two SSR markers linked to *Saltol* quantitative trait locus (QTL) (salinity

tolerant) were used to identify the genotype of rice samples. The results showed that BC15 and BC15/SKLo have strong tolerance to salinity. They are valuable sources for the breeding of salinity tolerant rice. Additionally, six SSR polymorphic markers RM 237, RM 518, RM 493, RM 10748, RM 562, and RM 20224 were found to be polymorphic. These markers can be used useful for classification of salt tolerance of rice, both cultivars and mutants. The results of phenotypic measurement and genetic analysis are correlated.

Chapter 3 determines the salinity tolerance and inheritance pattern of the elite mutant rice TBR1/KD18. The F_1 generation of the cross TBR1 (female cultivar) × KD18 (male cultivar) was treated with MNU to induce the first mutant generation M_1. M_1 was then self-pollinated to obtain M_2 and M_3 populations. Control populations were F_1 and $_{F2}$. Phenotypic, chemical, and genetic analyses were conducted in the M_2, M_3, F_1, F_2, TBR1, and KD18. A total of fifty SSR markers involved in growth parameters, yield, pest resistance, and the *Saltol* QTLs were used for genetic analysis. The results showed that the salinity tolerant *Saltol* QTLs, growth parameters, yield, pest resistance are maternally inherited from the female parent TBR1 in the M_2 generation and stabilized in the M_3 generation. Besides, antioxidant activities and contents of momilactones A and B might be maternally inherited. In contrast with the literatures, this is the first study to reveal that salinity tolerance of rice can be maternally inherited (normally paternally inherited). The MNU-induced maternal inheritance provides a simple protocol to finish progeny segregation in 2-3 generations (instead of 8-10 cycles in conventional breeding) and shorten breeding time for salt tolerant rice.

Chapter 4 focuses on the promising effects of magnesium on developing salt tolerance rice. From chapter 2, BC15 (salinity tolerant) and DT84DB (salinity susceptible) were selected and used as rice materials in chapter 4. The salinization was conducted in 7 days-old

seedlings with the supplement of $MgSO_4$ (0.5 mM). After treatment, physiological, antioxidant activities, and chemical properties of rice samples were investigated. The phytochemicals of rice materials including phenolic compounds as well as momilactones A and B were identified and quantified by High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography Electrospray Ionisation Mass Spectrometry (UPLC-ESI-MS), respectively. The results showed that exogenous application of Mg partially recovers the inhibited growth and improves the antioxidant activities as well as phenolic profiles of rice seedlings under salt stress. Momilactone A was only detected in salinity tolerant cultivar BC15 under control condition (a very low concentration that could not be measured, hence will be interpreted as undetectable). However, momilactone B were found in both tolerant and susceptible rice lines, in which, the amount of momilactone B of tolerant rice were higher than that of the susceptible one. It was also indicated that the amount of potential bioactive compound momilactone B was enhanced with Mg supplement in salt stressed rice at seedling stage. The results suggested that $MgSO_4$ is useful develop fertilizer for rice growing in saline soil. Additionally, *ρ*-coumaric acid, salicylic acid, ferulic acid, and momilactone B are involved in the tolerant ability of rice again salt stress. They can be used as promising agents to reduce salinity damages on rice production.

Key findings of the dissertation

The literatures showed that salinity tolerance of rice is nucleus inheritance (inherited from father). This study is the first to reveal a maternal inheritance in the salinity tolerance of rice. On the other hand, breeding program for salt-tolerant rice requires 8-10 years due to the complicated segregation in progenies. F_1 normally is crossed with father (backcross) and repeat in many generations to finish that segregation. The maternal inheritance induced by

MNU treatment in this study helps to finish the segregation in M_2 and M_3 generations, which can shorten breeding time from 8-10 cycles to 2-3 generations. However, the mutated rice should be sequenced and compared to parental genotypes to detect their genetical changes. The mechanism of the novel maternal inheritance should be clearly identified. Besides, magnesium is effective to reduce salinity-induced damages in rice seedlings by enhancing its inhibited growth, antioxidant activities, phenolic acids, and momilactone B. The results suggest that the salinity tolerance of rice can be improved by developing fertilizer with a supplementary $MgSO_4$. However, this application needs to be further investigated in rice field.

ABBREVIATIONS

A: Adenine

C: Cytosine

˚C: Celsius

cm: centimeter

Co. Ltd: Company Limited

DNA: Deoxyribonucleic acid

dNTPs: deoxynucleotide triphosphates

FAO: Food and Agriculture Organization of the United Nation

G: Guanine

g: gram

HCl: Hydrochloric acid

IRRI: International Rice Research Institute

M: Molar concentration

$MgCl_2$: Magnesium chloride

mm: milimeter

NaCl: Sodium chloride

NaOCl: Sodium hypochlorite

PVP: Polyvinylpyrrolidone

T: Thymine

Taq: Taq Polymerase

Tris: Tris (Hidroxymethyl) aminomethane

U: Udenin

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CHAPTER I.

GENERAL INTRODUCTION

1.1. Background

1.1.1. Rice production

Rice is one of the most important agricultural crops cultivated in at least 114 countries, in which, more than 50 countries have an annual rice production exceed 100,000 tons (FAO, 2020). People in most of Asia countries consider rice as their main food while some countries in Africa and South America consume rice with other cereals such as wheat and corn. Notably, rice plays a crucial role in human nutrition and calories intake, providing 20% of energy and 15% of protein consumed daily by humans (IRRI, 2020). Besides providing a huge amount of carbohydrate, rice is considered as high protein quality resource among cereals. Moreover, rice also provides minerals, vitamins, and fiber (IRRI, 2020).

Rice has been cultivated in diverse ecosystems: upland, irrigated, rainfed lowland, and flood prone throughout five continents (IRRI, 2020). According to FAO, in 2019, total rice-harvested area was 162 million hectares, and the global rice productivity was 504 million tons. Asia contributes to 90% rice production and consumption worldwide with about 80 % of the global rice growing area is located in eight Asian countries: China, India, Indonesia, Bangladesh, the Philippines, Vietnam, Thailand, and Myanmar (FAO, 2019).

Table 1.1. Top ten rice producing countries in 2019.

World rank	Countries	Rice production (Million tons)
1	China	140.65
2	India	117.51
3	Indonesia	37.95
4	Bangladesh	36.35
5	Viet Nam	29.18
6	Thailand	20.25
7	Myanmar	17.96
8	Philippines	12.63
9	Brazil	7.40
10	Pakistan	7.31

(FAO, 2020)

1.1.2. Challenges of current rice production

Being principal food for more than 3 billion people globally, rice contributes to the sustainable agricultural production and economy of many countries. However, rice cultivation today is facing some challenges. The current world's population was over 7.6 billion in 2020 and estimated to reach 8.3 billion in 2030 (FAO, 2020), especially, around 815 million people are affected by malnutrition (Richardson et al., 2018). The global population is supposed to grow continuously to about 9 billion in 2050 and food requirements is expected to raise by about 85% (FAO, 2020). To date, rice production needs to be improved to satisfy the human demand.

In the 2020s, climate change and its consequences has continuously affected to all living organisms such as plants, animals, and humans. Under undesirable climate variations, plants have suffered numerous stresses like waterlogging, drought, heat, cold, and salinity. According to IPCC (http://www.ipcc.ch/), the Earth's average temperature is expected to increase up to 4 °C at the end of the 21st century. The global warming is the main cause of extreme weather events which is expected to happen more frequently in the current decade. Drought, flood, temperature, and salinity stresses are key stresses affecting crop cultivations and threatening food productions. Particularly, the production of major crops has been dramatically reduced around the world (Ito et al., 2018). This reduction of crops productivity leads to higher risk of food security, especially in the rapid increase of the world's population.

1.1.3. Impact of salinity stress on rice production

Adversely effects of the climate change such as global warming and sea level rise are main causes of salt intrusion and salinity stress, which threaten food production. More than 80 million ha of land globally are affected by salinization, which accounts for approximately 6% of the global land area in over 100 countries (Figure 1.1). The productivity of main cereal crops such as wheat, maize, rice, barley can be reduced by 70% and the total cost loss is estimated to reach US $12 billion per year globally due to salinity stress (Jaiswal et al., 2019). In which, rice is one of the most affected crops because it is highly sensitive to salt conditions. Extremely high salt concentrations can lead to plant death through the combined effects of osmotic homeostasis, ionic homeostasis, and oxidative stress (Shannon, 1985). At high-moderate concentration, salinity may cause leaf burn and growth inhibitions or even lead to stomatal closure and reducing leaf size at the level of moderate to low salinities (Shannon,

1985; Tester and Davenport, 2003). The influenced physiological processes affect to all growth parameters, as a sequence, salt stress reduces rice productivity and quality.

Figure 1.1. World map representing countries with salinity problems.

(https://www.nsw.edu.au)

1.1.4. Rice breeding for salt tolerance

Since the first salt-tolerant rice variety *Pokkali* was introduced in 1939, a series of salt-tolerant rice were bred such as *Kala Rata 1-24*, *Nona Bokra*, *Bhura Rata*, *SR 26B*, *Chin.13*, and *349 Jhona* (India and the Philippines); *BRI*, *BR203-26-2*, *Sail* (Bangladesh); *FL530* (Thailand); *Mantaro rice*, *Kanto 51*, *Hama Minoru*, *Chikushiqing*, and *Lansheng* (Japan); *American Rice* (USA); *Dongjinbyeo, Ganchukbyeo, Gyehwabyeo, Ilpumbyeo, Seomjimbyeo, and Nonganbyeo* (South Korea); *VNIIR8207* and *Fontan* (Russia) (Bernstein et al., 1958; Ghose et al., 1959; Akbar et al., 1972; Fageria, 1985; Yeo et al., 1986; Heenan et al., 1988; Sun et al., 2017; Wang et al., 2019). More than 127,000 rice accessions

were collected worldwide by IRRI, providing a rich source for breeding rice salinity tolerance. From them, more than 100 varieties were identified as moderately to highly salt tolerance (Platten et al., 2013; Rahman et al., 2016).

During recent decades, tremendous efforts have been conducted to develop rice crops with higher salt tolerance include conventional breeding, and molecular breeding. In a conventional breeding, heterosis can be useful to generate progenies with greater traits compared to their parents. However, on the other hand, the heterosis in breeding is depressed. The great values of heterosis are performed in the first generation and gradually reduce over next generations. In rice, although the heterosis has been applied to improve crop yield, it is not easy to achieve the definite goal by conventional breeding because of the undesirable linkage drag in the progenies, which affects yield and grain quality of rice cultivars (Jeung et al., 2005).

Molecular breeding has developed to resolve the problems for the conventional breeding. Since DNA markers have been found as linked to *Saltol* QTLs, elite genes of salt tolerance enable to transfer in rice without the linkage drag (Mackill, 2007). Therefore, future rice cultivars possessing salt-tolerant can be created by molecular-assisted selection (MAS). Basically, MAS is developed based on transferring the target allele from donor line to recipient line and selecting against remaining donor introgressions. This process requires normally 8-10 years. Furthermore, despite MAS is considered as a modern method with the application of biotechnologies, it has some limitations. Firstly, MAS focuses on few major genes, while other unknown meaningful traits/DNA sections may less be studied (Lammerts van Bueren et al., 2010). Besides, molecular markers for complex traits are expensive to

develop, and breeding process needs personal knowledge and techniques in molecular science, statistics, and molecular lab facilities (Lammerts van Bueren et al., 2010). Additionally, the interaction between genotype and environment causes a variation in all agronomical traits, therefore, the success of MAS can be ensured under similar conditions with donor line. The obtained traits might lose in progenies because of unfavourable growing conditions.

Application of other biotechnologies are promised to maximize the success of breeding program. As a component of new discipline 'molecular breeding', transgenes can simplify the genetic architecture for desirable traits. Some target rice with abiotic stresses tolerance such as salinity tolerance were developed by transgenic previously. However, transgenic approach requires some specific conditions. The material should be genetically stable, the effect of new gene in organism chromosome and the unexpected genes transformed should be considered. The adaptation of foreign gene with the target organism is also complicated and needed to carefully be examined. Additionally, similar to MAS, the high requirement of molecular lab instruments as well as personal knowledge and techniques retard its further application.

To accelerate the above situations, mutation has drawn growing attention from rice breeders. The first advantage of mutation is shortening breeding time. The homozygosity of progenies can be taken after two or three generations, which cannot be obtained by the classical breeding and MAS. Mutation is also widely used in a variation of crops because it is cost-effective and straightforward. According to FAO/IAEA (2020), more than 3000 mutant varieties have been developed by mutagenesis, including 853 rice cultivars. The achievements of mutated application in rice are high yield, good quality, disease resistances,

and stress tolerances (FAO/IAEA 2020). Both physical and chemical-induced mutations have been applied for rice breeding. Among the most using mutation agents, N-methyl-N-nitrosourea (MNU) is widely used in cereal crops. This chemical factor has been achieved high frequency and extensively applied to breeding purpose (Suzuki et al., 2008).

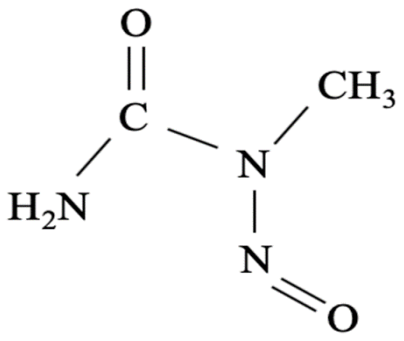


Figure 1.2. MNU's chemical structure (Tsubura et al., 2011)

1.1.5. Advances of molecular marker for breeding salt tolerant rice

Salinity tolerance of rice is controlled by polygenes (Mishra et al. 1998). Since quantitative trait loci (QTL) was detected, thousands of QTLs associated with desirable traits rice have been mapped. They distribute along 12 chromosomes. Among them, almost QTLs contributing to salinity tolerance in rice are located on chromosome 1. By using molecular markers, valuable QTL alleles can be tagged and introduced into elite cultivars by MAS. On the other hand, DNA markers contribute to the selection process by a quick and simple choosing target plants based on their genotypes. Simple sequence repeats (SSRs) are broadly applied to breeding process due to their effectiveness compared to other markers. SSRs randomly repeat a short nucleotide sequence. They are polymorphic and distributed throughout the genomes of plants and animals. Another important attribute of SSR loci is

their high level of allelic variation. The advantages of SSRs have been demonstrated during the last twenty years for the improvement of rice crops with elite important yield and quality attributing traits, as well as resistance to diseases and environmental stresses.

1.1.6. Segregation and maternal inheritance

Breeding program includes parental crossing, progeny testing, and pure line selection. Basically, SSRs are used as probers to distinguish the polymorphism in parental lines and analyse the genotype of progenies because they are co-dominant in inheritance. The mode of inheritance in progenies is very important (Verlues et al., 2006). Thanks to its specific characters, the breeders enable to transfer both expected genes from parents into the target cultivar.

With normal rice crossings, two alleles of parents present in the progeny (follow the Mendelian theory), and progenies receive genetic materials from both female and male parents. However, in some cases, non-Mendelian inheritance patterns were recorded (Brooker, 2009b). Non-Mendelian can be caused by many factors from genetic, physiological, and environmental aspects (Alheit et al., 2011; Reflinur et al., 2014). The non-Mendelian inheritance is called uniparental inheritance, maternal or paternal inheritances (Sato and Sato, 2013), of which, maternal inheritance occurs when an inherited trait is determined by its mother genotype without effects of paternal genotype (Russel 2010b). Maternal effects contribute to the phenotype of its offspring because they increase survival ability and facilitate range development of offspring in different novel environment (Duckworth, 2009; Räsänen and Kruuk, 2007).

Maternal inheritance usually happens in chloroplast genes (Russell, 2010b). In rice, the genes inherited follow chloroplast (from mother) mostly are low quality related traits while salinity tolerant genes are inherited from fathers. Maternal effect has observed in crossing between wild rice and domestic rice, O. *meridionalis* and O. *sativa* (Yu et al., 2018), O. *rufipogon* and O. *sativa* (Charlesworth, 2017). The maternal inheritance normally occurs in rice progenies which are generated from parents with far genetic distance.

1.1.7. Application of exogenous phytoprotectants

Along with developing tolerant cultivar by breeding and genetic engineering, detoxification of reactive oxygen species (ROS), maintenance nutrient homeostasis, and reduction of salt uptake accelerate some tolerant mechanisms of plant under salt stress condition (Wu et al., 2012). Using exogenous phytoprotectants for improving salt stress tolerance by osmoregulation, ROS, and ion homeostasis is one of the prominent strategies for improving salt stress tolerance in crop plants.

The role of osmoprotectants to confer salt stress tolerance in rice seedlings was found in several studies. Supplementation of Proline (Pro) increased photosynthetic pigments, antioxidants, as well as growth parameters of rice cultivars growing under salinity stress (Nounjan et al., 2012; Teh et al., 2015; Bhusan et al., 2016). Exogenous plant hormones such as abscisic acid (ABA), auxin, cytokinins (CK) are applied for enhancing rice tolerances to various kinds of abiotic stresses including salinity. ABA pre-treatment conferred salt tolerance in rice seedlings by supplying abundant energy and reducing Na^+ and Cl^- concentrations (Li et al., 2010; Gurmani et al., 2011). The plant growth regulator auxin (indole-3-acetic acid IAA) plays an important role in improving germination percentage of

seeds and grain quality of rice under salt stress condition (Kim et al., 2006; Javid et al., 2011). Another phytohormone cytokinin (kinetin) improves grain yield and quality of rice developed in salt conditions by improving sucrose and glucose content in rice grain (Javed et al., 2011). Signaling molecules such as nitric oxide (NO) and H_2O_2 inhibit injuries from oxidative stress (Uchida et al., 2002; Habid et al., 2016) while polyamines interacting with other biomolecules (hormones/signaling molecule/amino acids) plays pivotal roles during plant developmental and stress adaptation process (Rahman et al., 2017).

The effect of trace elements in prohibiting the negative impacts of abiotic stresses in plants has attracted more attention from researchers. Studies in the past showed that the application of micronutrients improved rice growth, dry matter, leaf area index, photosynthetic contents, and yield attributed traits under saline condition (Memood et al., 2009; Zayed et al., 2011; Farood et al., 2015). Several medium nutrients also have been used to enhance such as silicon and calcium (Zayed et al., 2011; Farood et al., 2015; Rahman et al., 2016). However, the research about other important medium nutrient magnesium for improving salinity tolerant rice have still limited. Moreover, the mechanism of signal and defence pathways of these supplements is still unclear, and the responses of rice are dependent on dose (Hasanuzzaman et al., 2014). Therefore, it is needed to study more about exogenous protections to carry out the alternative supply as well as their roles in reducing damages of salt stress.

1.2. Dissertation structure

Considering all above mentions, to develop the salinity tolerance of rice, and to cope with the unexpected climate change, the study was conducted to (1) identify the salinity

tolerance of rice mutants by phenotypic measurements and SSR markers, (2) determine the inheritance pattern of salinity tolerance and beneficial phytochemicals of rice mutants, (3) shorten breeding time by the possible maternal inheritance, and (4) improve salinity tolerance of rice by application of magnesium.

The dissertation is designed in five chapters as below:

Chapter 1 presents general introduction of the study, research objectives, and thesis organization.

Chapter 2 identifies salinity tolerance in rice mutants by phenotypic evaluation and simple sequence repeat markers.

Chapter 3 investigates the segregation of mutant population and the possibility of maternal inheritance of salinity tolerance and beneficial phytochemicals in rice.

Chapter 4 assesses the application of exogenous magnesium to improve salinity tolerance in rice.

Chapter 5 gives general discussions with the recent advances and challenges in developing rice tolerance to salinity stress. Research achievements, findings, as well as recommendations toward the improvement of salinity tolerant rice are also provided.

CHAPTER II.

IDENTIFICATION OF SALINITY TOLERANCE IN RICE MUTANTS BY PHENOTYPIC AND SIMPLE SEQUENCE REPEAT ANALYSES

2.1. Introduction

Rice is one of the most important crops grown and consumed globally. It ranks the third in agricultural production and provides daily meals for more than half of the world's population. However, ongoing climate change adversely threatens rice production. Both biotic and abiotic stresses cause a significant yield loss in large rice-growing areas, including high salinity, drought, heat, and cold. Among them, salinity stress is the main hazardous factor of rice productivity (Zeng et al., 2000).

Rice is highly sensitive to salty conditions, especially at the seedling stage. The inhibition in seedling is the initial step that leads to other consequences. The high concentration of toxic ions Na^+, induced by salinity, is the main cause of various physiological damages and inhibitory processes of plants. On the other hand, in a physiological view, it disturbs the uptake of potassium, which plays an important role in preserving membrane potential, enzyme activities, and cell turgor. A high Na^+/K^+ ratio increases the osmotic gradient that causes cellular dehydration. Na^+ also affects the activity of enzymes or proteins after entering the cytosol (Zeng et al., 2000). Besides this, high levels of salt stress are inversely correlated with photosynthesis and photoinhibition, as inducing abscisic acid synthesis leads to stomatal closure and reducing leaf size. All of the factors mentioned above directly influence rice seedling growth, leaf formation, and panicle emergence (Tester et al., 2003). Accordingly, salinity reduces rice productivity by a decline

in panicle length, number of spikelets per panicle, and number of grains (James et al., 2016). Additionally, caloric and nutritional values are also changed (Oladosu et al., 2016).

Tremendous efforts have been conducted to improve salinity tolerance and to ensure a sustainable rice production. In which the study on rice mutants as new elite materials is an impressive approach, since the high efficiency of mutagenesis has been widely documented and reflected in the enhancement of more than 3000 mutant varieties, including 828 rice cultivars, according to Food and Agriculture Organization/International Atomic Energy Agency Mutant Varieties Database (FAO/IAEA, 2020). Basically, mutation can be created by spontaneous mutagenesis or induced mutagenesis (chemical mutagenesis, UV radiation mutagenesis, and ionizing radiation) (Sikora et al., 2011). Induced mutagenesis has been extensively used for the genetic improvement of all organisms, including microbes, animals, and plants. It is reported that mutation induction is useful for generating potent rice lines. Furthermore, it performs cost- and time-effective strategies in the enhancement of expected characteristics of crops (Moradi et al., 2003; Hanyong et al., 2004). Along with physical mutagens, the chemical mutagens, such as ethyl-nitroso urea (MNU), have been utilized frequently for breeding purposes. MNU is an alkylating agent, which can covalently attach an alkyl group to a biomolecule under physiological conditions (aqueous solution, 37 °C, pH 7.4) (Maria et al., 2016). In rice, it is demonstrated to obtain high-frequency mutation and is effective for genetic approach (Zhang et al., 1995).

To accelerate the development of rice cultivation, enormous DNA markers related to *Saltol* quantitative trait loci (QTL) have been developed. Among them, simple sequence repeat (SSR) markers are broadly applied because of their dominances compared to other

markers. With 1 to 10 nucleotides, SSRs (or microsatellites) have used for genotyping plants over the past 20 years. They are abundant, distributed throughout the genome, and highly polymorphic. As multi-allele genetic markers, SSRs can also be replicated and transferred among relative species (Gregorio et al., 1997). SSR markers are valuable in breeding new rice cultivars to obtain elite important yields and quality attributing traits, as well as resistance to disease and environmental stresses (Ali et al., 2000). In previous studies, SSR markers performed as a useful indicator to identify salinity tolerance of rice (Zhang et al., 1995; Gregorio et al., 1997 ; Ali et al., 2000 ; Flowers et al., 2000; Prasad et al., 2000; Koyama et al., 2001; Lang et al., 2001; Bonilla et al., 2002; Lin et al., 2004; Niones, 2004; Ren et al., 2005; Walia et al., 2005; Sabouri et al., 2009; Thomson et al., 2010; Muhamad et al., 2011; Linh et al., 2012; Islam et al., 2012; Neelam et al., 2013; Babu et al., 2014; Mardani et al., 2014; Chowdhury et al., 2016; Ganie et al., 2016, Camilla et al., 2017; and Anh et al., 2019).

In recent decades, many advanced techniques have been made to enhance tolerant rice varieties which can adapt to environmental stresses such as submergence, drought, chill, high temperature, low pH, salinity, etc. Nevertheless, it is not easy to overcome the challenges of rice salt-tolerant enhancement, because of its complex mechanism. Although phenotype is the eventual expression of molecular compositions, it is adversely influenced by environmental impacts through various physiological and biological processes. Therefore, a combination of genetic and phenotypic investigation was a prior technique for the selection of the most promising genotype.

To date, the screening of salinity tolerance of rice mutants using both morphological analysis and SSR markers is only carried out sporadically, as mentioned above. This study

was therefore conducted to evaluate salinity-tolerant abilities of mutant rice lines, and search for potent SSR markers for breeding rice cultivars tolerant to salinity. Although SSR markers have been applied in many earlier research, especially in marker-assisted selection, in this study, we conducted the genetic analysis in new rice mutants, which were created by "respiratory mutation". It is the first time that their salt tolerances were assessed. Additionally, our previous research pointed out that rice mutants expressed better panicle and grain characters than their parents, such as panicle number per plant, full grain per plant, grain weight, and grain yield per ha (Anh et al., 2019). Additionally, greater performances of protein, amylose, and lipid contents were also found in these mutants when compared with those of parents (Anh et al., 2019). Therefore, findings of this study are useful for the development of target rice varieties, which are not only of high yield and quality, but also resistant to abiotic stresses, to cope with climate change.

2.2. Materials and Methods

2.2.1. Plant Materials

Rice samples were provided by Khai Xuan International Co. Ltd. and Agricultural Genetics Institute (Hanoi, Vietnam). Mutant rice was created by chemical mutation using N-Nitroso-N-methylurea (MNU) (Anh et al., 2018). Rice seeds of origin cultivars were soaked in 150 mM MNU for 3 h before drying, and kept in hermetic conditions before storage at 4 °C. The first mutated generation (M_1) was self-pollinated to obtain the second (M_2) generation. M_2 was used for further works.

In this research, ten rice materials, including mutant lines and parental generations, were used to evaluate their salinity tolerance. Their origins and information are provided in Table 2.1.

Table 2.1. The origins and classifications of rice cultivars and mutant lines

Samples	Origins	Classifications
B1	Bao thai	Cultivar
B2	DT84	Cultivar
B3	Bao thai/DT84	Mutant
B4	DT84DB	Mutant
B5	SKLo	Cultivar
B6	SKLo/BC15	Mutant
B7	BC15	Cultivar
B8	Khang dan/Wild rice	Mutant
B9	Bao thai DB	Mutant
B10	BC15/TBR1	Mutant

Initially, rice seeds were soaked in 0.1% NaOCl for 30 min. After washing several times in distilled water, the seeds were immersed in water at 30 °C for 3 days for germination. The germinated seeds then were grown in a floating tray (2 seeds per hole) in plant growth chamber (28 °C day; 25 °C night; 12 h light; 12 h dark). Culture solution was supplied with Yoshida's nutrient (Gregorio, 1997). The solution was salinized at the seedling stage (10 days after sowing) by NaCl powder at four levels of electrical conductivity (EC): 0 dS m^{-1}, 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1}, which describe the levels of salinity that damage crop growth (0–4 dS m^{-1}: slight salinity, 4–8 dS m^{-1}: moderate salinity, 8–12 dS m^{-1}: severe salinity

(United State Department of Agriculture). The time for saline treatment was 21 days, a sufficient period to reveal differences in growth and physiological traits. Trays without NaCl were considered as control (0 dS m^{-1}). During treatment, EC and pH (5.5) of the culture solution were checked daily by EC and pH meters. The salinity level (EC level) was maintained at 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1} by dissolving NaCl powder in the solution. The culture solution was renewed weekly. The treatment was carried out in 21 days, and the experiment was tri-replicated.

2.2.2. Phenotypic Evaluation

After 21 days of treatment, salinity tolerance of 10 rice materials was scored by standard evaluation score (SES) (Flowers et al., 2000), as shown in Table 2.2. Survivability was determined by the percentage of survived plants after treatment. Root lengths and plant heights of rice samples were measured in millimeters with the longest root and leaf (sample size =10). Phenotypic measurements were replicated 3 times. After drying in a hot air oven for 5 days at 40 °C, each individual plant was weighed and expressed in grams. Reductions in root length, plant height, and dry weight caused by salinity stress were calculated by the following formula:

$$\% \text{ reduction} = (P_{sample} - P_{control})/P_{sample} \times 100$$

where P_{sample} is the value of the sample growing in salt concentration (average data of levels 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1}) and $P_{control}$ is the value of the sample in non-salinity.

Table 2.2. Modified standard evaluation score (SES) of injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves whitish and rolled	Tolerant
5	Growth severely retarded most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dried, some plants are dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

2.2.3. Genotypic Analysis

The total DNA of rice was extracted by the cetyl trimethylammonium bromide (CTAB) method (Anh et al., 2018). Before applying for polymerase chain reaction (PCR), 0.8% agarose gel electrophoresis was used to check the quality of DNA. Forty-two SSR markers linked to Saltol were collected from the Gramene database and previous studies (Zhang et al., 1995; Gregorio et al., 1997 ; Ali et al., 2000 ; Flowers et al., 2000; Prasad et al., 2000; Koyama et al., 2001; Lang et al., 2001; Bonilla et al., 2002; Lin et al., 2004; Niones, 2004; Ren et al., 2005; Walia et al., 2005; Sabouri et al., 2009; Thomson et al., 2010; Muhamad et al., 2011; Linh et al., 2012; Islam et al., 2012; Neelam et al., 2013; Babu et al., 2014; Mardani et al., 2014; Chowdhury et al., 2016; Ganie et al., 2016, Camilla et al., 2017; and Anh et al., 2019). DNA was amplified using a Thermal Cycler Gen Atlas S machine. Each reaction contained 0.75 µL genomic DNA (100 ng), 1.5 µL PCR buffer (10 X), 1.2 µL

$MgCl_2$ (25 mM), 0.15 µL dideoxynucleotides (dNTPs) (1 mM), 0.75 µL each of forward and reverse SSR primers (5 µM), and 0.05 µL Taq DNA polymerase (5 U). The volume was increased up to 15 µL by nuclease free water. PCR conditions included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55–62 °C for 1 min (based on each primer annealing temperature), extension at 72 °C for 2 min, and finished by a final extension at 72 °C for 7 min. The amplification products were resolved on 3 % agarose gel in TBE buffer (0.5 X) and 2.5 µL of safe view under room temperature, at a constant voltage of 50 volts for 75 min. After running, the gel was visualized by AMZ System Science limited STAGE system ECX-F15M (Vilber Lourmat, Eberhardzell, Germany).

2.2.4. Data Analysis

Thirty plants from the three replications were selected in a completely randomized design to evaluate the phenotypic measurements. Phenotypic data were analyzed by one-way analysis of variance (ANOVA), analysis of variance for salt-tolerance parameters was conducted by two-way ANOVA, Minitab software version 16. Tolerant and susceptible lines were evaluated for genotypic identification by SSR markers. Genetic banding patterns were scored based on the presence and absence of a particular band. Heterozygosity (He) and polymorphic information content (PIC) values were calculated with a PIC calculator (The University of Liverpool).

2.3. Results

2.3.1. Phenotypic Performance

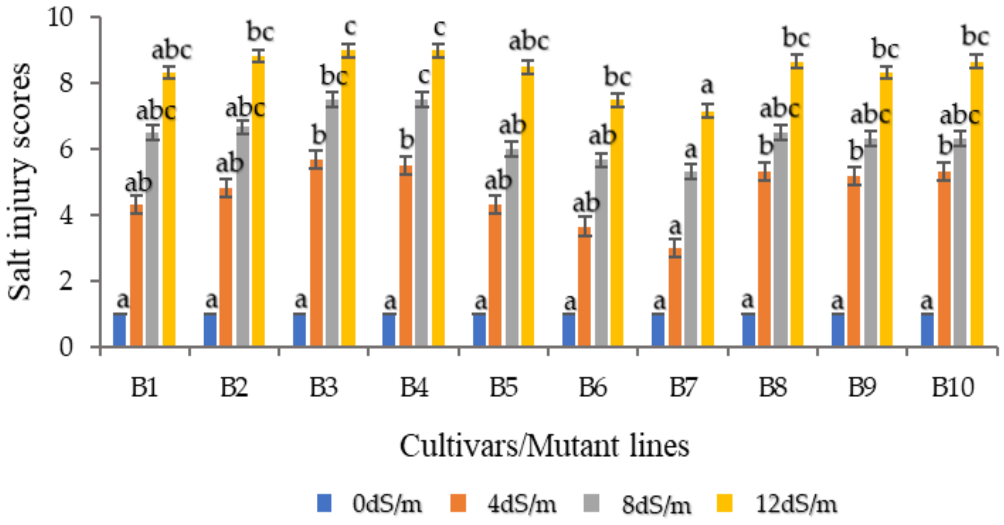


Figure 2.1. Salt injury scores of rice samples at different levels of salinity. Values represent mean of scores, Different letters in a salinity level indicate significant difference ($P<0.05$) by Tukey's test.

Reactions of rice plants to salinized solution showed prominent differences at the seedling stage (Figure 2.1). All the rice materials grew healthily in non-salinized conditions (0 dS m^{-1}). In salinized culture solutions, there was a classification of salt tolerance among 10 examined rice cultivars and lines. Results of measurements show that B7 had the lowest injury score in all three levels of salt stress (4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1}) (3.00, 5.33, and 7.17, respectively); followed by B6 with 3.67, 5.67, and 7.50, respectively. In contrast, the high scores were recorded in B3 (5.67, 7.00, 9.00) and B4 (5.50, 7.50, 9.50) at 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1}, respectively. Based on these results, B7 and B6 were determined as tolerant genotypes with lower scores than other samples, while B3 and B4 were susceptible.

Table 2.3. Survivability of samples under different levels of salinity stress.

Samples/ Salinity levels	Survivability (%)			
	0 (dS/m)	4 (dS/m)	8 (dS/m)	12 (dS/m)
B1	100	100	90.00 ± 0.00^{ab}	50.00 ± 0.00^{bc}
B2	100	100	86.67 ± 3.33^{abc}	46.67 ± 3.33^{c}
B3	100	100	73.33 ± 3.33^{c}	16.67 ± 3.33^{de}
B4	100	100	86.67 ± 3.33^{abc}	3.33 ± 3.33^{e}
B5	100	100	86.67 ± 3.33^{abc}	56.67 ± 3.33^{abc}
B6	100	100	93.33 ± 3.33^{ab}	63.33 ± 3.33^{ab}
B7	100	100	96.67 ± 3.33^{a}	66.67 ± 3.33^{a}
B8	100	100	83.33 ± 3.33^{abc}	46.67 ± 3.33^{c}
B9	100	100	80.00 ± 0.00^{bc}	43.33 ± 3.33^{c}
B10	100	100	83.33 ± 3.33^{abc}	26.67 ± 3.33^{d}

Values represent mean ± Standard error (SE). Different letters in a column indicate significant difference ($P<0.05$) by Tukey's Test.

Table 2.3 shows an extreme decrease in survivability of 10 genotypes after treatment. At all three levels of NaCl 4 dS m^{-1}, 8 dS m^{-1}, and 12 dS m^{-1}, survivability of B7 was the greatest (100%, 96.67%, and 66.6%, respectively); followed by B6 (100%, 93.33%, and 63.33%, respectively). B4 was found as the most salt-sensitive genotype, with only 3.33% plants survived at 12 dS m^{-1}. B3 was also susceptible to salinity condition, with 16.67% survived plants observed at the same salt level of 12 dS m^{-1}.

Table 2.4. Reductions of samples root length, plant height, and dry weight.

Samples	Reduction (%)		
	Root length	**Plant height**	**Dry weight**
B1	-5.88 ± 1.04^{cd}	11.15 ± 2.17^{ab}	29.66 ± 6.01^{ab}
B2	-2.21 ± 0.92^{c}	13.69 ± 3.68^{ab}	24.85 ± 4.18^{ab}
B3	2.90 ± 0.93^{ab}	20.34 ± 3.80^{a}	40.70 ± 4.90^{a}
B4	3.17 ± 0.67^{a}	16.31 ± 1.82^{ab}	29.76 ± 2.78^{ab}
B5	-5.51 ± 1.19^{cd}	10.83 ± 1.76^{ab}	17.07 ± 4.34^{b}
B6	-7.91 ± 1.19^{d}	8.57 ± 1.33^{b}	15.93 ± 3.56^{b}
B7	-7.92 ± 1.57^{d}	5.65 ± 0.78^{b}	14.42 ± 3.19^{b}
B8	-3.49 ± 0.88^{cd}	13.77 ± 1.95^{ab}	28.44 ± 5.34^{ab}
B9	-4.57 ± 1.42^{cd}	15.35 ± 1.41^{ab}	23.64 ± 3.64^{ab}
B10	-1.83 ± 0.39^{bc}	15.79 ± 2.97^{ab}	21.42 ± 2.80^{ab}

Values represent mean ± Standard error (SE). Different letters in a column indicate significant difference ($P<0.05$) by Tukey's Test.

Salt stress significantly impacts root length, plant height, and dry weight of examined rice (Table 2.4). In detail, the root length of B3 and B4 decreased, while root length of remaining rice increased. The largest root length reduction was recorded in B4 with 3.17%, followed by B3 with 2.9%. Conversely, roots of B7 and B6 developed by 7.92% and 7.91%, respectively. An extreme reduction was observed in plant height of rice materials under salinity stress (Table 2.4). The minimum decline was found in B7, followed by B6 (5.65%

and 8.57%, respectively). Plant height reduction of B3 was largest, with a percent of 20.34%, remarkably high compared to B4 (16.31%), as well as remaining samples. Similar phenomena were observed in dry weight decrease. Particularly, B3 and B4 presented noteworthy reductions in dry weight with 40.70% and 29.76%, respectively. On the contrary, B6 and B7 performed much lower percentages, in which B7 had the lowest value, at 14.42%.

Table 2.5. Analysis of variance for salt-tolerance parameters of rice.

Source of variation	Df	SIS	SUR	RLR	PHR	DWR
		Sum of squares				
Samples	9	91.93*	5298.90*	1255.35*	1447.31*	5155.20*
Salinity levels	2	128.67*	47426.00*	334.36*	2103.27*	10942.60*
Interaction	18	7.92	4902.20	87.12*	608.56*	1047.80
Error	60	5.16	2074.10	402.64	1254.22	723.30
Total	89	233.68	59701.20	2079.48	5413.36	17868.80

SIS: Salt injury score; SUR: Survivability; RLR: Root length reduction; PHR: Plant height reduction; DW: Dry weight reduction.

Under salt stress, variation of all parameters was highly significant in different samples at different salinity levels (Table 2.5). Results of analysis also show that salinity level is the most influential factor in the disparity of salt injury score, survivability, plant height, and dry weight reduction. Root length reduction was most affected by samples factor. Noticeably, there was a remarkable discrepancy observed in root length and plant height reductions of rice plants caused by the interaction of samples and salinity levels. No disparity

decrement was recorded in salt injury score, survivability, and dry weight reduction of examined rice, because of this combination.

Results of correlation analysis among five parameters are presented in Table 2.6. There are noticeable negative correlations between survivability and remaining parameters in which the maximum value was observed between survivability and root length reduction (−0.95). Additionally, the tolerant score was significantly positively correlated with root length, plant height, and dry weight reductions. The analysis also recorded the positive correlations between plant height and root length reductions, dry weight, and root length reductions, and dry weight and plant height reductions.

Table 2.6. Correlation matrix among tolerant parameters.

Parameters	**Parameters**				
	TS	**SUR (%)**	**RLR (%)**	**PHR (%)**	**DWR (%)**
TS	1				
SUR (%)	-0.93**	1			
RLR (%)	0.90**	-0.95**	1		
PHR (%)	0.94**	-0.87**	0.89**	1	
DWR (%)	0.79**	-0.70*	0.78**	0.82**	1

2.3.2. Genotypic Analysis

A set of 42 SSR markers were used to identify the differences between tolerant and sensitive genotypes. Among them, twenty-two markers showed clear DNA bands, and six markers were found to be polymorphic with amplified SSR loci, including RM 237, RM 518,

RM 493, RM 10748, RM 562, and RM 20224. All these markers could determine the difference between tolerant groups (B6 and B7) and susceptible groups (B3 and B4), as shown in Figure 2.2. SSR markers, location, number of alleles, heterozygosity (He), and polymorphism information content (PIC) are presented in Table 2.7.

Table 2.7. Polymorphic markers information.

SSR markers	Chromosome	Sequences*	Number of alleles	He	PIC
RM 237	1	CAAATCCCGACTGCTGTCC TGGGAAGAGAGCACTACAGC	3	0.65	0.58
RM 518	4	CTCTTCACTCACTCACCATGG ATCCATCTGGAGCAAGCAAC	2	0.49	0.37
RM 493	1	TAGCTCCAACAGGATCGACC GTACGTAAACGCGGAAGGTG	2	0.41	0.33
RM 10748	1	CATCGGTGACCACCTTCTCC CCTGTCATCTATCTCCCTCAAGC	3	0.65	0.58
RM 562	1	CACAACCCACAAACAGCAAG CTTCCCCCAAAGTTTTAGCC	2	0.41	0.33
RM 20224	2	AGTATGAAAGTCGGTGACGATGG GAGATGTCACGTCTTCACTTAGGG	3	0.65	0.58

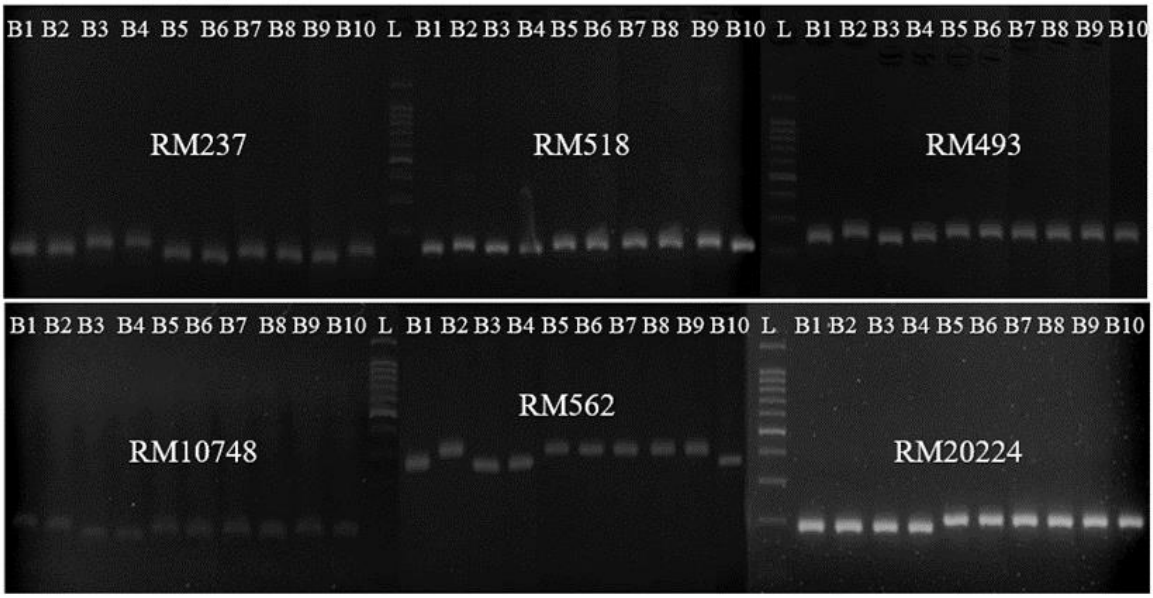


Figure 2.2. PCR products of SSR markers RM 237, RM 518, RM 493, RM 10748, RM 562 and RM 20224 (L: Ladder 100 bp).

2.4. Discussion

Salinity stress caused variations in survivability, plant height, root length, and dry weight of ten examined parameters. However, observed responses to salinity were different in different samples. Based on phenotypic performance, ten rice cultivars/lines can be classified into three groups: tolerant (B6 and B7), moderate-tolerant (B1, B2, B5, B8, B9, B10), and susceptible (B3 and B4) genotypes. The classification was carried out based on their performances in all measurements, including root length, plant height, and dry weight surveys, in which salt injury score and survivability are conclusive parameters. Notably, root length reductions of examined rice variably fluctuated. Negative values in root length reduction indicate that roots of these variety/mutant lines were elongated under saline treatment. This phenomenon maybe occurs because of their tolerant mechanisms adapting to salt concentrations. Previous researchers indicated that each rice variety has one or two salt

tolerance mechanisms, not all, and the response of plants to salt tolerance is a complex combination of individual factors (Yeo and Flowers 1984). Although Na^+ and K^+ contents in shoots and roots are different between tolerant varieties and sensitive varieties, these traits are mainly controlled by additive genes (Yeo and Flowers 1984). Therefore, the heritability of these traits is very low (Teng et al., 1994). On the other hand, the *Saltol* QTLs located on chromosome 1, which are tightly correlated with chlorophyll content and Na^+ and K^+ concentrations, as well as Na^+/K^+ ratios in rice shoots and roots (Thomson et al., 2010), were used for the genetic analysis. The results showed a clear distinction between tolerant genotype and susceptible genotype. Since phenotype represents the consequence of genotype–environment interactions ($P = G \times E$) in all living organisms, phenotype is the physical representation that positively correlates with genotype (under the same controlled environment). In this case, genes with specialized functions play a key role in a series of transcriptions and translations. Therefore, tolerant plants are able to protect themselves from saline damages by various physiological processes. Correlation analysis reflects that all morphological assessments contributed to salt injury score. Explanation of high correlation may be attributed to pleiotropic or linked genes (Makihara et al., 1999). Pleiotropism occurs when the expressions of more than one character are influenced by the same gene. In case of genes linkage, several genes located in the same chromosome are inherited together. This information is effective in rice breeding, due to the simultaneous selection of various expected characters based on the individual.

Although rice yield is adversely affected by salinity, rice has been considered as a suitable crop for reclamation of saline and sodic soil (Singh and Flowers, 2010), therefore, developing salinity tolerance in rice is a crucial key to ensure sustainable rice production.

Studies on rice tolerance mostly focus on seedling stage, because of its significant interaction with productivity (Yeo and Flowers 1984; Moradi et al., 2003; Singh and Flowers, 2010). Salt-tolerant rice at the seedling stage has a lower sodium concentration in maturity, compared to sensitive genotypes (Singh and Flowers, 2010); the desirable plants can be easily and quickly selected. On the other hand, rice salt-tolerant investigation in this stage is considered as a rapid method based on simple principles (Ali et al., 2014).

Salinity tolerance is a complex physiological trait, it is related to other traits (Walia et al., 2005) and controlled by different mechanisms (Moradi et al., 2003). Therefore, it is essential to evaluate rice genotypes. Application of molecular markers helps the breeders to select target plants in early stages by classifying genotypes based on the presence or absence of a particular marker locus and determine whether significant differences exist between them (Manikanda, 2013). Among these markers, SSR is considerable in accelerating rice breeding time (Collard et al., 2008). Therefore, SSR is the most widely used marker in major cereals (Garland et al., 1999; Gupta et al., 1999; Gupta and Varshney, 2000). Former research used SSR markers linked to the *Saltol* gene in chromosomes 1, 2, and 4, to identify tolerant as well as sensitive genotypes. In this study, genetic analysis was conducted by application of 42 Saltol-linked SSRs, distributed along 12 chromosomes. Among them, six markers, RM 493, RM 562, RM 10748, RM 20224, RM 237, and RM 518 are polymorphic, as they can classify tolerant and susceptible genotypes (others were not shown). Therefore, these markers are useful for classification of salt tolerance of rice. Similar results were observed in earlier research (Walia et al., 2005; Mohamadi et al., 2008); Muhamad et al., 2011; Linh et al., 2012; Islam et al., 2012; Neelam et al., 2013; Babu et al., 2014; Mardani et al., 2014; Chowdhury et al., 2016. The low polymorphic ratio may be because of the high genetic similarity between

examined rice. Analysis pointed out that these SSR markers expressed moderate polymorphic values. In particular, we found that they distinguish tolerant and sensitive rice in both mutant lines and cultivars. Although SSRs are co-dominant, they were scored as presence or absence markers, because examined cultivars (homozygous genotypes) are the result of breeding programs through various generations. On the other hand, homozygosity can be also obtained in rice mutants when they complete segregation.

It is significant to note that the mutant line originating from SKLo and BC15TB had higher yield than their parents (Anh et al., 2019). Also confirmed were their widely adapted abilities, as well as better characteristics, compared to parental performances (Anh et al., 2019). Furthermore, this mutant line was originated from elite cultivated parents in Northern Vietnam which are indicated to have good quality, high yield, and abiotic resistance (Successful Study: Bright Leaf Tolerant Variety BC15). Haplotypes developed from them are promised as valuable inheritances for crop improvement. However, the cross SKLo/BC15TB needs to be compared its characteristics with BC15TB, especially the production under saline stress. The further goal is to breed a new cultivar generation with higher quality, productivity, and wider adaptation to feasible conditions.

2.5. Conclusions

In this study, rice mutants were classified into three groups of salinity tolerance, including tolerant (SKLo/BC15TB, BC15TB), moderately tolerant (Bao Thai, DT84, SKLo, Khang dan/Wild rice, Bao Thai DB, BC15TB/TBR1), and susceptible groups (Bao Thai/DT84, DT84 DB). The markers RM 493, RM 562, RM 10748, RM 518, RM 237, and RM 20224 are the most polymorphic in salinity tolerance. Of them, RM 237, RM 10748, and

RM 224 show the highest polymorphism information (PIC = 0.58). The results suggest that BC15TB and its progeny BC15TB/SKLo are valuable sources for breeding rice tolerant to salinity. Findings of this study may help to simplify the breeding of salinity-tolerant rice to adapt to climate change.

CHAPTER III.

MATERNAL INHERITANCE OF SALINITY TOLERANCE AND BENEFICIAL PHYTOCHEMICALS IN RICE

3.1. Introduction

Salt is one of the main causes of land degradation worldwide with approximately 2000 million ha affected land being recorded every year, according to a study by Economics of Salt-Induced Land Degradation and Restoration (unu.edu/media-relations/releases). Salinity stress can reduce 70% yield loss of wheat, maize, rice and barley and the total cost of such loss in crop productivity can reach US $12 billion per year globally (Jaiswal et al.,2019). Half of the world population consumes rice (*Oryza sativa* and *Oryza glaberrima*) as a staple food (Menguer et al., 2017). By 2030, rice production needs to increase by 25% to feed the global population, however, rice growth and productivity is threatened by abiotic stresses including cold, drought, heat, flood, and salt (Menguer et al., 2017). Rice is one of the most salt sensitive crops with a threshhod of 3 dSm^{-1} for most currently cultivated varieties (Linh et al., 2016). At EC (electrical conductivity of its saturation extract) of 7.2 dSm^{-1}, 50% rice yield loss was recorded (Shrivastava and Kumar, 2015). Besides the effects from climate change, the dam construction on the main stream of important rivers such as the Mekong River in Southeast Asia has caused severe drought in the lower basin which induces the intrusion of sea water into the coastal deltas, resulting in serious reduction of rice yield (Yoshida et al., 2020). Therefore, the breeding of rice cultivars tolerant to salinity is required.

The salinity tolerance of rice has not been the priority in the breeding program until recent decades, where climate change including salinity stress is causing severe damage to

rice cultivation. Different from important agronomic traits such as growth, yield, quality, and pest tolerance, few genes/QTLs (quantitative trait locus) responsible for salinity tolerance have been discovered so far (Negrao et al., 2010). By conventional breeding, at least 10 years and huge cost (US $50–900 million) are required to develop salinity tolerant varieties (Jaiswal et al., 2019). Marker assisted selection (MAS) can reduce the breeding time to 3–6 years, however, it is difficult to identify all genetic markers relevant to QTLs/genes tolerant to salinity in rice. Among them, the *Saltol* QTL has been discovered as a principal QTL responsible for salinity tolerance in rice (Chowdhury et al., 2010; Thomson et al., 2010; Javed et al., 2011; Mardani et al., 2014). The introduction of the *Saltol* QTL to target rice cultivars have been extensively conducted (Babu et al., 2017), however, it causes great secessions from the F2 generation, which requires many cropping seasons (8–10 years), including both cross and backcross to stabilize the salinity tolerant trait (Babu et al., 2017; Xuan et al., 2019). The key genes involved in salinity tolerance are *OsCPK17, OsRMC, OsNHX1, OsHKT1;5*, and *SalT* (Negrao et al., 2012), in which *OsCPK17* increases a transient in cytosolic Ca^{2+} under salinity stress (Negrao et al., 2012); this gene also occurs with four QTLs linked to ion homeostasis (Negrao et al., 2011). *OsRMS* is a jasmonic acid-induced *DUF26* protein and is upregulated at the transcript level by high salinity (Rabbani et al., 2003). The two other principal genes, *OsHKT1;5* and *OSNHX1*, are involved in ion homeostasis to adjust osmotic pressure by keeping Na^+ away from the cytosol (Zhang et al., 2001). *OsHKT1;5* is responsible for retreiving Na^+ from the xylem sap to reduce Na^+ load in shoots and can balance low Na^+/K^+ shoot ratios in rice plants in salinity stress (Mian et al., 2011; Negrao et al., 2011). *OsNHX1* encodes a vacuolar (Na^+, K^+) H^+ antiporter placed in the tonoplast to allow efficient compartment of Na+ in the vacuole (Fukuda et al., 2004). In addition, *SalT* in the *Saltol* QTL,

which is localized in chromosome 1, is the first isolated and characterized key gene from the roots of salinity tolerant rice (Thomson et al., 2010). This gene is regulated by abscisic acid (ABA)-dependent and ABA-independent pathways, and it controls the expression with the production of osmoprotectants such as trehalose and proline (Negrao et al., 2012).

By cDNA microarray and RNA gel-blot analyses, 57 stress-inducible genes were found in rice; among them, 36, 62, and 43 genes were induced by cold, drought and ABA, respectively (Rabbani et al., 2003). Except for the five key genes *OsCPK17, OsRMC, OsNHX1, OsHKT1;5*, and *SalT*, many candidate genes might be also involved in salinity as well as other environmental stresses, although their functions still remain unknown. To date, all QTLs/genes determining important agronomic traits such as yield, quality, growth, pest and environmental tolerance are localized in the nucleus, while a few traits inherited from the reccurent parent (female cultivar) exhibited cytoplasmic effects in rice, including low temperature (Ratho and Pradhan, 1992), a low yield and width of the flag leaf (Tao et al., 2004), a low grain weight (Chandraratna and Sakai, 1960), a low protein content (Chang et al., 1974; Shi et al., 1996), chalkiness (Shu and Shen, 1988), a low cooking quality (Shi and Zhu, 1994), and low nutrient levels (Anh et al., 2018). The genetic variation in the cytoplasmic effects was only 2.41%–20.80%, whilst the maternal influence on the lysine content was greater than that on the protein content and index (Shi and Zhu, 1994).

In rice as well as other cereals, the environmental stress tolerant traits do not commonly associate with important agronomic traits, including yield and quality (Xuan et al. 2019). Therefore, conventional breeding results in great recombination and recessions of phenotypes and genotypes; laborious work and huge expenditure are required to select rice

lines which have acceptable yield and quality but are tolerant to pest and environmental stresses (Anh et al., 2018; Xuan et al., 2019). For instance, FL478 is a salt tolerant recombinant inbred line of IR66946-3R-178-11 developed from Pokkali (salinity tolerant) and IR29 (salinity susceptible), and it shows low quality parameters as compared with common commercial rice (Babu et al., 2017). Our laboratory observed that >97% kernel color of the FL478 was similar to that of Pokkali. In addition, the use of DNA molecular markers such as RFLP, RAPD, AFLP, ISSR, SSR, SNP, DarT and Retrotransposons are useful to reduce the time for selection, but they can not exhibit all QTLs/genes interactions involved in the selected traits (Anh et al., 2018; Xuan et al., 2019).

Our group has developed a protocol using N-methyl-N-nitrosourea (MNU) to treat rice seeds at low concentrations for three months prior to germination (Anh et al., 2018; Xuan et al., 2019). The MNU application induced uniparental inheritance of the yield attributing traits, including plant height, semi-dwarfism, amylose content, protein content, gel consistency, grain yield, and spikelet fertility (Anh et al., 2018; Xuan et al., 2019). In this study, we continuously examine whether the salinity tolerant *Saltol* QTL can also be uniparentally inherited by MNU treatment. The influence of the uniparental inheritance on induction of beneficial phytochemicals including total phenols and flavonoids, antioxidant activities, and momilactones A and B in the offspring lines were also evaluated.

3.2. Materials and Methods

3.2.1. Rice Materials and Salinity Treatment

TBR1 and KD18 are commercial rice in Vietnam. Both are *Indica* subtypes, where TBR1 performs a higher yield, protein content and lipid content compared to KD18. TBR1

is resistant and KD18 is susceptible to pests and diseases. In this study, TBR1 was used as the recurrent (female) cultivar, whilst KD18 was the male variety. They were provided by the Agricultural Genetics Institute, Hanoi, Vietnam. The field experiment was conducted near Hiroshima University, Higashi–Hiroshima, Hiroshima, Japan (270–280 m elevation; 33/25 °C day/night; humidity: 60%–65%; precipitation average: 1485 mm). The fertilizers, weeding, watering, and pesticides were provided by conventional methods in Japan. The original F_1 rice seeds (total 300 seeds) were obtained by crossing TBR1 (female) and KD18 (male) cultivars. Subsequently, the F_1 seeds were treated with the MNU as described previously (Anh et al., 2018; Xuan et al., 2019) to induce the first mutated generation M_1 (200 seeds). The M_1 seeds were kept in the dark for 3 months in a hermetic condition and stored at 4 °C before self-pollinating in a paddy field to obtain the second generation (M_2) seeds. The M_2 population (200 seeds) was continuously self-pollinated in rice fields to provide the third generation (M_3). After gemination, TBR1, KD18, F_1, F_2 (self-pollinated from F_1), M_2, and M_3 seeds were grown in a 0.5% agarose media supplied by Yoshida nutrient and placed in a plant growth chamber (28 °C day: 25 °C night; 12 h light: 12 h dark). Salinity was applied after five days of transplanting with a concentration of 12 dSm^{-1} NaCl to evaluate the salinity tolerant of the parental cultivars and progenies. The treatment without NaCl was considered as control (0 dSm^{-1}). During treatment, EC and pH (5.5) were checked and maintained daily.

3.2.2. Physiological Analysis of Salt Tolerance

After 21 days of treatment, salinity tolerant rice materials were scored by a standard evaluation score (SES) (Gregorio et al., 1997). Fifty plants from the five replications were

randomly selected to evaluate the phenotypic characteristics. Survivability was determined by percentage of survived plants. Root length and plant height of rice samples were measured with the highest one. Rice plants were weighed twice. Fresh weight was measured right after treatment and dry weight was determined after drying in hot air oven for 5 days at 40 °C.

3.2.3. Total Phenols, Total Flavonoids, and Antioxidant Activities

The roots, stems, and shoots of rice seedlings were harvested and transferred directly to laboratory. They were cleaned by tap water and rinsed many times with distilled water. After drying for 5 days at 40 °C, the mixture of roots, stems, and shoots of rice samples were ground into powder. This powder was then extracted by methanol after 3 days using a magnetic stirrer. After that, the extract was separated by hexane and finally dried by evaporator at 50 °C. The obtained powder was kept in methanol at 4 °C in the dark for further measurements. Total phenolic content (TPC) and ABTS•+ decolorization measurement were conducted following a method described by Quan et al., 2019a. Total flavonoid content (TFC) was estimated as detailed in Xuan et al., 2016. 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and nitric oxide (NO) scavenging assays were evaluated by a method described in Govindarajan et al., 2003. The antioxidant activity was calculated by the following formula:

$$\text{Antioxidant activity (\%)} = (1 - A_{sample}/A_{control}) \times 100\%$$

where A_{sample} is the absorbance of reaction with sample and $A_{control}$ is the absorbance of reaction with pure methanol. IC_{50} value is determined as the essential concentration to obtain 50% radical reduction.

3.2.4. Identification and Quantification of Momilactones A and B

Momilactone A (MA) and momilactone B (MB) were identified and quantified by UPLC-ESI-MS following a protocol described in Quan et al., 2019 using MA and MB purified from rice husk as the standards. The quantification of each momilactone was measured using a linear model through peak areas and retention times. Detection and quantitation limits of MA and MB were 0.68 and 2.05 ng/mL and 0.27 and 0.83 ng/mL, respectively.

3.2.5. Genetic Analysis

The total DNA in rice samples was extracted by cetyl-trimethyl ammonium bromide (CTAB) method (Xuan at al., 2019). Before applying for polymerase chain reaction (PCR), 0.8% agarose gel electrophoresis was used to check the quality of DNA. The DNA was amplified using a Thermal Cycler Gen Atlas S machine (Xuan et al., 2019). The amplification products were resolved in 3% agarose gel in tris borate EDTA (TBE) buffer (0.5 X) and 2.5 µl of safe view under room temperature at a constant voltage of 50 volts for 75 min. After running, the gel was visualized by AMZ System Science limited STAGE system ECX-F15M (Vilber Lourmat, Eberhardzell, Germany).

A total of fifty SSR markers which were reported previously to be involved in growth parameters, yield, pest resistance, and the *Saltol* QTL (salinity tolerant) (Wu et al., 2004; Fuentes et al., 2008; Nejad et al., 2008; Chowdhury et al., 2010; Thomson et al., 2010; Javed et al., 2010; Ashkani et al., 2011; Islam et al., 2012; Linh et al., 2012; Aliyu et al., 2013; Neelam et al., 2013; Mardani et al., 2014; Kioko et al., 2015; Nachimuthu et al., 2015; Singh

et al., 2015; Ganie et al., 2016; Boranayaka et al., 2018; Zhu et al., 2018; Wattoo et al., 2019; Xuan et al., 2019) were selected to evaluate the differences among genotypes of the recurrent TBR1 and male KD18 cultivars. These markers are distributed throughout 12 chromosomes of the rice genome (Supplementary Table S1). Twenty-one markers including RM 493, RM 518, RM 562, RM 1233, RM 10694, RM 10720, RM 10793, RM 10852, RM 13197, RM 149, RM 201, RM 202, RM 206, RM 207, RM 213, RM 219, RM 229, RM 432, RM 508, RM 587, and RM 589 are polymorphic (Supplementary Table S2), of which, RM 493, RM 562, RM 10694, RM 10720, RM 10793, RM 10852 are closely associated with the *Saltol* QTL located on chromosome 1. This QTL is identified for shoot Na+ concentration, shoot and root K^+ concentrations, and shoot and root Na-K ratio (Thomson et al., 2010). The marker RM 13197 is located on chromosome 2, which is influenced by seedling plant height, seedling survival, leaf chlorophyll content, and root K^+ concentration (Thomson et al., 2010). Markers RM 508, RM 587, and RM 589, which are associated with leaf diameter and root length, are located on chromosome 6 (Singh et al., 2015). Besides these, markers relevant to other *Saltol* QTLs, such as plumule (shoot) fresh weight and root Na–K ratio, are RM 149 (chromosome 8) and RM 201 (chromosome 9), respectively (Chowdhury et al., 2011; Javed et al., 2011). Other polymorphic markers were identified relating to growth parameter, grain yield and quality, and pest resistance, as well as water and nitrogen use efficiency (Ashkani et al., 2011; Aliyu et al., 2013; Boranayaka et al., 2018; Xuan et al., 2019). These polymorphic markers therefore were used to identify F_2, M_2 and M_3 genotypes.

3.2.6. Data Analysis

Chemical assessments were tr-replicated. Data were statistically analysed by analysis of variance (ANOVA). A linear model was used to calculate correlation between parental and progeny data. Reduction proportions of agronomical and chemical data were calculated by following formula:

$$\% \text{ reduction} = (P_{sample} - P_{control})/P_{sample} \times 100\%$$

where P_{sample} is value of sample growing in salt concentration 12 dS m^{-1} and $P_{control}$ is the value of sample in non-salinity.

3.3. Results

3.3.1. Salt Tolerance, Agronomical, and Phytochemical Performances of Rice Population

The results in Table 3.1 show that phenotypic performances among F_1 and F_2, and M_2 and M_3 are not remarkably different. In control conditions, no significant difference was observed in injury score, survivability, and growth parameters among TBR1, KD18, F_1, F_2, M_2 and M_3. In salinity stress condition, the injury score and survivability of the M_2 and M_3 were not significantly different from the recurrent parent TBR1. On the contrary, those of the F_1 and F_2 were between the values of the parents. The other growth parameters of the M_2 and M_3 including root length, shoot length, fresh weight, and dry weight were neither markedly different from TBR1 nor less affected from salinity than KD18 (Table 3.1), as they showed greater performances than those of the parents. In the control population (F_1 and F_2), these values were between the values of parental cultivars. Findings of table 1 indicate that the TBR1 cultivar was salinity tolerant whilst KD18 was salinity susceptible. The response

of M_2 and M_3 against salinity was as tolerant as that of the recurrent parent TBR1, thus, it was concluded that the M_2 and M_3 are also salinity tolerant. Therefore, the salinity tolerant characteristic of M_2 is uniparentally inherited from TBR1. To date, all reports in literature, such as Mishra et al., 1998 and Mohammadi et al., 2013, reported that the salinity tolerance trait in rice is polygenic, principally inherited from father cultivar following the Mendelian rules. However, in contrast, findings in table 1 show that the treatment of MNU can uniparentally control the salinity tolerant *Saltol* QTL in rice, conversely different from all research in literature.

Table 3.2 shows the changes of chemical components including TPC, TFC, and contents of MA and MB, and antioxidant activities (DPPH, ABTS, and NO levels). In the untreated conditions, the phytochemical contents and antioxidant activities vary among TBR1, KD18, F_1, F_2, M_2 and M_3, whilst no trace of MB was detected. Similar with phenotypic responses, no significant disparity was recorded in chemical compositions between F_1 and F_2, and M_2 and M_3. However, in the salinity stress condition, antioxidant activities of TBR_1, F_1, F_2, M_2 and M_3 were both significantly stronger than that of KD18, of which F_1 and F_2 show weaker antioxidant properties than TBR1.

Table 3.1. Phenotypic responses of examined rice in salinity stress.

NC	Samples	Injury score	SUR (%)	RL (mm)	SL (mm)	FW (mg)	DW (mg)
Control	TBR1	1.0±0.0d	100.0±0.0a	58.4±1.5ab	188.7±4.0a	83.0±3.0ab	25.0±1.0ab
	KD18	1.0±0.0d	100.0±0.0a	61.1±2.7ab	180.7±1.8ab	85.0±5.0ab	25.0±1.0ab
	F_1	1.0±0.0d	100.0±0.0a	61.1±1.8ab	181.5±2.5ab	86.0±3.0ab	25.0±1.0ab
	F_2	1.0±0.0d	100.0±0.0a	60.9±2.3ab	184.2±1.5ab	82.0±1.0ab	25.0±0.0ab
	M_2	1.0±0.0d	100.0±0.0a	60.9±1.0ab	187.6±4.6a	97.0±4.0a	27.0±1.0a
	M_3	1.0±0.0d	100.0±0.0a	62.7±1.5ab	185.6±2.3ab	92.0±3.0a	25.0±1.0ab
Stress	TBR1	5.9±0.2b	67.0±1.5b	63.1±3.0ab	160.0±4.4b	74.0±2.0bc	21.0±1.0bc
	KD18	7.2±0.1a	32.0±2.5c	52.7±2.4b	136.8±4.7d	60.0±5.0c	19.0±1.0c
	F_1	6.5±0.2ab	47.5±2.1bc	60.7±1.7ab	151.9±4.2c	68.2±4.0bc	20.0±1.0bc
	F_2	6.8±0.3a	50.8±1.5bc	60.5±4.5ab	155.6±4.5c	70.7±4.0bc	19.0±2.0c
	M_2	6.0±0.2b	59.0±1.0b	68.0±1.4a	165.7±4.0b	86.0±4.0bc	24.0±1.0ab
	M_3	6.0±0.5b	61.5±2.3b	65.1±3.5a	167.5±5.2b	88.6±3.0b	24.0±0.0ab

Values are means ± SE (standard errors); NC: Nutrition condition, SUR: survivability, RL: Root length, SL: Shoot length, FW: Fresh weight, DW: Dry weight; Mean with same letters in a column is not significantly different ($p < 0.05$).

Table 3.2. Changes in phytochemical properties of examined rice in salt stress.

NC	Samples	TPC (mg GAE g^{-1} DW)	TFC (mg RE g^{-1} DW)	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	NO (IC_{50} mg/mL)	MA (ng/g)	MB (ng/g)
Control	TBR1	1.6±0.1b	0.3±0.0d	1.5±0.1c	1.8±0.0b	0.9±0.0cd	59.9±1.3c	nd
	KD18	0.8±0.2c	0.4±0.0cd	2.4±0.1a	2.1±0.0a	1.8±0.1a	76.5±2.1b	nd
	F_1	1.6±0.2b	0.3±0.1d	1.9±0.0bc	1.9±0.2ab	1.5±0.1b	61.3±2.5c	nd
	F_2	1.5±0.1b	0.3±0.0d	1.9±0.1bc	1.9±0.1ab	1.5±0.1b	72.3±1.5bc	nd
	M_2	0.9±0.1bc	1.4±0.1b	2.0±0.0b	1.7±0.0b	1.1±0.1c	65.1±0.1c	nd
	M_3	0.7±0.2c	1.3±0.1b	1.9±0.0bc	1.7±0.1b	1.0±0.2c	67.8±0.5c	nd
Stress	TBR1	2.1±0.0a	0.6±0.0c	1.1±0.0d	1.0±0.0d	0.5±0.0d	21.4±2.7d	46.3±0.6a
	KD18	0.9±0.1bc	0.6±0.0c	2.3±0.1a	1.5±0.0c	1.5±0.1b	13.9±0.4d	nd
	F_1	1.5±0.0b	0.9±0.1bc	1.8±0.2bc	1.9±0.2ab	1.3±0.0bc	25.7±1.4d	nd
	F_2	1.5±0.2b	0.8±0.2bc	1.8±0.0bc	1.9±0.1ab	1.2±0.1bc	20.6±3.0d	nd
	M_2	1.5±0.1b	2.5±0.1a	1.1±0.1d	1.0±0.0d	0.7±0.0cd	104.7±3.1a	34.9±1.8b
	M_3	1.0±0.1bc	1.6±0.0ab	1.5±0.0c	1.2±0.1cd	0.5±0.2d	102.5±2.5a	20.5±1.5c

Values are means ± SE (standard errors); nd: not detected. TPC: total phenol content; TFC: total flavonoid content; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; ABTS: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; NO: nitric oxide; MA: momilactone A; MB: momilactone B. Mean with same letters in a column is not significantly different ($P < 0.05$).

No remarkable difference in antioxidant capacities between TBR1 and M_2 was observed (Table 3.2). The TPC and TFC values of the M_2, M_3, F_1, and F_2 individuals were either significantly higher or lower than that of its parent KD18 and recurrent parent TBR1 (Table 3.2). Subsequently, the content of MA in TBR1 was higher than KD18, but M_2 and M_3 show much greater content of MA than their parental cultivars do (104.7 and 102.5 ng/g, respectively). Interestingly, no trace of MB was found in KD18, but the salinity stress induced MB in TBR1 (46.3 ng/g), M_2 (34.9 ng/g), and M_3 (20.5 ng/g) (Table 2). Findings of Table 3.2 indicate that the MNU treatment also caused the uniparental inheritance of beneficial phytochemicals, including antioxidant activity and MA and MB from TBR1 to F_2 generation.

3.3.2. Correlation of Physiological Parameters between Progeny and Parental Lines

The distributions of examined traits of the M_2 population are shown in Figure 3.1. A high correlation of 0.81 ($R^2 = 0.6494$) was observed between M_2 and TBR1, while in contrary a much lower correlation of 0.45 ($R^2 = 0.2059$) was found between M_2 and KD18. The evidence indicates that agronomical and chemical properties of progenies are uniparentally inherited from the female cultivars.

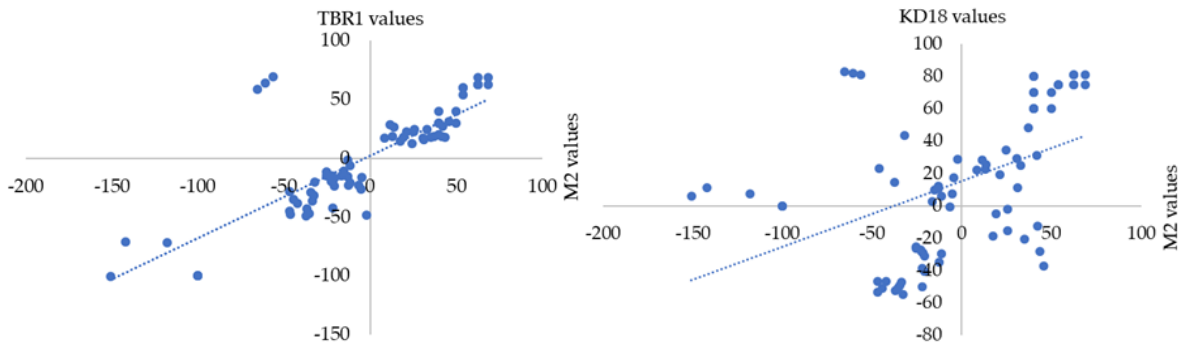


Figure 3.1. Distributions of phenotypic and chemical parameters between M_2 and TBR1 (R^2=0.6494); and M_2 and KD18 (R^2=0.2059).

3.3.3. Genetic Segregation of F_2, M_2 and M_3 Populations

A total of 228 rice plants were used to analyze the segregations in the F_2, M_2 and M_3 generations (Figure 3.2 and Figure 3.3). The results show that in the amplification of all the polymorphic SSR markers, the F_2 population possess both the male parent allele KD18 and the female parent allele TBR1 (following the Mendelian theory). However, both M_2 and M_3 generations are completely inherited (100%) from the recurrent parent TBR1 cultivar (Figure 3.3 a,b). It was observed that the preliminary treatment of MNU on the original seeds from the cross TBR1 × KD18 induced the salinity tolerance in the M_2 and M_3 genotypes, which are completely inherited from the recurrent parent TBR1 cultivar. The uniparental inheritance of salinity tolerance in the M_2 generation was completely stabilized in M_3 generation, with no segregation observed (Figure 3.3 b).

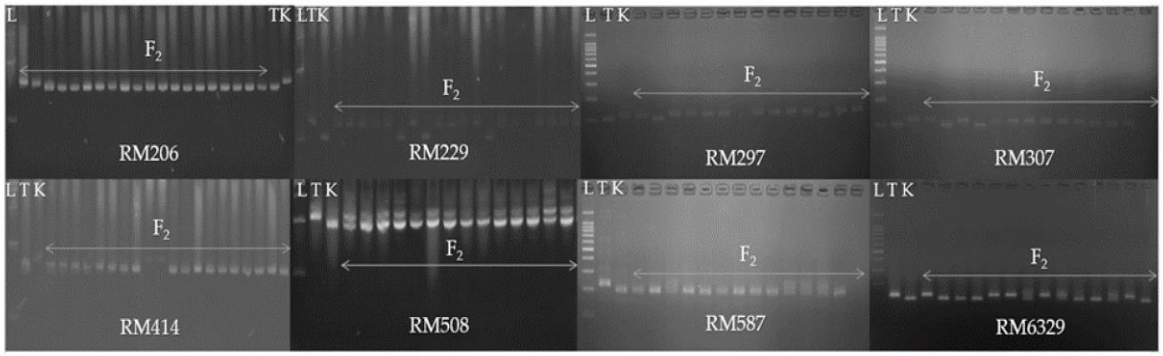


Figure 3.2. PCR products of F_2 generation by simple sequence repeat (SSR) markers RM206, RM229, RM297, RM307, RM414, RM508, RM587, and RM6329 (L: DNA ladder, T: TBR1, K: KD18, $F_{2:}$ F_2 progenies).

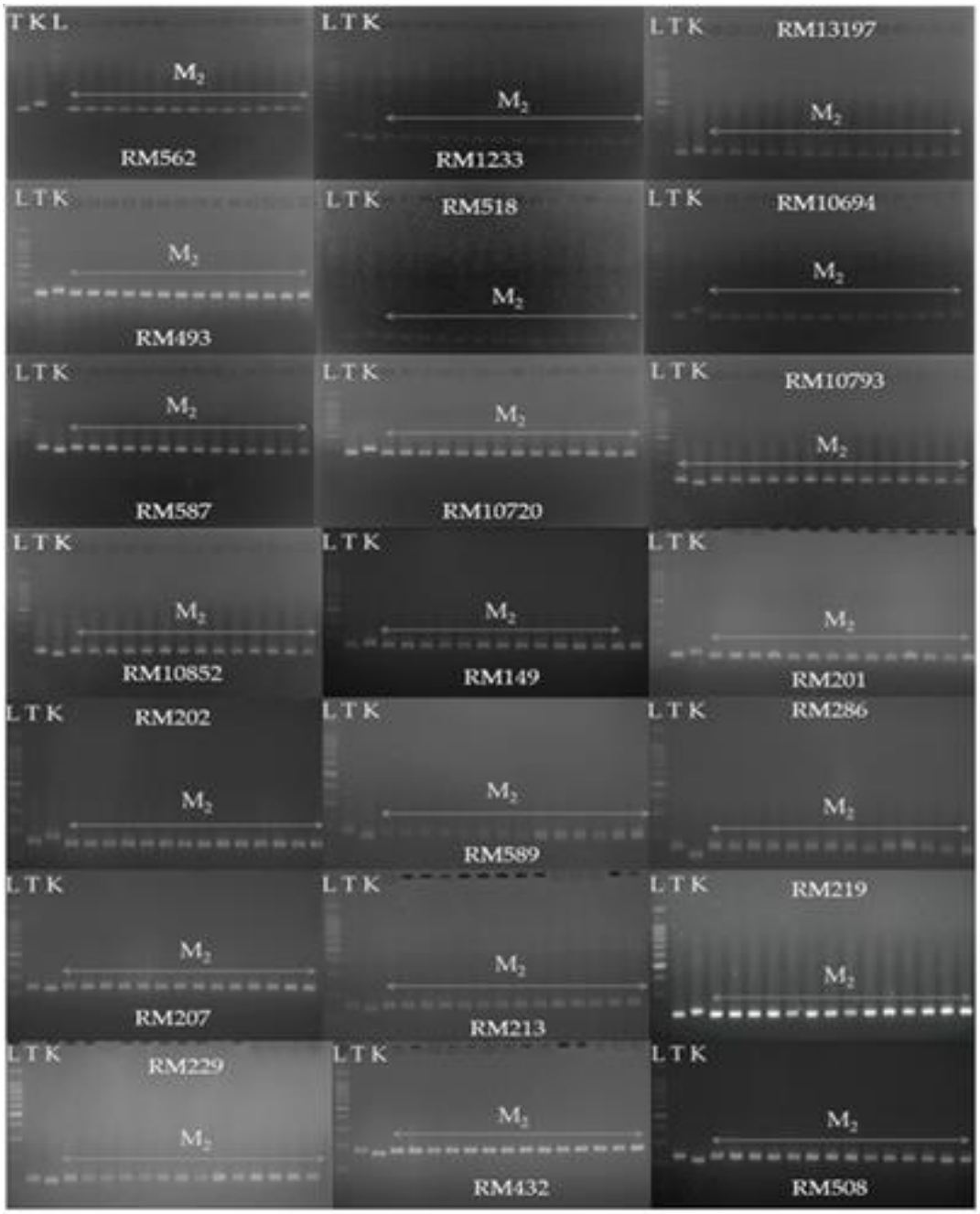


Figure 3.3. PCR products of M_2 population by SSR markers RM562, RM1233, RM13197, RM493, RM518, RM10694, RM587, RM10720, RM10793, RM10852, RM149, RM201, RM202, RM589, RM206, RM207, RM213, RM219, RM229, RM432, and RM508 (L: DNA ladder, T: TBR1, K: KD18, M_2: M_2 progenies).

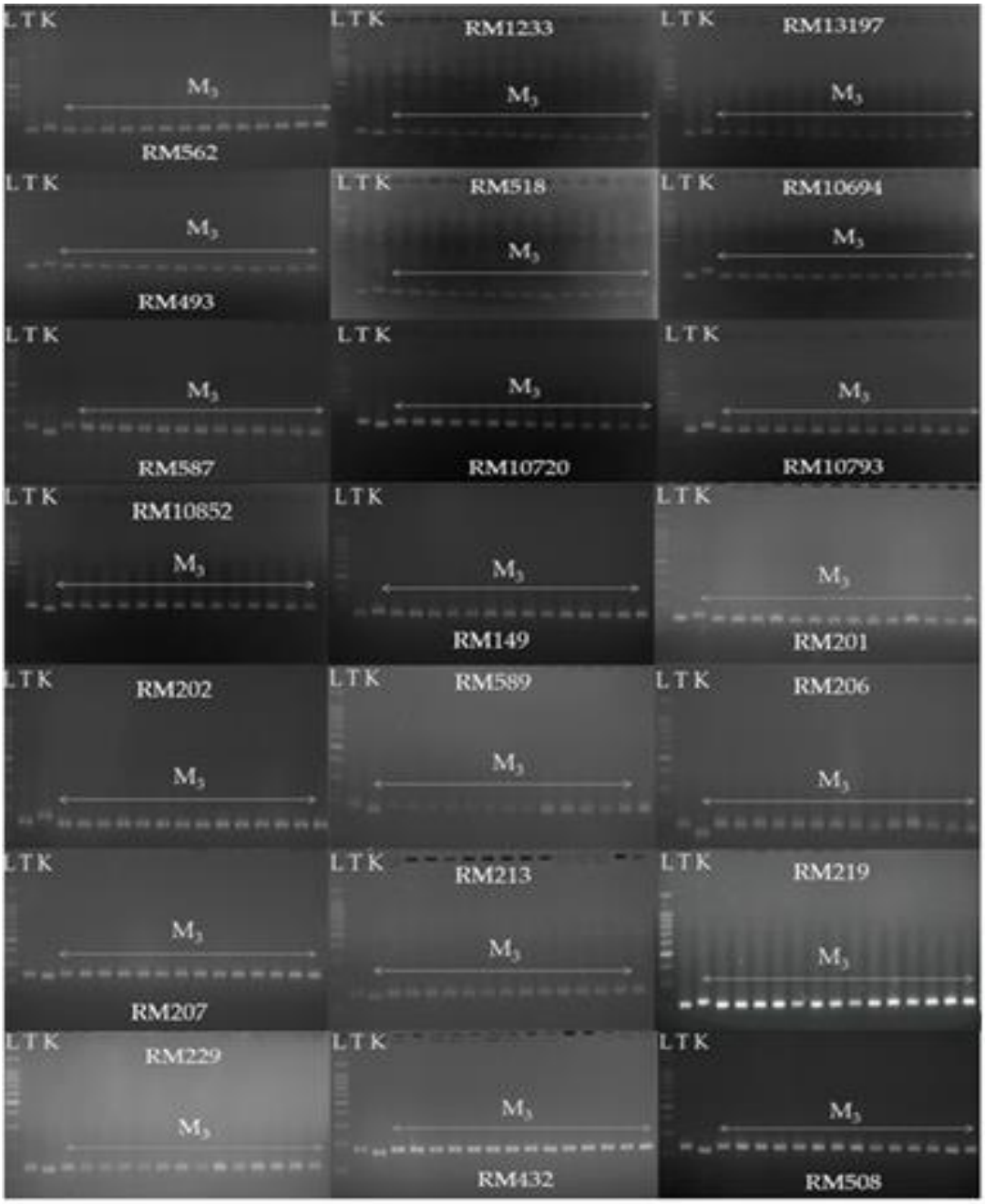


Figure 3.4. PCR products of M_3 population by SSR markers RM562, RM1233, RM13197, RM493, RM518, RM10694, RM587, RM10720, RM10793, RM10852, RM149, RM201, RM202, RM589, RM206, RM207, RM213, RM219, RM229, RM432, and RM508 (L: DNA ladder, T: TBR1, K: KD18, M_3: M_3 progenies).

3.4. Discussion

The preliminary treatment of MNU on the original seeds from the cross TBR1 × KD18 induces the uniparental inheritance of salinity tolerant *Saltol* QTL from TBR1 to the M_2 and M_3 generations in both phenotypes (Table 1 and Table 3.2) and genotypes (Figure 3.2 and Figure 3.3). The genotype of the M_3 was completely similar to M_2 (Figure 3). Following the salinity tolerance, antioxidant activities and contents of MA and MB were also uniparentally inherited (Table 3.2), although the associated genetic markers with QTLs/genes determining antioxidant activities and biosynthesis of MA and MB were not examined in this study.

In this study, the F_1 seeds are the progenies of the cross between TBR1 (female variety) × KD18 (male variety), and the M_1 generation was induced by treating F_1 with MNU treatment. The M_1 was self-pollinated to obtain M_2, and M_3 was induced by the M_2 self-pollination. The purpose of this was to check the stability of uniparental inheritance of the salinity tolerant *Saltol* QTL from the mother variety. In conventional breeding, the salinity tolerant cultivar should be the father cultivar. Because the salinity tolerant characteristics are determined by multiple genes/QTLs, they follow the Mendel rules with complicated segregation from the F_2 generation. Therefore, to stabilize the salinity tolerance, F_2 seeds are commonly crossed with the father variety (backcross) and repeated in many generations which requires huge amounts of fieldwork, time, and money (Pandit et al., 2010; Tiwari et al., 2016; Babu et al., 2017; Leon et al., 2017; Kumar et al., 2018; Singh et al., 2018; Xuan et al., 2019). All previous work used the donor (male) cultivars having the *Saltol* QTL in the breeding to introduce the salinity tolerance to the target cultivars. For instance, Babu et al., 2017 developed a rice line Pusa1734-8-3-3 having good salinity tolerance in the seedling stage compared with the FL478 donor (father cultivar). The salinity tolerance of Pusa1734-

8-3-3 was the BC_3F_4+ (>7 generations) from the cross Pusa Basmati 1121 (female cultivar) and FL478 (male cultivar). Similarly, Leon et al., 2017 developed different salinity tolerant introgression lines (Ils) from the BC_4F_4 (eight generations), but the salinity tolerance characteristic has not yet been stabilized. The treatment of MNU in this study induced the uniparental inheritance for the *Saltol* QTL, providing a simple protocol to develop rice lines tolerant to salinity.

To date, almost all known QTLs involved in salt-tolerance are located on chromosome 1 (Thomson et al., 2010). In this study, the selected nine SSR markers relevant to salinity tolerant are polymorphic and stationed on chromosomes 1, 2, 3, 4, 5, 7, 8, 10 and 11, in which the major SSR markers are located on the chromosome 1 (Supplementary Table S1). In this study, we did not carry out a vast screening on many SSR markers but selected only fifty known SSR markers related to important agronomic, pest resistant and salinity tolerant *Saltol* QTL, which have been already reported in previous research (Supplementary Table S1). These markers were used to distinguish the difference in parental genotypes associated with the phenotypes observed in Table 3.1. Subsequently, twenty-one SSR markers were found to be polymorphic and involved in salinity tolerant and elite agronomic traits (amylose content, plant height, spikelet fertility, brown plant hopper resistance, blast disease resistance) (Supplementary Table S1). Therefore, they were used to examine the F_2, M_2, and M_3 individuals. However, only nine SSR markers involved in *Saltol* QTL are polymorphic, including seven SSR markers locate on chromosome 1 (RM493, RM562, RM10694, RM10720, RM10793, RM10852, and RM13197), and two markers RM201 and RM149 located on chromosome 8 and 9, respectively (Chowdhury et al., 2010; Javed et al., 2011; Mardani et al., 2014) (Figure 3.2; Supplementary Table S2). Other polymorphic markers were

identified relating to plant height (RM202, RM206, RM219, RM229) (Xuan et al., 2019), leaf diameter (RM508) (Aliyu et al., 2013), spikelet fertility (RM202, RM206) (Xuan et al., 2019) spikelet number (RM229) (Xuan et al., 2019), grain yield (RM219, RM432) (Xuan et al., 2019), amylose content (RM219, RM432, RM508, RM587, RM589), gel consistency (RM589) (Xuan et al., 2019), blast resistance (RM1233, RM206, RM207, RM213) (Aliyu et al., 2013;Xuan et al., 2019), brown planthopper resistance (RM206) (Xuan et al., 2019), and water and nitrogen use efficiency (RM518) (Boranayaka et al., 2018) (Supplementary Table S2). Other SSRs relevant to the *Saltol* QTL are not polymorphic and they could not be used to identify uniparentally controlled by MNU treatment. Further treatments of MNU should be applied to enable the uniparental inheritance of more salinity tolerant genes/QTLs, such as *OsCPK17, OsRMC, OsNHX1, OsHKT1;5* genes (Negrao et al., 2012), except the *SalT* gene derived from the *Saltol* QTL.

It is proposed that only these nine SSR markers relevant to the *Saltol* QTL are responsible for the salinity tolerance of the TBR1 × KD18 cross, and momilactones MA and MB are determined by five known biosynthesis genes (*AK103462:* dehydrogenase gene and *CYP99A2* and *CYP99A3*: *P-450* genes form a chitin oligosaccharide elicitor, together with *OsKS4* and *OsCyc1*, the diterpene cyclase genes) clustered in chromosomes 2 and 4 (Shimura et al., 2007). Following the Mendelian genetic rules, because all these genes are located on chromosomes in the rice nucleus, the segregation ratios should be theoretically 1/1024 (5 genes) × 1/262,444 (9 genes) = 1/268,435,456 recombinants. Therefore, this study helps to reduce the huge laborious work, cost, and long breeding time that conventional breeding requires (Anh et al., 2018; Xuan et al., 2019).

In our previous study, a total of 28 polymorphic SSR markers were selected from 200 markers relevant to plant height, semi-dwarfism, amylose content, protein content, gel consistency, grain yield, and spikelet fertility were genotyped on the second generation of similar cross TBR1 (mother) × KD18 (father) (Xuan et al., 2019). These markers are distributed on chromosomes 1–7, 9, and 11. All phenotypes and genotypes of the abovementioned growth and quality characteristics were uniparentally inherited in the second generation (Xuan et al., 2019). In addition, PCR results showed identical results of this study, indicating that all the 28 SSR markers were completely inherited from the recurrent TBR1 cultivar, and no segregation was observed (Xuan et al., 2019). Combining the results obtained from this research, it can be concluded that by the prior treatment of MNU on F_1 seeds, important growth, quality traits, and the salinity tolerant *Saltol* QTL of the TBR1 cultivar can be uniparentally inherited to the F_2 and F_3 generations. Study on the ability of this promising rice source on other abiotic stresses is suggested. However, the subsequent generations should be strictly examined to ensure the stability of the uniparental inheritance. The mechanism of inducing this novel uniparental inheritance for salinity tolerance by MNU application needs to be further analyzed.

This study also shows that in salinity stress, rice plants promote the levels of antioxidant activities as well as content of MA and MB (Table 3.2). In a non-treated control, no trace of MB was observed, but in the salinity treated condition, TBR1, M_2, and M_3 induced MB as well as promoted MA content (Table 3.2). Although it is observed that phenotypically, antioxidant activities and induction of MA and MB in rice plants are also uniparentally inherited, polymorphic SSR markers related to antioxidant potentials and biosynthesis of MA and MB should be screened to evaluate whether their genotypes can be also uniparentally

inherited or not. However, this study highlights that the preliminary treatment of MNU can also induce levels of antioxidant activities and important phytochemicals including MA and MB in rice, and the induction of antioxidant activities and MA and MB is also uniparentally inhibited to the second generation (Table 3.2).

MA and MB were first isolated and identified by Kato et al., 1973 who reported that these two compounds are the growth inhibitors (allelochemicals) and phytoalexins (Cartwright et al., 1981; Toyomasu et al., 2008) in rice. For more than 40 years since 1973, scientists worldwide have acknowledged that MA and MB are allelochemicals and conducted various experiments on examining quantities of MA and MB in different rice cultivars, as well as in rice organs and the release from roots (Fukuta et al., 2007; Kato-Noguchi et al., 2010). However, the application of allelochemicals including MA and MB is questionable as the released amounts from the root leaches and plant parts are too low to inhibit growth of weeds, although they showed promising reduction on weed growth in laboratory experiments (Xuan et al., 2016). For MA and MB, we recently found that the two compounds are involved in drought and salinity tolerance in rice rather than allelochemicals (Xuan et al., 2016). In addition, MA and MB have the potential to control diabetes (Quan et al., 2019b), cancers (Chung et al., 2005; Kim et al., 2007; Joung et al., 2008), and skin diseases (Quan et al., 2019c). In this study, MB was not found in the control condition, however the salinity stress induced MB in the recurrent parent (TBR1), M_2, M_3, and MA in all tested samples (Table 3.2). Therefore, it is concluded that treatment of MNU induced uniparental inheritance for both the salinity tolerant *Saltol* QTL, as well as beneficial phytochemicals, including antioxidants and MA and MB in rice.

3.5. Conclusions

Findings of this study reveal that the principal salinity tolerant *Saltol* QTL trait in rice can be uniparentally controlled, along with beneficial phytochemical antioxidants and MA and MB in rice. The treatment of MNU aids to speed up and simplify the breeding of new rice cultivars, having both elite agronomic traits and tolerance to environment stresses. The question of whether QTLs/genes other than the *Saltol* QTLs such as *OsCPK17, OsRMC, OsNHX1, OsHKT1; 5* genes can also be uniparentally inherited by MNU treatment should be further examined.

CHAPTER IV.

IMPROVEMENT OF SALINITY TOLERANCE IN RICE BY EXOGENOUS MAGNESIUM APPLICATION

4.1. Introduction

In the upcoming years, climate change is predicted to continuously affect a large area of agricultural cultivation. Among the adverse consequences of climate change, sea level rise is the main cause of salt intrusion and salinity stress, which have threatened food production. Rice is one of the most vulnerable crops because it is highly sensitive to salt conditions. Extremely high salt concentrations may lead to plant death through the combined effects of osmotic homeostasis, ionic homeostasis, and oxidative stress (Shannon 1985). At high-moderate concentration, salinity causes leaf burn and growth inhibition or even leads stomatal closure and reduces leaf size at the levels from low to moderate salinities (Shannon, 1985; Tester and Davenport, 2003). These influenced physiological processes affect all growth parameters, as a consequence, salt stress reduces rice productivity.

Plant growth and development are adversely inhibited by toxic effects induced by salinity stress. Salt stress increases the formation of reactive oxygen species (ROS), which disrupts the antioxidant defense system and consequently causes oxidative stress. Developing ionic and osmotic balances as well as detoxifying ROS are some salt-stress tolerances of plants (Wu et al., 2012; Hasanuzzaman et al., 2013; Mishra et al., 2013). Plants have an antioxidant defense system to detoxify the overproduce of ROS. Besides, plants can produce the endogenous compounds to sustain osmotic balance by maintaining the water status and stabilizing protein and enzyme complexes (Iqbal et al., 2015).

Phenolics are secondary metabolites that are involved in stress protection of plants. The structure of phenolic compounds commonly is presented by one or more hydroxyl substituents attached directly to one or more benzene rings. According to the structures, phenolicscan be grouped into phenolic acids, flavonoids, stilbenoids, and lignans. Phenolic acids are considered as powerful antioxidants that can scavenge the damage from overproduced ROS in plants under abiotic stress (Bistgani et al., 2019). Particularly, the role of endogenous phenolic acids in plant-tolerant mechanism against abiotic stresses has been recorded in many plant species (Mahdavi et al., 2015; Sharma et al., 2016; Sharma et al., 2019; Wang et al., 2019). Additionally, it has been shown that other secondary metabolites momilactones A and B are involved in drought and salinity tolerances of rice (Xuan et al., 2016).

Application of exogenous phytoprotectants are impressive strategies to reduce salt-induced damages in plants. The roles of phytoprotectants including regulating antioxidant defense to detoxify overproduced ROS, improving ionic and osmotic balances have been reported in previous studies (Rahman et al., 2017). Magnesium (Mg) is the second most abundant element that is involved in basically all metabolic pathways (Hartwig et al., 2001). As a central atom of chlorophyll molecules, Mg is important for photosynthesis. Besides, Mg is used for energy synthesis, enzyme activators, ion transporters, and maintaining osmotic balance (Soetan et al., 2010). However, plant Mg nutrition has been consistently overlooked by botanists and agronomists in past decades, unlike other ions such as iron (Fe), zinc (Zn), iodine (I) and selenium (Se) (Guo et al., 2016). Especially, the application of Mg in reducing salt-induced damage in rice has not been well studied.

Considering the strategies discussed, this study was conducted to identify the positive potential of supplemental magnesium to develop salt tolerance in rice. The growth parameters, water status, antioxidant activities, phenolic profiles, and the amounts of momilactones A and B of rice seedlings under salt stress were also investigated.

4.2. Materials and methods

4.2.1. Materials and treatments

From the results in chapter 2, salinity tolerant cultivar BC15 and susceptible rice DT84DB were selected for experiment in this chapter. List of samples and treatments are provided in Table 4.1.

Table 4.1. List of samples and treatments.

Samples	Treatments
C1	BC15-Control
C2	BC15-Stress
C3	BC15-Stress ($MgSO_4$ 0.5 mM)
C4	DT84DB-Control
C5	DT84DB-Stress
C6	DT84DB-Stress ($MgSO_4$ 0.5 mM)

Rice seeds were soaked in 0.1% NaOCl for 30 min. After washing several times in distilled water, the seeds were immersed in water at 30 °C for 5 days for germination. The germinated seeds then were grown in a plant growth chamber (28 °C day; 25 °C night; 12 h light; 12 h dark). Culture solution was supplied with Yoshida's nutrient (Gregorio, 1997). The solution was salinized at the seedling stage (14 days after sowing) by NaCl powder at concentration 100 mM (moderate salinity, United State Department of Agriculture). Magnesium sulphate ($MgSO_4$) 0.5 mM was supplemented for rice seedlings in this step (from a pre-investigation conducted at 3 levels of $MgSO_4$ 0.25, 0.5, 0.75 mM, the concentration 0.5 mM of $MgSO_4$ showed the best result in reducing salt damages to rice growth and development, therefore, it was applied in the experiment). Trays without NaCl were considered as control (0 dS m^{-1}).

4.2.2. Phenotypic and chemical measurements

After 7 days of treatment, salinity tolerance of rice materials was scored by standard evaluation score (SES) (Flowers et al., 2000). Their growth parameters including root length, plant height, fresh weight, and dry weight were also measured. Root length and plant height of samples were measured with the longest root and leaf. Dried biomass of samples was extracted with methanol in 3 days using a magnetic stirrer. The extract was then separated by hexane and finally dried by evaporator at 50 °C. The obtained powder was kept in methanol at 4 °C in the dark for further measurements. Chemical analyses including total phenolic content (TPC), total flavonoid content (TFC); and antioxidant activities DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid),

NO (nitric oxide) assays of samples were conducted. Protocols for the chemical assessments were provided by Govindarajan et al., 2003; Xuan et al., 2016; and Quan et al., 2019a.

4.2.3. Identification and quantification of phenolic compounds, momilactones A and B

Twelve phenolic and relative compounds including gallic acid, catechol, protocatechuic acid, caffeic acid, chlorogenic acid, ρ-coumaric acid, salicylic acid, cinnamic acid, benzoic acid, and ferulic acid were used as standards. Phenolic profiles of samples were identified and quantified by High Performance Liquid Chromatography (HPLC) method (Quan et al., 2019b) with the following conditions: pump: PU-4180 RHPLC; controller: LC-Net II/ADC controller; detector: UV-4075 UV/Vis (Jasco, Tokyo, Japan); stationary phase: XBridge BEH Shield RP18 (USA); mobile phase: solution A (0.1% aqueous formic acid) and solution B (100% acetonitrile); program: 5% B during 0–2 min, 5-70% B during 2–12 min, 100% B from 12–16 min and maintained for 6 min, 100% B to 5% during 22–24 min, equilibration: 10 min; flow rate: 400 µL/min. The operation was carried out in 35 min under room temperature (24–26 °C). Momilactones A and B were identified and quantified by UPLC-ESI-MS (Quan et al., 2019a). Identification and quantification of phenolics compounds and momilactones A and B were carried out based on corresponding peak and its area compared to the curve built from the standard chemicals.

4.2.4. Data analysis

Physiological and chemical measurements was conducted with 3 replications. Data were analysed by analysis of variance (ANOVA).

4.3. Results

4.3.1. Phenotypic Performances

Table 4.2. Phenotypic responses of rice under salt stress.

Samples	Injury score	Root length (mm)	Plant height (mm)	Fresh weight (mg)	Dry weight (mg)
C1	1.00±0.00e	64.93±0.55ab	190.67±1.63a	97.57±0.64a	41.43±0.67a
C2	3.43±0.1c	65.37±1.44a	170.83±2.26b	79.67±0.58c	36.00±1.00bc
C3	3.00±0.00d	65.30±0.38a	175.10±2.70ab	90.93±0.41b	38.97±2.37b
C4	1.00±0.00e	59.80±2.17bc	187.43±3.60a	96.56±0.58a	39.67±0.41a
C5	5.23±0.07a	58.03±0.70c	145.63±0.20c	70.47±0.58d	26.67±0.88d
C6	4.73±0.03b	60.00±0.56abc	166.97±2.32bc	80.60±0.90c	28.57±1.33c

Values represent mean ± standard errors (SE); different letters in a column indicate significant difference ($P < 0.05$) by Tukey's test.

Salinity treatment decreased plant growth performances (root length, plant height, fresh weight, and dry weight) of all rice samples, in which, the changes in phenotypic responses of tolerant cultivar were smaller compared to those of susceptible rice (Table 4.2). Under salt stress, the injury score of C3 was lowest (3.00) while the highest score (5.23) was found in C5. C3 and C6 had better growth performances compared to C2 and C5, respectively. The results showed that samples performed lower injury score and better growth parameters with magnesium application.

4.3.2. Chemical Performance

Table 4.3. Total phenolic, total flavonoid contents, and antioxidant activities of rice under salinity stress.

Samples	TPC (mg GAE g^{-1} DW)	TFC (mg RE g^{-1} DW)	DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	NO (IC_{50} mg/mL)
C1	0.93±0.01b	0.26±0.00d	0.96±0.01c	1.24±0.02c	1.53±0.05c
C2	1.47±0.01a	0.41±0.00c	0.74±0.01b	0.97±0.01b	1.09±0.02a
C3	1.56±0.02a	0.64±0.01ab	0.37±0.07a	0.67±0.03a	0.79±0.06a
C4	0.96±0.01b	0.65±0.01a	2.05±0.01e	2.57±0.01f	2.89±0.05d
C5	0.76±0.02c	0.35±0.01c	1.54±0.04d	2.21±0.04e	2.29±0.08c
C6	0.89±0.04bc	0.58±0.01b	1.50±0.04d	2.05±0.01d	1.96±0.05c

Values represent mean ± standard errors (SE); different letters in a column indicate significant difference ($P < 0.05$) by Tukey's test.

Total phenolic content and antioxidant activities of tolerant variety were better than those of sensitive rice (Table 4.3). C3 had the highest total phenolic content (1.56 mg GAE g-1 DW) while the biggest total flavonoid content was found in C4 (0.65 mg GAE g-1 DW). C4 performed the strongest antioxidant capacities (in DPPH, ABTS, and NO assays). It was also found that rice plants obtained higher total phenolic content, total flavonoid content, and stronger antioxidant activities with Mg supplement (Table 4.3). Especially, antioxidant properties increased in tolerance but decreased in susceptible rice under salt stress.

4.3.3. Phenolic profiles

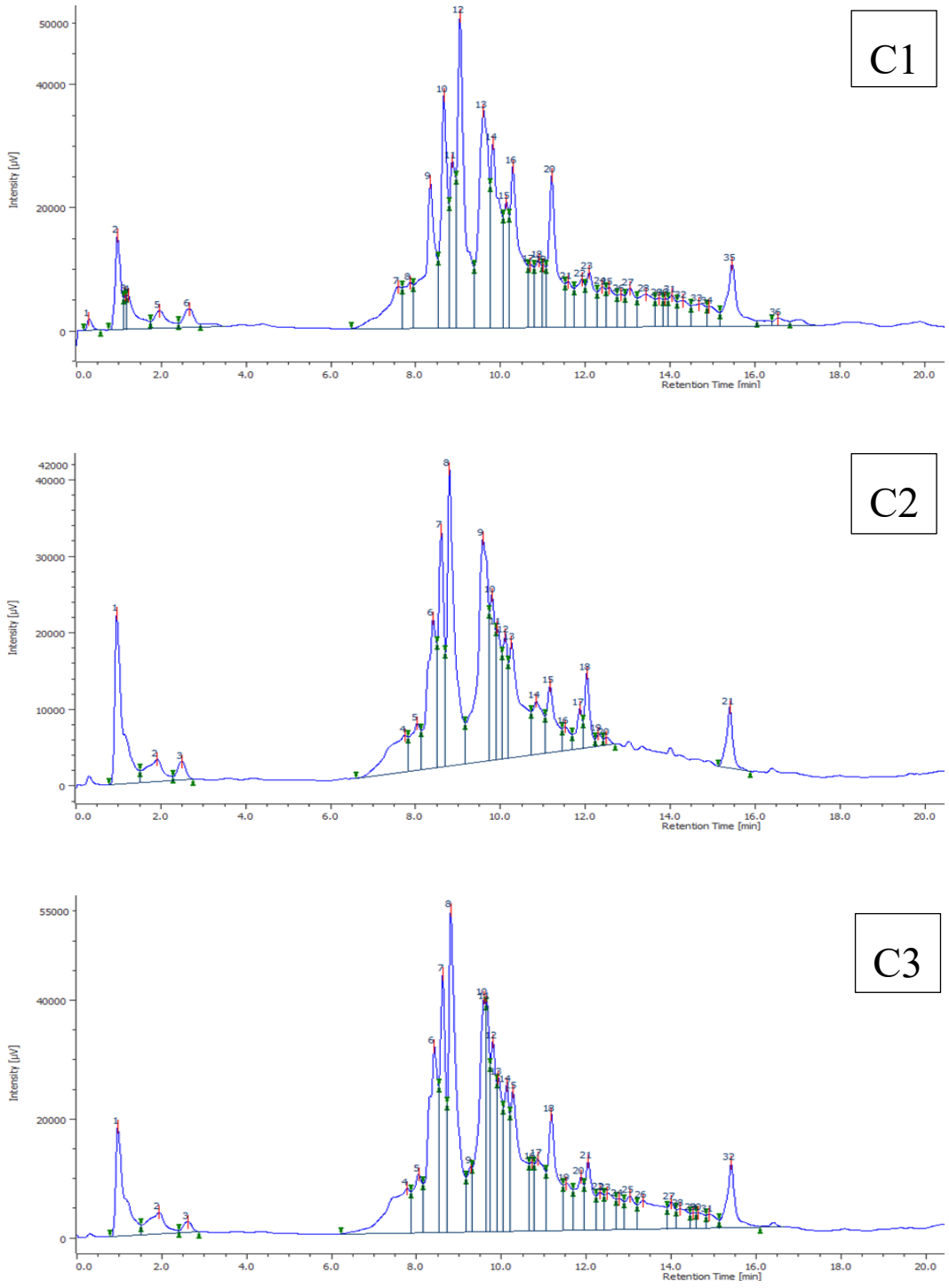


Figure 4.1. HPLC chromatograms of salinity tolerant rice BC15 at different treatments.

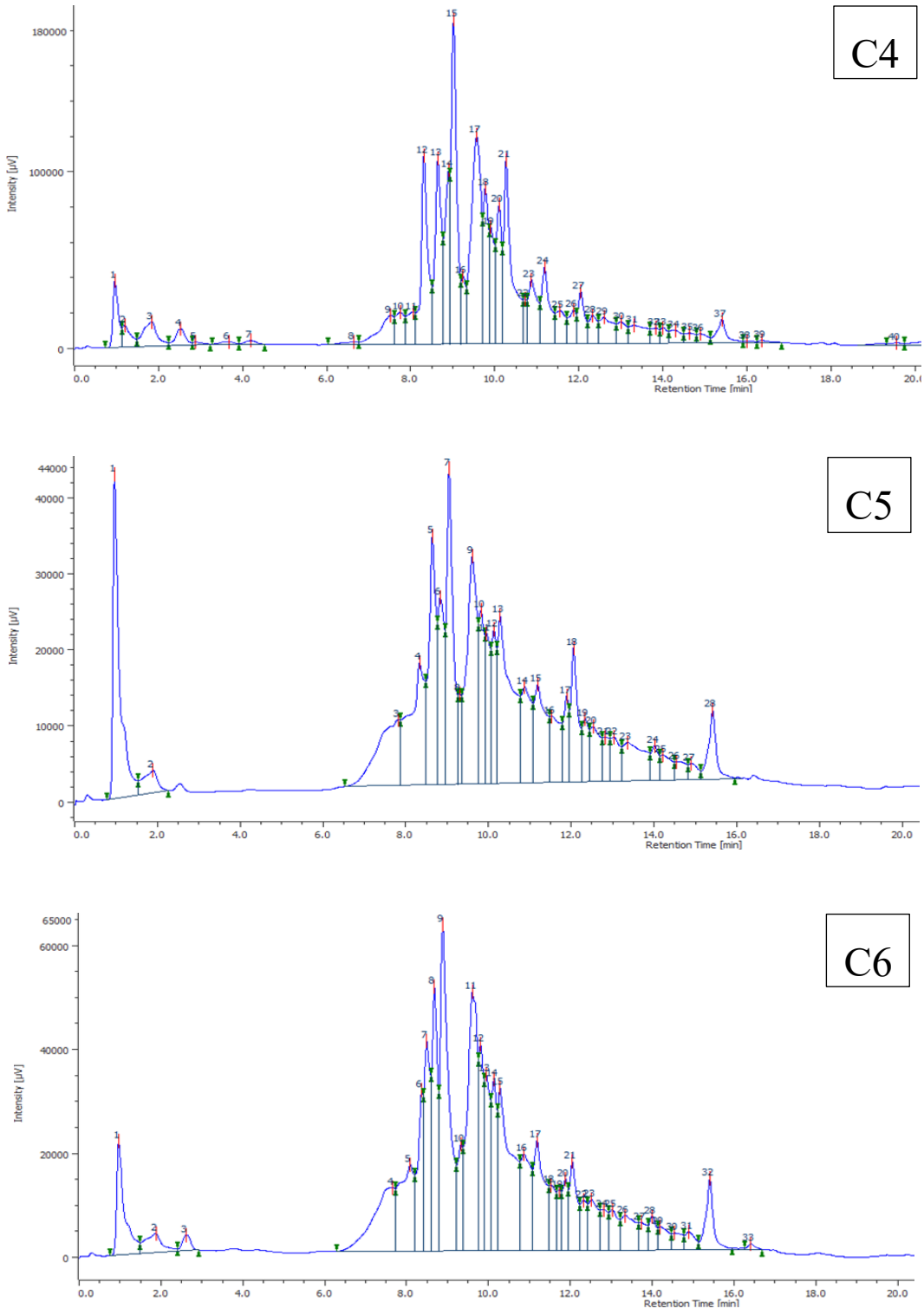
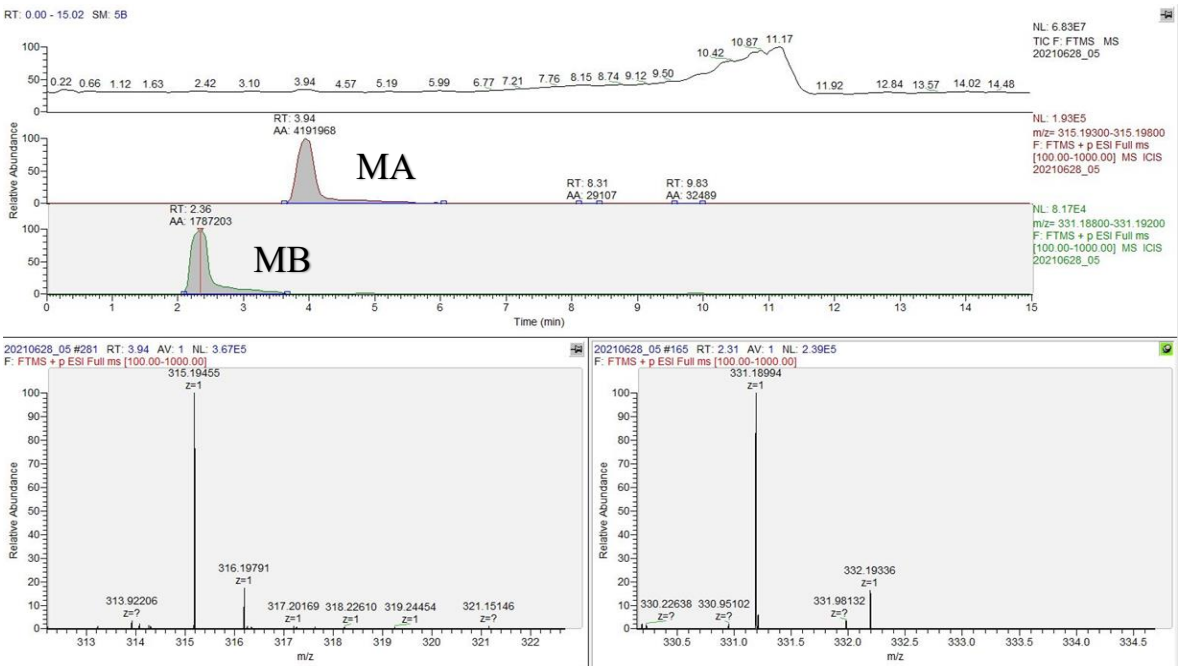
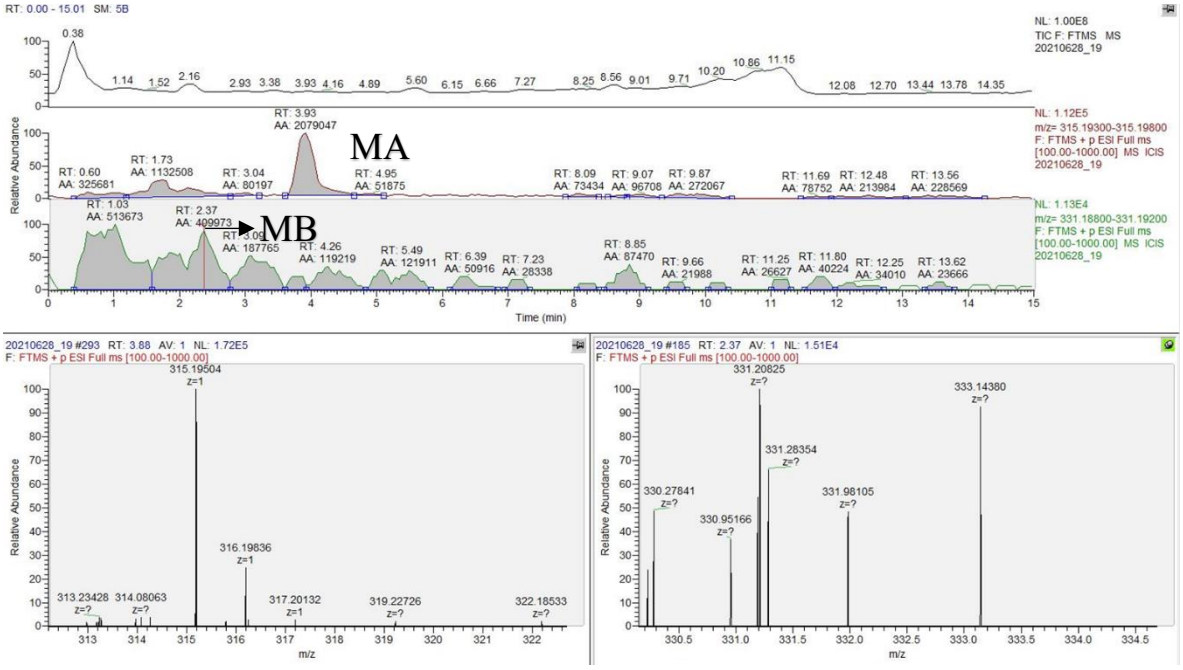


Figure 4.2. HPLC chromatograms of salinity susceptible rice DT84DB at different treatments.



(a)



(b)

Figure 4.3. UPLC chromatograms and mass spectrums of (a) MA and MB standards; and (b) C1.

Table 4.4. Phenolic profiles of rice under salinity stress (unit: µg/g DW).

Samples	Caffeic acid	*ρ*-Coumaric acid	Salicylic acid	Cinnamic acid	Benzoic acid	Ferulic acid	Tricin	MA (ng/g)	MB (ng/g)
C1	0.05±0.00b	0.12±0.01d	0.18±0.01d	0.04±0.00b	0.65±0.03d	0.11±0.00d	0.03±0.00cd	3.72±0.07a	1.45±0.11a
C2	0.01±0.00c	0.16±0.00c	0.29±0.00d	0.01±0.00d	0.62±0.02de	0.15±0.01c	0.02±0.00e	-	-
C3	0.04±0.00b	0.17±0.00bc	0.49±0.03c	0.02±0.00cd	1.00±0.04c	0.24±0.01b	0.09±0.00a	-	0.61±0.01b
C4	0.23±0.01a	0.47±0.01a	2.45±0.05a	0.07±0.01a	2.74±0.10a	0.34±0.01a	0.04±0.00bc	-	0.68±0.04b
C5	0.02±0.00c	0.09±0.00e	0.45±0.02c	0.02±0.00cd	0.42±0.02e	0.10±0.00d	0.03±0.00de	-	-
C6	0.01±0.00c	0.18±0.01b	0.76±0.01b	0.03±0.00bc	1.35±0.02b	0.16±0.00c	0.05±0.00de	-	0.53±0.01b

Values represent mean ± standard errors (SE);

'-': nonquantifiable (a very low concentration that could not be measured, henceforth will be interpreted as undetectable);

different letters in a column indicate a significant difference ($P < 0.05$) by Tukey's test.

As can be seen in Table 4.4, under control conditions, the amounts of phenolics in C6 (salinity susceptible) were significantly higher than those of C3 (salinity tolerant). However, salinity induced a dramatic reduction in phenolic amounts of sensitive rice samples (Table 4.4). Particularly, the quantity of *ρ*-coumaric, salicylic, and ferulic acids increased in tolerant variety but decreased in susceptible rice under salinity stress (Table 4.4). Volume of caffeic acid, cinnamic acid, benzoic acid, and tricin remarkably declined in all salt-seedlings. On the other hand, momilactone A was only detected in C1 (salinity tolerant cultivar-control) (Table 4.4). Momilactone B was found to be higher in C1, C3 (tolerant cultivar) than in C4, C6 (susceptible rice), respectively. The results indicated that under salt stress, the amounts of phenolics (except caffeic acid), and momilactone B in rice seedlings were improved with Mg application (Table 4.4).

4.4. Discussions

Salinity-induced osmotic stress inhibits plant growth by reducing water uptake capacity (Shabani et al., 2013). In this study, salt stress decreased root length, plant height, fresh weight, and dry weight in the rice seedlings; however, the reduction of these parameters were smaller in tolerant cultivar compared to susceptible rice. Similar salt-induced rice growth inhibition has been reported in previous studies (Munns, 2011; Minh et al., 2016; Rahman et al., 2016). Other research also recorded a less decline of growth parameters in tolerant variety compared to susceptible rice in salinized conditions (Suplick-Ploense et al., 2002; Kumar et al., 2009). However, supplementation of Mg in the current study restores rice biomass loss; improves its water status, antioxidant activities, phenolic contents, and momilactone B; and improves tolerant ability of the salt-stressed seedlings.

Under stress conditions, overproduction of ROS causes oxidative stress. In the current study, salt stress induced a significant reduction in antioxidant properties of salinity sensitive rice. However, the antioxidant activities of tolerant rice increased under stress. In an earlier study, salt tolerant plants were found to regulate ionic homeostasis, water status, and antioxidant defense system against the ROS (Hussain et al., 2012). In this study, exogenous Mg reduced the oxidative damage of all samples by enhancing their antioxidant capacities. Similar results were also found in the previous study of Sebastian et al., 2015 and Rahman et al., 2016 who stated that elements can be applied to inhibit abiotic stress-induced oxidative stress by reversing ROS production.

As the plant-specialized metabolites, phenolics have attracted attention from researchers due to their crucial functions in physiological processes throughout the plant life cycle, including responses to stress (Šamec et al., 2020). Plant phenolic acids are powerful antioxidants that can scavenge the overproduction of ROS in plants under different abiotic stresses (Bistgani et al., 2019). The stimulation of the biosynthetic pathways that induce the synthesis of phenolic acids is one cause of the activated plant's antioxidant system (Bistgani et al., 2019. In which, the accumulation of endogenous phenolic acids is determined as a mechanism of plant tolerance against abiotic stresses in many plant species including rice (Sharma et al., 2016; Sharma et al., 2019; Wang et al., 2019). Based on the up-regulation of key genes phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), phenolic biosynthesis is increased, resulting in the improvement of rice tolerance of abiotic stress (Sharma et al., 2016; Sharma et al., 2019).

In the current study, momilactone A was only detected in BC15 (tolerant cultivar) under control conditions at the seedling stage. However, momilactone B was identified in both tolerant and susceptible samples, in which, the amount of momilactone B in BC15 is higher than that of DT84DB (susceptible genotype). This result is collaborated with other researchers who stated that momilactones A and B are involved in salt tolerance of rice (Xuan et al., 2016). It was also shown that momilactone B has a greater correlation with rice salinity tolerance compared to momilactone A (Xuan et al., 2016). Especially, Mg induces a richer amount of momilactone B in rice seedlings under salt stress. Similarly, the quantity of phenolic compounds can also be enhanced with Mg application. The previous study indicated that Mg is involved in the phenolic and anthocyanin syntheses (Gershenzon et al., 1984; Nissim-Levi et al., 2007). Besides, the amount of *ρ*-coumaric, salicylic, and ferulic acids is built up in tolerant samples under salinity stress. These phenolic acids may contribute to the tolerant mechanism of rice against salt conditions. The antioxidant potential of these phenolic acids can be explained by their oxidation to respective phenoxyl radicals (Šamec et al., 2020). The accumulation of *ρ*-coumaric acid can inhibit oxidative pressure because their hydroxyl nature is a radical scavenger (Jamalian et al., 2013). Besides working as a free-radical scavenger, ferulic acid can also inhibit enzymes involved in free-radical generation and assist the activity of other scavenging enzymes (Kamila et al., 2018). Moreover, ferulic acid is able to enhance the plant cell wall and cell elongation (Wakabayashi et al., 1997). On the other hand, the accumulation of plant hormone salicylic acid also improved in tolerance to rice under salt stress. The role of salicylic acid in regulation of plant growth and development, as well as resistance to abiotic stress has been demonstrated. Especially, salicylic acid works as a hormone signaling disease resistance (Dempsey et al., 2017).

Studies in the past showed a potential of supplemented elements (Mn, Si, Zn) in the improvement of plant growth, photosynthesis, and antioxidant activities. (Iqbal et al., 1999; Kim et al., 2014; Rahman et al., 2017). However, the effect of these elements was mostly dose dependent (Rahman et al., 2017). On the other hand, different plants have different mineral demands, exceeding application of those elements can damage plant growth and development. Different from the above elements, Mg is a macronutrient of a plant. Plant requires a huge amount of Mg for its essential structure components as well as metabolisms. Mg is the central component of chlorophyll molecules and particularly essential to plants, with 75% of leaf Mg involved in protein synthesis and 15–20% of total Mg associated with chlorophyll pigments (White et al., 2009). Besides, this element is involved in many enzyme activities and the structural stabilization of tissues such as nucleic acids, proteins, cell membranes and walls (Cowan et al., 2002; Sreedhara et al., 2002; Marschner et al., 2012).

The crucial role of Mg in regulation of ROS, cation–anion balance, as well as cell turgor was determined (Marschner et al., 2012; Bose et al., 2013). Researchers stated that the special roles of Mg^{2+} in plants that cannot be found in other elements may be caused by its unique chemical properties such as great hydrated radius and preference in acting with oxygen (Moomaw et al., 2008). However, surprisingly, studies of Mg have mostly focussed on detoxification of heavy metals such as Al, Cd (Guo et al., 2016). Especially, application of Mg in reducing salt damages on rice is still limited. Results of this research showed that exogenous Mg enhanced the inhibited growth, water status, antioxidant activities, phenolic profiles, and momilactone B of salted rice seedlings. Moreover, the variation of Mg concentration in plants is highly heritable (Broadly et al., 2008). It is a potential strategy for breeding crops with Mg enrichment. On the other hand, the supplemented $MgSO_4$ in the

current study is the component of many plant fertilizers. It is suggested that this compound may be useful to develop fertilizer for rice growing in saline soil. However, application of exogenous Mg needed to be investigated in field scale.

4.5. Conclusions

The results of this study conclude that application of Mg reduced salt-induced damages by improving growth parameters, water status, antioxidant activities, phenolic acids, and momilactone B of the rice seedlings. Mg supplement may be useful to develop fertilizer for rice growing in saline soil. Moreover, *ρ*-coumaric acid, salicylic acid, ferulic acid, and momilactone B may contribute to rice tolerant ability against salt stress at seedling stage. The involvements of these compounds in recovery mechanisms of rice under salt stress should be further elaborated at different growth stages.

CHAPTER V.

GENERAL DISCUSSION

5.1. Advances and challenges in developing salinity tolerant rice

Cultivating around the world and feeding nearly fifty percent of the global population, the role of rice in maintaining food security is undoubted. Recently, it is needed to develop rice production because of the growing population. Besides, the negative impacts of climate change also threaten rice cultivation by a vast number of abiotic stresses such as heat, low pH, drought, and salinity stress. Rice is highly sensitive with salt conditions, resulting in remarkable yield loss and economic loss in affected cultivated land. Therefore, the breeding of salt-tolerant rice cultivars is considered as one of the most crucial keys to assure food security and agricultural economy in rice growing areas.

5.1.1. Cultural methods

Recently the cultural methods of crops cultivation including rice has developed to cope with the damages from climate change. The alteration of planting and harvesting time, collection of crops with short life cycles, crop rotation, irrigation techniques, and variation in cropping schemes obtained from famers trials helps to accelerate plant adaptation under climatic variation. Crop-management is also important to enhance crop development under various environmental stresses. Some management techniques choice of sowing time, planting density, and optimum irrigation practices were demonstrated as promising methods to lessen the effects of weather stresses on agricultural cultivation. Nevertheless, the cultural methods and support techniques are less sustainable.

5.1.2. Conventional breeding and marker-assisted breeding

Dominant conventional breeding based on selection of elite rice sources through various generations of crosses and marker-assisted selection also were applied to improve salinity tolerant rice. However, besides time consuming and laborious work, these efforts are also facing some other challenges. Firstly, salinity tolerance is controlled by numerous genes ("quantitative trait loci" -QTL) that generate complex physiological processes. It is difficult to identify the phenotypic characters which most contribute to salt tolerance. The evaluation of phenotypic parameters is greatly affected by human manipulation, and the rate of leaf death and plant death of different materials are different. Therefore, this evaluation system may not complete and accurately reflect the salt tolerance of rice varieties (Qin et al., 2020). On the other hand, the large size of chromosome regions identified by QTLs can lead to "linkage drag", which is related to undesirable traits in the products (Martinez et al., 2002). The identified QTLs are also influenced by genetic and environmental backgrounds (Flower et al., 2004). Another complication that needs to be considered for breeding salinity tolerant rice is that almost all materials with good tolerant ability have poor agronomic characteristics. Therefore, these sources are not potential donors in breeding programs for developing salinity tolerance. Although many new tolerant cultivars were bred successfully, the products do not possess the tolerant level as their parents and have poor grain quality, low yield, as well as photosensitivity (Ismail et al., 2007; Reddy et al., 2014).

5.1.3. Genetic engineering

The advance of modern biotechnologies promises a powerful tool in plant breeding programs. Genetic approach for developing salinity tolerant plants has focused on genes

encoding antioxidants (detoxification of ROS), ion transport, signal transduction and transcription, and protein synthesis (Hoang et al., 2016). To date, many genes have been introduced into rice to improve salinity tolerance, however, the commercialization of those rice has limited. The reasons why transgenic salt tolerant variety still has not reached the farmer are (1) salinity tolerance of rice is controlled by many genes while the genetic engineering has mostly transformed single gene or a few genes; (2) major salt tolerance genes have been isolated from QTLs which are inconsistent and variable in different genetic backgrounds and environments; (3) many transgenic plants do not display the expected transgene expression because of gene silencing (Hoang et al., 2016; Qin et al., 2020). On the other hand, both additive and non-additive genes are involved in controlling salinity tolerance in rice, which are complicated, low inherited, and largely influenced by environment (Moeljopawiro and Ikehashi, 1981). In fact, the gene that determines the major response to salinity stress has not yet been confirmed, and it is difficult to ensure that the salt tolerance can be effectively improved by transferring a single or a few genes. Furthermore, unpredictable gene silencing which can be occurred by the presence of foreign genes or additional copies of endogenous genes also affects the success of transgenic strategy (Hoang et al., 2016).

The recent technique genome editing has contributed to developing salt tolerant rice. Some rice lines created by this technique perform better characters such as salt tolerance or blast resistance. The advance of genome editing is using synthetic DNA-binding proteins that recognize specific DNA targets. Among recent genome modified techniques, clustered regularly interspersed short palindromic repeats (CRISPR)/Cas system has attracted scientists due to its design based on exploitation of prokaryote defense systems to remove foreign DNA.

Despite the prominent advantages genome modification strategies has demonstrated, it is limited by the presence of integrated sequences that must be removed by segregation in future generations (Hoang et al., 2016). On the other hand, the similar difficulty of transgenes and genome editing is that they are strictly prohibited in many countries. Therefore, licensing the products released from these technologies is complex, rigorous, and time-consuming. Furthermore, the safety of these modified plants toward ecosystem, biodiversity, especially human health is also concerned. It is a challenge to develop their products in a commercial market.

5.2. N-methyl-N-nitrosourea (MNU) -induced mutation

Rice domestication and the specific selection in breeding programs have led to narrowing of its genetic variation (Viana et al., 2019). Creating and increasing the genetic variability of rice is important for breeders to develop a new cultivar. Mutant databases are possible to elucidate genetic, physiological, and biochemical processes of rice (Viana et al., 2019). Therefore, mutagenesis is considered as one of the most important tools to improve rice.

In this study, rice mutants were developed by N-methyl-N-nitrosourea (MNU), a prominent chemical agent which has been widely used for cereal crops. A respiratory mutation was created by treating rice seeds in MNU (150 mM) in 3h and placing them in the dark with hermetic condition in 3 months. Rice mutants then were grown in a rice field near Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan for 4 years 2016–2019 to investigate their characteristics (temperature 33/25°C day/night; humidity 70%; precipitation average 1554 mm; source: http://www.hiroshima.climatemps.com/). The fertilizers, weeding,

watering, and pesticides were provided by conventional methods in Japan. Although these rice sources are Vietnamese rice, they adapt and develop well with Japan's climate. In fact, the climatic conditions in the rice cultivation season between Hiroshima and Vietnam were similar (climate conditions in Vietnam: temperature 35/28 °C day/night; humidity 75%; precipitation average 1700 mm; source: Vietnam government information). Besides, almost all rice lines/cultivars used in the study are generated from Chinese rice, and some of them are widely adapted to climatic conditions. After investigation in field scale, the rice population performed acceptable yield and quality traits (similar values as the population grown in Vietnam obtained), with some of them performing better characteristics with the population grown in Vietnam (Anh et al., 2019). Especially, the generated mutant populations performed better yield and quality compared to their parents (Anh et al., 2019). Therefore, these mutants are valuable sources for rice breeding programs.

5.2.1. MNU mutation mechanism

Different with physical agents (direct method) utilizing mostly electromagnetic radiation, chemical mutagens have been practiced by alkylating agents, intercalating agents and base analogues (Chikelu et al., 2010). Generally, both of them carry out the alteration of DNA in treated organisms, consequently, their appearances, traits and characteristics are also changed (Chikelu et al., 2010). MNU has been considered as a complete, powerful, and a direct-acting alkylating agent since the past decade (Tsubura et al., 2011; Satoh et al., 2010; Faustino-Rocha et al., 2015). By a specific nucleotide substitution started by methylation (Satoh et al., 2010), MNU is able to alter DNA structure (Lijinsky, 1987; Satoh et al., 2010). Researchers in the past reported that MNU alkylates specific nucleotides at high-density and

random distribution (Till et al., 2007), therefore it can modify the level of amino acid on mutants (Kumamaru et al., 1997). Particularly, it was found that MNU alters the bases G to A or A to G transition (Suzen et al., 1998). In a previous study, Kurowska et al. (2012) also carried out the G-A and C-T substitution in the MNU-induced mutation. Additionally, MNU is also able to create some process disruptions and lead to the change of metabolic mechanisms of the cells (Singla and Dhawan, 2010).

The effects MNU on creating point mutation have demonstrated in the last two decades. In this study, MNU treatment might create a change in the molecular level of rice and therefore lead to the variation in their genetic and agronomic traits. The alterations in growth parameters as well as yield and quality contributing traits in the same mutated population also were found in earlier studies (Anh et al., 2019; Xuan et al., 2019). On the other hand, point mutation is the most popular natural occurring mutation. Compared to other techniques which have a more serious effect on the DNA structure (chromosomal mutation) such as insertion, deletion, or inversion of sections of DNA; the induced point mutation is considered as a safer method.

5.2.2. Segregation and maternal inheritance

The segregation of botany in general follows a central law genetic theory (Mendelian rule) with the presence of two alleles from parents in the progeny (Ruvinsky, 2013). The progenies in this concept can receive the genetic materials from both female and male parents, ensuring their species development. Most genes follow this fundamental process. However, in some cases, genes are not controlled by Mendelian theory. This phenomenon is called non-Mendelian inheritance (Brooker, 2009). The non-Mendelian inheritance was found in DNA

of cytoplasmic organelles, which are called as uniparental inheritance, maternal or paternal inheritances (Sato and Sato, 2013) and can be caused by genetic, physiological, and environmental elements (Alheit et al., 2011; Reflinur et al., 2014). Maternal inheritance happens when the inherited trait is determined by the mother genotype without effects from father genotype (Russel 2010). The maternal inheritance can occur in mitochondrial and chloroplast genes and transfers from generation to generation. Maternal effects allow offspring to adapt with the variation of environment conditions (Galloway and Etterson, 2007). Although the maternal inheritance is uncommon in plants (Kumamaru et al., 1997), it can be discovered in populations of crossing between wild rice and domestic rice, *Oryza meridionalis* and O. *sativa* (Yu et al., 2018); O. *rufipogon* and O. *sativa* (Charlesworth, 2017). These populations are generated from genetically diverse parents.

Basically, salt tolerance in rice is nucleus inherited (paternal inheritance). In a breeding program for salinity tolerant rice, the female parent with good agronomical traits (such as good quality) normally is crossed with the salinity tolerant male parent. In which, their offspring inherit both alleles from father and mother followed Mendelian theory (Mishra et al., 1998). However, the current study (chapter 3) showed a contrasting case with normal Mendelian rule when all of progenies possess female parent allele without any male parent allele. By genetic analysis with polymorphic SSR markers related to salinity tolerance of rice, the mutated rice TBR1/KD18 was found to be completely inherited from the female parent TBR1. In addition, phenotypic performance of the TBR1/KD18 were also similar to TBR1.

This mutant population was crossed between two Indica cultivars, therefore the genetic distance between parental genotypes is close, and it is unable to create the maternal

inheritance by themselves. Studies in the past showed that non-Mendelian inheritance can be caused by linkage genes or pleiotropy (Brondani et al., 2002). In the mutant population TBR1/KD18, a putative gene linkage might be created by MNU-induced mutation, and this gene linkage might manage the segregation of the progenies leading to maternal inheritance (Xuan et al., 2019). Because the progenies of the cross normally follow Mendelian rule with complicated segregations from the second generation, it takes time to breed a new cultivar and finish the segregation. The progeny normally is crossed again with its parents (back cross) and repeats in many generations to finish the segregation. The induction of maternal inheritance in this study helps to shorten breeding time from 8-10 cycles to 2-3 generations (the segregation finishes in the second generation and stabilizes in the thirst generation). However, the mechanism of the putative gene linkage, and the potent maternal inheritance needs to be clearly identified.

5.3. Application of phytoprotectants

In recent decades, researchers have used phytoprotectants to enhance salinity tolerance of rice. Exogenous osmoprotectants (pro, glycine betaine, trehalose, sorbitol and ectoine); plant hormones (abscisic acid, auxin, cytokinin); signal molecule (nitric oxide, H_2O_2); and trace elements (Si, Zn, Mn) were found to contribute to rice salt-tolerance (Rahman et al., 2017). However, the success of these micronutrients applications was mostly dose dependent (Rahman et al., 2017). On the other hand, magnesium (Mg) is a macronutrient for plants. It is the centre component of chlorophyll molecules and plays an important role in plant photosynthesis. Besides, it is an abundant element that is involved in a vast physiological processes of plants. However, studies about the application of Mg in developing salinity tolerant rice have still been limited. In this study, Mg was used in the study to improve

the salinity tolerance of rice (chapter 4). The assessments showed a promising result that Mg recovered inhibited growth and improved the antioxidant, phenolic profiles, and momilactone B of the rice seedlings under salt stress.

The impacts of Mg in developing salinity tolerance of rice may be explained by its unreplaced role in plants. Salt stress leads to a vast amount of changes inside rice plants which damage plant tissues such as osmotic stress, ionic imbalance, and oxidative stress (Rahman et al., 2017). These alterations inhibit plant growth and development and finally affect rice yield and grain quality. Magnesium might contribute to salinity tolerance of rice by ion transporter and osmotic balance. The application of Mg might reduce the salt damage to rice by taking part or catalysing the physiological processes of plants under salt stress. Researchers stated that the magnesium ion ($Mg^{2+)}$ is the most abundant divalent cation within cells (Payandeh et al., 2013). It is also an important co-factor in the machines that replicate, transcribe, and translate genomic information (Hartwig et al., 2001). Mg^{2+} participates in regulating enzyme activity and targeting macromolecules to specific complexes or cellular locations (Payandeh et al., 2013). Therefore, it is considered as a pivotal component in metabolic networks and signal cascades (Payandeh et al., 2013). Additionally, the maintenance of proper Mg^{2+} homeostasis has been correlated with physiological well-being in cells. Mg^{2+} has also been identified as a key intracellular signal molecule during immune cell activation (Li et al., 2011). The unique role of Mg^{2+} in plants can be explained by its special chemical properties such as great hydrated radius and preference in acting with oxygen (Moomaw et al., 2008). On the other hand, Mg^{2+} can be associated with the phenolic and anthocyanin syntheses by its special and unique enzyme catalysis (Guo et al., 2016). In this study, application of Mg restores biomass loss and improves water status of the salt-

stressed seedlings. Besides, it helps to increase phenolic compounds, antioxidant activities, and improves momilactone B which is involved in salt tolerance of rice.

The Mg used in the current study is $MgSO_4$, which can be found in many commercial plant fertilizers. On the other hand, the variation of Mg^{2+} concentration in plant tissues is highly heritable (Broadly et al., 2008). It is a potential strategy for breeding crops with Mg enrichment. The finding of chapter 4 may be useful to develop rice salinity tolerance by adjustment of $MgSO_4$ in plant fertilizer to apply in saline soil or salt intrusion areas. However, more tests and investigations are needed to carry out the most suitable dose of fertilizer components to utilise it in field scale.

5.4. Findings and recommendations

5.4.1. Findings

1. The study carried out that the rice cultivar BC15 and its mutant progeny BC15/SKLo have strong salinity tolerance. They are promising sources for rice breeding to develop new salinity tolerant cultivar. Additionally, SSR markers RM 237, RM 518, RM 493, RM 10748, RM 562, RM 20224 are potent to screen salinity tolerant rice, both cultivar and mutant.

2. The literature showed that salinity tolerance of rice is paternally inherited. This is the first study to reveal a maternal inheritance in rice salt-tolerance. On the other hand, the breeding program for salt tolerant rice requires 8-10 years due to the complicated segregation in progenies. F_1 normally is crossed with father (backcross) and repeated in many generations to finish that segregation. The maternal inheritance induced by MNU treatment helps to finish

the segregation in M_2 and M_3 generations, which can shorten breeding time from 8-10 cycles to 2-3 generations.

3. Magnesium is effective to reduce salinity-induced damages in rice seedlings by enhancing its inhibited growth, antioxidant activities, phenolic acids, and momilactone B. The results suggest that the salinity tolerance of rice can be improved by developing fertilizer with a supplementary $MgSO_4$.

5.4.2. Recommendations

Besides the findings of this dissertation, the following recommendations should be considered for future works:

1. The yield and quality of elite mutant populations under salt stress should be investigated.

2. The mutated rice should be sequenced and compared to parental genotypes to detect their genetic changes. The mechanism of maternal inheritance should be clearly identified.

3. The investigation of magnesium application towards enhancing the salt tolerant ability of rice needs to be further investigated in field scale.

Although rice production has dramatically increased in recent decades, it has still challenged to meet the staple supply due to the rapid growing population and the unexpected impacts of climate change. The purpose of rice breeders is creating new rice sources with greater characteristics such as higher yield and wider adaptation to environmental conditions. More efforts need to be made to develop modern rice cultivation and maintain food security, economic growth, quality of life, and social stability.

REFERENCES

Akbar, M.; Yabuno, Y.; Nakao, S. Breeding for saline resistant varieties of rice. Variability for salt-tolerance among some rice varieties. *Jpn. J. Breed*. 1972, 22, 277–284.

Alheit, K.V.; Reif, J.C.; Maurer, H.P.; Hahn, V.; Weissmann, EA.; Miedaner, T.; Würschum, T. Detection of segregation distortion loci in triticale (x Triticosecale Wittmack) based on a high-density DArT marker consensus genetic linkage map. *BMC Genomics* 2011, 12, 380.

Ali, M.L.; Pathan, M.S.; Zhang, J.; Bai, G.; Sarkarung, S.; Nguyun, H.T. Mapping QTLs for root traits in a recombinant inbred population from two indica ecotypes in rice. *Theor. Appl. Genet.* 2000, 10, 756–766.

Ali, M.N.; Lucina, Y.; Saikat, G.A.; Rupak, G.; Somsubhra, C. Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. *Physiol. Mol. Biol. Plants* 2014, 20, 411–423.

Aliyu, R.E.; Adamu, A.K.; Muazu, S.; Alonge, S.; Gregorio, G.B. Validation of rice markers tagged to salinity stress. *Afr. J. Biotechnol.* 2013, 12, 3239–3243.

Anh, T.T.T.; Khanh, T.D.; Dat, T.D.; Xuan, T.D. Identification of phenotypic variation and genetic diversity in rice (*Oryza sativa* L.) mutants. *Agriculture* 2018, 8, 30.

Anh, T.T.T.; Xuan, T.D.; Huong, C.T.; Dat, T.D. Phenotypic performance of rice (*Oryza sativa* L.) populations induced by the MNU mutant on the adaptive characteristics. *J. Hort. Plant Res.* 2019, 5, 13–24.

Ashkani, S; Rafii, M.Y.; Sariah, M.; Akmar, A.S.N; Rusli, I; Rahim, H.A.; Latif, M.A. Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genet. Mol. Res.* 2011, 10, 1345–1355.

Babu, N.N.; Krishnan, S.G.; Bhowmick, P.K.; Vanaja, T.; Krishnamurthy, S.L.; Nagarajan, M.; Singh, N.K.; Prahu, K.V.; Singh, A.K. Maker based haplotype diversity of Satol QTL in relation to seedling stage salinity tolerance in selected genoypes of rice. *Indian J. Genet. Plant Br.* 2014, 74, 16–25.

Babu, N.N.; Krishnan, S.G.; Vinod, K.K.; Krishnamurthy, S.L.; Singh, V.K.; Singh, M.P.; Singh, R.; Ellur, R.; Rai, V.; Bollinedi, H.B.; Bhowmick, P.K.; Yadav, A.K.; Nagarajan, M.; Singh, N.K.; Prabu, K.; Singh, A.K. Marker aided incorporation of *Saltol*, a major QTL associated with seedling stage salt tolerance, into *Oryza sativa 'Pusa* Basmati 1121'. *Front. Plant Sci.* 2017, 8, 41.

Bado, S.; Forster, B.P.; Nielen, S.; Ali, A.M.; Lagoda, P.J.L.; Till, B.J. Plant mutation breeding: Current progress and future assessment. In: Plant Breeding Reviews, 1st ed.; Janick J., Ed.; Wiley-Blackwell, John Wiley &Sons, Inc. 2015.

Bernstein, A.L. and Hayward, H.E. Physiology of salt tolerance. *Annu. Rev. Plant Physiol.* 1958, 9, 25–46.

Bhusan, D.; Das, D.K.; Hossain, M.; Murata, Y.; Hoque, M.A. Improvement of salt tolerance in rice (*Oryza sativa* L.) by increasing antioxidant defense systems using exogenous application of proline. *Aust. J. Crop Sci.* 2016, 10, 50–56.

Bistgani, Z.E.; Hashemi, M.; Dacosta, M.; Craker, L.; Maggi, F.; Morshedloo, M.R. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis Celak*. *Ind. Crop. Prod*. 2019, 135, 311–320.

Bonilla, P.S.; Dvorak, J.; Mackill, D.; Deal, K.; Gregorio, G. RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Phillip. Agric. Sci.* 2002, 65, 68–76.

Boranayaka, M.B.; Lokesha, R.; Diwan, J.R.; Rajendragouda, P. Marker validation in F2 population of rice (*Oryza sativa* L.) for water and nitrogen use efficiency. *Int. J. Curr. Microbiol. App. Sci.* 2018, 7, 1275–1278.

Bose, J; Babourina, O.; Shabala, S; Rengel, Z. Low-pH and aluminum resistance in *Arabidopsis* correlates with high cytosolic magnesium content and increased magnesium uptake by plant roots. *Plant Cell Physiol.* 2013, 54, 1093–1104.

Broadley, M.R.; Hammond, J.P.; King, G.J.; Astley, D. Bowen, H.C.; Meacham, M.C.; Mead, A.; Pink, D.A.C.; Teakle, G.R., Hayden, R.M.; Spracklen, W.P.; White, P.J. Shoot calcium and magnesium concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in *Brassica oleracea*. *Plant Physiol.*, 146, 1707–1720.

Brondani, C.; Rangel, P.; Brondani, R.; Ferreira, M. QTL mapping and introgression of yield related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor. Appl. Genet.* 2002, 104, 1192–1203.

Brooker, R.J. Non-Mendelian inheritance. In: Genetics analysis and principles, 3rd ed.; Reidy, P.E., Ed.; McGraw-Hill, New York, USA, 2009.

Camilla, S.C.S.; Flávia, B.S.B.; Cinthia, S.R.; Marcela, P.M.; Amanda, M.M.; Antônio, R.N. Genetic and phenotypic parameters in the selection of upland rice genotypes. *Am. J. Plant Sci.* 2017, 8, 3450–3459.

Cartwright, D.W.; Langcake, P.; Pryce, R.J.; Leworthy, D.P.; Ride, J.P. Isolation and characterization of two phytoalexins from rice as momilactones A and B. *Phytochemistry* 1981, 20, 535–537.

Chandraratna, M.F.; Sakai, K.I. A biometrical analysis of matroclinous inheritance of grain weight in rice. *Heredity* 1960, 14, 365–373.

Chang, T.T.; Liu, F.H. Diallel analysis of protein content in rice. *Agron. Abstr.* 1974, 65.

Charlesworth, D. Origins of rice cytoplasmic male sterility genes. *Cell Res.* 2017, 27, 3.

Chikelu, M.; Rohul, A.; Souleymane, B. et al. Induced mutagenesis in plants using physical and chemical agents. In: Plant cell culture, essential methods, chapter: Induced mutagenesis in plants using physical and chemical; Davey, M.R., Ed.; Wiley-Blackwell, John Wiley and Sons Ltd., Chichester, Sussex, UK, 2010.

Chowdhury, A.D.; Haritha, G.; Sunitha, T.; Krishnamurthy, S.L.; Divya, B.; Padmavathi, G.; Ram, T.; Sarla, N. Haplotyping of rice genotypes using simple sequence repeat markers associated with salt tolerance. *Rice Sci.* 2010, 23, 317–325.

Chung, I.M.; Ali, M.; Hahn, S.J.; Siddiqui, N.A.; Lim, Y.H.; Ahmad, A. Chemical constituents from the hulls of *Oryza sativa* with cytotoxic activity. *Chem. Nat. Compd.* 2005, 41, 182–189.

Collard, B.C.Y.; Mackill, D.J. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Phil. Trans. B* 2008, 363, 557–572.

Cowan, J.A. Structural and catalytic chemistry of magnesium-dependent enzymes. *Biometals* 2002, 15, 225–235.

Dempsey, D.A. and Klessig, D.F. How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans ? *BMC Biol* 2017, 15, 23.

Duckworth, R.A. Maternal effects and range expansion: a key factor in a dynamic process? *Philos. Trans. R. Soc.* 2009, 364, 1075–1086.

Fageria, N.K.; Barbosa, M.P.; Gheyi H.R. Tolerance of rice cultivars to salinity tolerance. *Pesqui. Agropecu. Bras.* 1981, 16, 677–681.

Fageria, N.K. Salt tolerance of rice cultivars. *Plant Soil.* 1985, 88, 237–243.

FAO, 2020. Available online: *http://www.fao.org* (assessed on December 21, 2020).

FAO/IAEA. Available online: *http://mvgs.iaea.org/Search.aspx* (accessed on January 24, 2020).

Farooq, M.A.; Saqib, Z.A.; Akhtar, J. Silicon-mediated oxidative stress tolerance and genetic variability in rice (*Oryza sativa* L.) grown under combined stress of salinity and boron toxicity. *Turk J Agric For.* 2015, 39, 718–729.

Faustino-Rocha, A.I.; Ferreira, R.; Oliveira, P.A.; Gama, A.; Ginja, M. N-Methyl-N-nitrosourea as a mammary carcinogenic agent. *Tumor Bio.* 2015, 36, 9095–9117.

Flowers, T.J. Improving crop salt tolerance. *J. Exp. Bot.* 2004, 55, 307–319.

Flowers, T.J.; Koyama, M.L.; Flowers, S.A.; Sudhakar, C.; Singh, K.P.; Yeo, A.R. QTL: Their place in engineering tolerance of rice to salinity. *J. Exp. Bot.* 2000, 51, 99–106.

Fuentes, J.L.; Correa-Victoria E.J.; Escobar, F.; Prado, G.; Aricapa, G.; Duque, M.C.; Tohme, J. Identification of microsatellite markers linked to the blast resistance gene Pi-1(t) in rice. *Euphytica* 2008 160, 295–304.

Fukuda, A.; Nakamura, A.; Tagiri, A.; Tanaka, H.; Miyao, A.; Hirochika, H.; Tanaka, Y. Function, intracellular localization and the importance in salt tolerance of a vacuolar Na^+/H^+ antiporter from rice. *Plant Cell Physiol.* 2004, 45, 146–159.

Fukuta, M.; Xuan, T.D.; Deba, F.; Tawata, S.; Khanh, T.D.; Chung, I.M. Comparative efficacies in vitro of antibacterial, fungicidal, antioxidant, and herbicidal activities of momilatones A and B. *J. Plant Interact.* 2007, 2, 245–251.

Galloway, L.F. and Etterson, J.R. Transgenerational plasticity is adaptive in the wild. *Science* 2007, 318, 1134–1136.

Ganie, S.A.; Dey, N.; Mondal, T.K. Differential promoter methylation of salt tolerant and susceptible rice genotypes under salinity stress. *Funct. Genomic.* 2016, 8, 1–11.

Garland, S.H.; Lewin, L.; Abedinia, M.; Henry, R.; Blakeney, A. The use of microsatellite polymorphisms for the identification of Australian breeding lines of rice (*Oryza sativa*. L). *Euphytica* 1999, 108, 53–63.

Gershenzon, J. Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Phytochemical Adaptations to Stress; Timmermann, B., Steelink, C., Loewus, F., Eds.; Springer, US, 1984.

Ghose, R.L.M. and Butany, W.T. Botanical improvement of varieties-general characters of Indian varieties and the application of genetics to rice improvement. *Proc. Indian Acad. Sci. Sect. B.* 1959, 49, 287–302.

Govindarajan, R; Rastogi, S; Vijayakumar, M; Shirwaikar, A; Rawat, A.K.; Mehrotra, S; Pushpangadan, P. Studies on the antioxidant activities of Desmodium gangeticum. *Biol. Pharm. Bull.* 2003, 26, 1424–1427.

Gramene. Available online: *http://www.gramene.org/* (accessed on January 14, 2018).

Gregorio, G.B.; Senadhira, D.; Mendoza, R.D. Screening Rice for Salinity Tolerance, 3rd ed.; International Rice Research Institute, Los Baños, The Philippines, 1997.

Guo, W., Nazim, H., Liang, Z., Yang, D. Magnesium deficiency in plants: An urgent problem. *Crop J.* 2016, 4, 83–91.

Gupta, P.K.; Varshney, R.K. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 2000, 113, 163–185.

Gupta, P.K.; Varshney, R.K.; Sharma, P.C.; Ramesh, B. Molecular markers and their applications in wheat breeding. *Plant Breed.* 1999, 118, 369–390.

Gurmani, A.R.; Bano, A.; Khan, S.U.; Din, J.; Zhang, J.L. Alleviation of salt stress by seed treatment with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) optimizes ion and organic matter accumulation and increases yield of rice (*Oryza sativa* L.). *Aust. J. Crop Sci.* 2011, 5, 1278–1285.

Habib, N. Nitric oxide regulated improvement in growth and yield of rice plants grown under salinity stress: antioxidant defense system. *Appl Ecol Environ Res.* 2016,14, 91–105.

Hanyong, Y.; Xinghua, W.; Yiping, W.; Xiaoping, Y.; Shengxiang, T. Study on genetic variation of rice varieties derived from Aizizhan by using morphological traits, allozymes and simple sequence repeat (SSR) markers. *Chin. J. Rice Sci.* 2004, 18, 477–482.

Hartwig, A. Role of magnesium in genomic stability. *Mutat Res.* 2001, 18, 113–121.

Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int J Mol Sci.* 2013, 14, 9643–9684.

Hasanuzzaman, M.; Nahar, K.; Fujita, M. Silicon and selenium: two vital trace elements in conferring abiotic stress tolerance to plants. In: Emerging 172 Advances in International Rice Research technologies and management of crop stress tolerance, vol. 1– Biological techniques; Parvaiz, A., Rasool, S., Eds; Academic Press, New York, 2014.

Heenan, D.P.; Lewin, L.G.; Mccaffery, D.W. Salinity tolerance in rice varieties at different growth stages. *Aust. J. Exp. Agric.* 1988, 28, 343–349.

Hoang, T.M.L.; Tran, T.N.; Nguyen, T.K.T.; Williams, B.; Wurm, P.; Bellairs, S.; Mundree, S. Improvement of salinity stress tolerance in rice: Challenges and opportunities. *Agronomy* 2016, 6, 54.

Hussain, M.; Farooq, M; Shehzad, M; Khan, M.B; Wahid, A.; Shabir, G. Evaluating the performance of elite sunflower hybrids under saline conditions, *Int. J. Agric. Biol.* 2012, 14, 131–135.

Iqbal, M. and Aslam, M. Effect of Zn application on rice growth under saline condition. *Int. J. Agric. Biol.* 1999, 1, 362–365.

Iqbal, N.; Umar, S.; Khan, N.A. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *J Plant Physiol.* 2015, 178 ,84–91.

IRRI, 2020. Available online: *http://www.irri.or*g (assessed on December 21, 2020).

Islam, M.R.; Gregorio, G.B.; Salam, M.A.; Collard, B.C.Y.; Singh, R.K.; Hasan, L. Validation of Saltol linked markers and haplotype diversity on chromosome 1 of rice. *Mol. Plant Breed.* 2012, 3, 103–114.

Ismail, A.; Heuer, S.; Thomson, M.; Wissuwa, M. Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Mol. Biol.* 2007, 65, 547–570.

Ito, R.; Vasconcelos, H.L.; Feeley, K.J. Global climate change increases risk of crop yield losses and food insecurity in the tropical *Andes. Glob. Chang. Biol.* 2018, 24, 592–602.

Jaiswal, S.; Gautam, R.K.; Singh, R.K.; Krishnamurthy, S.L.; Ali, S.; Sakthivel, K.; Iquebal, M.A; Rai, A.; Kumar, D. Harmonizing technological advances in phenomics and genomics for enhanced salt tolerance in rice from a practical perspective. *Rice* 2019, 12, 89.

Jamalian, S; Gholami, M; Esna-Ashari, M.Abscisic acid-mediated leaf phenolic compounds, plant growth and yield is strawberry under different salt stress regimes, *Theor. Exp. Plant Physiol.* 2013, 25, 291–299.

James, S.D.; Susan, N.M.; John, J.M. Salinity stress effects on some morpho-physiological traits of selected rice (Oryza sativa L.) genotypes. *Int. J. Dev. Sustain.* 2016, 5, 74–86.

Javed, M.A.; Fahrul, Z.H.; Alina, W.; Faeza, M.S. Identification of QTLs for Morph-Physiological traits related to salinity tolerance at seedling stage in *Indica* rice. *Procedia Environ. Sci.* 2011, 8, 389–395.

Javid, M.G.; Sorooshzadeh, A.; Sanavy, S.A.M.M.; Allahdadi, I.; Moradi, F. Effects of the exogenous application of auxin and cytokinin on carbohydrate accumulation in grains of rice under salt stress. *Plant Growth Regul.* 2011, 65, 305–313.

Jeung, J.U.; Hwang, H.G.; Moon, H.P.; Jena, K.K. Finger printing temperate japonica and tropical indica rice genotypes by comparative analysis of DNA markers. *Euphytica* 2005, 146, 239–251.

Joung, Y.H.; Lim, E.J.; Kim, M.S.; Lim, S.D.; Yoon, S.Y.; Lim, Y.C.; Yoo, Y.B.; Ye, S.K.; Park, T.; Chung, I.M.; et al. Enhancement of hypoxia-induced apoptosis of human breast cancer cells via STAT5b by momilactone B. *Int. J. Oncol*. 2008, 33, 477–484.

Kamila, M.Z.; Dana, A.; Kołodziejczak, A.; Rotsztejn, H. Antioxidant properties of ferulic acid and its possible application. *Ski. Pharmacol. Physiol.* 2018, 31, 332–336.

Kato, T.; Kabuto, C.; Sasaki, N.; Tsunagawa, M.; Aizawa, H.; Fujita, K.; Kato, Y.; Kitahara, Y. Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedon Lett.* 1973, 39, 3861–3864.

Kato-Noguchi, H.; Hasegawa, M.; Ino, T.; Ota, K.; Kujime, H. Contribution of momilactone A and B to rice allelopathy. *J. Plant Physiol*. 2010, 167, 787–791.

Kim, S.J.; Park, H.R.; Park, E.; Lee, S.C. Cytotoxic and antitumor activity of momilactone B from rice hulls. *J. Agric. Food Chem.* 2007, 55, 1702–1706.

Kim, S.K.; Son, T.K.; Park, S.Y.; Lee, I.J.; Lee, B.H.; Kim, H.Y.; Lee, S.C. Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *J. Environ. Biol.* 2006, 27, 181–186.

Kim, Y.H.; Khan, A.L.; Waqas, M.; Shim, J.K.; Kim, D.H.; Lee, K.Y.; Lee, I.J. Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. *J. Plant Growth Regul.* 2014, 33, 137–149.

Kioko, W.F.; Musyoki, M.A.; Piero, N.M.; Muriira, K.G.; Wavinya, N.D.; Rose, L.; Felix, M.; Ngithi L.N. Genetic diversity studies on selected rice (*Oryza sativa* L.) populations based on aroma and cooked kernel elongation. *Phylogen. Evolution Biol.* 2015, 3, 4.

Koyama, M.L.; Levesley, A.; Koebner, R.M.D.; Flowers, T.J.; Yeo, A.R. Quantitative Trait Loci for component physiological trait determining salt tolerance in rice. *Plant Physiol.* 2001, 125, 406–422.

Kumamaru, T.; Sato, H.; Satoh, H. High-lysine mutants of rice, *Oryza sativa* L. *Plant breed.*1997, 116, 245–249.

Kumar, V.; Shriram, V; Nikam, T.D; Jawali, N; Shitole, M.G. Antioxidant enzyme activities and protein profiling under salt stress in *Indica* rice genotypes differing in salt tolerance. *Arch Agron Soil Sci.* 2009, 55, 379–394.

Kurowska, M.; Labocha-Pawłowska, A.; Gnizda, D.; Maluszynski, M.; Szarejko, I. Molecular analysis of point mutations in a barley genome exposed to MNU and Gamma rays. *Mutat. Res*. 2012, 738–739, 52–70.

Lammerts van Bueren, E.T.; Backes G, de Vriend H.; Østergard, H. The role of molecular markers and marker assisted selection in breeding for organic agriculture. *Euphytica* 2010, 175, 51–64.

Lang, N.T.; Li, Z.K.; Bui, C.B. Microsatellite markers linked to salt tolerance in rice. *Omonrice* 2001, 9, 9–21.

Li, F. Y.; Chaigne-Delalande, B.; Kanellopoulou, C. et al. Second messenger role for Mg^{2+} revealed by human T-cell immunodeficiency. *Nature* 2011, 475, 471–476.

Li, X.; Yang, M.; Chen, H.; Qu, L.; Chen, F.; Shen, S. Abscisic acid pretreatment enhances salt tolerance of rice seedlings: proteomic evidence. *Biochim. Biophys. Acta* 2010, 1804, 929–940.

Lijinsky, W. Carcinogenicity and mutagenicity of N-nitroso compounds. *Mol. Toxicol.* 1987, 1, 107–119.

Lin, H.X.; Zhu, M.Z.; Yano, M.; Gao, J.P.; Liang, Z.W. QTLs for Na and K uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet.* 2004, 103, 253–260.

Linh, L.H.; Linh, T.H.; Xuan, T.D.; Ham, L.H.; Ismail, A.M.; Khanh, T.D. Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *Int. J. Plant Genom.* 2012, 2012, 1–9.

Mackill, D.J. Molecular markers and marker-assisted selection in rice. In: Genomic assisted crop improvement; Varshney, R.K., Tuberosa, R., Eds; Springer, New York, 2007.

Mahdavi, V.; Farimani, M.M.; Fathi, F.; Ghassempour, A. A targeted metabolomics approach toward understanding metabolic variations in rice under pesticide stress. *Anal. Biochem.* 2015, 478, 65–72.

Makihara, D.; Tsuda, M.; Morita, M.; Hirai, Y.; Kuroda, T. Effect of salinity on the growth and development of rice (*Oryza sativa* L.) varieties. *Jpn. J. Trop. Agri.* 1999, 43, 285–294.

Manikanda, B. Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits, 3rd ed.; Springers, New Delhi, India, 2013.

Mardani, Z.; Rabiei, B.; Sabouri, H.; Sabouri, A. Identification of molecular markers linked to salt-tolerant genes at gemination stage of rice. *Plant Breed.* 2014, 133, 196–202.

Maria, L.C.V.; Luciane, S.; Augusto, L.D.; Carla de, F.M. Microsatellite markers: What they mean and why they are so useful. *Genet. Mol. Biol.* 2016, 3, 312–328.

Marschner, H. Mineral Nutrition of Higher Plants, 3rd ed.; Academic Press, London, 2012.

Martinez, V.A.; Hill, W.G.; Knott, S.A. On the use of double haploids for detecting QTL in outbred populations. *Heredity* 2002, 88, 423–431.

Mehmood, E.U.H.; Kausar, R.; Akram, M.; Shahzad, S.M. Is boron required to improve rice growth and yield in saline environment? *Pak. J. Bot.* 2009, 41, 1339–1350.

Menguer, P.K.; Sperotto, R.A.; Ricachenevsky, F.K. A walk on the wild side: *Oryza* species as source for rice abiotic stress tolerance. *Genet. Mol. Biol.* 2017, 40, 238–252.

Mian, A.A.; Prasad, S.; Maathuis, F.J.M. Improving crop salt tolerance: anion and cation transporters as genetic engineering targets. In: Plant Nutrition and Abiotic Stress Tolerant III Plant Stress 5; Anjum, N.A., Lopez-Lauri, F., Eds; Global Science Books, UK, 2011.

Mishra, B.; Singh, R.K.; Jetly, V. Inheritance pattern of salinity stress in rice. *J Genet. Breed.* 1998, 52, 325–331.

Mishra, P.; Bhoomika, K.; Dubey, R.S. Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive *Indica* rice (*Oryza sativa* L.) seedlings. *Protoplasma.* 2013, 250, 3–19.

Moeljopawiro, S. and Ikehashi, H. Inheritance of salt tolerance in rice. *Euphytica* 1981, 30, 291–300.

Mohamadi, N.; Arzani, A.; Rezai, A.M.; Singh, R.K.; Gregorio, G.B. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *Saltol* QTL. *Afr. J. Biotechnol.* 2008, 7, 730–736.

Mohammadi, R; Merlyn, S.M.; Genaleen, Q.D.; Glenn, B.G.; Rakesh, K.S. Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa) Plant Breed.* 2013, 133, 548–559.

Moomaw, A. S. ang Maguire, M. E. The unique nature of Mg^{2+} channels. *Physiology* 2008, 23, 275–285.

Moradi, F.; Ismail, A.M.; Gregorio, G.B.; Egdane, J.A. Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. *India J. Plant Physiol.* 2003, 8, 105–116.

Muhamad, A.J.; Fahrul, Z.H.; Alina, W.; Faeza, M.S. Identification of QTLs for Morph-Physiological traits related to salinity tolerance at seedling stage in *Indica* rice. *Procedia Environ. Sci.* 2011, 8, 389–395.

Munns, R. Genes and salt tolerance: bringing them together. *New Phytol.* 2005, 167, 645–663.

Munns, R. Plant adaptations to salt and water stress: differences and commonalities. *Adv Bot Res.* 2011, 57, 1–32.

Nachimuthu, V.V.; Muthurajan, R.; Duraialaguraja; S. et al. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa. Rice* 2015, 8, 30.

Neelam, S.; Hemant, R.K.; Praveen, S.; Sneh, L.P.; Ashwani, P. A suite of new genes defining salinity stress tolerance in seedlings of contrasting rice genotypes. *Funct. Integr. Genomic.* 2013, 13, 351–365.

Negrao, S., Courtois, B., Ahmadi, N., Abreu, I., Saibo, N., Oliveira, MM. Recent updates on salinity stress in rice: from physiological to molecular responses. *Crit. Rev. Plant Sci.* 2011, 30, 329–377.

Negrao, S.N.; Almadanim, M.C.; Pires, I.; Abreu, I.A; Maroco, J.; Courtois, B; Gregorio, G.B.; McNally, K.L.; Oliveira, M.M. New allelic variants found in key rice salt-tolerance genes: an association study. *Plant Biotechnology J.* 2012, 11, 87–100.

Nejad, M.N.; Arzani, A.; Rezai, A.M.; Singh, R.K.; Gregorio, G.B. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *Saltol* QTL. *Afr. J. Biotechnol.* 2008, 7, 730–736.

Niones, J.M. Five Mapping of the Salinity Tolerance Gene on Chromosome 1 of Rice (*Oryza Sativa* L.) Using Near Isogenic Lines. Master's Thesis, University of the Philippines, Los Banos, Laguna, Philippines, 2004.

Nissim-Levi, A; Ovadia, R.; Forer, I.; Oren-Shamir, M. Increased anthocyanin accumulation in ornamental plants due to magnesium treatment, *J. Hortic. Sci. Biotechnol.* 2007, 82, 481–487.

Nounjan, N. and Theerakulpisut, P. Effects of exogenous proline and trehalose on physiological responses in rice seedlings during salt-stress and after recovery. *Plant Soil Environ.* 2012, 58, 309–315.

Oladosu, Y.; Mohd, Y.R.; Norhani, A.; Ghazali, H.; Asfaliza, R.; Harun, A.R.; Gous, M.; Magaji, U. Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnol. Biotech. Eq.* 2016, 30, 1–16.

Payandeh, J.; Pfoh, R.; Pai, E. F. The structure and regulation of magnesium selective ion channels. *Biochim. Biophys. Acta* 2013, 1828, 2778–2792.

PIC Calculator. Available online: *https://www.liverpool.ac.uk/~kempsj/pic.html* (accessed on March 11, 2018).

Platten, J.D.; Egdane J.A.; Ismail, A.M. Salinity tolerance, Na^+ exclusion and allele mining of HKT1;5 in *Oryza sativa* and *Oryza glaberrima*: Many sources, many genes, one mechanism? *BMC Plant Biol*. 2013, 13, 32.

Prasad, S.R.; Bagali, P.G.; Hittalmani, S.; Shashidhar, H.E. Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr. Sci.* 2000, 78, 162–164.

Qin, H.; Li, Y.; Huang, R. (2020). Advances and challenges in the breeding of salt-tolerant rice. *Int. J. Mol. Sci* 2020, 21, 8385.

Quan, N.T.; Xuan, T.D. Foliar application of vanillic and p-hydroxybenzoic acids enhanced drought tolerance and formation of phytoalexin momilactones in rice. *Arch. Agron. Soil Sci.* 2018, 64, 1–16.

Quan, N.V.a; Xuan, T.D.; Tran, H.-D.; Thuy, N.T.D.; Trang, L.T.; Huong, C.T.; Yusuf, A.; Tuyen, P.T. Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of *Canarium tramdenum* Bark. *Molecules* 2019, 24, 605.

Quan, N.V.b; Thien, D.D.; Khanh, T.D.; Tran, H.-D.; Xuan, T.D. Momilactones A, B, and tricin in rice grain and by-products are potential skin aging inhibitors. *Foods* 2019, 8, 602.

Quan, N.V.c; Tran, H.-D.; Xuan, T.D.; Ahmad, A.; Dat, T.D.; Khanh, T.D; Teschke, R. Momilactones A and B are α-Amylase and α-Glucosidase Inhibitors. *Molecules* 2019, 24, 482.

Rabbani, M.A.; Maruyama, K.; Abe, H.; Khan, M.A.; Katsura, K.; Ito, Y.; Yoshikawa, K.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* 2003, 133, 1755–1767.

Rahman, A.; Hossain, M.S.; Mahmud, J.A.; Nahar, K.; Hasanuzzaman, M.; Fujita, M. Manganese-induced salt stress tolerance in rice seedlings: regulation of ion homeostasis, antioxidant defense and glyoxalase systems. *Physiol Mol Biol Plants.* 2016, 22, 291306.

Rahman, A.; Nahar, K.; Hasanuzzaman, M.; Fujita, M Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. *Front. Plant Sci.* 2016, 7, 609.

Rahman, A.; Nahar, K.; Mahmud, J. A.; Hasanuzzaman, M.; Hossain, S.; Fujita, M. Salt stress tolerance in rice: emerging role of exogenous phytoprotectants. In: Advances in international rice research; Li, J., Ed; InTech, Rijeka, 2017.

Rahman, A; Kamrun, N.; Jubayer, A. M.; Hasanuzzaman, M.; Hossain, M.S; Masayuki, F. Salt stress tolerance in rice: emerging role of exogenous phytoprotectants, advances in international rice research; Li, J. Ed.; IntechOpen, 2017.

Rahman, M.A.; Thomson, M.J.; Shah-E-Alam, M.; De Ocampo, M., Egdane, J.; Ismail, A.M. Exploring novel genetic sources of salinity tolerance in rice through molecular and physiological characterization. *Ann. Bot. Lond.* 2016, 117, 1083–1097.

Räsänen, K. and Kruuk, L.E.B. Maternal effects and evolution at ecological time scales. *Funct. Ecol.* 2007, 21, 408.

Ratho, S.N.; Pradhan, S.B. Cytoplasmically controlled cold tolerance in a cytoplasmic-genetic male sterile line of rice. *Euphytica* 1992, 58, 241–244.

Reddy, A.M.; Francies, R.M.; Rasool, S.N.; Reddy, V.R.P. Breeding for tolerance stress triggered by salinity in rice. *Int. J. Appl. Biol. Pharm. Technol.* 2014, 5, 167–176.

Reflinur.; Kim, B.; Jang, S.M.; Chu, S.H.; Bordiya, Y.; Akter, M.B.; Lee, J.; Chin, J.H.; Koh, H.J. Analysis of segregation distortion and its relationship to hybrid barriers in rice. *Rice* 2014, 7, 3.

Reflinur.; Kim, B.; Jang, S.M.; Chu, S.H.; Bordiya, Y.; Akter, M.B.; Lee, J.; Chin, J.H.; Koh, H.J. Analysis of segregation distortion and its relationship to hybrid barriers in rice. *Rice* 2014, 7, 3.

Ren, Z.H.; Gao, J.P.; Li, G.L.; Cai, X.L.; Huang, W.; Chaw, D.Y.; Zhu, M.Z.; Wang, Z.Y.; Luan, S.; Lin, H.Y. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat. Genet.* 2005, 37, 1141–1146.

Richardson, K.J.; Lewis, K.H.; Krishnamurthy, P.K.; Kent, C.; Wiltshire, A.J.; Hanlon, H.M. Food security outcomes under a changing climate: Impacts of mitigation and adaptaion on vulnerablity to food insecurity. *Clim. Chang.* 2018, 147, 327–341.

Russel, P.J. Extension of and deviations from Mendelian genetic principles. In: iGenetics a molecular approach, 3rd ed.; Wilbur, B., Ed.; Pearson Benjamin Cummings, San Francisco, USA, 2010.

Ruvinsky, I. Segregation. In: Brenner's encyclopedia of genetics; Maloy, S., Hughes, K., Eds.; Academic Press, Elsevier Inc, London, UK, 2013.

Sabouri, H.; Biabani, A. Toward the mapping of Agronomic characters on a rice genetic map: Quantitative Trait loci analysis under saline conditions. *Biotechnology* 2009, 8, 144–149.

Šamec, D.; Karalija, E.; Šola, I.; Vujčić Bok, V.; Salopek-Sondi, B. The role of polyphenols in abiotic stress response: the tnfluence of molecular structure. *Plants* 2021, 10, 118.

Sato, M. and Sato, K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *Biochim. Biophys. Acta, Mol. Cell Res.* 2013, 1833, 1979–1984.

Satoh, H.; Matsusaka, H.; Kumamaru, T. Use of N-methyl-N-nitrosourea treatment of fertilized egg cells for saturation mutagenesis of rice. *Breed. Sci.* 2010, 60, 475–485.

Sebastian, A. and Prasad, M.N. Iron- and manganese-assisted cadmium tolerance in *Oryza sativa* L.: lowering of rhizotoxicity next to functional photosynthesis. *Planta.* 2015, 241, 1519–1528.

Shabani, A; Sepaskhah, A.R.; Kamgar-Haghighi, A.A. Growth and physiologic response of rapeseed (*Brassica napus* L.) to deficit irrigation, water salinity and planting method. *Int J Plant Prod.* 2013, 7, 569–596.

Shannon, M.C. Principles and strategies in breeding for higher salt tolerance. *Plant Soil* 1985, 89, 227–241.

Sharma, A.; Thakur, S.; Kumar, V.; Kanwar, M.K.; Kesavan, A.K.; Thukral, A.K.; Bhardwaj, R.; Alam, P.; Ahmad, P. Pre-sowing seed treatment with 24-epibrassinolide ameliorates pesticide stress in *brassica juncea* L. through the modulation of stress markers. *Front. Plant Sci.* 2016, 7, 1569.

Sharma, A.; Yuan, H.; Kumar, V.; Ramakrishnan, M.; Kohli, S.K.; Kaur, R.; Thukral, A.K.; Bhardwaj, R.; Zheng, B. Cas-tasterone attenuates insecticide induced phytotoxicity in mustard. *Ecotoxicol. Environ. Saf.* 2019, 179, 50–61.

Shi, C.H.; Xue, J.M.; Yu, Y.G.; Yang, X.E.; Zhu, J. Analysis of genetic effects on nutrient quality traits in Indica rice. *Theor. Appl. Genet.* 1996, 92, 1099–1102.

Shi, C.H.; Zhu, J. Analysis of seed and maternal genetic effects for characters of cooking quality in *Indica* rice. *Chin. J. Rice Sci.* 1994, 8, 129–134.

Shimura, K.; Okada, A.; Okada, K.; Jikumaru, Y.; Ko, K.W.; Toyomasu, T.; Sassa, T.; Hasegawa, M.; Kodama, O.; Shibuya, N.; Koga, J.; Nojiri, H.; Yamane, H. Identification of a biosynthetic gene cluster in rice for momilactones. *J. Biol. Chem.* 2007, 23, 34013–34018.

Shrivastava, P.; Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* 2015, 22, 123–131.

Sikora, P.; Chawade, A.; Larsson, M.; Olsson, J.; Olsson, O. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *Int. J. Plant. Genom.* 2011, 2011, 1–13.

Singh, A.K.; Singh, P.K.; Arya, M; Singh, N.K.; Singh, U.S. Molecular screening of blast resistance genes in rice using SSR markers. *Plant Pathol. J.* 2015, 31, 12–24.

Singh, R.K. and Flowers, T.J. Physiology and Molecular Biology of the Effects of Salinity on Rice, 3rd ed.; CRC Press, Boca Raton, FL, USA, 2010.

Singla, N.; Dhawan, D.K. Modulation of carbohydrate metabolism during N-methyl N-nitrosourea induced neurotoxicity in mice: role of curcumin. *Neurochem. Res.* 2010, 35, 660–665.

Soetan, K.O; Olaiya, C.O; Oyewole, O.E. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* 2010, 4, 200–222.

Sreedhara, A. and Cowan, J.A. Structural and catalytic roles for divalent magnesium in nucleic acid biochemistry. *Biometals* 2002, 15, 211–223.

Successful Study: Bright Leaf Tolerant Variety BC15. Available online: *http://www.cuctrongtrot.gov.vn/TinTuc/Index/4351* (accessed on June 26, 2019).

Sun, M.F.; Yan, G.H.; Wang, A.M.; Zhu, G.Y.; Tang, H.S.; He, C.X.; Ren, Z.L.; Liu, K.; Zhang, G.Y.; Shi, W. et al. Research progress on the breeding of salt-tolerant rice varieties. *Barley Cereals Sci.* 2017, 34, 1–9.

Suplick-Ploense, M.R; Qian, Y.L.; Read, J.C. Salinity tolerance of Texas bluegrass, Kentucky bluegrass, and their hybrids, *Crop Sci.* 2002, 42, 2025–2030.

Suzen, S.; Jenkins, G.J.S.; Parry, J.M. Application of the restriction site mutation technique to N-methyl-N-nitrosourea-induced mutations in the rat. *Teratog., Carcinog. Mutagen.* 1998, 18, 171–182.

Suzuki, T.; Eiguchi, M.; Kumamaru, T.; Satoh, H. et al. MNU-induced mutant pools and high-performance TILLING enable finding of any gene mutation in rice. *Mol Genet Genom* 2008, 279, 213–223.

Tao, D.; Hu, F.; Yang, J.; Yang, J.; Yang, G.; Yang, Y.; Xu, P.; Li, J.; Ye, C.; Dai, L. Cytoplasm and cytoplasm-nucleus interactions affect agronomic traits in Japonica rice. *Euphytica* 2004, 135, 129–134.

Teh, C.Y.; Mahmood, M.; Shaharuddin, N.A., Ho, C.L. In vitro rice shoot apices as simple model to study the effect of NaCl and the potential of exogenous proline and glutathione in mitigating salinity stress. *Plant Growth Regul. 2015*, 75, 771–781.

Teng, S. Gene Tagging for Salt Tolerance in Rice (*Oryza sativa* L.). Ph.D. Thesis, The University of the Philippines, Los Banos, Laguna, Philippines, 1994.

Tester, M. and Davenport, R. Na^+ tolerance and Na^+ transport in higher plants. *Ann Bot* 2003, 91, 1–25.

Thomson, M.J.; Ocampo, M.; Egdane, J.; Rahman, M.A.; Sajise, A.G.; Adorada, D.; Tumimbang-Raiz, E.; Blumwald, E.; Seraj, Z.I.; Singh, R.K.; Gregorio, G.B.; Ismail, A.M. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. *Rice* 2010, 3, 148–160.

Till, B.J.; Cooper, J.; Tai, T.H.; Colowit, P.; Greene, E.A.; Henikoff, S.; Comai, L. Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biol. 2*007, 7, 1–12.

Toyomasu, T.; Kagahara, T.; Okada, K.; Koga, J.; Hasegawa, M.; Mitsuhashi, W.; Sassa, T.; Yamane, H. Diterpene phytoalexins are biosynthesized in and exuded from the roots of rice seedlings. *Biosci. Biotechnol. Biochem.* 2008, 72, 562–567.

Tsubura, A.; Lai, I. C.; Miki, H.; Sasaki, T; Uehara, N.; Yuri, T.; and Yoshizawa, K. Animal models of N-methyl-N-nitrosourea-induced mammary cancer and retinal degeneration with special emphasis on therapeutic trials. *In vivo* 2011, 25, 11–22.

Uchida, A.; Jagendorf, A.T.; Hibino, T.; Takabe, T. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.* 2002, 163, 515–523.

Viana, V. E.; Pegoraro, C., Busanello, C.; Costa de Oliveira, A. Mutagenesis in rice: the basis for breeding a new super plant. *Front. Plant Sci.* 2019, 10, 1326.

Wakabayashi, K; Hoson, T; Kamisaka S. Osmotic stress suppresses cell wall stiffening and the increase in cell wall bound ferulic and diferulic acids in wheat coleoptiles. *Plant Physiol.* 1997, 113, 967–973.

Walia, H.; Wilson, C.; Condamine, P.; Liu, X.; Ismail, A.M.; Zeng, L.; Wilson, C.; Condamine, P.; Lile, X.; Zing, L.; et al. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiol.* 2005, 139, 822–835.

Wang, C.L.; Zhang, Y.D.; Zhao, L.; Lu, K.; Zhu, Z.; Chen, T.; Zhao, Q.Y.; Yao, S.; Zhou, L.H.; Zhao, C.F., et al. Research status, problems and suggestions on salt-alkali tolerant rice. *China Rice.* 2019, 25, 1–6.

Wang, L.; Shan, T.; Xie, B.; Ling, C.; Shao, S.; Jin, P.; Zheng, Y. Glycine betaine reduces chilling injury in peach fruit by enhancing phenolic and sugar metabolisms. *Food Chem.* 2019, 272, 530–538.

Wattoo, J.I.; Liaqat, S.; Mubeen, H.; Ashfaq, M.; Shahid, M.N.; Farooq, A.; Sajjad, M.; Arif, M. Genetic mapping of grain nutritional profile in rice using basmati derived segregating population revealed by SSRs. *Int. J. Agric. Biol*. 2019, 21, 929–935.

White, P.J. and Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* 2009, 182, 49–84.

Wu, G.Q. and Wang, S.M. Calcium regulates K^+/Na^+ homeostasis in rice (*Oryza sativa* L.) under saline conditions. *Plant Soil Environ.* 2012, 58, 121–127.

Wu, J.L; Sinha, P.K; Variar, M.; Zheng, K.L.; Leach, J.E.; Courtois, B.; Leung, H. Association between molecular markers and blast resistance in an advanced backcross population of rice. *Theor. Appl. Genet*. 2004, 108, 1024–32.

Xu, Y.B.; Shen, Z.T. Maternal effect on chalkiness in rice kernels. *Rice Genet. Newsl.* 1988, 5, 111–113.

Xuan, T.D.; Anh, T.T.T.; Hoang-Dung, T.; Khanh, T.D., Dat, T.D. Mutation breeding of a N-methyl-N-nitrosourea (MNU)-induced rice (*Oryza sativa* L. ssp. *Indica*) population for the yield attributing traits. *Sustainability* 2019, 11, 1062.

Xuan, T.D.; Minh, T.N.; Anh, L.H.; Khanh, T.D. Allelopathic momilactones A and B are implied in rice drought and salinity tolerance, not weed resistance. *Agro. Sust. Dev.* 2016, 36, 52.

Alheit, K.V.; Reif, J.C.; Maurer, H.P.; Hahn, V.; Weissmann, EA.; Miedaner, T.; Würschum, T. Detection of segregation distortion loci in triticale (x *Triticosecale* Wittmack) based on a high-density DArT marker consensus genetic linkage map. *BMC Genomics* 2011, 12, 380.

Yeo, A.R. and Flowers T.J. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Aust. J. Plant Physiol.* 1986, 13, 161–173.

Yeo, A.R. and Flowers, T.J. Mechanisms of salinity resistance in rice and their role as physiological criteria in plant breeding. In: Salinity tolerance in plants-strategies for crop improvement; Staples, R.C., Toenniessen, G.A., Eds.; Wiley, New York, USA, 1984.

Yoshida, S.; Forno, D.A.; Cock, J.H.; Gomez, K.A. laboratory manual for physiological studies of rice, 3rd ed.; International Rice Research Institute, Los Baños, The Philippines, 1976.

Yoshida, Y.; Lee, H.S.; Trung, B.H.; Tran, H.-D.; Lall, M.K.; Kakar, K.; Xuan, T.D. Impacts of mainstream hydropower dams on fisheries and agriculture in lower Mekong basin. *Sustainability* 2020, 12, 2408.

Yu, X.; Zhao, Z.; Zheng, X.; Zhou, J.; Kong, W.; Wang, P.; Bai, W.; Zheng, H.; Zhang, H.; Li, J.; Liu, J.; Wang, Q.; Zhang, L.; Liu, K.; Yu, Y.; Guo, X.; Wang, J.; Lin, Q.; Wu, F.; Ren, Y.; Zhu, S.; Zhang, X.; Cheng, Z.; Lei, C.; Liu, S.; Liu, X.; Tian, Y.; Jiang, L.; Ge, S.; Wu, C.; Tao, D.; Wang, H.; Wan, J. A selfish genetic element confers non-Mendelian inheritance in rice. *Science* 2018, 360, 1130–1132.

Zayed, B.A.; Salem; A.K.M.; El Sharkawy, H.M. Effect of different micronutrient treatments on rice (*Oryza sativa* L.) growth and yield under saline soil conditions. *World J. Agric. Res.* 2011, 7, 179–184.

Zeng, L.; Shannon, M.C. Salinity effects on seedling growth and yield components of rice. *Crop Sci.* 2000, 40, 996–1003.

Zhang, G.Y.; Guo, Y.; Chen, S.L.; Chen, S.Y. RFLP tagging of a salt tolerance gene in rice. *Plant Sci.* 1995, 110, 227–234.

Zhang, H.X.; Blumwald, E. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nat. Biotechnol*. 2001, 19, 765–768.

Zhu, H.; Li, Y.; Liang, J. et al. Analysis of QTLs on heading date based on single segment substitution lines in rice (*Oryza Sativa* L.). *Sci. Rep.* 2018, 8, 13232.

APPENDIX

Supplementary Table S1. SSR markers used for distinguishing female parent (TBR1) and male parent (KD18)

No.	SSRs	Forward	Reverse	Chr.	Aneal. Temp.	References
1	RM10793	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC	1	55	Ganie et al. 2016, Chowdhury et al. 2010, Nejad et al. 2008, Islam et al. 2012
2	RM10720	GCAAACGTCTACGTGAGAAACAAGC	GCATGTGGTGCCTTAACATTTGG	1	55	Chowdhury et al. 2010
3	RM149	GCTGACCAACGAACCTAGGCCG	GTTGGAAGCCTTTCCTCGTAACACG	8	55	Chowdhury et al. 2010, Mardani, Xuan
4	RM10745	TGACGAATTGACACACCGAGTACG	ACTTCACCGTCGGCAACATGG	1	55	Ganie et al. 2016, Nejad et al. 2008
5	RM1287	GGAAGCATCATGCAATAGCC	GGCCGTAGTTTTGCTACTGC	1	55	Ganie et al. 2016, Nejad et al. 200, Islam et al. 2012
6	RM140	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	1	55	Ganie et al. 2016, Islam et al. 2012, Linh et al. 2012
7	RM10843	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG	1	55	Chowdhury et al. 2010
8	RM10825	GGACACAAGTCCATGATCCTATCC	GTTTCCTTTCCATCCTTGTTGC	1	55	Nejad et al. 2008
9	RM10764	AGATGTCGCCTGATCTTGCATCG	GATCGACCAGGTTGCATTAACAGC	1	55	Ganie et al. 2016
10	RM10694	TTTCCCTGGTTTCAAGCTTACG	AGTACGGTACCTTGATGGTAGAAAGG	1	55	Chowdhury et al. 2010
11	RM10852	GAATTTCTAGGCCATGAGAGC	AACGGAGGGAGTATATGTTAGCC	1	55	Chowdhury et al. 2010,
12	RM10748	CATCGGTGACCACCTTCTCC	CCTGTCATCTATCTCCCTCAAGC	1	55	Neelam et al. 2013
13	RM13197	AAACCCTCCGGCTCATTCTTGC	ACTCGAATCGTATCGGCTTGAGG	2	55	Thomson et al. 2010
14	RM127	GTGGGATAGCTGCGTCGCGTCG	AGGCCAGGGTGTTGGCATGCTG	4	55	Thomson et al. 2010
15	RM1233	GTGTAAATCATGGGCACGTG	AGATTGGCTCCTGAAGAAGG	11	55	Askani et al. 2012
16	RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	9	55	Javed et al. 2011 Xuan et al. 2019
17	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	2	55	Xuan et al. 2019
18	RM202	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA	11	55	Xuan et al. 2019
19	RM231	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	3	55	Zhu et al. 2018

No.	SSRs	Forward	Reverse	Chr.	Aneal. Temp.	References
20	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	1	55	Zhu et al. 2018
21	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	12	55	Zhu et al. 2018
22	RM214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	7	55	Wu et al. 2003
23	RM233	CCAAATGAACCTACATGTTG	GCATTGCAGACAGCTATTGA	2	55	Wattoo et al. 2019
24	RM289	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG	5	55	Chowdhury et al. 2010
25	RM222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	10	55	Chowdhury et al. 2010
26	RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11	55	Xuan et al. 2019
27	RM264	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	8	55	Chowdhury et al. 2010
28	RM20224	AGTATGAAAGTCGGTGACGATGG	GAGATGTCACGTCTTCACTTAGGG	6	55	Thomson et al. 2010
29	RM28102	CACTAATTCTTCGGCTCCACTTTAGG	GTGGAAGCTCCGAGAAAGTGC	12	55	Thomson et al. 2010
31	RM3867	TTGACTGGAACATCGAGCTC	ATCCCCTCTACACCGTACCC	3	55	Thomson et al. 2010
32	RM493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	1	55	Ganie et al. 2016, Chowdhury et al. 2010, Nejad et al. 2008, Islam et al., 2012, Linh et al. 2012
33	RM413	GTACGTAAACGCGGAAGGTG	TCCCCACCAATCTTGTCTTC	5	55	Askani et al. 2011
34	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG	6	55	Singh et al. 2015
35	RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	4	55	Boranayaka et al. 2018
36	RM5963	CGAAAAGTGGGAAGCAAATG	GCGTACCCCTAGTGGCTGTA	6	55	Nachimuthu et al. 2015
37	RM5926	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC	11	56	Fuentes et al. 2007
38	RM562	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC	1	55	Ganie et al. 2016, Nejad et al. 2008, Linh et al. 2012
39	RM6329	CCCTGGATGAAAAGCACAAG	GAAGTTGTAGATGCCCCATC	3	55	Thomson et al. 2010
40	RM7075	TATGGACTGGAGCAAACCTC	GGCACAGCACCAATGTCTC	1	50	Chowdhury et al. 2010, Neelam et al. 2013
41	RM8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA	1	55	Genie et al. 2016, Chowdhury et al. 2010, Nejad et al. 2008, Islam et al. 2012
42	RM339	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG	8	55	Kioko et al. 2015
43	RM207	CCATTCGTGAGAAGATCTGA	CACCTCATCCTCGTAACGCC	2	55	Xuan et al. 2019

No.	SSRs	Forward	Reverse	Chr.	Aneal. Temp.	References
44	RM213	ATCTGTTTGCAGGGGACAAG	AGGTCTAGACGATGTCGTGA	2	55	Xuan et al. 2019
45	RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	9	55	Xuan et al. 2019
46	RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	11	55	Xuan et al. 2019
47	RM432	TTCTGTCTCACGCTGGATTG	AGCTGCGTACGTGATGAATG	7	55	Xuan et al. 2019
48	RM508	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC	6	55	Xuan et al. 2019, Aliyu et al. 2013
49	RM587	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC	6	55	Xuan et al. 2019, Aliyu et al. 2013
50	RM589	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	6	55	Xuan et al. 2019, Aliyu et al.

Supplementary Table S2. Polymorphic SSRs for screening M_2 and M_3 generations

No.	SSRs	Chr.	Position	Map set	Related traits	References
1	RM 493	1	60-60 cM	CIAT SSR 2006	Shoot Na+ concentration	Thomson et al. 2010
2	RM 562	1	78.4-83.5 cM	Cornell SSR 2001	Shoot K+ concentration	Mohamadi-Nejad et al. 2008
3	RM 10694	1	10969872-10970066 bp	GANS 2009	Shoot Na–K ratio	Islam et al. 2012
4	RM 10720	1	11394704-11394908 bp	GANS 2009	Root K+ concentration	Chowdhury et al. 2010
5	RM 10793	1	12569890-12570013 bp	GANS 2009	Root Na–K ratio	Linh et al. 2012
6	RM 10852	1	13976520-13976691 bp	GANS 2009		Ganie et al. 2016
7	RM 13197	2	16439803-16439985 bp	GANS 2009	Seedling plant height Seedling survival Leaf chlorophyll content Root K+ concentration	Neelam et al. 2013 Thomson et al. 2010
8	RM 518	4	25.5-25.5 cM	CIAT SSR 2006	Water and nitrogen use efficiency	Boranayaka et al. 2018
9	RM 1233	11	112.9-112.9 cM	IRMI 2003	Blast disease resistance	Ashkani et al. 2011
10	RM 149	8	122.1–122.1 cM	CIAT SSR 2006	Plumule (shoot) fresh weight *Saltol* QTL Brown planthopper resistance Plant height Spikelet fertility	Mardani et al. 2013 Chowdhury et al. 2010 Xuan et al. 2019
11	RM 201	9	81.2–81.2 cM	Cornell SSR 2001	Root Na–K ratio Blast disease resistance Plant height Grain yield	Muhamad et al. 2011 Xuan et al. 2019
12	RM 202	11	42.1–42.1 cM	CIAT SSR 2006	Plant height Spikelet fertility	Xuan et al. 2019
13	RM 206	11	104.2–104.2 cM	CIAT SSR 2006	Blast disease resistance Brown planthopper resistance Plant height Spikelet fertility	Xuan et al. 2019
14	RM 207	2	191.2–191.2 cM	Cornell SSR 2001	Blast disease resistance	Xuan et al. 2019

No.	SSRs	Chr.	Position	Map set	Related traits	References
15	RM 213	2	186.4–186.4 cM	Cornell SSR 2001	Blast disease resistance	Xuan et al. 2019
16	RM 219	9	11.7–11.7 cM	Cornell SSR 2001	Amylose content Plant height Grain yield	Xuan et al. 2019
17	RM 229	11	77.8–77.8 cM	Cornell SSR 2001	Amylose content Plant height Spikelet number	Xuan et al. 2019
18	RM 432	7	43.5–43.5 cM	Cornell SSR 2001	Grain yield Amylose content	Xuan et al. 2019
19	RM 508	6	0–0 cM	Cornell SSR 2001	Leaf diameter Amylose content	Aliyu et al. 2013 Xuan et al. 2019
20	RM 587	6	10.7–10.7 cM	Cornell SSR 2001	Root length Amylose content	Aliyu et al. 2013 Xuan et al. 2019
21	RM 589	6	3.2–3.2 cM	Cornell SSR 2001	Root length Amylose content Gel consistency	Aliyu et al. 2013 Xuan et al. 2019