SALINITY TOLERANT RICE (*Oryza sativa* L.): PHENOTYPIC-GENOTYPIC EVALUATION OF RICE GENETIC RESOURCE

BY

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ABSTRACT

Salinity is one of the most severe environmental stresses that significantly reduce crop yield production worldwide. The present study was aimed to; 1) identify salinity tolerant genotypes at seedling stage in the Malaysian rice germplasm; 2) study the effects of salinity stress on rice seedling; 3) to determine the presence of salinity tolerant *Saltol* and SKC1 QTLs in the identified tolerant genotypes. Research was conducted using a split-plot design with three replications. The main plot was the level of salinity vis-a-vis normal freshwater at 0 dS m^{-1} (T1), and saline water at 4 dS m^{-1} (T2), 8 dS m^{-1} (T3), and 12 dS m^{-1} (T4), while the rice genotype was used as a sub-plot, respectively. A total number of 52 rice genotypes including four checks genotypes, namely Pokkali (tolerant), Nona Bokra (tolerant), IR29 (susceptible) and IR64 (susceptible) were screened. The salinity injury levels based on Standard Evaluation System (SES) scoring index and survival rate (SR) were recorded after two weeks of salinity stress treatment. Results on survival rate showed that MRQ76 completely survived 100% under 12 dS m^{-1} (T4) incomparable to both tolerant checks, Pokkali and Nona Bokra. Survival rate recorded the highest broad sense heritability with 0.954 followed by SES (0.69). Based on cluster analysis, the genotypes were classified into four different clusters. The Cluster I comprised MRQ76 as well as Pokkali as highly tolerant genotypes. Nona Bokra, Q70, Putra 1 and Jayanti were grouped in the Cluster II as tolerant genotypes while the rest of genotypes were classified as susceptible (Cluster III). All highly tolerant and tolerant genotypes identified were further analyzed for molecular screening. A total of 3 *Saltol* tightly linked SSR markers mainly RM1287, RM3412 and RM8094 were used in the screening. In addition, RM578, IM8748 and IM8854 markers were used in evaluating the presence of SKC1 QTL. Interestingly, based on those tightly linked markers, both *Saltol* and *SKC1* were absent in the MRQ76, Q70, Jayanti, and Putra 1, respectively. This result might suggest the presence of other salinity tolerant QTLs in those genotypes. Further study on reproductive stage salinity tolerance could be suggested in future.

Keywords: Salinity tolerant, rice, seedling stage, *Saltol* and *SKC1* QTLs

ملخص البحث

الملوحة هي واحدة من أشد الضغوط البيئية التي تقلل بشكل كبير من إنتاج غلة المحاصيل في جميع أنحاء العالم. هدفت هذه الدراسة إلى: 1) تحديد الأنماط الوراثية المتحملة للملوحة في مرحلة الشتلات في الأصول الوراثية SKC1 للأرز الماليزي؛ 2) دراسة آثار إجهاد الملوحة على شتلات الأرز؛ 3) تحديد وجود سالتول سالتول و المتحمل للملوحة في الأنماط الوراثية المتحملة التي تم تحديدها. أُجريت الأبحاث باستخدام تصميم QTLs قطعة أرض منقسمة مع ثلاث تكرارات. كانت قطعة الأرض الرئيسية عبارة عن مستوى الملوحة مقابل المياه 1-و8 ديسيس م ،(T2) 1-والمياه المالحة عند 4 ديسيس م ،(T1) 1-العذبة العادية عند 0 ديسيس م بينما استخدم النمط الوراثي للأرز كمخطط فرعي على التوالي. تم ،(T4) 1-و12 ديسيس م ،(T3) فحص ما مجموعه 52 نمطًا وراثيًا من الأرز بما في ذلك أربعة فحوصات، وهي بوكالي (متحمل)، ونونا بوكرا حساس). تم تسجيل مستويات الإصابة بالملوحة استنادًا إلى) IR64 حساس)، و) IR29 (متحمل)، و بعد أسبوعين من المعالجة (SR) ومعدل البقاء على قيد الحياة (SES) مؤشر درجات نظام التقييم القياسي قد نجا تمامًا بنسبة MRQ76 بإجهاد الملوحة. أظهرت النتائج المتعلقة بمعدل البقاء على قيد الحياة أن لا يقارن بكل من الشيكات المتسامحة وبوكالي ونونا بوكرا. سجل (T4) 1-100% تحت 12 ديسيس م .(0.69) SES معدل البقاء على قيد الحياة أعلى معدل توريث بالمعنى الواسع حيث بلغ 0.954 يليه ارتباطًا كبيرًا بين السمتين. وبناءً على التحليل العنقودي، تم تصنيف الأنماط SR و SES أظهر كل من وكذلك بوكالي كنماذج وراثية MRQ76 الوراثية إلى أربع مجموعات مختلفة. تألفت المجموعة الأولى من عالية التحمل. تم تجميع نونا بوكرا وكيو 70 وبوترا 1 وجايانتي في المجموعة الثانية كأنماط وراثية متسامحة بينما

صُنفت بقية الأنماط الوراثية إما على أنها حساسة (المجموعة الثالثة). تم تحليل جميع الأنماط الوراثية شديدة التحمل والمتحملة التي تم تحديدها لمزيد من الفحص الجزيئي. استُخدم في الفحص ما مجموعه 3 من صانعي RM8094. و RM3412 و RM1287 بشكل رئيسي SSR المرتبطين بإحكام SSR سالتول في تقييم وجود IM8854 و IM8748 و RM578 بالإضافة إلى ذلك، استُخدمت العلامات ومن المثير للاهتمام، استنادًا إلى تلك الواسمات ذات الارتباط الوثيق، كان كل من QTL SKC1. وجايانتي وبوترا 1 على التوالي. وقد تشير Q70 و MRQ76 غائبًا في كل من SKC1 السالتول و هذه النتيجة إلى وجود أنماط وراثية أخرى تتحمل الملوحة في تلك الأنماط الوراثية. يمكن اقتراح إجراء المزيد من الدراسات حول تحمل الملوحة في مرحلة التكاثر في المستقبل.

الكلمات المفتاحية: متحمل الملوحة، الأرز، مرحلة الشتلات، سالتول و SKC1 QTLs

APPROVAL PAGE

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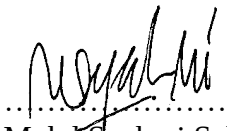

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DECLARATION

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This dissertation is dedicated to Che Selmah binti Ibrahim for laying the foundation of what I turned out to be in life.

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LIST OF FORMULA

LIST OF SYMBOLS

cm	Centimetres
°C	Degree celcius
g	Gram
ml	Milimeter
rpm	Revolution per minute
µg/ml	Microgram per mililiter
nm	Nanometer
µm	Micrometer
dsm^{-1}	Decisiemens per meter
MT	Metric tons

LIST OF ABBREVIATION

ANOVA	Analysis of Variance
BSH	Broad Sense Heritability
DAS	Day after sowing
DNMRT	Duncan New Multiple Range Test
EC	Electrical Conductivity
GA	Genetic Advance
GAM	Genetic Advance as percentage of mean
GCV	Genotypic Coefficient of Variance
LS	Leaves size
MSB	Mean square of blocks
MSE	Mean square of errors
MSG	Mean square of genotypes
MST	Mean square of treatments
NOL	Number of leaves
NPK	Nitrogen, Phosphorus, Potassium
PCV	Phenotypic Coefficient of Variance
PH	Plant height
RL	Root length
SAS	Statistical Analysis Software
SES	Standard Evaluation System
VE	Variance of error
VG	Genotypic variance
VP	Phenotypic variance

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Rice plays a crucial role in providing sustenance for a significant portion of the world's population, serving as a dietary staple for billions of individuals (Han et al., 2022). It has been estimated that rice yields need to be increased by about 70% by 2050 to meet the rising global demand for direct human consumption. Asia stands out as both the largest producer and consumer of rice. In Malaysia, rice holds immense cultural and dietary significance, with an average individual consuming approximately two and a half plates of rice per day. However, despite this demand, Malaysia only produced a total of 1.8 million metric tons (MT) of rice in 2016, achieving a self-sufficiency level (SSL) of only 70%. The increasing global population necessitates higher rice production to meet demand. Therefore, improving rice yield is crucial to ensure an adequate rice supply.

Ansari et al. (2015) reported that over 50% of reported crop damage is attributed to abiotic stresses, with salinity being one of the most severe environmental stresses. Salinity stress significantly impacts agricultural productivity by affecting plant growth and development, leading to substantial reductions in yield under prolonged exposure to high salt concentrations. (Gupta and Huang, 2014). This stress disrupts essential physiological processes in plants, including water uptake, nutrient absorption, and photosynthesis, resulting in stunted growth and diminished yields. Salinity stress can induce changes in various physiological and metabolic processes, depending on its intensity and duration. Generally, salinity interrupts rice growth and development, plant adaptation, and stress responses (Reddy et al., 2017). It has been demonstrated that rice plants are inherently susceptible to salt stress, particularly during the seedling and reproductive growth stages (Singh et al., 2007; Amirjani, 2011).

High salinity levels in the soil would affect seed germination, plant growth, and harvestable grain yield (Hussain et al., 2017). For example, a high accumulation of sodium and chloride ions leads to early senescence of older leaves (Hussain et al., 2017). Meanwhile, the ability of rice plants to withstand salt stress is important in ensuring rice yield production. It is crucial for plants to maintain a balance of sodium (Na^+) and potassium (K^+) ions within their cells to survive in saline environments. Increases in Na^+ ion concentration cause competition for transporter between K^+ and Na^+ ions, as they both share the same transporter, resulting in decreased K^+ ion uptake during salinity stress.

Overall identification of salt-tolerant rice genotypes based on morphological attributes had widely studied. Certain traditional cultivars and landraces have naturally developed tolerance to salt stress over generations through adaptation to thrive in salt-affected environments. This tolerance is a result of the continuous selection of individuals that exhibit better performance under high salinity conditions, leading to the accumulation of beneficial traits that enable these plants to withstand salt stress more effectively. Several traditional varieties including varieties from coastal areas of South Asia such as Nona Bokra (India), Pokkali (Sri Lanka), Getu (India), Kalarata 1-24 (India), Ketumbar (Indonesia) had been identified as tolerant traditional varieties (Negrao et al., 2011). These varieties, however, have poor agronomic characteristics, such as tall plant stature, poor grain consistency, low yield, and photosensitivity.

The selection and adoption of plant species with high salt tolerance have consistently been the preferred approach to enhance productivity in salt-affected soils (Soltabayeva et al., 2021). This selection process may rely on phenotypic and/or genotypic attributes. Relying solely on phenotypic attributes for the selection of salinity-tolerant varieties may not be reliable, as these attributes can be influenced by environmental factors. Hence, it is crucial to molecularly characterize various rice varieties for accurate analysis of genetic diversity. Therefore, SSR markers serve as valuable tools for characterizing salt-tolerant genotypes, being highly polymorphic, reproducible, co-dominant, and multi-allelic, making them well-suited for this purpose. (Kumari et al., 2019). SSR marker

analysis is valuable for identifying major gene loci associated with salt tolerance, providing valuable insights for plant breeders aiming to develop new rice cultivars. By targeting specific genetic regions linked to salt tolerance, breeders can strategically incorporate these genes into breeding programs to produce rice varieties with enhanced resilience to salinity stress, ultimately contributing to sustainable crop production in salt-affected areas.

Moreover, a significant Quantitative Trait Locus (QTL) for salinity stress responsiveness, known as Saltol, has been mapped to chromosome 1 in an F8 Recombinant Inbred Line (RIL) population resulting from a cross between Pokkali (salt-tolerant) and IR29 (salt-sensitive) rice varieties (Waziri, 2016). The Saltol QTL has been associated with the Na^+/K^+ ratio and salinity tolerance at the seedling stage, contributing to reduced Na^+ absorption, increased K^+ absorption, and a lower Na^+-to-K^+ ratio in rice shoots under salinity stress. Dependable molecular markers associated with Saltol also have been identified and employed for screening rice genetic resources to assess tolerance against salinity stress. (Kumari et al., 2019). This genetic mapping provides vital information for plant breeders aiming to comprehend the genetic basis of salt tolerance in rice and develop improved varieties through targeted breeding efforts.

Therefore, a high-throughput screening method that incorporates both phenotypic and genotypic assessments could be regarded as the most effective selection of diverse germplasms and genetic resource in the pre-breeding stage. The potential genotypes identified would later be used as donor parent in improving salinity tolerant of elite rice cultivars.

1.2 SIGNIFICANCE OF STUDY

The rapidly increasing global population has precipitated a surge in the demand for rice, necessitating a commensurate increase in its production. However, the looming threat of climate change, presenting formidable challenges, including the relentless rise in sea levels and the peril of floods, which imperil extant rice cultivation. The challenges posed by global climate change, such as the escalation of sea levels and the threat of floods, have cast a shadow over rice production worldwide. To illustrate, rice fields situated along coastal regions are susceptible to inundation by saltwater, leading to an elevation in both the salinity levels of the irrigated water and the soil. The incursion of seawater into these fields has resulted in a notable increase in soil salinity.

Furthermore, salinity presents a significant issue, impacting a third of the world's agricultural land (Kranto et al., 2016). Noteworthy research from the Malaysian Agriculture Research and Development Institute (MARDI) (Shahril, 2020, pers. comm.) and Universiti Kebangsaan Malaysia (UKM) (Noraziyah, 2020, pers. comm.) has revealed that the salinity levels in irrigation water collected from rice fields in Kg. Segantang Garam, Merbok, Kedah and Kg. Ban Pecah, Tanjung Piandang, Kuala Kurau, Perak ranged from 8-10 dS/m. In Pahang, rice fields near coastal areas, particularly in Kg Serandu, Pekan, and Pantai Sepat, Kuantan, recorded a salinity level of approximately 2-4 dS/m. These findings underscore the urgency in developing rice cultivars with salinity tolerance for our nation.

It is noteworthy that, to date, no salinity-tolerant rice cultivar has been introduced in Malaysia. Moreover, approximately 13,000 rice accessions in the National Rice Genebank, MARDI Seberang Perai, Penang remain underutilized in terms of their economic and genetic potential. Hence, this current study endeavors to assess the phenotypic-genotypic relationship of our local rice germplasm with regards to their capacity to withstand salinity stress during the seedling stage.

1.3 RESEARCH HYPOTHESIS

i. New salinity tolerant rice genotypes will be identified in the Malaysian rice germplasm.
ii. Salinity stress influences the morpho-physiological response of seedlings.
iii. The rice genotypes tolerant to salinity exhibited the presence of salinity tolerant *Saltol* and SKC1 QTLs.

1.4 RESEARCH OBJECTIVES

i. To identify salinity tolerant genotypes at seedling stage in the Malaysian rice germplasm.
ii. To study the effects of salinity stress on rice seedlings.
iii. To determine the presence of salinity tolerant *Saltol* and SKC1 QTLs in the identified tolerant genotypes.

CHAPTER TWO

LITERATURE REVIEW

2.1 RICE (*Oryza sativa* L.)

2.1.1 Origin, Distribution, Taxonomy and Classification

Rice belongs to the genus *Oryza*, family: Poaceae (Gramineae), tribe: Oryzeae. The genus *Oryza* has two cultivated species (*Oryza sativa; Oryza glaberrima*) and other 22 wild species of domesticated rice (Jena & Nissila, 2017), which widely distributed throughout the tropics and subtropics of the world. The origin of rice probably begins about 130 million years ago as a wild grass. *O. sativa* is mainly cultivated in the Asiatic region, Africa, Europe, Oceania, and North, Central, and South America, whereas *O. glaberrima* in West Africa. Asian rice is believed to be domesticated about 10000 years ago, meanwhile for African rice is about 3000 years ago (Sangeetha et al., 2020). The common ancestor of all rice species is *Oryza perennis*. *Oryza sativa*, commonly known as Asian rice, is derived from the perennial *Oryza rufipogon* and the annual *Oryza nivara*. On the other hand, *Oryza glaberrima*, also called African rice, originates from the perennial *Oryza longistaminata* and the annual *Oryza barthii*. The genus has both diploid (2n = 24), with genomic formula AA, along with tetraploid (2n = 48) species (Vijay & Roy, 2013). *Oryza sativa* consists of two major subspecies known as *Oryza japonica* and *Oryza indica*. Roychoudhury (2020) stated that *japonica* was firstly domesticated, while *indica* was emerged subsequently by adopting domestication alleles from *japonica*. The two subspecies are differed and classified as they had a distinct leaf morphology and seed traits. *Japonica* rice is characterized by its sticky texture and short grains, typically cultivated in dry fields. On the other hand, *Indica* rice is known for its non-sticky nature and long grains, predominantly grown in submerged fields. (Garris et al. 2005). Additionally, *javanica* type was identified as third subpopulation of *O.sativa* based on morphological, cytological and genetic analyses. Referring to United States Department of Agriculture (USDA), *Oryza sativa* is classified into taxonomical order as Table 1.

Table 1. The taxonomic classification of rice (retrieved from https://plants.usda.gov)

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae/Gramineae
Genus	*Oryza* L.
Species	*Oryza sativa* L.

2.1.2 Rice Morphology

Rice is primarily a self-pollinating plant, but cross-pollination facilitated by wind also possible. Rice plants are in the range of 1 and 1.8-meter-tall depending on the soils. Rice is typically cultivated in heavier soils with good water retention capacity. The rice plant is classified as an annual grass, characterized by round, hollow, jointed culms, sessile leaf blades, and terminal panicles. It has a wide range of heights, from dwarf varieties measuring 0.3 to 0.4 meters tall to floating varieties reaching heights of over 7 meters. The commercial variety usually ranges from 1 to 2 m tall.

2.1.3 Growth Stages and Development

In Malaysia, the majority of rice cultivation is carried out through the transplanting method. Young rice seedlings are typically transplanted by hand into rice fields. The paddy plant is a distinctive short-term crop with a maturity period ranging from 90 to 140 days after germination (Omar et al., 2019). Rice growth is typically divided into three main phases: vegetative, reproductive, and ripening. Each phase consists of several stages of growth. In the vegetative phase, there are four stages: emergence, seedling development, tillering, and internode elongation. (Dunand and Saichuk, 2005). Similarly, the reproductive phase of growth is subdivided into three stages which include pre-booting, booting, heading. The last phase of rice growth, which is the ripening phase is subdivided into grain filling and maturity stages.

The germination or seed emergence stage starts right after seed sowing until the seed emerged. Most carbohydrates in a seed are stored in a tissue known as the endosperm, with the embryo comprising the remaining portion. Germination initiates through the absorption of water. Consequently, the seed expands, increases in weight, and commences the process of converting carbohydrates into sugars, thereby activating the embryo (Moldenhauer and Slaton, 2006). Seedling growth commences with the emergence of the primary leaf, which occurs shortly after the coleoptile is exposed to light and splits open at its tip. During this stage, the rice seedling is developing seminal roots and leaves. Typically, the seedling possesses five leaves and undergoes rapid root system development. Subsequently, it enters the tillering stage, which spans from the emergence of the first tiller until the maximum tiller count is achieved. Tillers originate from the auxiliary buds of the nodes and supplant the leaves as they grow and mature. During this phase, tillering occurs vigorously, and the plant experiences significant elongation. Following this, tillers continue to proliferate as the plant progresses into the subsequent stage known as stem elongation. The elongation stage begins before panicle initiation. The leaves continue elongated until the time to initiate panicle.

During the reproductive phase, the initial stage is panicle initiation, during which the panicle within the stem grows to approximately 1/8 inch. Subsequently, the panicle continues its growth and development within the stem. The heading stage commences when the panicle emerges from the end of the rice stem. Following this, the flowering stage begins within one to five days after heading. The final phase is the ripening stage, characterized by the filling of the grain. The grain filling process comprises the milk, dough, and mature grain stages. The milk grain stage is identifiable when a milky white substance starts accumulating shortly after heading. Meanwhile, the dough stage occurs about a week later after milky grain stage. The mature grain stage is when the rice grain first become firm and the grain moisture at this time is around 30 percent. The time for grain to ripen up for harvesting purpose is depending on the weather (Counce et al., 2015).

2.1.4 Rice Industry

The rice industry's contribution to Malaysia's agricultural sector reflects its significance within the country's economy. Despite efforts to increase domestic production, the reliance on rice imports highlights the importance of addressing challenges such as productivity constraints and self-sufficiency goals. As global demand for rice continues to grow, strategies to enhance production efficiency and achieve greater self-sufficiency will remain key priorities for Malaysia's rice industry.

In 2017, Asian countries accounted for over 80% of global rice consumption, and this demand is expected to rise further. Malaysia produced a total of 2.7 million metric tons (MT) of paddy in 2016, achieving only 70% self-sufficiency. With 31 million consumers exceeding the national domestic rice production, Malaysia had to import the remaining 821,869 MT, primarily from Thailand and Vietnam, to meet rice demand (Omar et al., 2019). By 2020, the rice industry contributed 2.5 percent to Malaysia's agricultural sector, indicating a slight increase from the previous year.In 2020, the rice industry contributed 2.5 percent to the agricultural sector in Malaysia, reflecting a slight increase compared to the preceding year.

In 2019, Malaysia produced around 1.88 million metric tons of rice. (Statista Research Department, 2022). Malaysia is a significant player in the global rice industry. In terms of production, the country harvested approximately 2.4 million metric tonnes of paddy rice in 2020 (Department of Agriculture Malaysia, 2021). This indicates a steady production level, with efforts in place to maintain self-sufficiency and food security. On the consumption side, Malaysia is a notable consumer of rice, with an average per capita consumption of around 85 kilograms per year (Department of Statistics Malaysia, 2021). This high consumption rate underscores the critical role rice plays in the Malaysian diet. Despite being a significant producer, Malaysia does import a portion of its rice to meet domestic demand. In 2020, the country imported approximately 33% of its total rice consumption (Department of Agriculture Malaysia, 2021). This highlights the importance of a balanced approach to both domestic production and international trade to ensure a stable rice supply.

2.1.5 Impact of Climate Change on Rice Production

The consequences of climate change in Malaysia certainly disturbed the agricultural sector and in particular rice, the country's staple food. For example, the extreme occurrence of droughts and floods could significantly affect paddy and rice production. (Firdaus et al. 2020). According to (Abas et al. 2017), rice yields are expected to fall by 10% for every 10°C increase in temperature. Other rice production constraints include increased salinity in coastal granary areas causes by the incursion of seawater as well as challenges of pests and disease (Herman et al. 2015). Similarly, the increase in sea levels would lead to flooding and salt intrusion (Abas et al., 2017), thus increasing soil salinity. It is reported that, over 50% of reported crop damage is attributed to abiotic stresses, including drought, high and low temperatures, salinity, submergence, and oxidative stress (Ansari et al.,2015), Therefore, the fact that climatic changes will increase the severity of soils affected by salt is prone to disrupt food security globally (Riaz et al., 2019).

2.2 SALINITY STRESS

Under normal soil conditions, the pH typically ranges from 4.5 to 7.5, with electrical conductivity (EC) levels below 4 dS m^{-1}, exchangeable sodium percentage (ESP) below 15, and sodium absorption ratio (SAR) below 15. These conditions create an optimal environment for nutrient availability and plant growth. In contrast, salt-stressed soils exhibit higher concentrations of soluble salts, characterized by an EC exceeding 4 dS m^-1, an ESP below 15, and an SAR below 15. Salt stress occurs when an excess of salts in the soil solution inhibits plant growth or, in severe cases, leads to plant death (Horie et al., 2012).

2.2.1 Osmotic Stress

Plants experience osmotic stress immediately upon exposure to elevated salt levels outside their roots. This stress leads to the inhibition of water uptake, cell expansion, and lateral bud development. (Horie et al., 2012). Generally, osmotic stress reduces the water uptake by roots and triggers internal dehydration. Besides to decrease in water absorption capacity of the root system, the leaves also lose water due to the high accumulation of salt in the soil. Osmotic stress also causes stomatal closure, resulting in less evaporation and overall water transport. It leads to the higher accumulation of reactive oxygen species (ROS), damaging various cellular components and macromolecules eventually causes death to the plant (Qin et al., 2020).

2.2.2 Ionic Stress

Excess salt in soils led to ionic stresses which disrupted metabolism and reduced the productivity of crops (Siringam et al., 2011). Long-term continuous transport of salt into transpiring leaves leads to very high Na^+ and Cl^- concentrations (Reddy et al., 2017). The Na^+/ K^+ homeostasis plays important role in survivability during salinity stress. Reddy et

al. (2017) proposed that the Na^+ exclusion systems consist of several cell membrane transporters such as H^+ pump ATPases, Na^+/H^+ antiporters, and high-affinity uptake ion K^+. In saline conditions, the binding of Na^+ to GIPC (glycosyl inositol phosphorylceramide) regulates the activity of Ca^{2+} channels, resulting in an increase in the cytoplasmic concentration of calcium ions (Ca^{2+}) in plants.. Moreover, the excess salt is transported either onto the vacuole or sequestered into older leaves tissue, which is eventually died to protect the plant against stress from salinity (Gupta & Huang, 2014). Xie et al. (2020) also stated that the vital mechanisms underlying salt tolerance in plants involve reducing the effect of ionic stress. It can be done by minimizing the Na^+ accumulation on the cytosol, transpiring leaves. Hence, reducing sodium (Na+) transport to the shoots is essential to improve the salinity tolerance of rice plants. (Horie et al., 2012).

2.3 EFFECT OF SALINITY STRESS ON RICE

Rice is classified as a salt-sensitive plant, experiencing significant reductions in growth under salt stress, leading to lower production in saline environments compared to non-saline ones (Tahir et al., 2013). Salinity disrupts rice growth, development, and stress responses, with the early seedling stage being particularly susceptible compared to the tillering stage (Reddy et al., 2017; Hussain et al., 2017). Salt stress impacts various aspects of rice growth and development, including seed germination, seedling growth, leaf size, shoot and root length, tiller number, flowering, spikelet number, and overall productivity (Hussain et al., 2017). The total dry weight of rice significantly decreases under salt stress during the seedling stage (Chunthaburee et al., 2015). Additionally, the osmotic effect of salinity stress leads to premature leaf senescence, characterized by leaf rolling, chlorosis, or necrosis at the tips and margins of older leaves (Wankhade et al., 2013).

2.3.1 Effects on Rice Growth

High salinity levels in soil and water can have detrimental effects on the growth and productivity of rice crops. Salinity stress interferes with various physiological processes in rice plants, leading to reduced germination rates, stunted growth, leaf burn and necrosis, decreased photosynthesis, and ultimately, lower yields. The presence of excess salts disrupts the osmotic balance within rice seeds, hindering germination (Munns & Tester, 2008). Furthermore, high salt concentrations in the soil inhibit water uptake by plant roots, causing water stress and impeding cell elongation, which results in stunted growth (Fang et al., 2008). Salinity stress also disrupts the uptake and transport of essential nutrients such as potassium and magnesium, leading to leaf damage and chlorosis (Zhu, 2001). Reduced photosynthetic efficiency due to salt-induced stomatal closure and damage to chloroplasts further limits carbohydrate production in rice plants (Chaves et al., 2009). Consequently, the cumulative effects of salinity stress often led to decreased rice yields (Hasegawa et al., 2000). Additionally, weakened rice plants under salinity stress are more susceptible to pest infestations and diseases, exacerbating yield losses (Manschadi et al., 2006). Efforts to mitigate salinity stress in rice cultivation include the development of salt-tolerant varieties, soil amendments, and improved irrigation management practices (Wang et al., 2003).

2.3.2 Effects on Rice Grain Development

Salinity stress profoundly impacts rice grain development, affecting both grain yield and quality. High salt concentrations in the soil and irrigation water disrupt the physiological processes crucial for grain formation, leading to decreased grain size, weight, and ultimately, lower yields (Ismail et al., 2007). Salinity-induced osmotic stress and water deficit during critical stages of grain filling can impair the transport of assimilates to developing grains, reducing starch accumulation, and compromising grain filling (Zeng et al., 2006). Additionally, salt stress alters the composition of grain constituents, affecting nutritional quality and market value. For instance, excessive sodium accumulation in grains can render them unsuitable for human consumption (Roy et al., 2014). Mitigating the

adverse effects of salinity on rice grain development requires integrated approaches such as breeding for salt tolerance, optimizing irrigation management, and utilizing soil amendments to alleviate salt stress in rice-growing environments.

2.3.3 Effects on Rice Photosynthesis

Salinity stress significantly affects rice photosynthesis, which is crucial for plant growth and productivity. High salt concentrations in the soil and irrigation water disrupt various physiological processes involved in photosynthesis, leading to reduced photosynthetic rates and impaired carbon assimilation (Chaves et al., 2009). One of the primary mechanisms through which salinity inhibits photosynthesis is by inducing stomatal closure, which reduces the uptake of carbon dioxide (CO2) necessary for photosynthetic carbon fixation (Chaves et al., 2009; Munns & Tester, 2008). Additionally, salt stress can damage chloroplast structure and function, affecting the efficiency of light capture and electron transport during photosynthesis (Chaves et al., 2009). As a result, rice plants under salinity stress often exhibit decreased chlorophyll content, altered chloroplast ultrastructure, and impaired photosynthetic electron transport (Chaves et al., 2009; Munns & Tester, 2008). Mitigating the negative effects of salinity on rice photosynthesis requires strategies aimed at improving salt tolerance and optimizing environmental conditions to support photosynthetic activity in saline environments.

2.3.4 Effects on Nutrient Accumulation and Transport

Rice plants experience decreased photosynthetic efficiency due to disruptions in the photosystem II (PSII) complex under salt stress. Furthermore, the presence of Na+ and Cl– ions damages chlorophyll content in rice leaves, potentially inhibiting electron transport in PSII. Elevated salt levels result in reduced plant growth by impairing CO2 assimilation, leading to stomatal closure and subsequently lower intracellular CO2 partial pressure, ultimately reducing the photosynthetic rate (Amirjani, 2011). The intracellular sodium ions

accumulated during salt stress alters the ratio of potassium to sodium, thereby affecting the bio-energetic processes of photosynthesis. (Sudhir and Murthy, 2004).

2.4 TOLERANCE RESPONSE MECHANISM OF SALINITY STRESS

Rice seedlings have evolved intricate mechanisms to withstand salinity stress, crucial for their adaptation and survival in saline environments. One of the primary strategies involves the regulation of ion homeostasis to minimize the toxic effects of excess salt accumulation within plant tissues. This includes mechanisms such as ion exclusion from roots and sequestration into vacuoles, thereby preventing the buildup of sodium (Na+) and chloride (Cl-) ions in sensitive tissues and maintaining cellular ion balance (Platten et al., 2013). Furthermore, rice seedlings synthesize compatible solutes like proline and glycine betaine to counteract osmotic stress induced by high salt levels, thereby preserving cell turgor and functionality (Singh et al., 2015). Another crucial aspect of rice seedling tolerance to salinity stress involves the activation of antioxidant defense mechanisms. Salinity stress triggers the production of reactive oxygen species (ROS), which can cause oxidative damage to cellular components. In response, rice plants upregulate antioxidant enzymes such as superoxide dismutase and catalase to scavenge ROS and protect against oxidative stress (Singh et al., 2015). This antioxidative defense system helps mitigate the harmful effects of salinity-induced oxidative damage, allowing rice seedlings to maintain cellular homeostasis and functionality.

Furthermore, recent research has highlighted the role of molecular and genetic factors in enhancing rice seedling tolerance to salinity stress. Studies have identified specific genes and signaling pathways involved in the rice plant's response to salinity stress, including those related to ion transport, osmotic regulation, and antioxidant defense (Singh et al., 2015). By comprehending the molecular mechanisms that cause salinity tolerance in rice seedlings, researchers can develop strategies for breeding salt-tolerant varieties and implementing effective management practices to enhance rice production in saline environments.

2.5 PRE-BREEDING STRATEGIES TO IMPROVE SALINITY TOLERANCE IN RICE

2.5.1 Screening of Salinity Tolerance in Rice Germplasm

Acquaah (2012) defined phenotypes as the product of the interaction of genes and the environment. Plant phenotyping, which involves evaluating expressed traits influenced by genetic makeup and environmental changes, is a crucial process in crop improvement programs. (Sankaran, 2016). Selecting superior individuals or identifying regions of the genome that control a trait often involves conducting hundreds or even thousands of measurements and require more time, space and resources. (Walter et al., 2015). This calls for high-throughput phenotyping, which has been and is still most widely accomplished by quantitative and qualitative assessments performed by plant breeders. Furthermore, assessing genotypes using high-throughput phenotyping at an early growth stage would save resources and time. (Kakar et al., 2019).

Developing salinity-tolerant rice varieties necessitates the use of dependable screening techniques. (Gregorio et al., 1997) on the morpho-physiological response of rice under salinity stress. According to Anshori et al. (2018), various screening techniques had previously been developed at the germination stage, hydroponics which serves as the most effective and rapid screening at the vegetative phase, and saline soil screening technique that reflects the actual environment of salinity stress. Furthermore, various screening methods have been employed to measure salinity tolerance in rice, encompassing morphophysiological traits such as shoot weight, shoot Na+ concentration, the ratio of shoot Na+/K+, leaf injury and survival rate, leaf area, and bypass flow in the root. (Hairmansis et al., 2014).

Nevertheless, the basis of plant breeding is genetic diversity. Thus, it is important to understand and assess crop management, crop selection improvement, crop germplasm

usage, genome structure detection, and the transfer of desirable traits to other plants (Sohrabi et al., 2012). Furthermore, assessing genetic diversity and identifying superior genotypes are critical elements of any crop improvement program (Muti, Hoque, Islam, Siddique, & Islam, 2020). According to Senguttuvel et al. (2016), crop genetic improvement is determined by the magnitude of genetic variation and the heritability of economically important traits. Consequently, it is of utmost importance for the initiations of an efficient breeding program, such as rice, to estimate variability using parameters such as the genotypic-phenotypic coefficient of variation, heritability, and genetic advance. Rice genotypes respond distinctly to salinity stress. Therefore, it is essential to have sufficient heritable variability among genotypes in achieving the desired degree of genetic adaptation of rice to salinity (Senguttuvel et al., 2016).

2.5.2 QTLs/Gene Mapping for Salinity Tolerance in Rice

Quantitative trait loci (QTL) are defined as genes that control quantitative traits (Acquaah, 2012). Salinity tolerance is a complex and polygenic trait that is controlled by quantitative traits loci (QTLs) (Batayeva et al., 2018). Genetic modifications are necessary for elite rice cultivars through the introduction of new genes or QTL pyramids in developing salt-tolerant lines for cultivation in salt stress environment (Jena & Nissila, 2017). Despite the significance of developing salinity tolerance in rice, only a limited number of quantitative trait loci (QTL) have been mapped using techniques such as microsatellite or Simple Sequence Repeat (SSR) markers, Amplified Fragment Length Polymorphism (AFLP), and even Restriction Fragment Length Polymorphism (RFLP) in various populations. (Islam et al., 2011). The identification of Quantitative Trait Loci (QTL) associated with salinity stress has also facilitated progress in breeding for salt tolerance. A remarkable effort in identifying a significant quantitative trait locus (QTL) contributes to salt tolerance in rice (Hoang et al., 2016). According to Waziri et al. (2016), the QTL mapping allowed breeders in understanding the genetic control of the salt tolerance mechanism, with the possibility of developing salt tolerant varieties by accurately transferring QTL into widely known elite varieties.

In rice, ion homeostasis is the primary trait through which salt-responsive QTLs confer salt tolerance. (Ganie et al., 2019). Major salinity tolerance QTL was mapped includes Saltol that control Na^+/K^+ uptake ratio (Tariq et al., 2019) and qSKC-1 regulates the shoot potassium ion concentration (SKC) in a salinity-tolerant genotypes under salinity stress and encodes a high-affinity potassium ion transporter (HKT)-type sodium transporter. (Gao & Lin, 2013). However, several studies have reported the detection of another salt-tolerant quantitative trait locus (QTL) in certain wild rice cultivars. (Ren et al., 2005).

Previously, a Recombinant Inbred Line (RIL) population was developed at IRRI between the indica varieties IR29 and Pokkali and utilized in a QTL study. AFLP genotyping was conducted on a set of 42 sensitive and 38 tolerant RILs to distinguish the salinity tolerance genetic components in the tolerant landrace Pokkali. Consequently, it resulted in identification of a notable Quantitative Trait Locus (QTL) associated with the balance of sodium and potassium ions (Na–K ratio) and the seedlings' ability to tolerate salt stress during early growth stages known as *Saltol.* This locus is located on chromosome 1, alongside several smaller QTLs located on different chromosomes. (Thomson et al., 2010). It is also confirmed that FL478, one of the RILs showed salt tolerance higher than or comparable to the tolerant parent, Pokkali. The *Saltol* QTL regulates the Na+ / K+ uptake ratio and explains 64.3 to 80.2% of the phenotypic variation in salt tolerance. (Nejad et al., 2010).

Saltol was mapped from Pokkali/IR29 crosses on chromosome 1 in an F8 Recombinant Inbred Line (RIL) population (Waziri et al., 2016). The fine mapped of the backcross of Pokkali/IR29 using near isogenic lines (NILs) also confirmed the Saltol QTL in the segment of chromosome 1 (Niones, 2004). According to Islam et al. (2011), RM8094 was identified to be strongly associated with salinity tolerance, with statistical significance at $P<0.001$.

Besides, Ren et al. (2005) also recorded that eight QTLs were discovered to be responsible for the variation in potassium (K+) and sodium (Na+) content, among which SKC1 emerged as a major QTL influencing the shoot content of both K+ and Na+. Additionally, eleven QTL for salt tolerance were identified on chromosomes 1,4,6,7, and 9 in a crossing population of Nona Bokra (salinity tolerant) and Koshihikari (susceptible), which includes major QTL, qSNC-7 that control shoot Na+ concentration and qSKC-1 for shoot K^+ concentration (Quan et al., 2018). By using F2 populations derived from a cross between Nona Bokra and Koshihikari cultivars the *SKC-1* QTL was mapped on chromosome 1 (Gao & Lin, 2013).

Enhancing rice production in coastal areas has been pursued through the development of salinity-tolerant varieties (Bhowmik et al., 2009). Additionally, the advancement of salt-tolerant rice varieties is expected to address rising food demands amid climate change impacts (Huong et al., 2020). The identification of salinity-tolerant genotypes is closely linked to environmental conditions, selection criteria, and methodologies (Anshori et al., 2018). Traditional approaches to selecting salt-tolerant plants face challenges due to significant environmental influences and low narrow-sense heritability of salt tolerance (Gregorio et al., 1997). Consequently, the development of accurate and rapid screening techniques has been hindered, but DNA/molecular markers hold promise for efficient evaluation and selection of plant materials.

Genotyping plays a crucial role in plant breeding as it involves analyzing a large number of plant individuals with diverse genetic variations, which is essential for breeding and improvement efforts (Kim et al., 2020). This method utilizes DNA/molecular markers to select specific traits and determine their association with desired traits in target plants (Seyum et al., 2019). By genotyping tightly linked markers with traits, it becomes possible to predict the phenotypes of a large selection at an early stage of their development, thereby reducing the time required for selection (Hayward et al., 2015). Marker-assisted selection (MAS) exemplifies the application of single marker-trait associations in crop breeding, facilitating the efficient selection of breeding lines for incorporating desirable traits into commercial crop accessions, including high-throughput screening of resulting progeny.

Genotyping technology encompass PCR-based molecular markers like random amplified length polymorphism (RAPD) and amplified fragment length polymorphism (AFLP), as well as sequence-based PCR markers such as simple sequence repeat (SSR) and the latest molecular marker, single nucleotide polymorphisms (SNPs). (Kim et al., 2020). SSR has emerged as a widely used type of codominant molecular marker in genetic analysis and applications in plant breeding. Besides, SSR markers or microsatellite markers have also been proven to be ideal for genetic mapping, analysis of DNA fingerprinting, and genotypes study (Ahmed et al., 2019). Islam et al. (2011) stated that DNA/molecular markers have been recognised as potentially useful tools for rice crop improvement, particularly in abiotic stresses.

Molecular markers serve as crucial tools for characterizing salt-tolerant genotypes. DNA fingerprinting utilizing SSR markers has played a significant role in identifying genes associated with salt tolerance (Ahmed et al., 2019). SSR markers are considered the most suitable for the specific characterization of genotypes for salt tolerance due to their high polymorphism, reproducibility, co-dominance, and multi-allelic nature (Gholizadeh & Navabpour, 2014; Kumari et al., 2019). SSR markers provide valuable information for understanding the genetic basis of salt tolerance in plants. Their high polymorphism enables the detection of genetic variations among different genotypes, while their reproducibility ensures consistent results across experiments. Additionally, SSR markers exhibit co-dominant inheritance, allowing for the precise determination of allele frequencies in populations. Their multi-allelic nature further enhances their utility in capturing the genetic diversity associated with salt tolerance traits. Overall, SSR markers play a crucial role in the precise characterization and selection of salt-tolerant genotypes in plant breeding programs.

CHAPTER THREE

METHODOLOGY

The research was divided into two parts: firstly, screening for salinity tolerance in the Malaysian rice resources, and secondly, the phenotypic-genotypic evaluation of the tolerant rice based on the screening conducted in the first part.

3.1 SCREENING FOR SALINITY TOLERANT GENOTYPES IN MALAYSIAN RICE RESOURCES

3.1.1 Plant Material and Experimental Design

The seeds of modern and traditional Malaysian rice genotypes were acquired from the IIUM Rice Collection (Salleh et al, 2020a). All seeds were originally obtained from the National Rice Genebank, MARDI Seberang Perai, Penang. The salinity tolerant genotypes mainly Pokkali and Nona Bokra and susceptible genotypes IR29 and IR64 were used as check variety. List of plant materials were summarized in Table 2.

Table 2. List of rice genotypes

Genotype	Local/variety name	Country of origin	Category
V1	Alek	Malaysia	Landraces
V2	Bario Halus	Malaysia	Landraces
V3	Basmati370	India	Traditional basmati
V4	Berjer	Malaysia	Landraces
V5	Biris	Malaysia	Landraces
V6	Boewani	Suriname	Landraces
V7	CICA4	Colombia	Cultivar

V8	Haiboq	China	Landraces
V9	IR29	Philippines	Cultivar
V10	IR64	Philippines	Cultivar
V11	IR64-SUB1	Philippines	Cultivar
V12	IR77(NIL)	Philippines	Breeding lines
V13	Jarom Mas	Malaysia	Landraces
V14	Jawi Kuning	Malaysia	Landraces
V15	Jayanti	Malaysia	Landraces
V16	Ketian Gedjih	Indonesia	Landraces
V17	Krian B	Malaysia	Landraces
V18	Kuku Balam	Malaysia	Landraces
V19	Kuku Belang	Malaysia	Landraces
V20	Kunyit	Malaysia	Landraces
V21	Kurau Wangi	Malaysia	Landraces
V22	Liwagu Antap	Malaysia	Landraces
V23	Madu	Malaysia	Landraces
V24	Mahsuri	Malaysia	Traditional variety
V25	Merah Wangi	Malaysia	Landraces
V26	MR219	Malaysia	Cultivar
V27	MR253	Malaysia	Cultivar
V28	MR297	Malaysia	Cultivar
V29	MR5	Malaysia	Cultivar
V30	MR82	Malaysia	Cultivar
V31	MRQ74	Malaysia	Aromatic cultivar
V32	MRQ76	Malaysia	Aromatic cultivar
V33	Nona Bokra	India	Landraces
V34	Padi Gunong	Malaysia	Landraces
V35	Paginkano	Malaysia	Landraces
V36	Pak Kedek	Malaysia	Landraces
V37	Pokkali	India	Landraces
V38	Pulut Malaysia 1	Malaysia	Cultivar
V39	Putra 1	Malaysia	Cultivar
V40	Putra 2	Malaysia	Cultivar
V41	Q70	Malaysia	Breeding lines
V42	Rengan Wangi	Malaysia	Landraces
V43	Santap Wangi	Malaysia	Landraces
V44	Selayang	Malaysia	Landraces
V45	Serandu1	Malaysia	Cultivar
V46	Tangkai Mas	Malaysia	Landraces
V47	UKM-5	Malaysia	Cultivar
V48	UKMRC2	Malaysia	Cultivar
V49	UKMRC8	Malaysia	Cultivar
V50	UKM-6	Malaysia	Cultivar

The experiment was conducted at the Glasshouse and Nursery Complex, International Islamic University Malaysia (IIUM), Kuantan Campus, Pahang, utilizing a split-plot design. Salinity stress levels were designated as the main plot, while rice genotype was assigned as the sub-plot (Figure 1). There were four different levels of salinity treatment including control mainly at 0 dS m^{-1} (T1), 4 dS m^{-1} (T2), 8 dS m^{-1} (T3), and 12 dS m^{-1} (T4). Fifty rice accessions of the modern and traditional genotypes were evaluated based on a method developed by Senanayake et al. (2017).

3.1.2 Planting Procedure and Salinity Treatments

All seeds were soaked in water for 24 hours before incubation for another 48 hours following (Salleh et al., 2020b). The pre-germinated seeds were sown in a planting tray of 104 holes containing about 10g of topsoil. Each genotype was replicated with 11 seeds per row for each treatment per block. Two planting trays were immersed in a multipurpose (188 x 59 x 6.5 cm) tray containing water of about 6 cm depth throughout the experimental period per each treatment and block. The seedlings were allowed for adaptation in normal soil conditions for a week. Besides, NPK 15:15:15 fertilizer was also applied after two weeks of planting. Saline water at 0, 4, 8, 12 dS/m were prepared following Senanayake et al. (2017) and added into the multipurpose tray based on their designated treatment. The salinity stress treatment was imposed seven days after sowing (DAS) by replacing freshwater with saline water according to the designated treatments. The salinity level of water and soil was monitored on daily basis starting from transplanting until harvest maturity and measured based on the Electrical Conductivity (EC) meter using COMBI EC 5000, STEP Systems GmbH, 90451 Numberg, Germany.

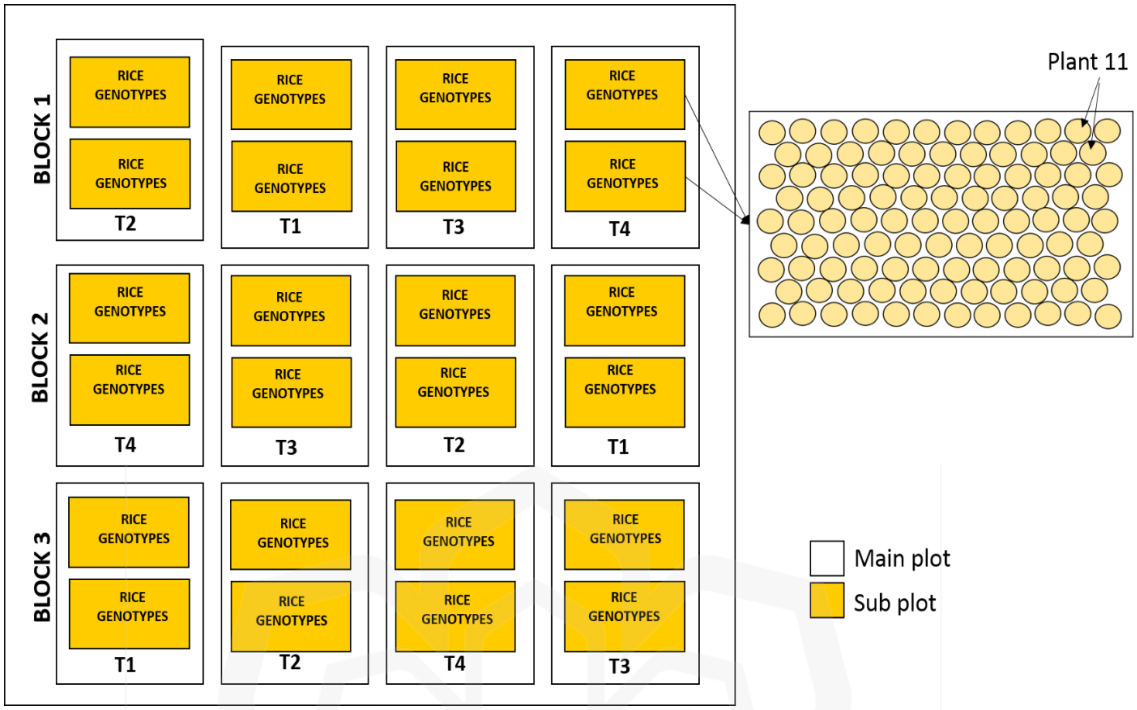

Figure 1. The experimental design and layout

Figure 2. Rice seedlings 7 days after transplant

3.1.3 Data Collection

3.1.3.1 Phenotyping Scoring Index

The phenotyping scoring index assessment was conducted to evaluate the sensitivity and tolerant ability of rice genotypes to salinity stress based on visual symptoms according to IRRI, (2013) with modification by Mondal et al. (2016) as shown in Table 3.

Table 3. Modified Standard Evaluation System (SES) for rice

Score	Observation	Rating
1	Normal growth, no leaf symptoms	Highly Tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded with most leaves are rolled and few elongating	Moderately Tolerant
7	Complete cessation of growth with most leaves dry and some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

3.1.3.2 Survival Rate

The survival rate (SR) was computed based on the following formula:

$$Survival\ Rate = \frac{No.of\ survive\ seedling}{Total\ number\ of\ seedlings}$$

Equation 1

3.1.4 Statistical analysis

The analysis of variance (ANOVA) was conducted using SAS 9.2 followed by mean comparison analysis using Duncan Multiple Range Test (DMRT) at $p<0.05$. Genetic variation was estimated by computing genotypic and phenotypic variance, error variance, and genotypic-phenotypic coefficient of variation, broad-sense heritability and expected genetic advance (GA) will be computed based on the following formula as mentioned by Sohrabi et al., (2012).

a) Genotypic variance:

$$\sigma_g^2 = \frac{MSG - MSE}{r}$$

Equation 2

Whereas MSG is the mean square of accessions, MSE is the mean square of error, and r is number of replications.

b) Phenotypic variance:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Whereas σ_g^2 is the genotypic pvariance and σ_e^2 is the mean square of the error.

Equation 3

c) Phenotypic coefficient of variance (PCV)

$$PCV\ (\%) = \frac{\sqrt{\sigma_p^2}}{\underline{X}} \times 100$$

Whereas σ_p^2 is the phenotypic variance and $\underline{X}$ is the mean of the trait.

Equation 4

d) Genotypic Coefficient of Variance (GCV)

$$GCV\ (\%) = \frac{\sqrt{\sigma_g^2}}{\underline{X}} \times 100$$

Whereas σ_g^2 is the genotypic variance and $\underline{X}$ is the mean of the trait.

Equation 5

e) Broad Sense Heritability

$$h_B^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Whereas σ_p^2 is the phenotypic variance and σ_g^2 is the genotypic variance.

Equation 6

f) Expected Genetic Advance (GA)

$$GA(\%) = K \times \sigma_p^2 \times h_B^2 \times 100$$

GA is a percent of the mean assuming selection of the superior 5% of accession.

$$GA(\%) = K \times \frac{\sqrt{\sigma_p^2}}{\underline{X}} \times h_B^2 \times 100$$

Whereas K is a constant, $\frac{\sqrt{\sigma_p^2}}{\underline{X}}$ is the phenotypic standard deviation, h_B^2 is the heritability and $\underline{X}$ is the mean of traits.

Equation 7

3.2 PHENOTYPIC – GENOTYPIC EVALUATION OF SALINITY TOLERANT GENOTYPES

3.2.1 Phenotypic Evaluation of Salinity Tolerant Genotypes

Phenotypic evaluation of the salinity tolerant genotypes was conducted using the same protocol as the previous screening for salinity tolerant genotypes.

3.2.1.1 Plant Material

Table 4. List of genotypes in the phenotypic evaluation of salinity tolerant genotypes

Gen	Genotypes name	Remarks
1	Nona Bokra	Tolerant check genotype
2	Pokkali	
3	IR29	Susceptible check genotype
4	IR64	
5	Jayanti	Identified as tolerant genotypes in the previous screening
6	MRQ76	
7	Putra 1	
8	Q70	

3.2.1.2 Morphological parameter

Morphological parameters including plant height (PH), leaf length (LL), leaf width (LW), leaf size (LS), number of leaves (NOL) (Table 5), and survival rate (SR) were evaluated for early seedling stage screening. All data was recorded prior to and after 7 days of salinity treatment imposition.

Table 5. List of morphological parameters

	Parameter	Description
1.	Plant height (PH)	The height was measured from the basal to the shoot tip
2.	Leave length (LL)	The length of the second youngest shoot/leaf
2.	Leaf size (LS)	Multiplying leaf length with the leaf width
5.	Number of leaves (NOL)	Number of leaves per seedling

3.2.2 Genotypic Evaluation of Salinity Tolerant Genotypes

3.2.2.1 DNA Extraction

The leaves were homogenized in a modified cetyltrimethylammonium bromide (CTAB) buffer within 2.0 mL microcentrifuge tubes and pulverized using a homogenizer according to the DNA extraction method outlined by Murray and Thompson (1980). The quantity of extracted DNA was subsequently measured using the NanoDrop™ Spectrophotometer (ThermoFisher Scientific). The DNA was then diluted to approximately 100 ng/µL using 1X Tris/EDTA (TE) buffer.

3.2.2.2 Polymerase Chain Reaction (PCR)

A total of 6 SSR markers mainly RM3412, RM8094, RM1287, RM578, IM8784, and IM8854 were tested in the polymorphism study. PCR components were prepared in PCR tubes. Specifically, a PCR master mix was created for each SSR marker. This mix consisted of 25µL of 2x PCRBIO Taq Mix, 0.5 µL of 10 mM forward and reverse primers, and 5.8

µL of double deionized water. Each tube was then loaded with approximately 9.0 µL of the PCR master mix and 2 µL of 100 ng/µL DNA template. Amplification reactions were conducted using a thermocycler with the following profile: initial denaturation at 95ºC for 4 minutes, followed by 30 cycles of denaturation at 95ºC for 15 seconds, annealing at 55ºC for 15 seconds, elongation at 72ºC for 15 seconds, and a final extension at 72ºC for 7 minutes. The optimal annealing temperature expected size of PCR product, and the forward and reverse sequences of each primer were obtained from the gramene.org database, as detailed in Table 6. The negative and positive control of the experiment has been added.

Table 6. Details of molecular marker used in the genotypic study.

QTL	Marker	AT (°C)	Expected size (bp)	Forward primer sequence	Reverse primer sequence	Remarks
Saltol	RM1287	55	154	GGAAGCATCA TGCAATAGCC	GGCCGTAGTTT TGCTACTGC	Flanking
	RM8094	55	209	AAGTTTGTACAC ATCGTATACA	CGCGACCAGTAC TACTACTA	Peak marker
	RM3412b	55	110	TCATGATGGATC TCTGAGGTG	GGGAGGATGCA CTAATCTTTC	Peak marker
SKC1	RM578	55	225	GGCGTCGTGTTT TCTCTCTC	CAAAAAGGAGG AGCAGATCG	Peak marker
	IM8784	55	219	AATGGACATCTT CTCAGCAA	AGTAAGGGAAC AACGACGA	Peak marker
	IM8854	55	167	CGATGAACCCCT ATTAATCT	GGTGATGGAATC GGACAG	Peak marker

3.2.2.3 Gel Electrophoresis

The amplicons were separated using 1.5% agarose gel at 100A and 180V for 40 minutes, then visualized using the ChemiDoc MP gel documentation system. The ladder utilized was PCRBIO IV, spanning from 100bp to 1500bp, as illustrated in the Figure 3.

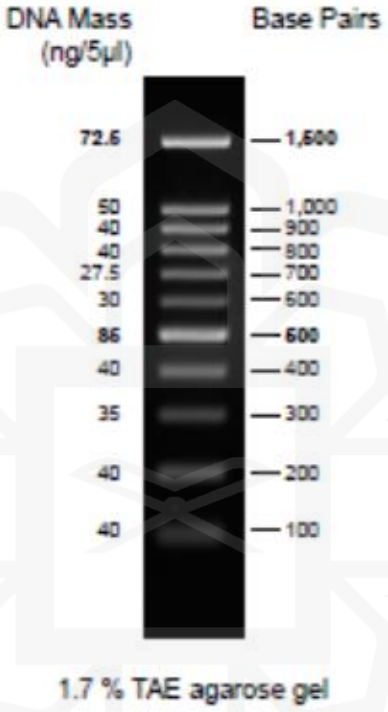

Figure 3. PCRBIO Ladder IV, 1.7 % TAE agarose gel (Retrieved from pcrbio.com)

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter is divided based on the objective of the study. Analysis of variance (ANOVA) was conducted for all morphological traits. Genetic analysis was performed to assess the heritability and potential genetic advancements of the studied traits based on their variance components. This data can assist breeders in identifying effective traits for selection criteria in salinity tolerant rice breeding programs.

4.1 Response of Rice Genotypes to Salinity Stress

Data for phenotyping scoring and survival rate for all 50 rice genotypes studied were analyzed. Analysis of variance at $p < 0.0001$ revealed highly significance differences among rice genotypes, salinity treatments and their intersection (genotypes x treatment) for the traits studied. For both traits, SES scoring, and survival rate were significantly different among rice genotypes, salinity treatments, and the salinity treatment x genotypes (TxG) intersection as shown in Table 7.

Table 7. ANOVA table for SES and survival rate of 50 rice genotypes.

Source of variation	SES				Survival Rate			
	Sum square	Mean square	F-Value	Pr > F	Sum square	Mean square	F-Value	Pr > F
Treatment (T)	5273.035	1757.6783	53.08	<.0001	70.101	23.367	59.96	<.0001
Genotype (G)	4853.765	99.056	2.99	<.0001	40.553	0.828	2.12	<.0001
TxG	9154.712	62.276	1.88	<.0001	131.383	0.894	2.29	<.0001

4.1.1 Phenotyping Scoring Index based on Standard Evaluation System (SES)

All genotypes recorded score 1.00 under normal condition at 0 dS m^{-1} (T1), while under T2 (4 dS m^{-1}), the SES score was ranged between 1.00 (Pokkali and Q70) and 9.40 (UKMRC8) as shown in Table 8. Under 8 dS m^{-1} (T3), the tolerant check, Pokkali consistently showed the lowest SES score at 2.33, while the highest score at 9.00 was showed by several genotypes mainly Basmati370, Biris, Merah Wangi, and MR82. Lower score indicating tolerant ability as opposed to high score (up to 9.00) indicating susceptibility. Based on the SES score in Table 8, two genotypes showed moderately tolerant to tolerant score incomparable to tolerant checks, Pokkali and Nona Bokra under high salinity treatment at 12 dS m^{-1} (T4). The SES score recorded for MRQ76 at 3.85 was comparable to Pokkali of score 3.00, indicating tolerant ability to salinity. Meanwhile, Jayanti was comparable to Nona Bokra with recorded SES score of 5.00 indicating moderately tolerant score. Recent study by Lutambi et al. (2024) on Eastern and Southern Africa rice genotypes indicated that the tolerant checks mainly Pokkali, FL478, and Nona Bokra recorded SES score of 1.0 to 1.7 indicating high tolerant ability to salinity at 12 dS/m, followed by genotype K5, Intsindagira bigega, and ZX117 with SES score of between 3.0-3.4, respectively.

Table 8. The SES scoring of 50 rice genotypes under levels of salinity treatments.

GEN	GENOTYPE	T1 (0 dS m^{-1})	T2 (4 dS m^{-1})	T3 (8 dS m^{-1})	T4 (12 dS m^{-1})
V1	Alek	1.00a	6.94bcdefghi	8.21 abcdefg	8.94a
V2	Bario Halus	1.00a	5.00jklmn	6.99gfhijk	9.00a
V3	Basmati370	1.00a	6.21bcdefghijk	9.00a	9.00a
V4	Berjer	1.00a	7.67abcd	7.85 abcdefgh	9.00a
V5	Biris	1.00a	6.53bcdefghij	9.00a	9.00a
V6	Boewani	1.00a	7.33bcdefg	8.64abc	8.70ab
V7	CICA4	1.00a	3.42nopqr	8.52abcd	9.00a
V8	Haiboq	1.00a	3.65mnopq	6.33ijkl	6.93ef
V9	IR29	1.00a	5.42ghijklm	8.67abc	9.00a
V10	IR64	1.00a	5.27hijklmn	7.83 abcdefgh	8.87ab
V11	IR64-SUB1	1.00a	5.67efghijkl	8.87ab	8.73ab

V12	IR77(NIL)	1.00a	5.67efghijkl	8.20 abcdefg	8.87ab
V13	Jarom Mas	1.00a	5.67efghijkl	8.70abc	8.45abc
V14	Jawi Kuning	1.00a	4.03lmnopq	6.58hijkl	8.88ab
V15	Jayanti	1.00a	1.67rst	3.67n	5.00g
V16	Ketian Gedjih	1.00a	4.70jklmnop	7.02fghijk	7.91bcd
V17	Krian B	1.00a	5.92defghijkl	5.32lm	7.72cde
V18	Kuku Balam	1.00a	7.06cdefgh	7.36cdefghij	8.27abc
V19	Kuku Belang	1.00a	6.93bcdefghi	8.27 abcdefg	9.00a
V20	Kunyit	1.00a	5.67efghijkl	7.77 abcdefgh	9.00a
V21	Kurau Wangi	1.00a	6.27cdefghijk	8.40abcde	9.00a
V22	Liwagu Antap	1.00a	5.48ghijklm	6.93ghijk	9.00a
V23	Madu	1.00a	8.33ab	8.09 abcdefg	8.94a
V24	Mahsuri	1.00a	4.82klmno	6.04jklm	9.00a
V25	Merah Wangi	1.00a	7.55abcde	9.00a	8.82ab
V26	MR219	1.00a	5.53ghijklm	8.73abc	9.00a
V27	MR253	1.00a	6.37cdefghijk	8.33 abcdef	8.67ab
V28	MR297	1.00a	5.40hijklm	8.07 abcdefg	8.73ab
V29	MR5	1.00a	7.43bcdef	7.60bcdefghi	8.00abcd
V30	MR82	1.00a	5.80defghijkl	9.00a	8.87ab
V31	MRQ74	1.00a	3.00opqrs	5.00m	8.78ab
V32	MRQ76	1.00a	2.45qrst	3.24no	3.85h
V33	Nona Bokra	1.00a	1.30st	3.06no	5.00g
V34	Padi Gunong	1.00a	7.84abc	8.70abc	9.00a
V35	Paginkano	1.00a	6.39 cdefghijk	8.45abcd	8.93a
V36	Pak Kedek	1.00a	6.03cdefghijk	7.06efghijk	8.76ab
V37	Pokkali	1.00a	1.00t	2.33o	3.00i
V38	Pulut Malaysia1	1.00a	5.67efghijkl	7.93 abcdefg	9.00a
V39	Putra1	1.00a	2.88pqrst	3.67n	7.22def
V40	Putra2	1.00a	4.33klmnopq	7.00fghijk	8.07abcd
V41	Q70	1.00a	1.00t	5.92klmn	6.42f
V42	Rengan Wangi	1.00a	6.45bcdefghijk	8.15abcdef	9.00a
V43	Santap Wangi	1.00a	6.21cdefghijk	6.21jklmn	9.00a
V44	Selayang	1.00a	5.12ijklmn	7.05efghijk	9.00a
V45	Serandu1	1.00a	6.16cdefghijk	8.07abcdefg	9.00a
V46	Tangkai Mas	1.00a	4.94jklmn	7.18defghij	9.00a
V47	UKM-5	1.00a	2.82pqrst	6.33ijkl	8.60abc
V48	UKMRC2	1.00a	7.4bcdef	7.93 abcdefg	8.33abc
V49	UKMRC8	1.00a	9.40a	7.87 abcdefg	8.47abc
V50	UKM-6	1.00a	5.60fghijkl	7.83 abcdefgh	8.33abc

4.1.2 Survival Rate of Rice Genotypes under Salinity Treatments

The survival rate of all genotypes was 100% under normal condition at 0 dS m^{-1} (T1) as shown in Table 9. However, under low level of salinity stress at 4 dS m^{-1} (T2), there was a variation in the survival rate of rice genotypes with the lowest score at 21% was recorded in Madu (V23). Moreover, several genotypes particularly Basmati370, Biris, Merahwangi, and MR82 were completely died with 0.00% survival rate under moderate level of salinity at 8 dS m^{-1} (T3). In addition, under the highest level of salinity treatment of 12 dS m^{-1} (T4), the highest survival rate at 100% was recorded in Pokkali and MRQ76, followed by Jayanti (83%), Nona Bokra (80%), and Q70 (73%), whilst the remaining genotypes were either completely died (0.00% survival rate) or lower than 70% (Table 9). Lutambi et al. (2024) also reported that Pokkali and Nona Bokra recorded significantly higher survival rate under 12 dS m^{-1} salinity level as compared to other genotypes.

Table 9. The survival rate of 50 rice genotypes under levels of salinity treatments.

GEN	GENOTYPE	T1 (0 dS m^{-1})	T2 (4 dS m^{-1})	T3 (8 dS m^{-1})	T4 (12 dS m^{-1})
V1	Alek	100% a	61% def	39%fghijk	3%f
V2	Bariohalus	100% a	100% a	69%abcdef	0.00%f
V3	Basmati370	100% a	91% abc	0.00%k	0.00%f
V4	Berjer	100% a	39%fgh	52%bcdeghi	0.00%f
V5	Biris	100% a	91%abc	0.00%k	0.00%f
V6	Boewani	100% a	61%def	18%hijk	15%def
V7	CICA4	100% a	100% a	24%ghijk	0.00%f
V8	Haiboq	100% a	93%ab	70%abcdef	15%def
V9	IR29	100% a	94%ab	7%jk	0.00%f
V10	IR64	100% a	93%ab	33%fghijk	7%f
V11	IR64SUB1	100% a	87%abcd	7%jk	13%def
V12	IR77(NIL)	100% a	100% a	40%efghijk	7%f
V13	Jarommas	100% a	87%abcd	15%ijk	24%def
V14	Jawikuning	100% a	100% a	88%abcd	6%f
V15	Jayanti	100% a	100% a	87%abcd	83%ab
V16	Ketiangedjih	100% a	91%abc	61%abcdefgh	45%cde

V17	Krianb	100% a	78%abcde	93%ab	45%cde
V18	Kukubalam	100% a	52%efg	65%abcdefg	27%def
V19	Kukubelang	100% a	80%abcd	36%fghijk	0.00%f
V20	Kunyit	100% a	88%abcd	58%abcdefgh	0.00%f
V21	Kurauwangi	100% a	79%abcd	23%ghijk	0.00%f
V22	Liwaguantap	100% a	94%ab	84%abcde	0.00%f
V23	Madu	100% a	21%h	45%defghi	0.03%f
V24	Mahsuri	100% aa	97%a	90%abc	0.00%f
V25	Merahwangi	100% a	64%def	0.00%k	9%f
V26	MR219	100% a	80%abcd	20%hijk	0.00%f
V27	MR253	100% a	73%abcde	25%ghijk	0.07%f
V28	MR297	100% a	100% a	47%cdefghi	13%def
V29	MR5	100% a	60%def	40%efghijk	10%f
V30	MR82	100% a	80%abcd	0.00%k	7%f
V31	MRQ74	100% a	100% a	87%abcd	7%f
V32	MRQ76	100% a	100% a	100% a	100% a
V33	Nonabokra	100% a	100% a	100% a	80%ab
V34	Padigunong	100% a	34%gh	15%ijk	0.00%f
V35	Paginkano	100% a	67%bcde	21%ghijk	4%f
V36	Pakkedek	100% a	97%a	61%abcdefg	12%ef
V37	Pokkali	100% a	100% a	100% a	100% a
V38	Pulutmalaysia1	100% a	100% a	53%bcdefghi	0.00%f
V39	Putra1	100% a	100% a	93%ab	40%cd
V40	Putra2	100% a	100% a	87%abc	47%cd
V41	Q70	100% a	93%ab	91%abc	73%abc
V42	Renganwangi	100% a	67%bcde	42%efghij	0.00%f
V43	Santapwangi	100% a	79%abcd	94%ab	0.00%f
V44	Selayang	100% a	97%a	72%abcdef	0.00%f
V45	Serandu1	100% a	80%abcd	47%cdefghi	0.00%f
V46	Tangkaimas	100% a	97%a	73%abcdef	0.00%f
V47	UKM-5	100% a	100% a	70%abcdef	20%def
V48	UKMRC2	100% a	80%abcd	53%bcdefghi	13%def
V49	UKMRC8	100% a	67%bcde	60%abcdefgh	27%def
V50	UKM-6	100% a	87%abcd	60%abcdefgh	47%cd

4.2 QUANTITATIVE GENETICS ANALYSIS

In this study, the phenotypic variances of both SES score and Survival rate (SR) were slightly higher than the genotypic variances, as shown in Table 10. Similarly, the phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV). The small differences between the genotypic and phenotypic coefficients of variation suggest an environmental effect on all traits. According to Teklu et al. (2014), PCV and GCV values greater than 20 were considered high, while values less than 10 were deemed low. The differences between PCV and GCV for both traits were small. As noted by Tuhina et al. (2015), higher differences between PCV and GCV indicate greater environmental effects on the traits, suggesting that selection based on those traits may not be effective.

Table 10. Quantitative genetic analysis of SES and SR under high salinity treatment (T4)

TRAITS	MEAN	VG	VE	VP	PCV	GCV	BSH	GA	GAM
SES	8.89	1.80	0.22	2.03	16.01	15.09	0.88791	2.60	29.29
SR	0.19	0.07	0.03	0.09	164.87	138.37	0.70438	0.45	239.23

Notes. VG: Variance Genetic, VE: Variance environment, VP: Variance Phenotype, PCV: Phenotypic coefficient variation, GCV: Genotypic coefficient variation, BSH: Broad-sense heritability, GA: Genetic advanced, GAM: Genetic advanced by percentage of mean.

The broad sense heritability recorded for both SES score and Survival rate (SR) were 88% and 70% respectively. A higher heritability value suggests that the trait can be inherited by the next generation, indicating that selection based on these traits would be effective and efficient in the breeding program. Mahmood et al. (2004) also identified plant height as highly heritable in previous studies.

However, measuring BSH alone may not be very useful as it requires considering the influence of both additive and non-additive genes. Therefore, genetic advances serve as another important indicator to achieve the expected results for the trait of interest in a population after selection (Salleh et al, 2020). Nonetheless, genetic advance as a percent of the mean (GAM) provides a more precise result compared to GA alone and could be further categorized as low (0-10%), moderate (10-20%), and high (≥20%) (Adhikari et al., 2018). Previous study by Salleh et al. (2020) also indicated that BSH need to be paired together with GAM due to the fact that BSH could not specifically measure the additive gene action as compared to narrow sense heritability (NSH). In the computation of BSH (the formula; BSH = VG/VP), variance genetic (VG) which consist of dominant (VD), additive (VA), and epistasis (VI) gene actions are being grouped together as VG = VD + VA + VI. In contrast, in the computation of NSH (formula as NSH = VA/VP), the VG has been partitioned and equated specifically as VA by removing other types of VG mainly VD and VI, respectively.

Thus, high levels of heritability and genetic advance as a percentage of the mean can serve as the basis for selection based on morphological traits. According to Akinola et al. (2019), high heritability combined with high genetic advance observed in agronomic traits indicates that these traits are mainly under genetic influence and can be scored based on their phenotypic performance. In this study, both SES and SR exhibited high heritability and genetic advance and were not greatly influenced by the environment, as evidenced by the relationship between PCV and GCV. Therefore, selection based on these traits would lead to improvements in genotypes.

4.3 CLUSTER ANALYSIS

The cluster analysis was conducted based on SES and SR in order to identify the tolerant genotypes (Table 11 and Figure 4). Result of the cluster analysis classified the rice genotypes into three different groups as shown in Table 11. Group 1 consisted of the Pokkali and MRQ76. MRQ76 is identified as highly tolerant genotypes incomparable to Pokkali the tolerant checks. The second group indicated the tolerant group which comprises of Nona Bokra (another tolerant check), Q70, Putra 1 and Jayanti. The remaining genotypes were grouped together in group 3 as highly susceptible.

Table 11. Cluster analysis of rice genotypes under high salinity treatment (T4)

GROUP	GENOTYPES
Group 1 : Highly tolerant genotypes	37 (Pokkali) 32 (MRQ76)
Group 2: Tolerant genotypes	41 (Q70) 39 (Putra 1) 33 (Nona Bokra) 15 (Jayanti)
Group 3 : Susceptible genotypes	Remaining 44 genotypes

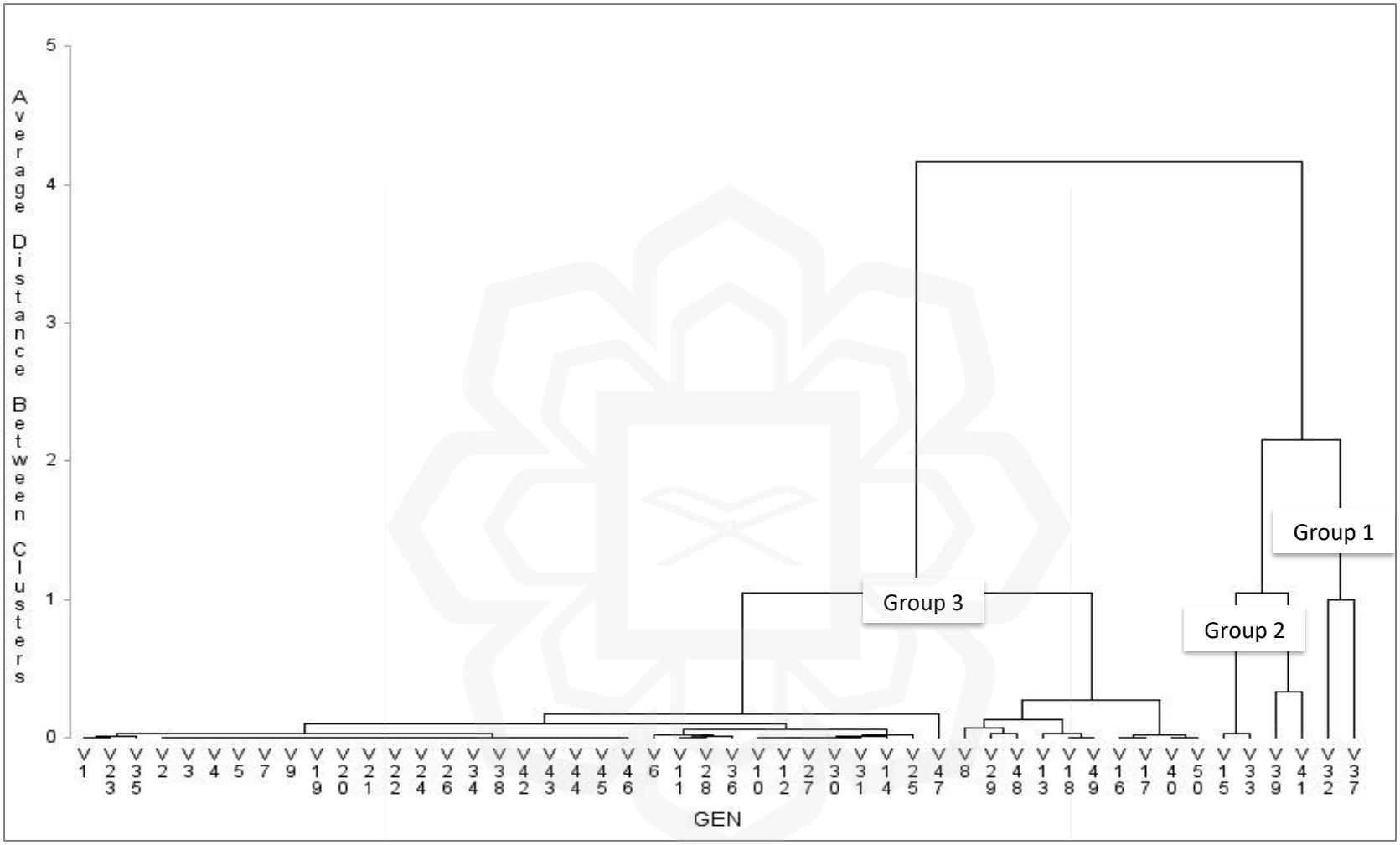

Figure 4. Cluster analysis of 50 rice genotypes under 12 dS m^{-1} (T4)

4.4 PHENOTYPIC – GENOTYPIC EVALUATION OF SALINITY TOLERANT GENOTYPES

4.4.1 Phenotypic Evaluation

Phenotypic evaluation of four newly identified tolerant genotypes (MRQ76, Jayanti, Q70 and Putra 1) including two susceptible checks (IR64 and IR29) and two tolerant checks (Pokkali and Nona Bokra) were completed and further analyzed. Table 12 showed the ANOVA table for SES score and Survival rates of 8 genotypes studied. In addition, Figure 5 and Figure 6 showed the increasing trend of SES score and survival rate (SR) of the studied genotypes as the level of salinity increases.

Table 12. Anova table for SES score and survival rate of 8 genotypes

Traits		**SES**					**Survival rate**			
Source of variation	**Df**	**Sum square**	**Mean square**	**F Value**	**Pr > F**	**Df**	**Sum square**	**Mean square**	**F Value**	**Pr > F**
Treatment	3	363.090	121.030	199.81	<.0001	3	2.7302	0.910	59.50	<.0001
Genotypes	7	193.402	27.629	45.61	<.0001	7	3.056	0.437	28.54	<.0001
Treatment x genotypes	21	97.146	4.626	7.64	<.0001	21	3.222	0.153	10.02	<.0001

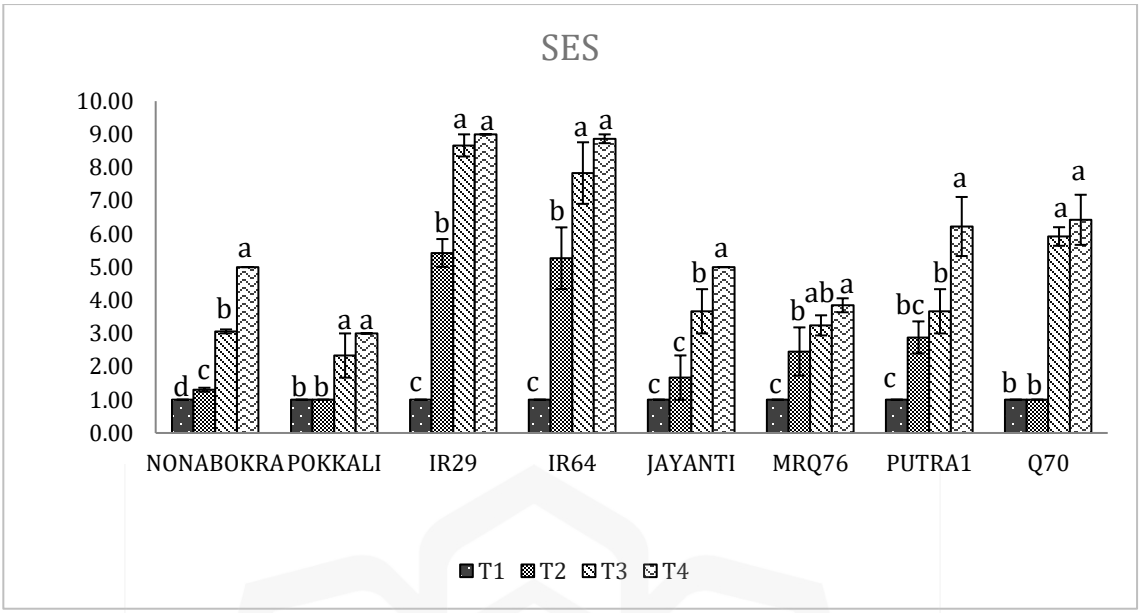

Figure 5. SES score of 8 genotypes under 4 different levels of salinity treatment. The small different letters are statistically different at ($p \leq 0.05$) among level of salinity stress, T1: salinity level at 0 dS m^{-1} ; T2: salinity level at 4dS m^{-1} ; T3: salinity level at 8 dS m^{-1} ; T4: salinity level at 12 dS m^{-1}

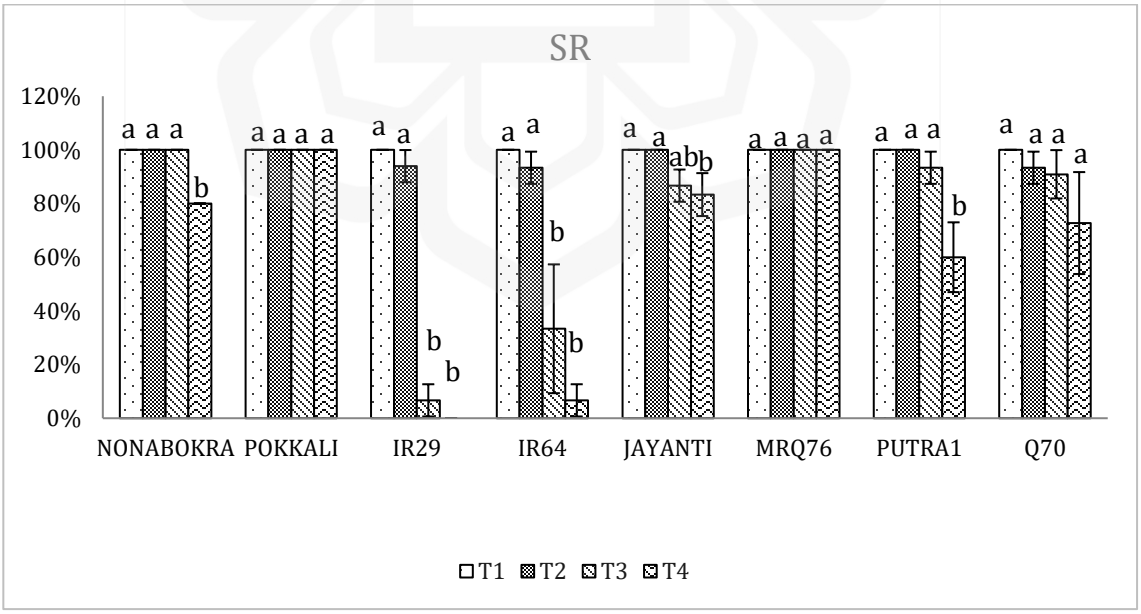

Figure 6. Survival rate (SR) of 8 genotypes under 4 different levels of salinity treatment

Under all three levels of NaCl 4 dS m^{-1}(T2), 8 dS m^{-1} (T3), and 12 dS m^{-1}(T4), survivability of MRQ76 was the greatest (100% survived); followed by Jayanti (100%, 87%, and 83%, respectively). Jayanti showed a significant difference between 4dS and 12dS. Next, there is no significant difference on the survivability rates of Q70 between the three levels of NaCl. The survivability rates recorded for Q70 were 93%, 91% and 73% respectively. Meanwhile, Putra 1 showed a significant difference between T3 and T4 with survivability of 93% and 27% respectively. For the susceptible check (IR29 and IR64) showed a decrease in survivability rate at 8 dS m−1 and 12 dS m−1.

In the case of morphological performance on selected genotypes, the increment of salinity levels generally resulted on a reduction of seedling growth and physiological parameters. In the present study, salinity stress reduced plant height (Figure 7), leaves size (Figure 8), number of leaves (Figure 9), and leaf size (Figure 10). The morphological performance on 4 genotypes including 2 tolerant check and 2 susceptible checks were analysed. Figure 7 showed the mean of plant height of 12 rice genotype under normal fresh water condition (T1), low salinity stress treatment at 4 ds m-1 EC (T2), moderate salinity stress treatment at 8 ds m^{-1} EC (T3) and high salinity stress treatment at 12 ds m^{-1} EC (T4). Generally, the trend of decreasing in plant height could be seen as level of salinity stress increases (Figure 7). Among all genotypes, maximum reduction in plant height was recorded in IR29 (100%) followed by IR64 (31.93%), respectively. The least reduction however was recorded in MRQ76 and pokkali with negative 56.6% and only 9.8% of height reduced respectively.

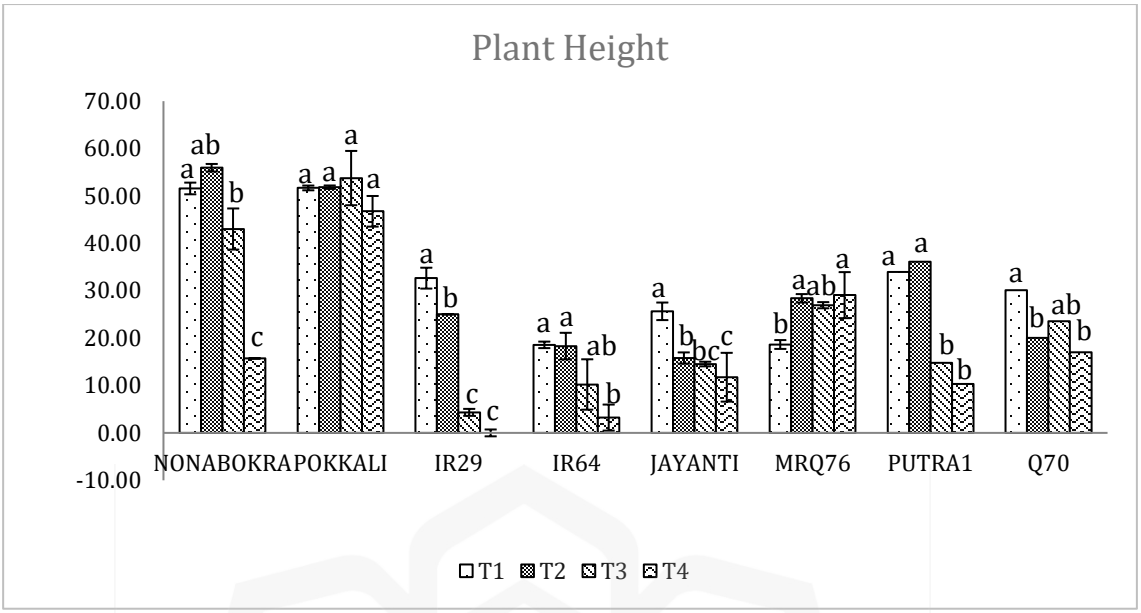

Figure 7. Plant height on 8 genotypes under 4 different levels of salinity treatment.

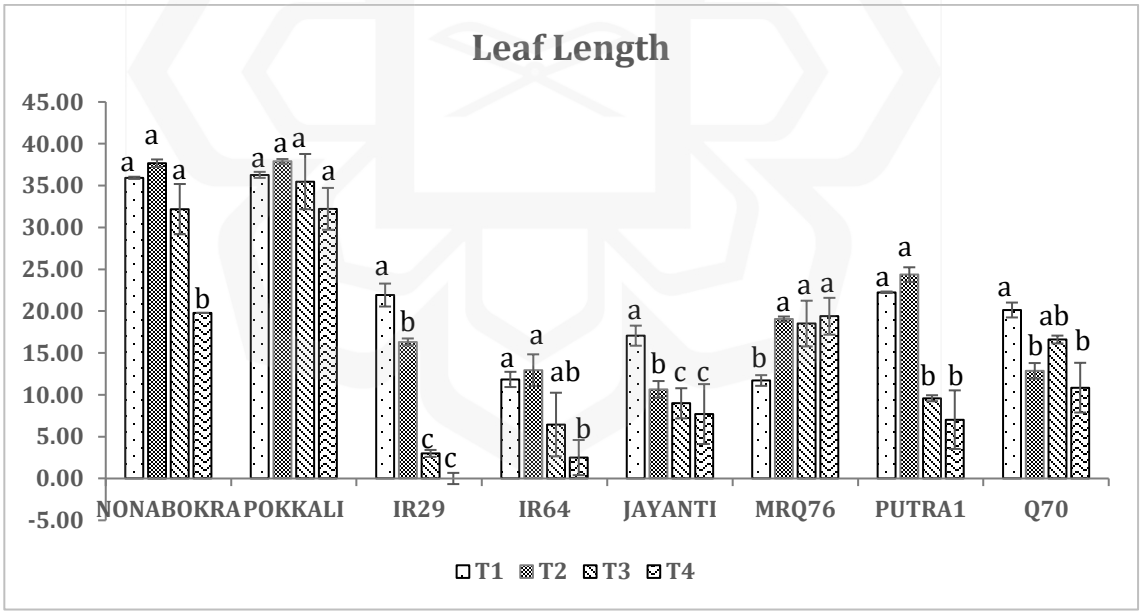

Figure 8. Leaf length on 8 genotypes under 4 different levels of salinity treatment

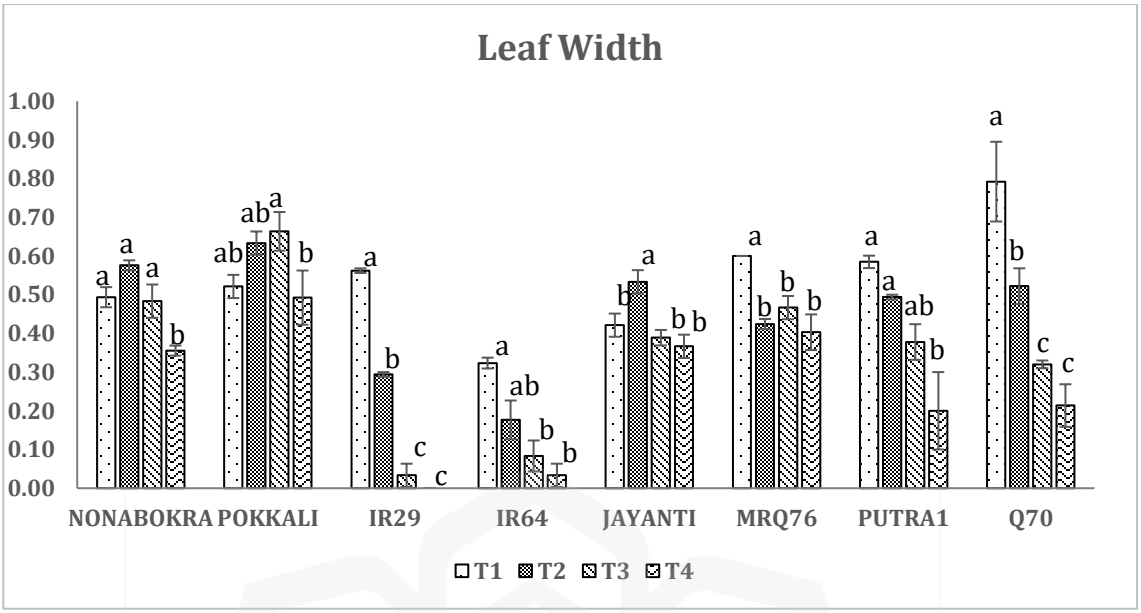

Figure 9. Leaf width on 8 genotypes under 4 different levels of salinity treatment

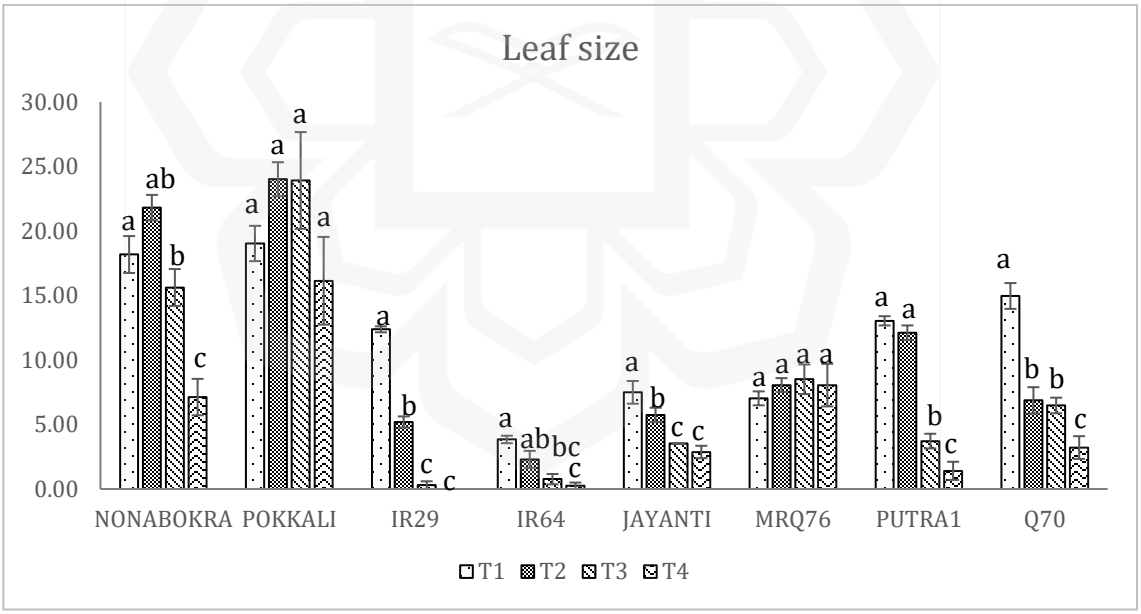

Figure 10. Leaf size on 8 genotypes under 4 different levels of salinity treatment

Figure 10 displayed the mean of leave size of 8 rice genotypes at seedling stage under all treatment. In general, the trend of leaves size reduction could be seen in all genotypes as the level of salinity treatment increases. Leaf size reduction could be used as an indicator for salinity stress of the rice seedling. As described by Hussain et al. (2017), salinity stress would affects plant metabolism, particularly on the photosynthesis rate and leaf cell multiplication. Significant reduction in leaf size would ultimately reduce photosynthesis rate thus may lead to the death of the leaves.

Moreover, the number of leaves decreased as level of salinity increases (Figure 11). Similar finding reported by Wankhade et al. (2013), which the salinity caused a significant reduction of the number of leaves at the developmental stage. Khanam et al. (2018) also stated that salinity extremely disturbed the production of leaf. The decreases of leaves number in severely salinity stress condition probably as a result of sodium chloride accumulation in the cell wall and cytoplasm of the older leaves. Low leaves number also may due to inhibition of the formation of leaf primordia under salt stress (Alamgir and Ali, 2006).

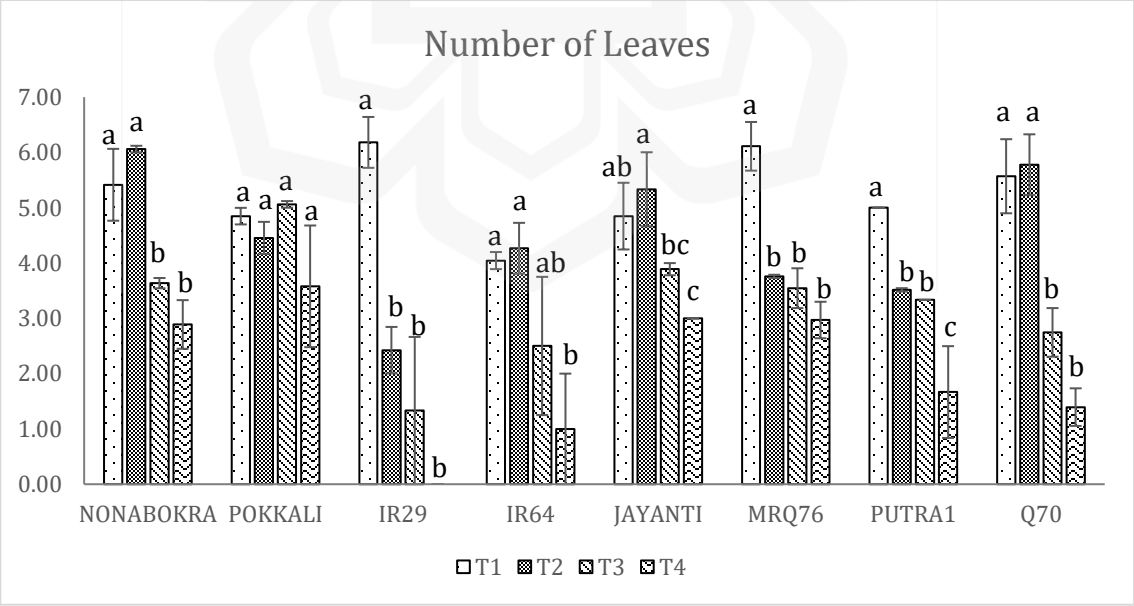

Figure 11. Number of leaves on 8 genotypes under 4 different levels of salinity treatment

4.4.2 Genotypic Evaluation

Table 13 demonstrates that all three SSR markers exhibited high polymorphism, with more than one allele present at each specific locus. The alleles present in each genotype are in the homozygous form (i.e., AA, BB, CC, DD, EE, etc.). This is because all genotypes have been selfed for at least two generations, thus could be considered homozygous. Moreover, SSR markers are widely utilized for fingerprinting and diversity studies of rice cultivars and their wild relatives due to their high polymorphism rate. In this study, six different alleles were recorded for RM1287 and RM8094, while RM3412B displayed seven different alleles. According to Thomson (2010), RM8094 and RM3412 were identified as foreground markers within the Saltol QTL region, while RM1287 was deemed the most informative marker flanking the Saltol region. The allelic sizes of RM1287, RM3412, and RM8094 in the reference genotype, Pokkali, were confirmed and validated to fall within the expected range of PCR product sizes at 154 bp, 110 bp, and 210 bp, respectively. However, none of the genotypes exhibited the presence of the Saltol QTL.

For SKC1 marker, RM578 showed monomorphisms for all genotypes (Table 14). Monomorphic markers cannot differentiate the DNA between or among the genotypes. Even though RM578 is reported as the peak marker of SKC1 QTL, in our study, the result showed monomorphism, therefore, we cannot confirm that the SKC1 QTL presented in the identified genotypes. Meanwhile for the other markers, IM8784 and IM8854 are the polymorphic marker 6 allele respectively. However, the identified tolerant genotype did not show the presence of SKC1 markers. Result of the present study is corroborated with previous study by Lutambi et al. (2024) which reported that the genotype ZX117 which shows tolerance to salinity stress do not possesses salinity tolerant QTL, Saltol in their genotyping study. This might suggest that other QTL or genes are also contributed to tolerance to salinity stress in rice, although yet to be identified and reported.

Table 13. The allelic profile of Saltol markers

GENOTYPE / MARKER	*Saltol*					
	RM1287 (bp)	Allele	RM3412B (bp)	Allele	RM8094 (bp)	Allele
Nona Bokra	183	CC	152	BB	196	FF
Pokkali (Reference genotypes for *Saltol* markers)	200	AA	110	AA	210	AA
IR29	158	BB	116	CC	203	EE
IR64	154	DD	114	CC	200	EE
MRQ76	152	EE	146	DD	213	BB
Putra1	162	FF	133	EE	207	DD
Jayanti	158	BB	103		205	
Q70	162	FF	127	GG	236	CC

Table 14. The allelic profile of SKC1 markers

GENOTYPE / MARKER	*SKC1*					
	RM578 (bp)	Allele	IM8748 (bp)	Allele	IM8854 (bp)	Allele
Nona Bokra (Reference genotypes for *SKC1* markers)	225	AA	137	AA	164	AA
Pokkali	225	AA	135	BB	157	CC
IR29	225	AA	133	CC	160	BB
IR64	225	AA	134	CC	170	DD
MRQ76	225	AA	135	BB	156	EE
Putra1	225	AA	138	DD	174	DD
Jayanti	225	AA	127	EE	170	FF
Q70	225	AA	128	FF	161	CC

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

In conclusion, our study identified four tolerant genotypes within the Malaysian rice germplasm, notably MRQ76, Jayanti, Putra 1, and Q70. These genotypes exhibited a higher survival rate (SR) of over 80%, except for Q70 and Putra 1, which showed a SR of 73% and 60% respectively, indicating a significant reduction in seedling growth performance under salinity stress of 12 dS/m. Despite observing polymorphism among the tolerant genotypes, our genotyping study using closely linked foreground markers revealed the absence of Saltol and SKC1 QTLs in these four tolerant genotypes identified in the present study which are MRQ76, Jayanti, Putra 1, and Q70. Moving forward, it is imperative to conduct further studies on the agronomic performances of these newly identified genotypes, particularly focusing on their reproductive and maturity stages. Additionally, future research endeavors should prioritize the identification of novel QTLs associated with salinity tolerance in MRQ76, Jayanti, Putra 1, and Q70, aiming to enhance our understanding and exploitation of genetic resources for improved rice cultivation in saline environments. Moreover, further study on the mechanism of salinity tolerance associated with those novel QTLs might be also conducted.

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APPENDIX

Appendix 1. Screening salinity tolerant - morphological data of 50 genotypes

1a. Plant height

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	41.45bcdef(±3.6)	18.87 ijklm (±0.2)	10.10 fghijk (±1.9)	0.70ij (±0.7)
V2	Bariohalus	34.68ghijklm (±2.4)	33.88bcde (±1.9)	17.23 bcdefghi (±2.1)	0.00j (±0)
V3	Basmati370	43.20bcd (±0.2)	34.20bcde (±2.3)	0.00k (±0)	0.00j(±0)
V4	Berjer	42.79bcd (±1.3)	12.95mno (±1.2)	14.49 cdefghij (±0.83)	0.00j(±0)
V5	Biris	35.81 efghijklm (±0.8)	30.32cdefg (±3.5)	0.00k (±0)	0.00j(±0)
V6	Boewani	39.73 cdefghi (±0.1)	23.00hijk (±1.3)	4.55ijk (±0)	3.29fghij (±1.8)
V7	CICA4	40.80cdefg (±0.1)	35.89bc (±0.3)	5.45hijk (±0.8)	0.00j (±0)
V8	Haiboq	42.14bcde(±1.8)	35.33bcd (±2.3)	19.35 bcdefg (±4.4)	13.47cdef (±1.2)
V9	IR29	32.65 jklmn (±1.2)	25.03fghi (±0.8)	4.33ijk (±4.3)	0.00j (±0)
V10	IR64	18.58rs (±0.5)	18.32jklm (±0.4)	10.22 fghijk (±5.7)	3.23fghij (±3.23)
V11	IR64SUB1	17.81rs (±1.6)	16.32lm (±0.97)	6.30 ghijk (±6.3)	4.97efghijk (±4.97)
V12	IR77(NIL)	24.03pqr (±0.8)	21.23ijkl (±0.8)	12.63 defghijk (±6.3)	6.37defghij (±6.37)
V13	Jarommas	44.25bc (±0.9)	33.99bcde (±2.5)	3.42jk (±1.8)	4.61efghij (±1.42)
V14	Jawikuning	43.15bcd (±0.2)	38.77b (±3.8)	26.45bc (±0.6)	1.08hij (±1.08)
V15	Jayanti	25.66opq (±2.2)	15.80lmn (±0)	14.53 cdefghij (±0.73)	11.74cdefg (±0.7)
V16	Ketiangedjih	35.36 fghijklm (±4.1)	30.37cdefg (±0.7)	21.37bcdef (±8.3)	14.51cde(±5.6)
V17	Krianb	31.48lmn (±4.7)	24.32ghij (±3.0	28.98b (±2.5)	11.08cdefghi (±2.0)
V18	Kukubalam	39.76cdefgh(±2.9)	16.96 klm (±3.4)	18.33 bcdefgh (±1.8)	8.38cdefghij (±4.2)
V19	Kukubelang	47.09ab(±0.2)	24.56 ghij (±0.3)	8.65 fghijk (±3.9)	0.00j (±0)
V20	Kunyit	27.12nop(±2.0)	20.43 ijkl (±3.02)	14.24 cdefghij (±2.1)	0.00j (±0)
V21	Kurauwangi	37.96cdefghijk(±1.3)	28.44efgh(±2.2)	9.66 fghijk (±9.7)	0.00j(±0)
V22	Liwaguantap	34.88ghijklm(±2.5)	29.22defgh (±3.3)	21.38bcdef (±3.03)	0.00j(±0)
V23	Madu	31.61klmn(±0.87)	6.51p (±1.8)	11.02 efghijk (±0.99)	0.55ij(±0.55)

V24	Mahsuri	32.04klmn(±2.5)	31.09cdef (±1.2)	25.76bcd (±3.3)	0.00j (±0)
V25	Merahwangi	38.78cdefgh(±2.2)	20.02 ijkl (±3.1)	0.00k (±0)	2.23ghij (±1.3)
V26	MR219	17.11s(±0.3)	15.60lmn (±1.4)	10.37fghijk (±5.7)	0.00j (±0)
V27	MR253	17.18s(±2.2)	16.36lm (±0.3)	4.28ijk (±4.28)	4.30efghij (±4.3)
V28	MR297	18.61rs (±1.3)	16.38lm (±0.7)	11.26 efghijk (±5.7)	5.10efghij (±5.1)
V29	MR5	18.20rs (±1.1)	17.66klm (±0)	9.57 fghijk (±4.8)	8.55cdefghij (±8.5)
V30	MR82	16.71s(±1.0)	16.02lm (±0.5)	0.00k (±0)	4.50efghij (±4.5)
V31	MRQ74	18.11rs(±0.3)	17.57klm (±0.03)	13.87 cdefghij (±0.2)	1.44ghij (±1.4)
V32	MRQ76	18.66rs (±0.7)	28.40efgh (±2.8)	26.95bc (±5.3)	29.07b (±2.7)
V33	Nonabokra	51.58a (±1.4)	55.95a (±1.5)	43.02a (±3.6)	15.71cd (±1.8)
V34	Padigunong	40.40cdefg (±6.5)	9.12op (±3.7)	5.67hijk (±5.67)	0.00j (±0)
V35	Paginkano	39.04cdefghij (±1.5)	21.92ijkl (±0.3)	8.99 fghijk (±8.99)	0.73ij (±0.7)
V36	Pakkedek	33.84ijklm (±0.06)	24.62 (±0.9)	17.24 bcdefghi (±6.4)	2.07ghij (±1.2)
V37	Pokkali	51.69a (±1.4)	51.85a (±1.1)	53.77a (±4.4)	46.76a(±3.3)
V38	Pulutmalaysia1	15.94s(±0.2)	15.50lmn (±0.7)	9.47 fghijk (±4.8)	0.00j(±0)
V39	Putra1	33.98hijklm (±1.9)	36.14bc (±1.2)	14.76 cdefghij(±0.5)	10.30cdefghij (±5.2)
V40	Putra2	21.27qrs(±0.4)	19.13 ijklm (±0.6)	17.35 bcdefghi (±0.9)	11.51cdefgh (±5.8)
V41	Q70	30.10mno (±0.9)	20.08 ijkl (±0.89)	23.59bcde (±0.97)	17.01c (±4.9)
V42	Renganwangi	39.38cdefghi (±1.1)	18.47jklm (±2.2)	11.02 efghijk (0.4)	0.00j (±0)
V43	Santapwangi	37.44 defghijkl (±1.2)	25.17fghi (±1.9)	26.35bc(±3.1)	0.00j (±0)
V44	Selayang	37.61defghijkl(±1.2)	32.18cde(±0.8)	20.35 bcdef(±1.9)	0.00j (±0)
V45	Serandu1	19.78qrs(±1.4)	17.31klm(±0.4)	11.35 efghijk (±5.8)	0.00j (±0)
V46	Tangkaimas	38.87cdefgh (±1.9)	35.16bcd (±2.5)	21.05bcdef (±6.3)	0.00j (±0)
V47	UKM-5	36.05 efghijklm (±0.2)	36.68bc (±0.5)	20.89 bcdef (±5.2)	5.97defghij (±5.9)
V48	UKMRC2	20.09qrs (±1.2)	14.31nop (±0)	10.31ghijk (±5.9)	4.80efghij (±4.8)
V49	UKMRC8	19.19rs (±0.97)	18.54jklm (±0.5)	18.67 bcdefghi (±1.1)	5.64defghij (±5.64)
V50	UKM-6	18.16rs (±1.1)	16.29lm(±1.9)	16.07 bcdefghi (±0.5)	9.13cdefghij (±4.6)

1b. Plant height reduction

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	0.00	53.84bc (±3.9)	75.42abcde(±4.6)	98.01a (±1.9)
V2	Bariohalus	0.00	1.63 (±7.5)	49.48 abcdefghi (±8.1)	100.00a (±0)
V3	Basmati370	0.00	20.78 ghijklm (±5.7)	100.00a (±0)	100.00a (±0)
V4	Berjer	0.00	69.50ab (±3.7)	66.19abcdefgh (±0.9)	100.00a (±0)
V5	Biris	0.00	15.66hijklmn (±8.1)	100.00a(±0)	100.00a (±0)
V6	Boewani	0.00	42.10cdefg (±3.2)	88.56abc(±0.03)	91.69abc (±4.6)
V7	CICA4	0.00	12.05ijklmno (±0.8)	86.64abc (±1.9)	100.00a (±0)
V8	Haiboq	0.00	15.44hijklmn (±8.6)	54.76 abcdefghi(±8.1)	67.79 abcd (±3.8)
V9	IR29	0.00	23.32ghijkl (±0.43)	87.66abc (±12.3)	100.00a (±0)
V10	IR64	0.00	1.11lmno (±4.62)	44.16 bcdefghij (±30.8)	81.70 abcd (±18.3)
V11	IR64SUB1	0.00	7.74 jklmno (±3.7)	70.11abcdefg (±29.9)	68.71 abcd (±31.2)
V12	IR77(NIL)	0.00	11.58 ijklmno (±3.3)	48.45 abcdefghi (±25.9)	73.07 abcd (±26.9)
V13	Jarommas	0.00	23.35ghijkl (±3.9)	92.30ab (±4.2)	89.65 abcd (±3.1)
V14	Jawikuning	0.00	10.22 ijklmno (±8.3)	38.68 bcdefghij (±1.7)	97.50ab (±2.5)
V15	Jayanti	0.00	37.41cdefg (±5.9)	42.97 bcdefghij (±2.2)	54.01 abcde (±1.2)
V16	Ketiangedjih	0.00	11.64 ijklmno (±10.7)	40.72 bcdefghij (±19.1)	54.95 abcde (±20.6)
V17	Krianb	0.00	21.39 ghijklm (±7.3)	5.43ij (±9.9)	60.98 abcd (±12.9)
V18	Kukubalam	0.00	55.76bc (±11.03)	54.00abcdefgh (±1.7)	77.65 abcd (±12.5)
V19	Kukubelang	0.00	47.85cd (±0.7)	81.69abcd (±8.1)	100.00a (±0)
V20	Kunyit	0.00	22.47 ghijkl (±15.5)	48.03 abcdefghij (±4.4)	100.00a (±0)
V21	Kurauwangi	0.00	25.33fghijk (±3.5)	72.67abcdef (±27.3)	100.00a (±0)
V22	Liwaguantap	0.00	14.54hijklmno (±13.3)	39.02 bcdefghij (±5.6)	100.00a (±0)
V23	Madu	0.00	79.69a (±4.93)	64.92abcdefgh (±3.9)	98.21a (±1.8)
V24	Mahsuri	0.00	2.08 klmno (±6.8)	20.16ghij (±4.5)	100.00a (±0)
V25	Merahwangi	0.00	48.91cd (±4.9)	100.00a (±0)	94.25ab (±3.6)
V26	MR219	0.00	8.48 jklmno (±10.01)	38.35 cdefghij (±34.3)	100.00a (±0)
V27	MR253	0.00	1.58 klmno (±12.4)	73.92abcde(±26.1)	79.81 abcd (±20.1)
V28	MR297	0.00	11.32 ijklmno (±5.9)	38.64 bcdefghij (±30.1)	75.46 abcd (±24.5)
V29	MR5	0.00	7.00 jklmno (±7)	47.63 abcdefghij (±26.1)	57.67 abcde (±0)
V30	MR82	0.00	3.65 jklmno (±4.8)	100.00a (±0)	71.52 abcd (±28.5)

V31	MRQ74	0.00	2.96klmno (±1.4)	23.43 defghij (±0.3)	91.76abc (±8.2)
V32	MRQ76	0.00	-51.56p (±9.1)	-42.78k (±22.4)	-56.07g (±15.3)
V33	Nonabokra	0.00	-8.78o (±5.9)	16.83hij(±4.8)	69.31 abcd (±4.4)
V34	Padigunong	0.00	78.08a (±8.9)	82.63abc (±17.4)	100.00a (±0)
V35	Paginkano	0.00	43.65cdef (±2.8)	78.62abcd (±21.8)	98.26a (±1.7)
V36	Pakkedek	0.00	27.26efghij (±2.6)	49.12abcdefghi (±18.9)	93.88ab (±3.4)
V37	Pokkali	0.00	-0.47lmno (±3.7)	-3.86jk (±6.5)	9.68f (±4.7)
V38	Pulutmalaysia1	0.00	2.85 klmno (±3)	40.05 bcdefghij (±30.0)	100.00a (±0)
V39	Putra1	0.00	-6.60no (±2.2)	56.48abcdefgh (±0.9)	67.94 abcd (±16.1)
V40	Putra2	0.00	9.95 ijklmno (±3.6)	18.29hij (±5.3)	44.97cdef (±28.01)
V41	Q70	0.00	33.00defgh (±4.9)	21.62fghij (±0.4)	42.52def (±17.9)
V42	Renganwangi	0.00	53.01bc (±5.7)	71.96abcdefg (±1.2)	100.00a (±0)
V43	Santapwangi	0.00	32.90defghi (±3.2)	28.93defghij (±10.3)	100.00a (±0)
V44	Selayang	0.00	14.38hijklmno (±0.63)	45.80 bcdefghij (±5.08)	100.00a (±0)
V45	Serandu1	0.00	11.23 ijklmno (±8.7)	46.40 bcdefghij (±27)	100.00a (±0)
V46	Tangkaimas	0.00	9.32 ijklmno (±6.2)	47.21 abcdefghij (±14.2)	100.00a (±0)
V47	UKM-5	0.00	-1.78mno (±1.9)	41.97 bcdefghij (±14.7)	83.40abcd (±16.6)
V48	UKMRC2	0.00	46.14cde(±0)	51.61abcdefgh (±26.3)	73.82abcd (±26.2)
V49	UKMRC8	0.00	2.57 klmno (±7.8)	5.13ij (±13.6)	72.24abcd (±27.8)
V50	UKM-6	0.00	8.90jklmno (±14.1)	10.71ij(±6.9)	49.84 bcde (±26.5)

1c. Leaf length

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	28.49bcde (±2.5)	13.12 jklmn (±0.2)	7.02 ghijklmn (±1.5)	0.51i (±0.5)
V2	Bariohalus	25.14cdefghijk (±1.7)	23.29bcd (±1.6)	12.50 bcdeghijk (±1.5)	0.00i (±0)
V3	Basmati370	26.34 bcdefghi (±1.4)	22.48cde (±1.5)	0.00n (±0)	0.00i(±0)
V4	Berjer	29.75bc (±0.9)	8.66nop (±0.9)	9.45 cdeghijklm (±0.8)	0.00i(±0)
V5	Biris	24.17 efghijkl (±0.2)	20.39cdefg (±2.8)	0.00n (±0)	0.00i(±0)
V6	Boewani	20.83jklmn (±0.1)	13.15 jklmn (±0.2)	3.12lmn (±0)	2.02fghi(±1.1)
V7	CICA4	26.26bcdefghi (±0.2)	24.67bc (±0.8)	3.73klmn (±0.6)	0.00i (±0)
V8	Haiboq	25.57bcdefghij (±0.9)	23.80bcd (±2.02)	13.69bcdefgh (±3.3)	9.50cde (±0.8)
V9	IR29	21.92 ijklm (±0.1)	16.32ghijkl (±0.5)	3.00lmn (±3)	0.00i (±0)
V10	IR64	11.84q (±0.4)	12.95 jklmn (±0.3)	6.45 ghijklmn (±3.3)	2.50efghhi (±2.5)
V11	IR64SUB1	11.20q (±1.2)	11.44lmn (±0.6)	4.53ijklmn (±4.5)	3.75defghi (±3.75)
V12	IR77(NIL)	15.87opq (±0.3)	14.87hijklm (±0.6)	8.74 eghijklmn (±4.3)	4.40 cdefghi (±4.4)
V13	Jarommas	30.47b (±0.5)	23.38bcd (±1.6)	2.38mn (±1.3)	2.98efghi (±0.9)
V14	Jawikuning	29.17bcd (±0.7)	27.59b (±2.4)	17.16bcde (±1.4)	0.62hi (±00.61)
V15	Jayanti	17.07nop (±1.4)	10.66 jklmn(±0.4)	8.99 deghijklmn (±0.4)	7.70cdefgh (±0.7)
V16	Ketiangedjih	22.59 ghijklm (±3.5)	20.43bcde (±0.8)	14.35bcdefgh (±5.9)	8.62cdef (±3.9)
V17	Krianb	21.58ijklmn (±3.5)	16.92fghijk (±2.3)	20.62b (±1.6)	7.68 cdefgh (±1.5)
V18	Kukubalam	26.49bcdefghi (±2.7)	12.21 klmn (±2.7)	13.45bcdeghij (±0.7)	5.13 cdefghi (±2.9)
V19	Kukubelang	37.78a (±2.0)	20.22cdefg (±3.4)	5.15 hijklmn (±2.2)	0.00i (±0)
V20	Kunyit	19.19mno (±1.4)	14.38ijklm (±2.2)	9.03 deghijklmn (±0.9)	0.00i (±0)
V21	Kurauwangi	24.55 defghijkl (±1.2)	19.29defgh (±1.8)	6.97 ghijklmn (±6.9)	0.00i (±0)
V22	Liwaguantap	23.20fghijklm (±1.4)	20.19cdefg (±2.3)	14.59bcdefg (±1.1)	0.00i(±0)
V23	Madu	20.58klmn (±1.6)	4.38p (±1.4)	7.37 ghijklmn (±0.5)	0.40i(±0.4)
V24	Mahsuri	23.02fghijklm (±1.7)	21.29bcde (±1.2)	17.95bcd (±1.4)	0.00i (±0)

V25	Merahwangi	27.77bcdef (±2.1)	13.79jklmn (±1.9)	0.00n (±0)	1.63fghi (±0.9)
V26	MR219	11.82q (±0.5)	10.83mno (±1.0)	7.63ghijklmn (±4.3)	0.00i (±0)
V27	MR253	11.87q (±1.6)	11.96klmn (±1.4)	2.94lmn (±2.9)	3.00efghi (±3)
V28	MR297	12.03q (±0.8)	10.93mno (±0.4)	7.28 ghijklmn (±3.6)	3.52efghi (±3.5)
V29	MR5	12.67q (±0.8)	12.83 klmn (±0)	6.61 ghijklmn (±3.3)	5.65 cdefghi (±0)
V30	MR82	11.35q (±0.7)	11.38lmn (±0.3)	0.00n (±0)	3.07efghi (±3)
V31	MRQ74	11.57q (±0)	11.07mno (±0.1)	9.02 deghijklmn (±0.1)	0.91ghi (±00.9)
V32	MRQ76	11.71q (±0.9)	19.06defghi (±1.9)	18.52bc (±3.8)	19.39b (±2.1)
V33	Nonabokra	35.93a (±1.2)	37.68a (±0.9)	32.19a (±1.8)	19.79b (±3.6)
V34	Padigunong	25.75bcdefghi (±4.3)	6.05p (±2.4)	4.29jklmn (±4.2)	0.00i (±0)
V35	Paginkano	26.53bcdefghi (±0.9)	14.75hijklm (±0.1)	6.03 ghijklmn (±6.0)	0.42hi (±0.42)
V36	Pakkedek	23.21 fghijklm (±0.3)	16.33ghijkl (±0.7)	11.90 bcdeghijkl (±4.2)	1.32fghi (±00.8)
V37	Pokkali	36.29a (±0.6)	37.90a (±0.2)	35.48a (±2.7)	32.22a (±2.2)
V38	Pulutmalaysia1	10.99q (±0.1)	11.17mno (±0.6)	7.11 ghijklmn (±3.6)	0.00i (±0)
V39	Putra1	22.25hijklm (±0.1)	24.37bc (±0.9)	9.59 cdeghijklm (±0.4)	7.03cdefghi (±3.5)
V40	Putra2	14.07pq (±0.4)	12.79 jklmn (±0.4)	12.15 bcdeghijkl (±0.8)	8.24cdefg (±4.2)
V41	Q70	20.14lmno (±0.9)	12.87 jklmn (±0.9)	16.61bcdef (±0.7)	10.85cd (±2.9)
V42	Renganwangi	26.87bcdefgh (±0.8)	12.02klmn (±1.3)	7.74fghijklm (±0.4)	0.00i (±0)
V43	Santapwangi	27.30bcdefg (±0.9)	17.69efghij (±1.7)	18.46bc (±2.4)	0.00i (±0)
V44	Selayang	26.10bcdefghi (±0.9)	22.58cde (±0.9)	12.15 bcdeghijkl (±0.8)	0.00i (±0)
V45	Serandu1	13.57pq (±0.9)	11.92klmn (±0.4)	7.36 ghijklmn (±3.7)	0.00i (±0)
V46	Tangkaimas	27.85bcdef (±1.5)	23.29bcd (±1.8)	13.35 bcdeghij (±4.2)	0.00i (±0)
V47	UKM-5	25.15cdefghijk (±0.8)	24.08bcd (±0.1)	14.70bcdefg (±3.2)	4.16deghi (±4.2)
V48	UKMRC2	13.22pq (±0.4)	9.44op (±0)	7.33 ghijklmn (±4.2)	2.72efghi (±2.7)
V49	UKMRC8	12.65pq (±0.6)	12.71 jklmn (±0.2)	12.22 bcdeghijkl (±0.8)	3.89defghi (±3.9)
V50	UKM-6	12.60pq (±0.9)	11.05mno (±1.0)	11.12cdeghijklm (±0.6)	6.04cdefghi (±3.1)

1d. Leaf width

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	0.55bcdef (±0.04)	0.19b (±0)	0.08 qrstuvw (±0.006)	0.01d (±0.006)
V2	Bariohalus	0.47 cdefghi (±0.02)	0.38b (±0.01)	0.18jklmno (±0.009)	0.00d (±0)
V3	Basmati370	0.42 ghijklmno (±0.01)	0.36b (±0)	0.00 w (±0)	0.00d (±0)
V4	Berjer	0.46 defghijk (±0.01)	0.11b (±0.01)	0.12nopqrst (±0.006)	0.00d (±0)
V5	Biris	0.40 hijklmno (±0)	0.19b (±0.04)	0.00w (±0)	0.00d (±0)
V6	Boewani	0.40 hijklmno	0.11b (±0.006)	0.02vw (±0)	0.03d (±0.001)
V7	CICA4	0.46 defghijk (±0)	0.39b (±0.006)	0.05 rstuvw (±0.013)	0.00d (±0)
V8	Haiboq	0.55bcde (±0)	0.49b (±0.07)	0.21ghijkl (±0.013)	0.20c (±0.02)
V9	IR29	0.56bcd (±0.02)	0.29b (±0.006)	0.03 uvw (±0.03)	0.00d (±0)
V10	IR64	0.32mno (±0.01)	0.18b (±0.05)	0.08 qrstuvw (±0.04)	0.03d (±0.03)
V11	IR64SUB1	0.30o (±0)	0.16b (±0.03)	0.03 uvw (±0.03)	0.03d (±0.03)
V12	IR77(NIL)	0.35jklmno(±0.005)	0.15b (±0.03)	0.08 qrstuvw (±0.03)	0.03d (±0.03)
V13	Jarommas	0.50 bcdefgh (±0)	0.23b (±0.01)	0.03uvw (±0.01)	0.05d (±0.01)
V14	Jawikuning	0.47 cdefghi(±0.03)	0.39b (±0.03)	0.21hijklm (±0.005)	0.01d (±0.006)
V15	Jayanti	0.42 ghijklmn (±0.03)	0.53ab (±0.03)	0.39cde (±0.02)	0.37b (±0.03)
V16	Ketiangedjih	0.56bcde (±0.02)	0.35b (±0.013)	0.23ghij (±0.05)	0.24c (±0.11)
V17	Krianb	0.41 ghijklmn (±0.04)	0.24b (±0.02)	0.39cd (±0.003)	0.20c (±0.09)
V18	Kukubalam	0.54bcdef (±0.09)	0.18b (±0.04)	0.14lmnopq (±0.006)	0.05d (±0.02)
V19	Kukubelang	0.50 bcdefgh (±0)	0.22b (±0)	0.06rstuvw (±0.03)	0.00d (±0)
V20	Kunyit	0.56bcd (±0.09)	0.34b(±0.02)	0.12nopqrs (±0.01)	0.00d (±0)
V21	Kurauwangi	0.51bcdefgh (±0.03)	0.25b (±0)	0.06rstuvw (±0.06)	0.00d (±0)
V22	Liwaguantap	0.48 cdefghi (±0.006)	0.44b (±0.05)	0.23ghijk (±0.02)	0.00d (±0)
V23	Madu	0.40 hijklmno (±0.05)	0.07b (±0.03)	0.10 opqrstu (±0.01)	0.00d (±0)
V24	Mahsuri	0.44efghijkl (±0.03)	0.32b (±0.02)	0.24fghi (±0.03)	0.00d (±0)
V25	Merahwangi	0.43fghijklm (±0.03)	0.13b (±0.02)	0.00 w (±0)	0.02d (±0.01)

V26	MR219	0.34lmno (±0.03)	0.17b (±0.063)	0.07 qrstuvw (±0.03)	0.00d (±0)
V27	MR253	0.33lmno (±0.01)	0.18b (±0.02)	0.03uvw (±0.03)	0.03d (±0.03)
V28	MR297	0.31no (±0.006)	0.14b (±0.008)	0.07 qrstuvw (±0.03)	0.03d (±0.03)
V29	MR5	0.37ijklmno (±0.01)	0.16b (±00.16)	0.07 qrstuvw (±0.03)	0.05d (±0.05)
V30	MR82	0.37ijklmno (±0.03)	0.22b (±0.09)	0.00w (±0)	0.03d (±0.03)
V31	MRQ74	0.49 bcdefgh (±0.01)	0.52ab (±0)	0.29fg (±0.03)	0.02d (±0.02)
V32	MRQ76	0.60b (±0)	0.42b (±0.01)	0.47bc (±0.03)	0.40a (±0.04)
V33	Nonabokra	0.49 bcdefgh(±0.02)	0.58ab (±0.01)	0.48b (±0.04)	0.36b (±0.013)
V34	Padigunong	0.42 ghijklmn (±0.04)	0.09b (±0.03)	0.03uvw (±0.03)	0.00d (±0)
V35	Paginkano	0.49 bcdefgh (±0.04)	0.15b (±0.017)	0.04tuvw (±0.04)	0.01d (±0.006)
V36	Pakkedek	0.50bcdefgh (±0.003)	0.31b (±0.003)	0.15klmnopq (±0.07)	0.03d (±0.02)
V37	Pokkali	0.52bcdefg (±0.03)	0.63a (±0.03)	0.66a (±0.05)	0.49a (±0.07)
V38	Pulutmalaysia1	0.32mno (±0.01)	0.18b (±0.02)	0.07 qrstuvw (±0.03)	0.00d (±0)
V39	Putra1	0.58bc (±0.02)	0.49ab (±0.007)	0.38de (±0.05)	0.20c (±0.1)
V40	Putra2	0.37ijklmno (±0.02)	0.21b (±0.05)	0.13mnopqr (±0.02)	0.07d (±0.03)
V41	Q70	0.79a (±0.1)	0.52ab (±0.05)	0.32ef (±0.02)	0.21c (±0.06)
V42	Renganwangi	0.47 cdefghi (±0.02)	0.23b (±0.04)	0.08opqrstuvw (±0.003)	0.00d (±0)
V43	Santapwangi	0.43fghijkl (±0.04)	0.26b (±0.03)	0.25fgh(±0.05)	0.00d (±0)
V44	Selayang	0.47 cdefghi (±0.01)	0.39b (±0.01)	0.17jklmno (±0.02)	0.00d (±0)
V45	Serandu1	0.30o (±0)	0.13b (±0.03)	0.07 rstuvw (±0.03)	0.00d (±0)
V46	Tangkaimas	0.48 cdefghi(±0.02)	0.40b (±0.04)	0.16jklmnop (±0.04)	0.00d (±0)
V47	UKM-5	0.50 bcdefgh (±0)	0.41b (±0.003)	0.19hijklmn (±0.04)	0.03d (±0.03)
V48	UKMRC2	0.33lmno (±0.01)	0.13b (±0.06)	0.07 qrstuvw (±0.03)	0.03d (±0.03)
V49	UKMRC8	0.35klmno (±0.02)	0.20b (±0.006)	0.10opqrstu (±0)	0.03d (±0.03)
V50	UKM-6	0.33lmno (±0.03)	0.12b (±0.02)	0.10opqrstu (±0.1)	0.07d (±0.03)

1e. Leaf size

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	15.67abc (±2.0)	4.25hijklm (±0.37)	1.40 hijklmno (±0.29)	0.10g (±0.1)
V2	Bariohalus	11.48defghijk (±0.8)	8.43bcdefgh (±0.18)	3.10efghijklmno (±0.41)	0.00g (±0)
V3	Basmati370	11.02fghijkl (±1.1)	8.99bcdefg (±0.59)	0.00o (±0)	0.00g (±0)
V4	Berjer	13.74cdefgh (±0.8)	2.40jklm (±0.18)	2.00 ghijklmno (±0.68)	0.00g (±0)
V5	Biris	9.69ijklm (±0.1)	4.47hijklm (±1.1)	0.00o (±0)	0.00g (±0)
V6	Boewani	8.33klmn (±0.06)	2.48jklm (±0.18)	0.31no (±0)	0.36fg (±0.19)
V7	CICA4	12.00 cdefghijk (±0.3)	9.59bcdefg (±0.16)	0.80klmno (±0.27)	0.00g (±0)
V8	Haiboq	14.12cdefg (±0.2)	12.58b (±2.1)	3.80efghijk (±0.54)	2.80cdef (±0.26)
V9	IR29	12.38 cdefghi (±0.23)	5.20 ghijklm (±0.43)	0.30no (±0.3)	0.00g (±0)
V10	IR64	3.84op (±0.29)	2.30jklm (±0.67)	0.76klmno (±0.38)	0.25g (±0.25)
V11	IR64SUB1	3.36p (±0.36)	1.84lm (±0.42)	0.45no (±0.45)	0.38fg (±0.38)
V12	IR77(NIL)	5.57nop (±0.05)	2.34jklm (±0.50)	0.99jklmno (±0.51)	0.44fge (±0.44)
V13	Jarommas	15.23bcd (±0.27)	6.52 defghijk (±0.34)	0.53mno (±0.29)	0.59fge (±0.14)
V14	Jawikuning	13.64 cdefgh (±1.1)	11.39bc (±1.43)	4.28defgh (±0.69)	0.06g (±0.06)
V15	Jayanti	7.49lmno (±0.88)	5.74 defghijklm (±0.56)	3.52efghij (±0.03)	2.87cde f (±0.48)
V16	Ketiangedjih	12.69 cdefghi (±1.73)	8.22bcdefghi (±0.77)	5.72de (±2.65)	4.58c (±2.1)
V17	Krianb	10.09 hijklm (±1.6)	5.58 efghijklm (±0.69)	9.15c (±1.61)	3.40cd (±1.41)
V18	Kukubalam	14.85bcdef (±3.7)	4.04hijklm (±1.15)	2.76 efghijklmno (±0.097)	1.10def g (±0.59)
V19	Kukubelang	18.89a (±1.0)	5.82 defghijklm (±1.3)	0.93ijklmno (±0.46)	0.00g (±0)
V20	Kunyit	10.42ghijklm (±1.67)	5.55 efghijklm (±0.6)	1.93ghijklmno (±0.1)	0.00g (±0)
V21	Kurauwangi	12.81 cdefghi (±1.35)	6.52defghijk (±0.49)	1.73hijklmno (±1.73)	0.00g (±0)
V22	Liwaguantap	11.18efghijkl (±0.85)	9.64bcdefg (±1.72)	3.95defghi (±0.59)	0.00g (±0)
V23	Madu	8.40jklmn (±1.77)	1.64lm (±0.9)	1.69 hijklmno (±0.11)	0.04g (±0.04)
V24	Mahsuri	10.32ghijklm (±1.02)	7.78cdefghi (±0.51)	4.80defg (±0.72)	0.00g (±0)
V25	Merahwangi	12.18 cdefghij (±1.9)	2.86jklm (±0.31)	0.00o (±0)	0.39fg (±0.25)

V26	MR219	4.01op (±0.18)	2.03klm (±0.92)	0.76klmno (±0.43)	0.00g (±0)
V27	MR253	3.94op (±0.65)	2.20klm (±0.34)	0.29no (±0.29)	0.30g (±0.3)
V28	MR297	3.68op (±0.23)	1.54lm (±0.12)	0.73klmno (±0.36)	0.35fg (±0.35)
V29	MR5	4.66op (±0.35)	2.04klm (±8.15)	0.66lmno (±0.33)	0.57efg (±0.57)
V30	MR82	4.18op (±0.56)	2.57jklm (±1.11)	0.00o (±0)	0.31g (±0.31)
V31	MRQ74	5.68nop (±0.11)	5.58 efghijklm (±0.05)	2.62fghijklmno (±0.27)	0.18g (±0.18)
V32	MRQ76	7.03mnop (±0.55)	8.06cdefghi (±0.54)	8.51c (±1.15)	8.05b (±1.6)
V33	Nonabokra	18.18a (±1.43)	21.81a (±0.99)	15.62b (±1.43)	7.13b (±1.4)
V34	Padigunong	11.18efghijkl (±2.9)	1.57lm (±0.66)	0.86jklmno (±0.86)	0.00g (±0)
V35	Paginkano	12.99cdefghi (±0.7)	3.81ijklm (±0.73)	1.21 ijklmno (±1.21)	0.08g (±0.08)
V36	Pakkedek	11.73 defghijk (±0.27)	5.36fghijkm (±0.51)	3.20efghijklmn (±1.93)	0.31g (±0.19)
V37	Pokkali	19.03a (±1.4)	24.01a (±1.32)	23.91a (±3.75)	16.14a (±3.4)
V38	Pulutmalaysia1	3.56p (±0.2)	2.02klm (±0.29)	0.71klmno (±0.35)	0.00g (±0)
V39	Putra1	13.03 cdefghi (±0.36)	12.12bc (±0.56)	3.71efghijkl (±0.57)	1.41defg (±0.7)
V40	Putra2	5.15nop (±0.39)	2.63jklm (±0.71)	2.29 fghijklmno (±00.8)	0.82fge (±0.42)
V41	Q70	14.96bcde (±1)	6.87defghij (±1.02)	6.47cd (±0.91)	3.21cd (±0.89)
V42	Renganwangi	13.09 cdefghi (±0.4)	4.44hijklm (±0.86)	1.49 hijklmno (±0.14)	0.00g (±0)
V43	Santapwangi	11.92 cdefghijk (±1.38)	5.99 defghijkl (±1.15)	5.09def (±1.2)	0.00g (±0)
V44	Selayang	12.17 cdefghij (±0.13)	9.13bcdefg (±0.13)	2.89 efghijklmno (±0.19)	0.00g (±0)
V45	Serandu1	4.11op (±0.24)	1.58lm (±0.38)	0.74klmno (±0.37)	0.00g (±0)
V46	Tangkaimas	13.49 cdefghi (±1.17)	9.78bcdef (±1.56)	2.93 efghijklmno (±0.88)	0.00g (±0)
V47	UKM-5	12.58 cdefghi (±0.39)	9.99bcde (±0.09)	4.03defghi (±1.06)	0.42fg (±0.42)
V48	UKMRC2	4.41op (±0.31)	1.31m (±9.1)	0.38mno (±1.22)	0.27g (±0.27)
V49	UKMRC8	4.38op (±0.27)	2.56jklm (±0.76)	1.22hijklmno (±0.075)	0.39fg (±0.39)
V50	UKM-6	4.25op (±0.71)	1.32m (±0.26)	1.11ijklmno (±0.06)	0.60fge (±0.31)

1f. Number of leaves

GEN	GENOTYPE	T1	T2	T3	T4
V1	Alek	6.42bc (±1.3)	1.00nopq (±0)	0.61ijklmn (±0.06)	0.03d
V2	Bariohalus	4.91efghij (±0.003)	3.12fghijklmno (±0.53)	1.49 defghijklmn (±0.14)	0.00d
V3	Basmati370	4.87efghij (±0.06)	2.97ghijklmnop (±0.08)	0.00n (±0)	0.00d
V4	Berjer	4.52 fghijkl (±0.12)	0.85opq (±0.24)	0.61 ijklmn (±0/03)	0.00d
V5	Biris	4.94 defghi (±0.03)	1.85klmnopq (±0.24)	0.00n (±0)	0.00d
V6	Boewani	5.00defghi (±0)	1.22mnopq (±0.023)	0.36klmn (±0)	0.21d
V7	CICA4	5.00 defghi (±0)	3.67efghijkl (±0.12)	0.24mn (±0.03)	0.00d
V8	Haiboq	7.12ab (±0.14)	6.23ab (±0.86)	2.36 bcdefghijkl (±0.36)	1.68bcd
V9	IR29	6.18bcd (±0.46)	2.42hijklmnopq (±0.42)	1.33fghijklmn (±01.3)	0.00d
V10	IR64	4.04ghijkl (±0.15)	4.27 cdefghij (±0.46)	2.50 bcdefghijkl (±1.25)	1.00cd
V11	IR64SUB1	4.43 fghijkl (±0.23)	3.87defghijk (±0.19)	1.33fghijklmn (±1.33)	1.33bcd
V12	IR77(NIL)	4.40 fghijkl (±0.5)	4.47 cdefgh (±0.06)	2.02 bcdefghijklmn (±1.15)	1.33bcd
V13	Jarommas	5.00 defghi (±0)	2.40hijklmnopq (±0.4)	0.30lmn (±0.16)	0.27d
V14	Jawikuning	4.33 fghijkl (±0.33)	3.33fghijklmn (±0.18)	2.45 bcdefghijk (±0.27)	0.06d
V15	Jayanti	4.85efghij (±0.6)	5.33cdef (±0.67)	3.89abc (±0.11)	3.00ab
V16	Ketiangedjih	4.96 defghi (±0.43)	1.82klmnopq (±0.09)	1.15 ghijklmn (±0.15)	1.55bcd
V17	Krianb	4.64fghijkl (±0.42)	2.25ijklmnopq (±0.43)	2.82bcdefgh (±0.39)	0.89cd
V18	Kukubalam	4.62fghijkl (±0.57)	1.03nopq (±0.15)	1.00hijklmn (±0)	0.45d
V19	Kukubelang	5.20defgh (±0.013)	1.42lmnopq (±0.29)	0.33lmn (±0.13)	0.00d
V20	Kunyit	3.45kl (±0.36)	2.03jklmnopq (±0.37)	1.01hijklmn (±0.23)	0.00d
V21	Kurauwangi	4.67fghijkl (±0.33)	2.21ijklmnopq (±0.73)	0.40jklmn (±0.4)	0.00d
V22	Liwaguantap	3.94hijkl (±0.307)	2.58ghijklmnopq (±0.15)	1.86 bcdefghijklmn	0.00d
V23	Madu	4.33 fghijkl (±0.24)	0.30q (±0.12)	0.45jklmn	0.03d
V24	Mahsuri	4.82fghijk (±0.18)	2.94ghijklmnop (±0.15)	2.55bcdeghij	0.00d
V25	Merahwangi	5.24defgh (±0.24)	0.91opq (±0.18)	0.00n	0.15d
V26	MR219	3.82hijkl (±0.96)	4.40 cdefghi (±0.2)	2.67bcdefghi	0.00d
V27	MR253	3.61jkl (±0.20)	4.53 cdefghi (±0.29)	1.33fghijklmn	1.33bcd

V28	MR297	4.20ghijkl (±0.11)	4.53 cdefghi (±0.37)	3.07abcdefgh	1.33bcd
V29	MR5	3.85ijkl (±0.08)	3.63efghijkl (±2.985)	2.60bcdefghi	1.00cd
V30	MR82	4.07hijkl (±0.18)	4.56cdefghi (±0.29)	0.00n	1.33bcd
V31	MRQ74	8.00a (±0)	7.56a (±0.55)	3.44abcdef	0.11d
V32	MRQ76	6.11bcde (±0.44)	3.76defghijk (±0.03)	3.55abcde	2.97ab
V33	Nonabokra	5.42cdefg (±0.66)	6.06bc (±0.06)	3.64abcd	2.89ab
V34	Padigunong	4.67fghijkl (±0.904)	0.58q (±0.24)	0.15n	0.00d
V35	Paginkano	4.79fghijk (±0.39)	1.24mnopq (±0.12)	0.39jklmn	0.04d
V36	Pakkedek	4.94 defghi (±0.03)	2.21ijklmnopq (±0.21)	1.79 cdefghijklmn	0.18d
V37	Pokkali	4.85efghij (±0.15)	4.45 cdefghi (±0.29)	5.06a	3.58a
V38	Pulutmalaysia1	3.53kl (±0.29)	4.07 cdefghijk (±0.067)	2.60bcdefghij	0.00d
V39	Putra1	5.00 defghi (±0)	3.52efghijklm (±0.03)	3.33abcdefg	1.67bcd
V40	Putra2	4.13ghijkl (±0.17)	4.67cdefgh (±0.33)	4.08ab	2.67abc
V41	Q70	5.57cdef (±0.67)	5.78bcde (±0.55)	2.75 abcdefg	1.39bcd
V42	Renganwangi	4.50 fghijkl (±0.16)	1.09nopq (±0.27)	0.58ijklmn	0.00d
V43	Santapwangi	4.03hijklhijkl (±0.03)	1.94jklmnopq (±0.07)	1.82 bcdefghijklmn	0.00d
V44	Selayang	4.24 ghijkl (±0.24)	2.39hijklmnopq (±0.13)	1.69 defghijklmn	0.00d
V45	Serandu1	3.93hijkl (±0.067)	4.20 cdefghij (±0.11)	2.58bcdefghij	0.00d
V46	Tangkaimas	5.00 defghi (±0.53)	3.27fghijlmn (±0.27)	1.55 defghijklmn	0.00d
V47	UKM-5	5.00 defghi (±0)	4.00 cdefghijk (±0.18)	1.39efghijklmn	1.33bcd
V48	UKMRC2	3.94hijkl (±0.33)	3.63efghijkl (±0.63)	2.53 bcdefghijkl	1.33bcd
V49	UKMRC8	4.20ghijkl (±0.11)	4.92cdefg (±0.083)	4.33ab	1.33bcd
V50	UKM-6	3.96hijkl (±0.15)	4.22 cdefghij (±0.11)	4.00ab	2.67abc

Appendix 2. Phenotypic Evaluation of Tolerant genotypes

2a. ANOVA TABLE

Traits			Plant Height		
Source of variation	Df	Sum square	Mean square	F Value	Pr > F
Treatment	3	4005.782	1335.261	65.71	<.0001
Genotypes	7	15070.854	2152.979	105.96	<.0001
Treatment x genotypes	21	4127.502	196.548	9.67	<.0001

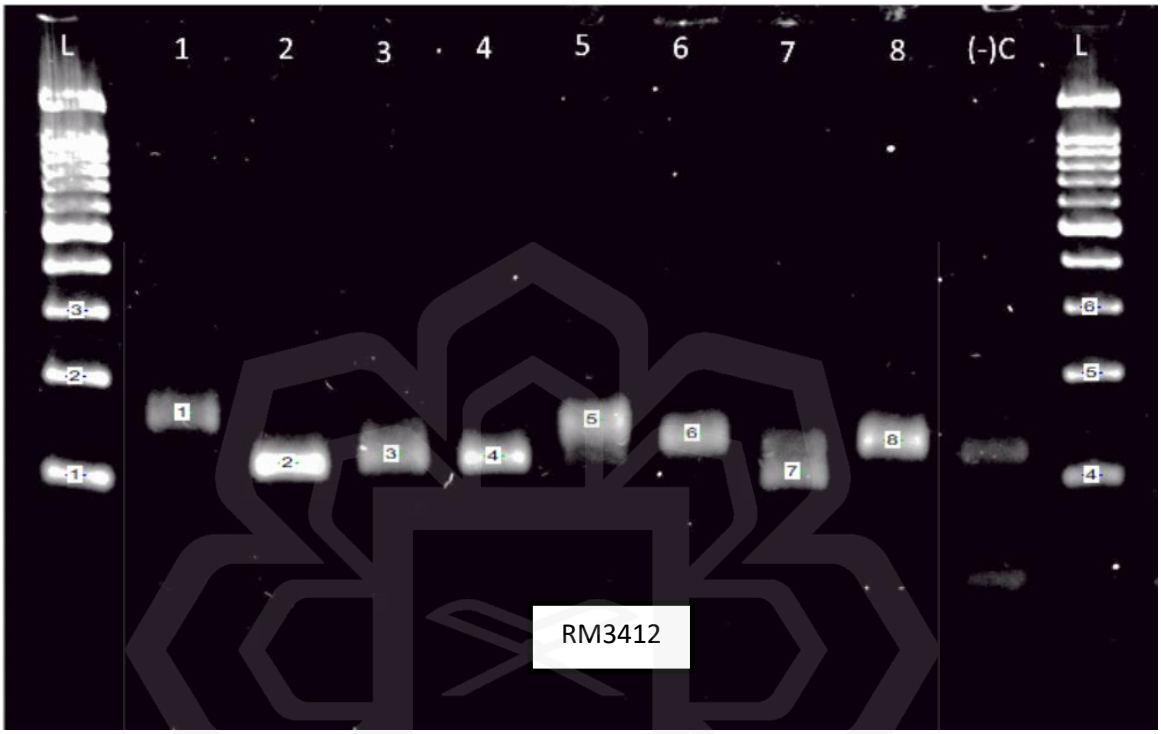
Traits			Leaf length		
Source of variation	Df	Sum square	Mean square	F Value	Pr > F
Treatment	3	1508.666	502.889	56.08	<.0001
Genotypes	7	8360.812	1194.402	133.20	<.0001
Treatment x Genotypes	21	1453.851	69.231	7.72	<.0001

Traits			Leaf width		
Source of variation	Df	Sum square	Mean square	F Value	Pr > F
Treatment	3	1.0744	0.358	75.35	<.0001
Genotypes	7	1.674	0.239	50.33	<.0001
Treatment x Genotypes	21	0.788	0.037	7.88	<.0001

Traits			Leaf size		
Source of variation	Df	Sum square	Mean square	Sum square	Mean square
Treatment	3	726.175	242.058	726.175	242.058
Genotypes	7	3323.619	474.802	3323.619	474.802
Treatment x Genotypes	21	662.693	31.557	662.693	31.557

Traits			Number of Leaves		
Source of variation	Df	Sum square	Sum square	Sum square	Sum square
Treatment	3	140.117	140.117	140.117	140.117
Genotypes	7	46.580	46.580	46.580	46.580
Treatment x Genotypes	21	53.316	53.316	53.316	53.316

Appendix 3. Gel photos for genotyping experiment

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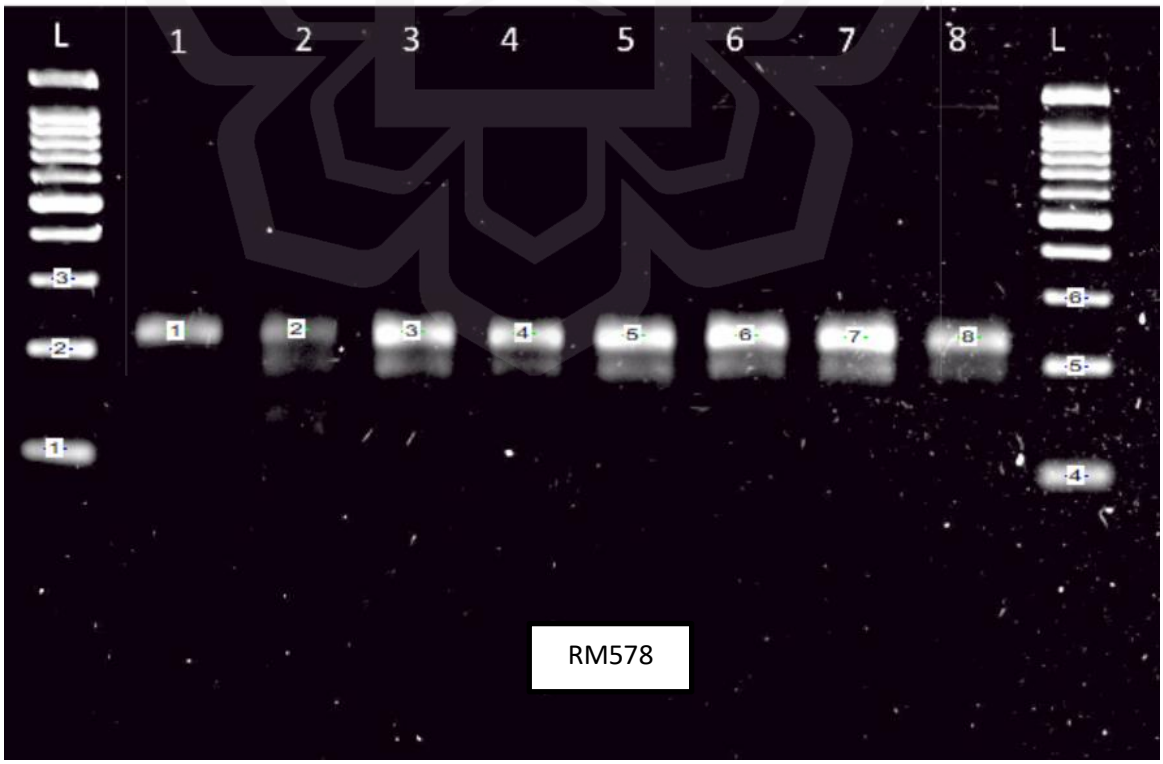
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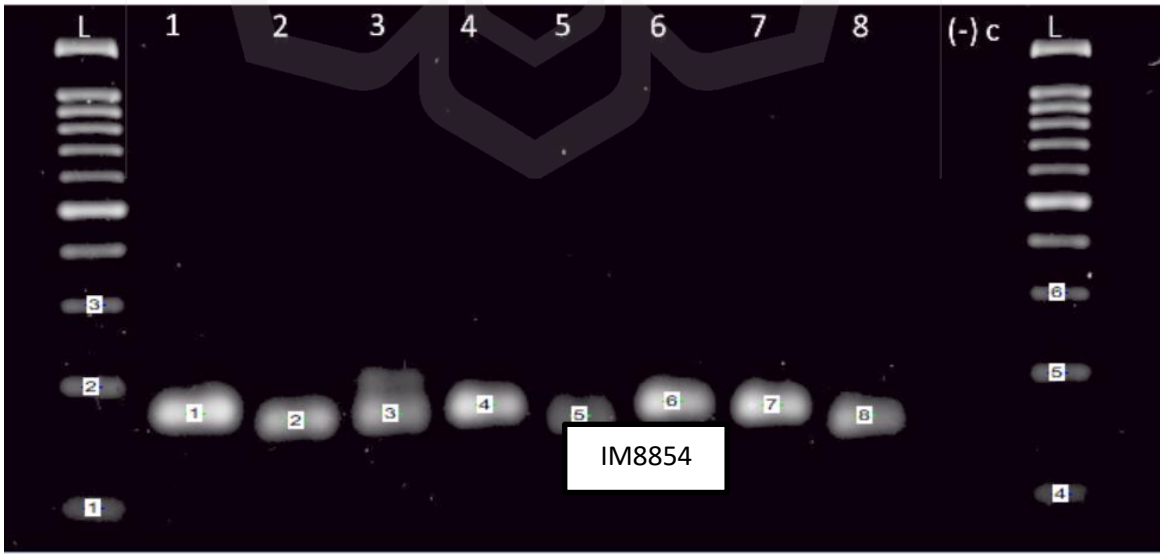
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