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THESIS

MOLECULAR BREEDING OF IMPROVED SIN-THWE-LATT FOR SUBMERGENCE TOLERANCE, BACTERIAL LEAF BLIGHT RESISTANCE, GRAIN AROMA AND SALT TOLERANCE

THIDA

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Sin-Thwe-Latt-108 (STL108), an improved line of Sin-Thwe-Latt showed intermediate salt tolerance when tested in salt prone areas in Myanmar. It posses the *Saltol* inherited from Pokkali (PK). Molecular breeding of improved STL108 with *Sub1*, *Xa21* and *badh2* was carried out at Rice Gene Discovery Unit (RGDU) for four years. Twenty nine BC_2F_5 (BILs) carrying the three target genes were successfully developed with similar cooking quality characters as recurrent parent STL108. All BC_2F_5 lines showed submergence tolerant, bacterial blight resistant and grain aroma characters but varied for salinity tolerance. Genome scan and association analysis indicated that salt tolerance traits were not only controlled by the *Saltol* but also involved genetic factors located on other locations especially on a long arm of chromosome 1. The selected BILs with high level of submergence and salinity tolerance, bacterial blight resistance and grain aroma were selected and will be evaluated at salt affected and submergence areas in Myanmar in the future. Therefore, Quantitative trait loci (QTL) for salt tolerance related traits at seedling and reproductive stages was studied using F_6 recombinant inbred lines (RILs) derived from KDML105 and PK. Eighteen putative QTLs were identified on chromosomes 1, 6 and 11. QTLs for salt injury score (SIS), survival days after treatment (SvDAT), and percent plant survival (PPS), were mapped on a long arm of chromosome 1. It is different location from the *Saltol* locus that used for molecular breeding in our study. However, QTL associated with $Na^+$and K^+ ion in the flag leaf of reproductive stage were detected on *Saltol* region, a short arm of chromosome 1. So it can be concluded that chromosome 1 is the most important for salt tolerance related traits and the salinity tolerance in rice may be controlled by several genes which correspond to different mechanisms contributing tolerance ability. We investigated the Myanmar germplasm for salinity tolerance. Two hundred and forty two accessions of Myanmar rice were screened with 100 mM NaCl at seedling stage. Five accessions collected from Ayeyarwaddy and Rakhine region showed high level of salt tolerance at seedling stage as the same SIS with standard check PK. The selected accessions are prospective for future salt tolerant breeding programs in Myanmar.

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Thida

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LIST OF ABBREVIVATIONS

BB	=	bacterial leaf blight
cm	=	centimeter
cM	=	centimorghan
dS/m	=	deci siemens per meter
DBIOM	=	dry biomass
DPNWT	=	dry panicle weight
DRWT	=	dry root weight
DSWT	=	dry shoot weight
EC	=	electric conductivity
FGP	=	filled grain percent
FBIOM	=	fresh biomass
FPNWT	=	fresh panicle weight
FRWT	=	fresh root weight
FSWT	=	fresh shoot weight
ha	=	hectares
kg	=	kilogram
Mb	=	mega base pairs
µl	=	microliter
µM	=	micromolar
mg/g	=	milligram per gram
ml	=	milliliter
mM	=	millimolar
min	=	minute
ng	=	nanogram
PNL	=	panicle length
K^+	=	potassium ion
qSt1b	=	QTL for salt tolerance on long arm chromosome1 (b)
QTL	=	quantitative trait loci

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LIST OF ABBREVIATIONS (Continued)

SIS	=	salt injury score
SISPN	=	salt injury score for panicle
SKC1	=	shoot potassium content1
SSR	=	simple sequence repeat
NAKR	=	sodium and potassium ratio(NA^+/K^+)
NaCl	=	sodium chloride
NA^+	=	sodium ion
SvDAT	=	survival days after treatment
TGWT	=	thousand grain weight
TGN	=	total grain number
UFG	=	unfilled grain percent

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MOLECULAR BREEDING OF IMPROVED SIN-THWE-LATT FOR SUBMERGENCE TOLERANCE, BACTERIAL LEAF BLIGHT RESISTANCE, GRAIN AROMA AND SALT TOLERANCE

INTRODUCTION

Rice, most loved cereal of Asia, feeds the majority of the world's population. More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people and about two-third of the world are poor live (Khush and Virk, 2000). In the world's rice production areas, rice crop has been affected by a number of biotic and abiotic stresses (Srinivasan and Gnanamanickam, 2005). In Asia, 21.5 million ha (both cultivated and barren) are salt-affected, of which 12 million ha are saline and 9.5 million ha are alkaline-sodic. These figures are alarming and require intensified efforts to minimize climate change-induced yield losses today and in the future (Jagadish *et al.*, 2012). Salinity and other associated soil problems are major constraints for rice production in most humid and sub-humid coastal and inland climates of Asia (Ismail *et al.*, 2007, Ismail and Tuong 2009). An anticipated effect of global warming is an increase in area affected and severity of salt stress, both in coastal and inland ecosystems. In coastal areas, an increase in salt intrusion has already been observed in some of the low-lying deltas such as in South Bangladesh, Vietnam and Myanmar (Wassmann *et al.*, 2004). In inland areas, salt deposition is expected to increase as a consequence of increased evapotranspiration and water shortage with rising temperatures. (Mackill *et al.*, 2010).

Flood is a significant problem for rice farming, especially in the lowlands of South and South-east Asia. Approximately 15 - 20 million ha of rice-growing areas are submergence-prone. On the other hand, coast lines of South and South-east Asia, Africa, Caribbean Islands, and the Indian and Pacific Ocean small island groups are identified as the most vulnerable areas of the world because of sea level rise and salt water inundation (Nicholls and Cazenave 2010).

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Bacterial blight disease (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most destructive diseases of rice throughout the world. Yield losses in severely infected fields ranging from 20 to 30%, but it can reach as high as 80% and grain quality (Khush *et al*., 1989; Noh *et al*., 2007). BB is a major threat to rice rainfed and irrigated lowland ecosystems in Myanmar. Large-scale and long-term cultivation of released Myanmar rice varieties have continuously caused the outbreak of BB since 1983 (Win *et al*., 2013).

Hence, developing rice varieties that can withstand the expected changes in climatic factors is very important and will have a large impact on the socio-economic and political stability of rice-producing regions of the world. Rising sea level will reclaim some of the rice lands in the coastal regions, increase salinity intrusion, and impede drainage leading to more flooding problems in low-lying areas. These damaging effects can be effectively addressed through plant breeding. Rice breeding has been a very successful activity in the past few decades, particularly in favorable areas. During the 1970s, modern, high-yielding varieties (HYVs) were rapidly adopted in irrigated and favorable rainfed lowland areas (Mackill *et.al*, .2010).

Rice fields suffering salt damage area is accounted for one fifth of all cultivated area in the continent. This salt damage can be reduced via managing soil and water, as well as modified chemical. However, it's hard to come true for the high cost and its ineffectiveness. Therefore, it is one of the effective ways to ensure the food safety production of the salinization rice area and improve the ecological environment to cultivate salt-tolerant rice variety and do the salt tolerance researches deeply in rice (Hu *et al*, 2012). Many scientists are trying to improve varieties and solve the environmental stress problems for the crop production by doing research on conventional and molecular breeding methods.

Giora Ben-Ari and Uri Lavi (2012) stated that the advantages of Marker Assisted Selection (MAS) results from the fact that many of the traits of interest to breeders are not easily assessed. Thus, selection, which is based on linked DNA markers, is much more efficient. Selection based on markers can be carried out at an

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early age (plantlets); therefore, it has the potential to significantly reduce the number of individuals assessed by the breeder, thus reducing costs. MAS has greater potential for efficient gene pyramiding; namely, combining several important genes in one cultivar. At the same time MAS does not reduce the time of the breeding project because the selected plants need to be tested and evaluated in the field. The length of the evaluation process depends mainly on the length of the juvenile period of each species.

Agriculture in Myanmar, dominated by rice cultivation, generates a direct or indirect economic livelihood for over 75% of the population. Rice is the principal human food resource and primary foreign exchange earner of Myanmar. It is an important crop for Myanmar, which has the highest per capita consumption of rice in the world: more than 210 kg per person per year. Rice cultivation covers eight million hectares (ha), 66% of total cultivated area (http://faostat.fao.org, 2009). FAO (2012) estimated 8,150,000 ha could be harvested and 33,000,000 Tonns could be produced in Myanmar.

As an origin of rice cultivation, Myanmar is one of the countries which have been found high in genetic diversity of rice and a part of the crop diversity area, and it also have heterogeneous geographical and ecological conditions such as hills and mountains. Geographically, Myanmar has six distinct regions: central dry zone, coastal strip region, delta region of Ayerwady and Sittaung rivers, eastern mountain region, northern mountain region, and western mountain region. As a staple food and crop with potential for export, rice is grown extensively in all of the six regions under all agro-ecological conditions. There are 5770 accessions of rice germplasm collected and conserved in Myanmar Seed Bank Project in 2000 (Saw *et al.*, 2006). The environment stress tolerant rice should be searched among the diverse germplasm varieties of Myanmar for the stress area.

Now a day, rice is grown in Myanmar under a wide range of ecologies ranging as irrigated lowland (18%), flood prone lands (14.3%) upland (14.9%) and rainfed lowland (52.8%) which is the largest and account of total rice areas (FAO, 2002).

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Under the rainfed lowlands, 48% area is unfavorable for rice production including submergence, deepwater, drought, upland and salt affected area. Salinity soil occupied 5%, submergence area occupied 6%. And flood prone area was 14.3% of total cultivated area. Salinity and submergence problems have greatly reduced the grain yield (Myanmar Agriculture Service, 2003). Rice diseases, insect pests and weeds are major biotic constraints for increasing productivity of rice. Among them, bacterial blight disease is a serious disease. Garcia *et al*. (1998) reported that bacterial blight was major disease in 80% of the township surveyed with 2.8% affected area and estimated losses of 398 kg ha^{-1}.

Costal saline areas occasionally experience submergence with saline water due to flash floods. In delta regions of Myanmar, sea water penetrates deeper the rivers and streams so that the problem of salinity of irrigation water occurs. This is one of the severe problems for rice production in Myanmar. In the inland area, saline and alkaline stresses are occurred in that area because of using the ground water and irrigation water to grow rice. So, salinity and submergence are serious problems of Myanmar rice production.

According to UNESCAP (2009), in Myanmar, problem soils occupy an area of nearly 1 million ha, representing about 7.8 percent of the total cultivable land. Of the area of problem soils, about 68.75 percent (660,000 ha) comprises saline and alkaline soils, although most of them are currently under cultivation. The remaining problem soil area comprises acid sulphate, degraded, peat and swampy soils. Therefore saline and alkaline soils are the predominant problem soil in Myanmar (www.unescap.org/rural/doc/sads/myanmar.PDF).

A significant amount of rice production could be increased from such areas by developing and introducing appropriate high yielding rice varieties with dual tolerance to salinity and submergence stresses. Mackill and group reported in 2010 that for coastal areas in the wet season, both salinity and submergence are problems. Fortunately, both *Sub1* (submergence tolerant gene) and *Saltol* (Salinity tolerant QTL)

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can be combined in the same variety, and these lines combine tolerance to both stresses.

Sin-Thwe-Latt (STL) is one of the mega and popular High Yield Varieties (HYV) in Myanmar among rice growing farmers for its market preference. According to circulation data report by Department of Agriculture, Ministry of Agriculture and Irrigation, Myanmar, STL occupied 289,205.36 hectares which is 4.6% of total rice growing area in 2013 wet season and it could give the average yield, 4.04 tons per hectare. This variety is grown across the country especially in the rice bowl area of Myanmar such as Ayeyawaddy, Pegu, Yangon, Mon, Mandalay, Magwe ,Taninthayi and Rakhine regions. Department of Agricultural Research, Yezin, Myanmar has collaborated research with BIOTEC, Rice Gene Discovery Unit (RGDU), Kasetsart University, Thailand, to improve Myanmar popular rice variety Sin-Thwe-Latt (IR53936-60-3-2-1) by marker-assisted selection to get *Saltol* (Salinity tolerant QTL) from PK under Mekong region Project since 2003 - 2004. The salt tolerant improved Sin-Thwe-Latt elite lines were validated in target areas but those are still lack of *Sub1*, *Xa21* and non aroma.

In this regards we would like to incorporate the traits: submergence tolerance, bacterial leaf blight resistance, grain aroma and salt tolerance into the improved STL.

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OBJECTIVES

General objective

To incorporate submergence tolerance, bacterial leaf blight resistance, and grain aroma into improved Sin-Thwe-Latt, salt tolerance elite line BC_3F_4 Yn3220-108-2-3-1 by incorporating with *Sub1, Xa21* and *badh2* genes through molecular marker assisted selection.

Specific objective

1. To incorporate *Sub1*, *Xa21* and *badh2*, genes from IR57514 BC_4F_4 RGD07343-13-21-9 (RGD309) into salt tolerant improved Sin-Thwe-Latt elite line BC_3F_4 Yn3220-108-2-3-1(STL108) through molecular marker assisted breeding.

2. Identification of quantitative trait loci (QTL) related to salinity tolerant.

3. Screening for salt tolerant at seedling stage in rice germplasm collected from different geographical areas of Myanmar including Ayeyarwady division, Rakhine State and dry zone area, middle of Myanmar.

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LITERATURE REVIEW

Myanmar Germplasm

Myanmar is geographically located between latitudes 09˚32′ to 28˚31’ N and longitudes 92˚10′ to 101˚11′E and is situated in South East Asia. The country is bounded on the northwest and west by India and Bangladesh, on the north and north-east by China, on the east and south-east by Laos and Thailand, on the south by the Andaman Sea and the Bay of Bengal (http://www.myanmar-embassy-tokyo.net/about.htm). Geographically, Myanmar has seven distinct regions namely: Northern Mountain Region, Western Mountain Region, Eastern Mountain Region, Central Dry Zone, Southern Coastal Strip Region, Western Coastal Strip Region and Delta Area of Ayeyawady and Sittaung rivers.

Rice, which constitutes more than half of the total sown acreage, is grown in the different climatic conditions and various topography of the country. Possessing prominent adaptability to the wide range of growing conditions, rice can be grown throughout the year. The irrigated rice is grown where irrigation system exists mostly in the dry zone area of the country. Geographically, rice cultivation increased occurred mostly in the deltas, which has 55% of the total rice sown areas, the coastal strips (14%), Central dry zone (13%) and the mountain area (18%) (Aung, 2004).

Myanmar is an important reservoir of genetic variation of cultivated species, the in situ and ex situ preservation of which is becoming a priority in the field of plant genetic resources. Genetic diversity depends on the environmental heterogeneity and various other factors, which are agro-ecological conditions, a long history of cultivation and ethnological complexity in Myanmar (Aung, 2004). Myanmar rice germplasms are greatly varied in grain morphological and physiological characters. Beale (1915) classified five groups of Myanmar rice as Emata (A), Letywezin (B), Ngasein (C), Meedon (D), and Byat (E) based on the grain length/width ratio of rice. The Emata and Letywezin groups have relatively long, thin grains whereas the

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Ngasein, Meedon and Byat groups are classified as bold, short-medium grain types. Emata, Letywezin and Ngasein are hard and translucent, and the Meedon and Byat groups are softer and more opaque. On the other hand, Myanmar farmers classify rice based on time of sowing, water regime and growth duration. Myanmar rice cultivars could be classified into four types based on time of sowing, premonsoon rice (sowing in March and harvest in July), monsoon rice (sowing in mid-June and harvest in October or November), late monsoon rice (sowing in August and harvest in January), and "mayin" or winter rice (sowing in November and harvest in March). Classification based on water regime includes three groups, irrigated, upland, and rainfed. Classification based on the growth duration comprises of three groups, "kaukyin" (early maturing variety-duration of growth 140 - 145 days) that ripens in October, "kauklat" (medium maturing variety 150 - 170 days) that matures in November, and "kaukkyi" (late maturing variety 170 - 200 days) that ripens in December. These facts suggest that the wide diversity of Myanmar rice germplasm is an important source for various breeding purposes as breeding materials (Win, 1991).

Stresses on rice

Rice (*Oryza sativa L.*) is the most important food crop in the world, which accounts for more than 21% of the calorific needs of the world's population and up to 76% of the calorific intake of the population of South East Asia (Ma *et al.*, 2007 ; Melissa *et al.*, 2009). Rice production employs one billion people and is essential for the economic development of rural areas in India, Bangladesh and Southeast Asia and provides rural employment and prosperity (Sudharani *et al.*, 2012).

Though significant improvement in productivity has been achieved over the years, a series of biotic and abiotic stresses limits its productivity worldwide. Abiotic stresses alone contribute to 50% of the total yield losses. Among abiotic stresses, salinity, drought and extreme temperatures are major barriers to limit rice crop production (Mohammadi-Nejad *et al.*, 2008). Abiotic and biotic stresses are believed to cause major problems in agriculture by reducing crop growth and productivity. Because of their sessile nature, plants must endure adverse environmental conditions

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and consequently evolve a variety of responses to acclimatize to environmental stresses. During evolution, plants have developed sophisticated mechanisms to sense the subtle changes of growth conditions, and trigger signal transduction cascades, which in turn activate stress responsive genes and ultimately lead to changes at the physiological and biochemical levels. Rice, a staple crop for over half of the world's population, is sensitive to a variety of abiotic stresses, including salinity, drought, submersion and cold (Lafitte *et al*., 2004). Researchers from all over the world have made great efforts in understanding the mechanisms of responses to abiotic stresses in rice. A greater understanding of the physiology and molecular biology of stress tolerance may provide a useful platform to improve stress-tolerant rice varieties (Gao *et al*., 2007).

The prospect of global warming resulting from accumulation of greenhouse gasses is causing major concern, especially in connection with its potential effect on rice production (Wassmann *et al*., 2009b). Mackill *et al*. (2010) stated that while the rise in CO_2 concentration would be expected to have a beneficial effect, the overall results will be negative in the tropics, where most of the world's poor live. Rising sea level will reclaim some of the rice lands in the coastal regions, increase salinity intrusion, and impede drainage leading to more flooding problems in low-lying areas. Increasing frequencies of both drought and floods will result from more erratic rainfall and extreme weather events. Higher temperatures will also have a negative effect on rice production. High temperature can affect rice growth and development at all stages, and particularly if it occurs during pollination. These damaging effects can be effectively addressed through plant breeding. Rice breeding has been a very successful activity in the past few decades, particularly in favorable areas. During the 1970s, modern, high-yielding varieties (HYVs) were rapidly adopted in irrigated and favorable rainfed lowland areas. However, these varieties were not ideal for the unfavorable areas, and most farmers in these areas continued to grow low-yielding varieties. Breeding for tolerance to abiotic stresses was rapidly expanded during this time.

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However, these varieties are invariably intolerant to the current major abiotic stresses that are likely to be further aggravated by climate change, such as drought, submergence, and salinity. These stresses reduce yields in millions of hectares in rice production areas; and modern HYVs have had limited impact in these areas. The scenario of climate change is leading to a convergence of technology developed for unfavorable rainfed environments with the need for future adaptation of rice varieties to the changing climate. Rice varieties tolerant to the major abiotic stresses (drought, flooding, salinity and high temperature) will provide some protection against the adverse effects of climate change. Rapid progress has been made in developing stress tolerant varieties, and they are being rapidly disseminated to farmers in unfavorable growing environments. Fortunately, both *Sub1* and *Saltol* can be combined in the same variety, and these lines combine tolerance to both stresses (Mackill *et al*., 2010).

1. Salt stress

The global climate change is feared to promote rapid soil degradations in agricultural lands worldwide. Soil salinization is one of the serious soil degradations, which can arise from natural causes and human-mediated activity such as irrigation in arid and semi-arid regions. Approximately 20% of the irrigated lands in the world are presumably affected by soil salinization (Yeo, 1999). Salinity stress significantly reduces growth and productivity of glycophytes, which are the majority of agricultural products. About 6.5% (831 million ha) of the world's total area (12.78 billion ha) is affected by salt in soils. Area under salt stress is on the increase due to many factors including climate change, rise in sea levels, excessive irrigation without proper drainage in inlands, underlying rocks rich in harmful salts etc., Vast areas of land are not utilized due to salinity and alkalinity problems (Sanker *et al*., 2011).

The term "salinity" represents all the problems of the soil accumulating excessive salts, which can be categorized into sodic (or alkaline) and saline soils . Sodic soils having a poor soil structure generally spread over arid and semi-arid regions, retaining high concentrations of Na^+ at the exchangeable site of clay particles in the soil, which shows high pH (greater than 8.5) with a high

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exchangeable sodium percentage (ESP > 15). Saline soils can be generally found in arid regions, estuaries, and coastal fringes, which are dominated by Na^+ ions with electrical conductivity (EC) more than 4 dS/m that corresponds to approximately 40 mM NaCl (IRRI, 2011; Munns and Tester, 2008). Moreover, saline soils exhibit ESP of < 15 and much lower pH values than the sodic soils (IRRI, 2011).

According to Reynolds *et al*. (2001), soil salinity (accumulation of salts in the surface zone) has a number of causes, which differ in different geological and climatic regions. The causes can be natural, be due to clearing of native vegetation (dry land salinity), or due to irrigation. Salinity is often accompanied by other soil properties, such as sodicity, alkalinity, or boron toxicity, which exert their own specific effects on plant growth. Salinity is an environmental factor that greatly affects plant growth and development and is a major constraint for crop production. This stress is complex and causes a number of determinant effects. Among them ionic and water constraints constitute the most important. The water constraint even called osmotic pressure is characterized by difficulties to absorb water. The ionic constraint interferes with the uptake of nutrients, and causes direct toxicity due to the ions Na^+ and Cl^-.

Salinity also interferes with the structure of the soil, causes an indirect stress and increases the sensitivity to diverse biotic stresses (Araya *et al*., 1991). To limit the effect of salt in plant productivity, amelioration and utilisation of salt-affected soils are needed. Two approaches are used to solve the problem: by using technical approaches (water and soil management) and biological approaches. Financial difficulties as well as the environmental injuries caused by desalinization programms oriented many research programs to the biological approach, a posteriori in this direction to screen and develop novel plants with increased salt tolerance and better ability to grow in saline areas. Among species, rice (*Oryza sativa*) may play a major role because of its role as 2nd most consumed cereal in the world, and on the other hand its capacity to survive a long submerging time. Since the genome of rice became completely sequenced, rice is increasingly becoming the model plant for cereals. To create rice tolerant lines capable to grow and minimize the toxicity

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effects induced by salt stress and capable to improve the productivity, it is necessary to identify the molecular mechanisms involved in the tolerance or the sensitivity of plants to salt (Diédhiou, 2006).

1.1 Morphological symptoms of salinity stress on rice

Most of the parameters like low tillering, spikelet sterility, less floret per panicle, low 1000 grain weight and leaf scorching, are affected uniformly under both sodicity and salinity, however it is not a thumb rule (http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_for_salt_tolerance.htm). Major symptoms are: white leaf tip followed by tip burning (salinity), leaf browning & death (sodicity), stunted plant growth, low tillering, spikelet sterility, (papery), low harvest index, less florets per panicle, less 1000 grain weight, low grain yield, change in flowering duration, leaf rolling, white leaf blotches, poor root growth and patchy growth in field.

Figure 1 The symptoms of salinity stress (leaf tip burning, spikelet sterility)

Source: http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_ for_salt_ tolerance.htm

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1.2 Effect of salinity on rice and importance of growth stage

Gregorio *et al.* (1997) emphasized that salinity symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish leaf tip, drying of leaves and also reduction in root growth, stunted growth and stem thickness leading to complete cessation of growth and dying of seedlings. Extremely high salt stress conditions cause severe damage to plants, while moderate to low salt stress affects the plant growth rate along with most of the growth and yield parameters like low tillering, stunting, spikelet sterility, less florets per panicle, low 1000-grain weight and leaf scorching etc.

Rice is relatively tolerant during germination, becomes very sensitive during early seedling stage, gains tolerance during active tillering, but becomes sensitive during panicle initiation, anthesis and fertilization and finally relatively more tolerant at maturity (Makihara *et al.*, 1999; Singh *et al.*, 2004). Studies have shown that a very poor correlation exists between tolerances at seedling stage with that during reproduction, suggesting that tolerance at these two stages is regulated by a different set of genes (Moradi *et al.*, 2003). The reproductive stage is crucial as it ultimately determines the grain yield. However, the importance of the seedling stage cannot be undermined as it affects crop establishment. Salinity reduces the growth of plant through osmotic effects, reduces the ability of plants to take up water and this causes reduction in growth. There may be salt specific effects. If excessive amount of salt enters the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduces the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002).

Alam *et al.* (2004) attributed the possible reasons for decrease in the shoot and root growth in salinized plants as reduction of photosynthesis, which in turn limits the supply of carbohydrates needed for growth and reduction of turgor in expanding tissues resulting from lowered water potential in root growth medium. Although rice is one of the most important food crops in the world, both economically

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and nutritionally, it ranks among the most sensitive to salinity (Maas and Grattan, 1999). Not only is rice considerably less tolerant to salinity than wheat, but salinity affects its reproductive development quite differently.

Lauchli and Grattan (2007) reviewed that rice sensitivity to salinity varies considerably from one growth stage to the next. In terms of grain yield, rice is tolerant during germination (Heenan *et al.*, 1988), sensitive to salinity during emergence and early seedling growth, becomes more tolerant later on in vegetative development, and then can become sensitive again during reproductive growth (Pearson and Bernstein, 1959; Flowers and Yeo, 1981; Khatun and Flowers, 1995; Abdullah *et al.*, 2001). The vegetative shoot biomass of rice, on the other hand, is often affected much less than reproductive growth (except for young seedlings) (Khatun and Flowers, 1995; Munns *et al.*, 2002). Field and greenhouse studies showed that salinity had a negative impact on stand establishment and adversely affected a number of yield components and even delayed heading (Grattan *et al.*, 2002). In one study, investigators found linear decreases in several yield components with increased salinity including the percent of sterile florets, tillers per plant and spikelets per panicle which translated into larger reductions in grain weight per plant at a given salinity (Zeng and Shannon, 2000) (Figure 2). However these investigators suggested that seedling emergence and early seedling growth stages were most sensitive to salinity, as was the 3-leaf panicle stage.

Being aware that rice response to salinity is a combination of the level of salinity, the duration of exposure and timing of exposure, Lee *et al.* (2004) proposed a salt stress index that incorporates these factors. Using solution cultures, they found that the growth of rice was reduced over three times more with NaCl than synthetic sea water and that rice was two times more sensitive to salinity at the seedling stage than it was at the tillering stage. This not only implies that the tolerance of rice varies with stage of growth but it is strongly affected by the composition of the root media, particularly when NaCl is used as the sole salinizing salt.

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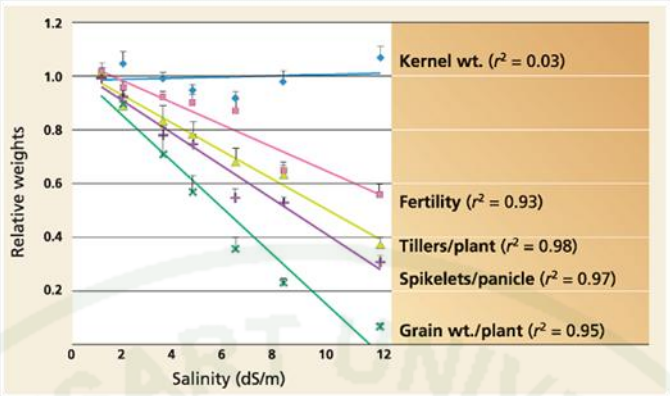

Figure 2 Relationship between salinity and various yield components of rice (*Oryza sativa* L. cv M-202) Fertility is inversely proportional to sterility.

Source: Grattan *et al*. (2002) originally adapted from Zeng and Shannon (2000)

1.3 Different kinds of tolerance mechanisms to salt stress in plants (Glycophytes)

Plants have to cope with two major stresses under high salinity, osmotic stress and ionic stress (Figure 3). The former stress immediately comes over plants in accord-ance with a rise in salt levels outside the roots, which leads to inhibitions of water uptake, cell expansion and lateral bud development (Munns and Tester, 2008). The latter stress phase develops later when toxic ions such as Na^+ ion accumulate in plants particularly in leaves over the threshold, which leads to an increase in leaf mortality with chlorosis and necrosis, and a decrease in the activity of essential cellular metabolisms including photosynthesis (Yeo and Flowers, 1986; Glenn *et al*., 1999). Rice is the most salt sensitive among cereals (Munns and Tester, 2008). In rice, it has been observed that the rate of Na^+ uptake into shoots mediated by the intrusive apoplasticion transport is considerably high under salinity stress (Yeo *et al*., 1987; Yadav *et al*., 1996; Ochiai and Matoh, 2002).

Munns and Tester (2008) explained the effects of salinity stress that to understand the physiological mechanisms responsible for the salinity tolerance, it is

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necessary to know whether their growth is being limited by the **osmotic effect** of the salt in the soil, or the **toxic effect** of the salt within the plant. In the simplest analysis of the response of a plant to salinity stress, the reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Table 1).

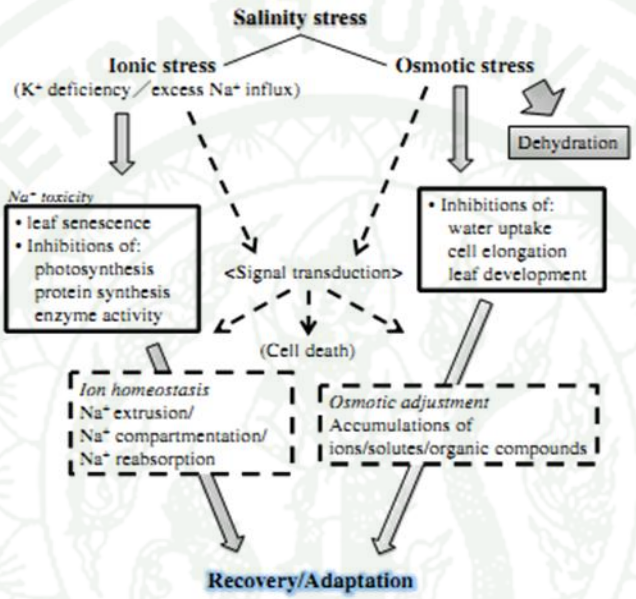

Figure 3 A schematic summary of the stresses that plants suffer under high salinity growth condition and the corresponding responses that plants use in order to survive these detrimental effects

Source: Horie *et al*. (2012)

Table 1 Effect of salinity stress on plants

Effect of stress	**Osmotic stress**	**Stress due to high leaf Na^+ (ionic stress)**
speed of onset	rapid	slow
primary site of visible effect	decreased new shoot growth	increased senescence of older leaves

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Moreover, Munns and Tester (2008) stated that in the first, osmotic phase, which starts immediately after the salt concentration around the roots increases to a threshold level, the rate of shoot growth falls significantly. The threshold level is approximately 40 mM NaCl for most plants, or less for sensitive plants like rice and *Arabidopsis*.

1.4 Quantitative trait loci (QTL) studies for salinity tolerance

Salt tolerance and its sub-traits were found determined by multiple genes supporting the complexity of this trait. QTLs were found associated with yield and yield components under both saline and non-saline environments. It was found out that the major determinants of yield vary with the environmental conditions and quantitative traits normally exhibit a large environmental x genotype interaction. Also, salinity tolerance QTL identified vary with the stage of plant development. Manneh (2004) identified molecular markers that are linked with yield and yield components. Identifying QTLs in relation to physiological attributes of a salt tolerant crop may pinpoint a physiological trait that may contribute to salt tolerance and eventually to yield under stress. QTL associated with physiological traits mentioned above have also been reported. Several AFLP markers were found associated with QTLs for various aspects of the presence of sodium and potassium in the shoots of rice. The QTLs identified governed shoot concentration by means of shoot dry weight, ion uptake in the roots, high sodium and potassium uptake and sodium/potassium ratio. Chromosomes 1, 4, 6 and 9 were found associated with high Na^+, high K^+ and Na^+/K^+ ratio (Koyama *et al*., 2001). Lin *et al*. (2004) found 8 QTLs for shoot and root traits relating to Na^+ and K^+ uptake. Two QTLs have large effects for shoot Na^+ concentration and shoot K^+ concentration that were located on chromosome 7. In the same study, root and shoot QTLs were not mapped in the same locations. Physiologically complex traits governed by few quantitative trait loci suits QTL analysis. So far, genes, cluster of genes or even the regulatory genes may govern QTL itself and these remains to be elucidated. Finding genes and regulatory genes in a QTL is necessary to understand what lies beneath QTL identified for salt tolerance (Flowers, 2004). QTLs related to K^+ absorption, Na^+ absorption, Na-K ratio, shoot

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and root K and Na concentrations and traits such as survival days of seedlings, seedling vigor scoring and dry mass were mapped in chromosomes 1, 3, 4, 6, 7, 9, 10 and 12 (Gregorio *et al*., 2002; Koyama *et al*., 2001; Lin *et al*., 2004; Prasad *et al*., 2000; Zhang *et al*., 1995). Siangliw (2013) described that in the long arm of chromosome 1 where *qSt1b* was located, QTLs for phenotypic responses to salinity tolerance like SIS, SDS and PPS from soil culture technique were located.

2. Submergence stress

Submergence stress is a major constraint to rice production during monsoon flooding season in lowlands and rainfed ecosystem. The visible symptoms of injury caused by complete and sustained submergence include phase of faster elongation by leaves accompanied by yellowing of other leaves and slow growth in dry mass of roots and shoots. After water levels fall, the whole shoot, may collapse and later can die (Jackson and Ram, 2003).

Plants require water for growth but excess water that occurs during submergence or water logging is harmful or even lethal. A submerged plant is defined as "plant standing in water with at least part of the terminal above the water or completely covered with water" (Catling, 1992). Submergence subjects plants to the stresses of low light, limited gas diffusion, effusion of soil nutrients, mechanical damage, and increased susceptibility to pests and diseases (Ram *et al*., 1999). Basically, flooding (submergence) can be classified into "flash flooding" and "deep-water flooding" in accordance with the duration of flooding and the water depth (Bailey-Serres *et al*., 2010; Catling, 1992; Jackson and Ram, 2003). Flash flooding, which generally lasts less than a few weeks, is caused by heavy rain but the depth is not very deep. On the other hand, deep water flooding, which lasts for several months, occurs during the rainy season, and the water depth reaches several meters (Catling, 1992; Hattori *et al*., 2011).

Rainfed lowland and deepwater rice are together account for approximately 33% of global rice farmlands: 50 million hectares of the estimated 150 million

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hectares of rice fields worldwide in 2004 - 2006 (Huke and Huke, 1997). About two thirds of the shallow and intermediate rainfed lowland rice lands are in India, Thailand, and Bangladesh; other countries with a high percentage of rainfed lowland rice farms include Nepal, Myanmar, Laos, and Cambodia. Lowland rice is typically cultivated in paddies of 5 to 25 cm of standing water, which are highly vulnerable to Monsoon flash floods of 50 cm or more that can rapidly and completely submerge plants. Of the low land rainfed rice farms worldwide, over 22 million hectares are vulnerable to flash flooding, representing 18% of the global supply of rice (Khush, 1984).

2.1 Different types of flooding stress

Mackill *et al*. (2010) reviewed that flooding is a major stress constraint to rice production, especially in rainfed lowland areas of the tropics. The flooding of the major river basins and deltas of Asia has provided the sustenance for the rice production that has been a prominent feature of the region for millennia. However, this flooding is also a cause of yield fluctuations because of erratic rainfall patterns and poor drainage of many rice fields (Ismail *et al*., 2010). For convenience, this flooding can be classified into four types depending on the plant traits and varietal types that are adapted to the conditions:

a) Flooding during germination (anaerobic germination; AG): a problem when direct seeding is practiced and heavy rains result in submergence before germination.

b) Flash flood (submergence): plants are completely submerged for up to 2 weeks. Submergence tolerance is required for this condition.

c) Stagnant flooding (medium deep or semi-deep): flooding occurs for a longer duration, more than 2 weeks and often several months, at depths up to 50 cm. Varieties tolerant of stagnant flooding conditions are required.

d) Deeper stagnant flooding (deepwater or floating rice): water depth increases throughout the season to depths above 50 cm and often a meter or more. Varieties with tall plant height or rapid internode elongation are required (Mackill *et al*., 2010).

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In any particular field, more than one of these situations can occur in the same season or in different seasons. Therefore, it is preferable that varieties developed for flood-prone areas have a combination of tolerance traits when possible. This is feasible with the exception that varieties developed for deepwater areas usually need to have rapid elongation ability, and this trait is probably incompatible with submergence tolerance conferred by the *Sub1* gene.

2.2 Mechanism of submergence

The physiological mechanisms provided to the tolerant rice determine plant survival and also recovery under flooding period. Submergence tolerant plant exhibited several morphological adaptations such as decreased chlorosis of tissues and reduced elongation growth in order to save carbohydrates and energy for maintenance processes including the protective antioxidant systems. Submergence stress cause the elongation rate of leaves and stem in some plant species. Under flash flooding, few characters were identified as playing a key role in submergence tolerance in rice, the most critical are: maintenance of high carbohydrate concentration, optimum rates of alcoholic fermentation and energy conservation by maintaining low elongation growth rates during submergence. Protective mechanisms as the up regulation of antioxidant system and low synthesis or sensitivity to ethylene during submergence were also found to be useful (Sarkar *et al*., 2006).

Nishiuchi *et al*. (2012) reviewed that many low land rice cultivars, despite having an ability of internal aeration, are still sensitive to complete submergence. Their leaves and stems moderately elongate under complete submergence to reach the air-water interface, but their elongation growth can exhaust energy reserves and cause death when the flooding depth is deep and the flooding period is long (Bailey-Serres *et al*., 2010). However, some cultivars use two distinct strategies of growth controls to survive under submerged conditions. One of the strategies is a quiescence strategy [i.e. the low-oxygen quiescence syndrome (Colmer and Voesenek, 2009), in which shoot elongation is suppressed to preserve carbohydrates for a long period (10 - 14 days) under flash- flood conditions.

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Submergence tolerant cultivars can restart their growth during de-submergence by using preserved carbohydrates. Another strategy is an escape strategy [i.e. the low-oxygen escape syndrome (Bailey- Serres and Voesenek, 2008; Colmer and Voesenek, 2009)] which involves fast elongation of internodes to rise above the water level and is used by deep water rice cultivars. Both strategies depend on ethylene responsive transcription factors (Hattori *et al*., 2009; Xu *et al*., 2006).

Catling (1992) defined submergence tolerance as "the ability of a rice plant to survive 10 - 14 days of complete submergence and renew its growth when the water sub-sides; there is no stem elongation during submergence." Under this definition, submergence tolerance indicates flash-flood tolerance. Generally, the seedlings of many lowland rice cultivars elongate their leaves to get oxygen at the water's surface under sub merged conditions. However, because this shoot elongation requires large amounts of energy, most rice cultivars (i.e. flash-flood-intolerant cultivars) have poor ability to recover fully after the water recedes and eventually sustain severe damage or die (Jackson and Ram, 2003). By contrast, the flash-flood-tolerant East Indian rice cultivar FR13A shows restricted shoot elongation and reduced energy consumption under submergence (Setter and Laureles, 1996). The energy in FR13A plants are preserved during submergence, and upon de-submergence their growth can be restarted by using this energy (Fukao *et al*., 2006). There is a negative correlation between shoot elongation and survival rate under complete submergence (Setter and Laureles, 1996).

2.2.1 Flash flood tolerance

The other type of flood is a flash flood, a surge flood that appears suddenly, but lasts no longer than a few weeks. A severe flash flood can damage rice plants at the seedling stage. If the rice has grown sufficiently to remain above water level, it can breathe and avoid drowning. However, flash floods often occur at the rice seedling stage, submerging the seedlings, which then drown (Figure 4a). When a flash flood occurs at the seedling stage, common rice seedlings elongate their leaves to avoid drowning. As the result, the seedlings consume energy while in the water and

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wilt and die after the water recedes (Figure 4a). In contrast, submergence-tolerant (flash flood tolerant) rice show stunting and survive in water for a few weeks, avoiding the energy consumption associated with plant elongation. Submergence-tolerant rice restarts its growth with conserved energy after the flood has receded (Figure 4b) (Nagai *et al*., 2010).

The genes at the *Submergence1* (*Sub1*) locus, which confer submergence tolerance, were identified by positional cloning (Xu *et al*., *2006*). In the *Sub1* region, three similar genes encode the AP2/ERF domain: *Sub1A*, *Sub1B* and *Sub1C*. These are located tandemly on chromosome 9 in the submergence tolerant variety FR13A.The *Sub1A* and *Sub1C* are robustly induced in the tolerance cultivars in response to submergence (Mackill, 2006; Ruanjaichon *et al*., 2008). The growth of plants carrying *Sub1A* is restricted while submerged, avoiding the energy consumption associated with plant elongation. After the water subsides, the plant restarts its growth using unused, accumulated energy. It is expected that repressed energy consumption would be observed in plants carrying *Sub1A* during submergence. Ethanolic fermentation is necessary for acclimation to transient stress, including low oxygen and submergence stress (Schwartz, 1969; Rahman *et al*., 2001).

The submergence tolerant rice restricts its growth while in water. Fukao and Bailey-Serres (2008) showed the mechanism by which *Sub1A* regulates plant growth. Slender rice-1 (SLR1) and SLR1 Like-1 (SLRL1) are repressors of GA signaling. The amount of SLR1 protein is elevated after submergence in submergence-tolerant rice (M202-*Sub1*), but not in submergence-intolerant rice (M202). Additionally, more SLRL1 protein accumulated in submergence-tolerant rice (M202-*Sub1*) than in submergence-intolerant rice (M202). These results suggest that the restriction of growth by submergence-tolerant rice is due to the accumulation of SLR1 and SLRL1 through *Sub1A* (Figure 4c) (Nagai *et al*., 2010).

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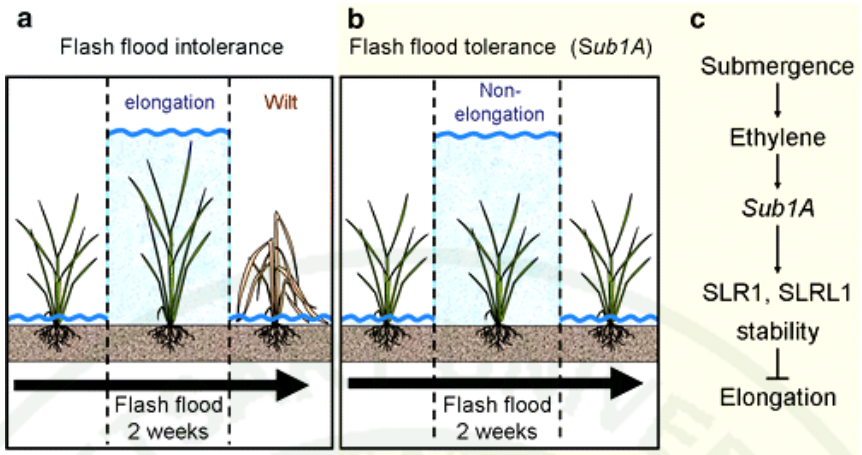

Figure 4 Model of flash flood tolerance and plant hormones in rice. **a** The strategy of flash flood intolerant rice. A normal rice seedling elongates its leaves during a flash flood to avoid drowning; as a result, it wilts and dies after the water recedes. **b** The strategy of flash flood tolerance at the rice seedling stage. Flash flood tolerant rice stops growing so as not to consume energy while submerged. The plant restarts its growth using accumulated energy after the water recedes. **c** The scheme of flash flood tolerance. Flash flood tolerant rice carries *Sub1A*, which promotes the accumulation of SLR1 and SLRL1, negative regulators of GA signaling, and inhibits internodes elongation.

Source: Nagai *et al*. (2010)

2.3 *SUB1*'s journey from landrace to modern cultivar

As a coping strategy, farmers have traditionally cultivated chronically flood-prone lowlands with landraces that can endure 10 days or more of complete submergence and resume growth upon de-submergence (Catling, 1992). However, these submergence tolerant landraces produce less than 2 ton (t) of grain ha^{-1}, paling in comparison to the 6 - 8 t of grain ha^{-1} yields of advanced semi-dwarf varieties. Unfortunately, the popular "mega-varieties" grown in large areas of Asia are sensitive to complete submergence and usually die within 7 days of complete inundation. A challenge recognized by breeders in the 1970s was the need to improve yields in the

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rainfed lowlands by introduction of submergence tolerance to high yielding varieties (Mackill *et al*., 1996). Landraces with unusual flooding and submergence tolerance were first reported in the early 1950s and systematically screened in the 1970s. The accessions FR13A and FR43B from Orissa, India and Kurkaruppan, Goda Heenati, and Thavalu from Sri Lanka were recognized for their resilience to complete submergence (Mackill *et al*., 1996). FR13A stood out as extremely submergence tolerant; 100% of 10-day-old seedlings survived 7 days of complete submergence (Hille Ris Lambers and Vergara, 1982). However, FR13A lacks other agronomic attributes. It is photoperiod sensitive, tall, and provides low yields of poor quality grain. None the less, the recognition of landraces with pronounced submergence tolerance raised hopes that this trait could be introduced into advanced breeding lines to reduce yield loss from flash flooding. Although strides towards this goal began in the 1980s (Mohanty and Chaudhary, 1986; Haque *et al*., 1989), it was not until the mid 1990s that submergence tolerance from the FR13A derived breeding line IR49830-7-1-2-2 was successfully introduced into productive short to intermediate stature lines (Mackill *et al*., 1993; Mishra *et al*., 1996). Fifty-day-old plants of these breeding populations showed prolonged submergence tolerance under greenhouse and field conditions.

2.4 Mapping and molecular characterization of *SUB1*

Until the mid-1990s, the genetic control of submergence tolerance remained ambiguous. Several studies suggested that it was a typical quantitative trait (Mohanty *et al*., 1982; Mohanty and Khush, 1985; Haque *et al*., 1989). Molecular mapping allowed the identification of the major QTL SUBMERGENCE 1 (*SUB1*) on chromosome 9, contributing up to 70% of phenotypic variation in tolerance (Xu and Mackill, 1996). Several independent studies confirmed the major chromosome 9 QTL and identified other minor QTLs that accounted for less than 30% of the phenotypic variation in tolerance (Nandi *et al*., 1997; Toojinda *et al*., 2003; Siangliw *et al*., 2003).

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2.5 Breeding for *Sub1* mega-varieties

Although complete submergence is a common natural disaster that damages rice production in many rice-growing areas throughout the world, all commercially important cultivars are intolerant to the stress. The identification of the *SUB1* QTL enabled its transfer by marker-assisted back crossing (MABC) into the farmer preferred varieties (Xu *et al.*, 2004; Mackill, 2006). The gene level analyses of the *SUB1* region resolved single nucleotide polymorphisms within *SUB1A* and *SUB1C* that could be used for molecular markers and in precision breeding (Neeraja *et al.*, 2007; Septiningsih *et al.*, 2009). Using MABC, a small genomic region containing *SUB1A* has been introgressed into modern high-yielding varieties, such as Swarna, Samba Mahsuri, IR64, Thadokkam1 (TDK1), CR1009, and BR11 (Septiningsih *et al.*, 2009). Micro-satellite markers that were polymorphic between the two parents were used to ensure that the recurrent parent genome was combined with the *Sub1* region originally from FR13A on chromosome 9. Multiple evaluations of submergence tolerance in the green house and farmers 'fields confirmed that all "*Sub1*" lines exhibit significantly greater tolerance to complete submergence as compared with their original parents (Sarkar *et al.*, 2009; Septiningsih *et al.*, 2009; Singh *et al.*, 2009). SSR marker RM219 and codominant PCR based marker RM464A were selected for submergence tolerance and were found to be linkded to *Sub1* by 3.4 and 0.7 cM respectively in further tested indica and japonica rice (Xu et al., 2004). Toojinda and group in 2003 and 2005 reported that molecular markers that were tightly linked with *Sub1*, flanking *Sub1*, and unlinked to *Sub1* were used to apply foreground, recombinant and background selection, respectively. Siangliw *et al.* (2003) stated that the close association between tightly linked markers of the tolerance locus on chromosome 9 and submergence tolerance in the field demonstrates considrable promise of using markers in lowland rice breeding programmes for selecting increased submergence tolerance.

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3. Bacterial leaf blight (BB) disease in rice

Bacterial blight is found worldwide and is particularly destructive in Asia during the heavy rains of the monsoon season. In many Asian countries, bacterial blight has become endemic on rice following repeated cultivation. Reductions in rice yield may be as high as 50% in fields where the crop is severely infected and infection at the tillering stage can lead to total crop losses. More commonly, however plants are affected at the maximum tiltering stage and yields are reduced 10 - 20% (Ou, 1985).

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive bacterial diseases of rice in irrigated and rain-fed lowland ecosystem. It is one of the oldest known diseases and was first reported in Fukuoka, Japan as early as 1884 in rice. Subsequently, its incidence has been reported from different parts of Asia, Northern Australia, Africa and United States of America. In 1909, masses of bacteria were isolated from the acidic turbid dewdrops of infected rice leaves, and the disease was reproduced by inoculating healthy leaves with these dewdrops. Shortly thereafter its aetiology as a bacterial disease was established, and the causal agent was isolated and classified as *Bacillus oryzae* (Mizukami and Wakimoto, 1969). The bacterium was renamed *Pseudomonas oryzae* and later *Xanthomonas oryzae* (Ishiyama, 1922). In 1978, it was reclassified as *X. campestris* pv. *oryzae* (Dye *et al.*, 1978). In 1990, Swings proposed this species *X. campestris* pv. *oryzae* to change the name on the basic of phenotypic, genotypic, and chemotaxonomic data. Finally, the causal agent of BB of rice was reclassified as *X. oryzae* pv. *oryzae* (Swings *et al*., 1990).

3.1 Causal organism

The causal organism of the disease is *Xanthomonas oryzae* pv. *oryzae*, a rod-shaped with a polar flagellum, 0.4 - 0.8 x 1.5 - 2.9 µm occurring single or in pairs. The organism is gram negative and non-spore forming (Ishiyama, 1922). Bacterial cells are surrounded by mucous capsules. Colonies are circular, convex, whitish yellow to straw yellow later, with smooth surface and entire margin, viscid

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and opaque against transmitted light. The yellow pigments are soluble in water. The bacteria multiply inside the leaf tissues and invade the vascular system. Some ooze out from water pores. The bacterium has many strains with different ability (virulence) to infect rice plants. Some strains are weak and cause only small lesions while others are virulent, causing large lesions on the same variety. The bacterium infects the plants by entering leaf tissues through natural opening, growth opening at the base of leaf sheaths caused by emergence of new roots, and through leaf of root wounds caused by certain management practices during transplanting. Wounds on rice leaves are also favorable for entry of the pathogen. The infection seems more successful in the case of entry of the pathogen through wound sites than natural openings.

3.2 Symptom

Bacterial blight is a vascular disease resulting in a systemic infection of rice (Mew, 1987). There are three types of symptoms; wilting (kreset), leaf blight, and pale yellow leaves. Kreset is the result of systemic infection that is common in the tropics in young plants and during the tillering stage of susceptible cultivars. In this stage, leaves of infected plants wilt, roll up, turn grey-green and wither, and entire plants finally die. The bacterium spreads through the vascular system to the growing point of the young plants and infects the base of other leaves. Individual tiller or plant may die. If the plants are alive from this infection, they look stunted and yellowish. The most prominent symptom of leaf blight is wavy elongated lesions, which develops along the leaf margins (Figure 5). The lesion enlarges in length and width, and may have wavy margins. It turns a whitish-straw color from its initial water-soaked grayish or yellowish hue in 1 - 2 weeks. Leaf blight may occur at all growth stages, but it is common from maximum tillering until maturity. As the disease advances, the lesions may cover the entire blade which turns white and later grayish. Lesions extend to the leaf sheaths and reach the lower internodes of susceptible varieties. Drops of bacterial ooze can be seen in the morning on young lesions. Bacterial ooze may be observed in humid and warm conditions. On panicles, the disease causes grey to light brown lesions on glumes causing infertile and low quality

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grains. The third symptom, pale or yellow symptom is also the results of systemic infection at the tillering stage. At this infection, bacteria are found in the internodes and crowns of affected stems, but not in the leaf itself (Ou, 1985; Goto, 1992).

Figure 5 The bacterial leaf blight disease symptom occur on adult plant

Source: www.ricethailand.go.th/rkb/data_005/Image

3.3 Bacterial leaf blight resistance gene *Xa21*

Currently, more than 38 BB resistance genes have been identified in cultivated and wild rice and some of these have been transmitted to modern rice varieties (Gu *et al*., 2008; Korinsak *et al*., 2009b; Korinsak *et al*., 2009c; Guo *et al*., 2010; Miao *et al*., 2010; Bhasin *et al*., 2012).

The dominant resistant gene, *Xa21* was transferred from wild rice species *O. longistaminata* to IR24 (Khush *et al*., 1989). *Xa21* confers resistance to pathotypes from the Philippines and India at post-seedling growth stage. *Xa21* located on chromosome 11, is one of the most preferred genes for improving resistance in rice against bacterial blight because it confers broad spectrum resistance to most isolates of *X. oryzae* pv. *oryzae* (Ikeda *et al*., 1990; Khush *et al*., 1989; 1991). *Xa21* gene has been tagged with molecular markers (Ronald *et al*., 1992) and using marker assisted breeding (Sharama *et al*., 2001; Singh *et al*., 2001) and by transformation. This *Xa21* gene was cloned and encoded a receptor-like kinase with LRR in the presumed extracellular domain, a transmembrane domain and serine-threonine protein kinase domain (Song *et al*., 1995). Currently, PB7-8, a functional marker derived from *Xa21*

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gene has been very effective for selecting BB resistance *Xa21* gene in rice (Chunwongse *et al.*, 1993).

Win *et.al* (2013) reported that resistant alleles of *xa5, Xa21* and *xa33* from RG-9 were successfully transferred into Manaw-Thu-Ka introgressed line MK-75 by using marker-assisted backcrossing (MAB) method. They confirmed the resistance of those introgression lines, carrying *xa5, xa33* and *Xa21* genens, against to Thai and Myanmar *Xoo* isolates. The single resistance gene-introgressed lines carrying *xa5* had higher resistance against to Thai *Xoo* strains, while lines carrying *Xa21* showed higher level of resistance to Myanmar *Xoo* strains.

In general, in rice adult plants, resistance exhibits race and cultivar specificity and is affected by environmental conditions. At later growth stages were usually increased in a race non specific manner (Mew, 1987). Studies of the bacterial blight resistance response conditioned by the resistance gene *Xa21* indicate that *Xa21* mediated resistance to *Xoo* is not expressed in the early stages of development (Mazzola *et al.*, 1994). However, after 21days post-germination, the *Xa21* line exhibits resistance and as the plant matures further, resistance continues to increase (Century *et al.*, 1999).

4. Multiple abiotic stress tolerant rice

Abiotic stresses such as heat, drought, submergence and salinity are the major factors responsible for significant annual rice yield losses. Although abiotic stresses have been addressed individually, their occurrence is often in combination in farmers' fields, causing incremental crop losses. Mittler (2006), using a novel 'stress combination matrix', has illustrated the interactions between different abiotic stresses such as heat and drought, and heat and salinity. The combined stress increased the negative effect on crop production. For example, in response to heat stress, plants open their stomata to maintain a cooler canopy microclimate through transpiration, but under combined heat and drought stress, the sensitive stomata are closed to prevent loss of water, which further increases the canopy/tissue temperatures

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(Rizhsky *et al.*, 2002; 2004). A similar phenomenon occurs under combined heat and salinity stress (Moradi and Ismail, 2007). It was thus concluded that the study of abiotic stress combinations involves a 'new state of abiotic stress' rather than just a sum of two different stresses (Mittler, 2006). Therefore, the need to develop crop plants with high level of tolerance for a combination of stresses is advocated. In support of this hypothesis, recent research has highlighted physiological, biochemical and molecular connections between heat and drought stress (Rang *et al.*, 2011; Jagadish *et al.*, 2011a; 2011b). N22, an Aus landrace mentioned earlier to be heat tolerant, was consistently the best performer under combined heat and drought stress at the physiological (Rang *et al.*, 2011) and molecular levels (Jagadish *et al*., 2011b). Furthermore, a strong antioxidant scavenging mechanism in the panicles of N22 was documented in response to severe drought stress (Selote and Chopra, 2004). However, the poor combining ability of N22 is a major bottleneck and there is an urgent need to identify other alternative donors for increasing heat and drought tolerance in rice.

In response to the actual farmer field situation, progress achieved in understanding and developing independent abiotic stress tolerance is being exploited to combine tolerances (for example, heat and drought; salinity and submergence) to address emerging environmental problems across a wide range of rice ecosystems (Jagadish *et al.*, 2012).

Major sources of salt tolerance of these varieties include the landrace cultivars Pokkali (PK) and Nona Bokra (NB). For coastal areas in the wet season, both salinity and submergence are problems. Fortunately, both *Sub1* and *Saltol* can be combined in the same variety, and these lines combine tolerance to both stresses (Mackill *et al.*, 2010).

Development of 'two-in-one' rice varieties with dual tolerance for salinity and submergence is a step-ahead (Mercado *et al.*, 2010). Rice genotypes are invariably exposed to frequent submergence during wet season, especially in the coastal areas. Usually, stagnant water is saline to certain degrees and exerts more stress to rice than submergence with non-saline water. Hence, an ideal rice variety for

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the coastal areas needs tolerance for salinity and submergence. Considering this, we have successfully integrated both salinity and submergence tolerance in one genetic background using molecular marker-assisted selection (Jagadish *et al.*, 2012).

Field testing of the two-in-one genotypes in IRRI fields confirmed their superiority under salinity and submergence stresses compared with the checks. IR84649-81-4-B-B, a rice genotype with dual tolerance, has comparable survival and yield after exposure to complete submergence for 2 weeks as against Swarna-*Sub1* and IR64-*Sub1*. Moreover, it performed well under target environments in the coastal areas of Orissa and Bangladesh (Jagadish *et al.*, 2012).

Quantitative Trait Loci

A trait refers to a genetically determined characteristic, which could be anything of phenotypes. Two kinds of traits, Mendelian and quantitative, are distinguished. A Mendelian trait is determined by a single gene (or few genes), following classical Mendelian inheritance patterns, such as 3:1 for a phenotypic ratio from a trait controlled by a single dominant gene in an F_2 family. In contrast, multiple genes could determine a quantitative trait and its value is continuous, such as plant height and human weight. Quantitative traits are very common and are important both in applied and theoretical studies. For example, increasing crop production or plant disease resistance all requires the manipulation of quantitative traits.

QTL mapping has been carried out for various traits in many species. The theory of QTL mapping was first described by (Sax, 1923), who noted that seed size in bean, a complex trait, was associated with seed coat color, monogenically-controlled trait. Modern QTL mapping is derived from this idea, with the key innovation being that defined sequences of DNA act as the linked monogenic markers. New interest was generated when studies with rice, maize and tomatoes demonstrated that some markers explained much of the phenotypic variance of complex characters (Tanksley, 1993).

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Data for QTL mapping usually have two components: marker data and trait values. Marker data includes marker genetic map position and marker genotype. Trait values can be continuous, such as disease leaf area, or they may be categorical, such as leaf size denoted by large, medium and small. Sample size needs to be considered when planning the experimental design. With a greater sample size, detection of QTL with smaller effect is more efficiently (Young, 1996; Manly and Olson, 1999; Vanavichit *et al*., 2005;).

1. Genetic Mapping and Linkage Analysis

The techniques of genetic mapping and linkage analysis were developed in 1911 by Morgan and his graduate student Sturtevant and are still used today in much the same way but with far more advanced techniques. The basis of genetic mapping is the phenomenon of "crossing-over" of chromosomes during meiosis, where homologous chromosomes exchange sections of their gene sequence. The tendency of two genes to recombine is used as a measurement of their linkage and distance on a genetic map. For example, two genes that recombine often are far apart on a genetic map and two that rarely recombine are said to be "linked" and are very close together on a genetic map. To determine trait recombination frequencies and form a genetic map, a mapping population must first be produced. The first step in producing a mapping population is selecting two genetically divergent parents (that will still produce viable progeny). Often one common cultivar and one wild parent are selected as they are likely to be the most divergent. The two selected parents are screened for polymorphism with the markers that are to be mapped to be sure that the progeny will produce recombinants. The mapping population is then produced by crossing the two parents to form an F_1 hybrid population which is selfed to produce an F_2 population which can be used for mapping (Gupta *et al*., 1999). The initial cross will produce a uniform, heterozygous population with each plant contain one chromosome from each parent. During meiosis, the homologous chromosomes may or may not, cross-over and form recombinants. The expected ratio of phenotypes for the F_2 population is the classic Mendelian 1:2:1, it is the divergence from this ratio that determines the amount of linkage between genes.

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2. Maps and map construction

A genetic map describes orders and positions of identifiable landmarks on DNA. Theses landmarks might be genes or genetic markers. Two types of map are commonly used in practice, genetic and physical maps. For QTL studies both are extensively used for fine mapping and physical characterization of QTL.

A genetic map and a physical map provide similar information on marker or gene order along the chromosomes. Estimating recombination frequency between two positions generates a genetic map. In contrast, having the complete sequence makes it possible to determine directly the order and spacing of the genes, which is a type of physical map (Weeks and Lange, 1987). Software has also been developed to construct genetic maps; a popular one is MAPMAKER by Lander *et al.* (1987). Assembling sequences or DNA fragments into contigs allows construction of a physical map. Two strategies are commonly used for genome sequencing: hierarchical sequencing and shotgun sequencing. Hierarchical sequencing works as a top-down approach: it starts with cutting and cloning the genome into large ordered DNA fragments. These are then sequenced, typically by sub-cloning many smaller overlapping fragments of each large clone, sequencing these and assembling the sequences into a large sequence contig representing the whole original clone. In contrast, shotgun sequence is a bottoms-up approach: small fragments of genomic DNA from the whole genome are sequenced and these are assembled into a genomic sequence using computer algorithms (Tammi *et al*., 2002).

Molecular marker technologies permit plant geneticists to construct high-density genetic maps for any species amenable to genetics and use them for detecting, mapping, and estimating the effects of QTL. The analysis involves testing DNA markers throughout a genome for the likelihood they are linked with a QTL. Individuals in an appropriate mapping population (F_2, backcross, recombinant inbred) are analyzed for DNA marker genotypes and the phenotype of interest (Young, 1996). For each DNA marker, the individuals are split into classes according to marker genotype. Mean and variance parameters are calculated and compared among the

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classes. A significant difference between means suggests that there is a relationship between the DNA marker and the trait of interest. In other words, the DNA marker is probably linked to a QTL. Since the traits of interest are, by nature, genetically complex, environmental factors and genetic background potentially have an enormous impact on results. This is one of the most powerful applications of QTL mapping (i.e. analyzing gene x gene and gene x environment interactions), but it also means that many large, time-consuming experiments need to be carried out to analyze a system thoroughly.

Finally, QTL mapping, like any genetic study, is only as good as its phenotypic scoring method. In studies of disease resistance, factors all the way from a suitable inoculum to difficulties in quantitative estimation of resistance make QTL mapping more challenging. Fortunately, powerful computer software programs are now available to analyze QTL mapping results (Nelson, 1997; Manly *et al*., 2001; Broman *et al*., 2003; Wang *et al*., 2005) and better DNA marker systems have been developed to simplify the technique and increase marker density.

3. QTL mapping methods

Various statistical methods have been developed for QTL mapping. The most commonly used methods for QTL mapping are based on the maximum-likelihood method. From simple to more complicated, three approaches are commonly used: single marker analysis (SMA), simple interval mapping (SIM); and simple composite interval mapping (sCIM) (Broman, 2001; Manly and Olson, 1999; Vanavichit *et al*., 2005; Young, 1996).

3.1 Single marker analysis (SMA)

SMA tests the association between marker genotypes and trait values using t-tests, ANOVA models or regression. In other words, it tests trait value differences among markers groups. SMA is the least informative of the analyses, because recombination, as well as the additive and the dominant effects of a QTL may

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be confounded. SMA often fails to give reliable estimates of numbers and positions of QTL and the magnitude of their effects.

3.2 Simple Interval mapping (SIM)

In 1961, introduced simple interval mapping and a mathematical treatment of this method was presented by Lander and Botstein (1989). SIM uses two observable flanking markers to construct an interval within which to search for QTL along the chromosomes. A map function, either Haldane or Kosambi, is used to translate from recombination frequency to distance or vice visa. Then, a LOD score is calculated at each increment in the interval. Finally, the LOD score profile is calculated for the whole genome. When a peak has exceeded a threshold value, there is evidence that a QTL has been found at that location.

3.3 Simple Composite interval mapping (sCIM)

Jansen and Stam (1994) and Zeng (1994) developed sCIM. This method is an extension of SIM that places certain markers into the model as cofactors. CIM fits parameters for a target QTL in one interval while simultaneously fitting partial regression coefficients for background markers to account for variance caused by non target QTL. In theory, sCIM gives more power and accuracy than simple IM because the effects of other QTL are not present as residual variance.

Marker assisted selection (MAS)

Plant breeding describes methods for the creation, selection, and fixation of superior plant phenotypes in the development of improved cultivars suited to needs of farmers and consumers. Primary goal of plant breeding with agricultural and horticultural crops have typically aimed at improved yields, nutritional qualities, and other traits of commercial value. Predicted population growth and pressure on the environment, traits relating to yield stability and sustainability should be a major focus of plant breeding efforts. These traits include durable disease resistance, abiotic

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stress tolerance and nutrient and water-use efficiency (Mackill *et al*., 1999; Slafer *et al*., 2005; Trethowan *et al*., 2005). Despite optimism about continued yield improvement from conventional breeding, new technologies such as biotechnology will be needed to maximize the probability of success (Ortiz, 1998; Ruttan, 1999; Huang *et al*., 2002).

The advantages of MAS results from the fact that many of the traits of interest to breeders are not easily assessed. Thus, selection, which is based on linked DNA markers, is much more efficient. Selection based on markers can be carried out at an early age (plantlets); therefore, it has the potential to significantly reduce the number of individuals assessed by the breede thus reducing costs. MAS has greater potential for efficient gene pyramiding; namely, combining several important genes in one cultivar. At the same time MAS does not reduce the time of the breeding project because the selected plants need to be tested and evaluated in the field. The length of the evaluation process depends mainly on the length of the juvenile period of each species (Giora Ben-Ari and Uri Lavi, 2012).

DNA markers can be used to detect the presence of allelic variation in the genes for desired traits by using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased. The use of DNA markers in plant breeding is called marker-assisted selection (MAS) and is a component of the new discipline of ‘molecular breeding’. Molecular markers are especially advantageous for agronomic traits that are otherwise difficult to tag such as resistance to pathogens, insects and nematodes, tolerance to abiotic stresses, quality parameters and quantitative traits (Collard and Mackill, 2008).

1. Marker-assisted backcrossing

Backcrossing is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety. In most cases, the parent used for backcrossing has a large number of desirable attributes but is deficient in only a few characteristics (Allard, 1999). The use of DNA markers in backcrossing

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greatly increases the efficiency of selection. Three general levels of marker-assisted backcrossing (MAB) were described by Holland (2004). In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as 'foreground selection' (Hospital and Charcosset, 1997). This may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures. Furthermore, recessive alleles can be selected, which is difficult to do using conventional methods. The second level involves selecting BC progeny with the target gene and recombination events between the target loci and linked flanking markers referred to as 'recombinant selection'. The purpose of recombinant selection is to reduce the size of the donor chromosome segment containing the target locus (i.e. size of the introgression). This is important because the rate of decrease of this donor fragment is slower than for unlinked regions and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent, referred to as 'linkage drag' (Hospital, 2005). Using conventional breeding methods, the donor segment can remain very large even with many BC generations (Ribaut and Hoisington, 1998; Salina *et al*., 2003). By using markers that flank a target gene, linkage drag can be minimized. Since double recombination events occurring on both sides of a target locus are extremely rare, loss of vigor of the lines. Recombinant selection is usually performed using at least two BC generations (Frisch *et al*., 1999b).

The third level of MAB involves selecting BC progeny with the greatest proportion of recurrent parent (RP) genome, using markers that are unlinked to the target locus referred to as 'background selection'. Background selection refers to the use of tightly linked flanking markers for recombinant selection and unlinked markers to select for the RP (Hospital and Charcosset, 1997; Frisch *et al*., 1999b). Background markers are useful because the RP recovery can be greatly accelerated. With conventional backcrossing, it takes a minimum of six BC generations to recover the RP and there may still be several donor chromosome fragments unlinked to the target gene. Using markers, it can be achieved by BC_4, BC_3 or even BC_2 (Visscher *et al*., 1996; Hospital and Charcosset, 1997; Frisch *et al*., 1999 a; 1999b), thus saving two to four BC generations.

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2. Marker-assisted pyramiding

Pyramiding is the process of combining several genes together into a single genotype. Pyramiding may be possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene. Using conventional phenotypic selection, individual plants must be evaluated for all tested traits. Therefore, it may be very difficult to assess plants from certain population types (e.g. F_2) or for traits with destructive bioassays. DNA markers can greatly facilitate selection because DNA marker assays are non-destructive and markers for multiple specific genes can be tested using a single DNA sample without phenotyping. The most widespread application for pyramiding has been for combining multiple disease resistance genes (i.e. combining qualitative resistance genes together into a single genotype). The motive for this has been the development of 'durable' or stable disease resistance since pathogens frequently overcome single gene host resistance over time due to the emergence of new plant pathogen races. The combination of multiple genes (effective against specific races of a pathogen) can provide durable (broad spectrum) resistance (Kloppers and Pretorius, 1997; Shanti *et al*., 2001; Singh *et al*., 2001). The incorporation of quantitative resistance controlled by QTLs offers another promising strategy to develop durable disease resistance.

Rice quality

Quality of rice is not always easy to define as it depends on the consumer and the intended end use for the grain. (http://www.betuco.be/rijst/Rice%20Quality.pdf). Rice quality is combination of physical and chemical characteristics. The *physical* characteristics of grain (before cooking) are whole and broken grains, shape and size of the grain, color of grain, chalkiness, weight, damaged and discolored kernels, foreign material like dirt, stones, and moisture content of the grain (http://www.knowledgebank.irri.org/ericeproduction/quality_01.htm). The chemical characteristics are about how the grain looks after cooking and how it feels when it is eaten such as gelatinization temperature, amylase content, gel consistency, texture (how it feels when eaten) and aroma (how it smells).

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Rice grain quality includes processing quality, appearance quality, nutritional quality, cooking and eating quality, among which cooking and eating quality is higher acceptance by most consumers. The cooking and eating quality of the rice grain is one of the most serious problems in many rice producing areas of the world. Aroma is the highest desired trait of good quality rice and amylose content is important determinant of cooking and eating quality. Aromatic or scented rice fetching premium price in international market is characterized by its natural fragrance or pleasant aroma and good eating quality. Grain appearance, processing behavior, cooking and eating quality are directly related to three physico-chemical properties of rice grain starch making up 90% of total dry weight of the endosperm, namely amylose content (AC), gel consistency (GC) and gelatinization temperature (GT).

1. Aroma

Aromatic or fragrant rice is rice with natural chemical compounds which give it a distinctive scent. It can be used just like conventional rice for cooking, but adds a new dimension of flavor and aroma to meals. The demand for aromatic rice has increased markedly in recent years to the extent that consumers are willing to pay a premium price for aromatic rice. The Jasmine type rice of Thailand and the Basmati rice of India and Pakistan are the aromatic cultivars commonly sold in world trade. This rice is highly valued throughout Asia (Baishya *et al*., 2000) and also have wider acceptance in Europe, Australia (Reinke *et al*., 1991), USA and the Middle East (Shobha Rani *et al*., 2006). Although most of the trade is from Thailand, India and Pakistan, aromatic rice is cultivated and prized in many other countries of the world.

Aromatic or scented or fragrant rice, a special group of rice that emits natural fragrance in the field at flowering, harvesting, in storage, during milling, cooking and eating has been cultivated mainly in South and South-East Asian countries from ancient time. This specialty rice can be defined as those with different grain shape, size, color, chemical composition and cooking characteristics compared with the common long-, medium- or short-grain type. Aromatic rice has a relatively diverse range of aroma that is much more dominant than in non-aromatic cultivars.

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The scent or natural fragrance in the kernel is the much valued quality factor. The most popular aromatic rices are Basmati rice from India and Jasmine rice from Thailand (Vanavichit, 2007).

The aroma of both aromatic and non-aromatic rice is composed of a complex mixture of odor-active compounds. Several odor-active compounds in cooked aromatic rice have been determined using odor units (Buttery *et al*., 1983). Although several volatile flavor compounds have been identified to be responsible for aroma such as, Carbohydrate, Ketone, Aldehyde, Acetaldehyde, Alcohol, (Mahatheeranont *et al*., 2001), the most potent compound of aroma in Basmati and Jasmine-style fragrant rice is pop-corn like flavor compound 2-acetyl-1-pyrroline (2AP) (Buttery *et al*., 1983; Lorieux *et al*., 1996; Nadaf *et al*., 2006). In rice, 2AP has a lower odor threshold (0.02 ng/l) than many other volatile compounds and the concentration is very low (Buttery *et al*., 1983). The concentration of 2AP in milled Basmati rice showed (0.06 ppm) and non-aromatic rice did not show any present of 2AP (Nadaf *et al*., 2006).

A wide range of 2AP concentrations have been observed in both fragrant and non-fragrant varieties in different studies. These differences may be due to the different rice varieties studied, differences in extraction procedure or quantification of 2AP, environmental influences on the level of fragrance such as temperature and salt and drought stress (Yoshihashi *et al*., 2005), harvest time or storage conditions of the rice (Bhattacharjee *et al*., 2002; Yoshihashi *et al*., 2005), whether the rice was milled or unmilled (Buttery *et al*., 1983; Philpot *et al*., 2005) or timing/level of nitrogenous fertilizer application to the growing plants (Wilkie and Wootton, 2004). 2AP can also be found in other plants, for example, Pandan leaves (*Pandanus amaryllifolius* Roxb.) that contain 2AP at concentrations 10 times higher than the fragrant rice varieties (Buttery *et al*., 1983), bread flower (*Vallaris glabra*) (Wongpornchai *et al*., 2003) and soybean (Masuda, 1989). Moreover, some microorganisms have also been reported to have abilities to produce 2AP (Romanczyk *et al*., 1995).

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Lorieux *et al*. (1996) and Bradbury *et al.* (2005a) confirmed that 2-acetyl-1-pyrroline (2AP) and aroma were perfectly correlated and mapped at the same locus and 2AP concentration in aromatic rice is 12 times higher than that of common rice. Many researchers reported that a single nuclear recessive gene controlled aroma in Basmati and Jasmine-style aromatic rice (Ahn *et al*., 1992; Buttery *et al*., 1983; Bradbury *et al*., 2005a; Wanchana *et al*., 2005). Bradbury *et al.* (2005b) has been identified aroma is recessive traits due to an eight base pair deletion and three SNP in exon seven of the gene which encodes a putative betaine aldehyde dehydrogenase 2 (*badh2*) on chromosome 8 of *Oryza sativa* largely controlled by the level of 2AP. Additionally, an aromatic allele, a 3-bp insertion in exon 13 of Os2AP was found as a major allele in aromatic rice varieties from Myanmar (Myint *et al*., 2012).

2. Amylose content

Amylose content of rice endosperm is a major determinant of cooking and eating quality (*Oryza sativa* L.) The ratio of amylose to total starch, measured as amylose content, varies from cultivar to cultivar; it means 18 - 32% in indica rice and 10 - 22% in japonica. The high amylose levels are usually associated with dry, fluffy, and separate cooked rice grain (Juliano, 1985). According to the proportion of amylose in total starch of endosperm, rice varieties are classified as waxy (0 - 2%), very low (5 - 12%), low (12 - 20%), intermediate (20 - 25%) and high (25 - 33%) (Juliano *et al*., 1981). The inheritance of amylose content have revealed one major gene and several modifiers gene with high amylose content incompletely dominant over low and intermediate amylose content (Sano, 1984; Kumar and Khush, 1986; Kumar *et al*., 1987). It is genetically controlled by a multiple series at the *Waxy* locus in non-waxy rice isolates (Kumar *et al*., 1987) and at least two functional alleles at *Waxy* locus, Wx^a and Wx^b which controlled not only the level of the gene product but also amylase content in endosperm starch. Wx^a was predominant in *Indica* subspecies, Wx^b was predominant in *Japonica* type and Wx^a produces higher level of *Waxy* protein and amylose than Wx^b (Sano, 1984).

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3. Gel consistency (GC)

The GC is responsible for softness of cooked rice. Index of cooked rice texture is evaluated by gel-consistency. Varieties with similar amylose content softer gel-consistency are the prefer type by most consumers. The inheritance of GC was controlled by one gene and the short and hard gel consistency was dominance over long and soft gel consistency (Chen and Li, 1981). However, the major *Wx* gene plus several minor genes and/ or modifier governed the expression of GC (Lanceras *et al.*, 2000; Tian *et al.*, 2005). Many studies for GC showed that three QTLs on chromosome 6 and 7 with their interaction (Lanceras *et al.*, 2000), three QTLs on chromosome 1, 2 and 6 (Tian *et al.*, 2005) contributed for GC variation. On the other hand, many reported papers examined that *Wx* was not responsible for GC and some minor genes involved in governing GC. Their QTLs analysis for GC indicated that two QTLs on chromosome 2 and 7 with positive additive effects (He *et al.*, 1999).

4. Gelatinization Temperature (GT)

Gelatinization temperature, time required for cooking is physical property of cooked rice. GT affects water absorption, volume expansion and kernel elongation, the quality and quantity of starch and GT strongly influence the cooking and eating quality of rice. The length elongation of cooked rice are probably inherited in a polygenic fashion and those QTLs detected on chromosome 2, 3 and 6 (Ge *et al.*, 2004) and a major and a minor on chromosome 6 (He *et al.*, 1999), three QTL on chromosome 2 and 6 (Lanceras *et al.*, 2000), two QTLs on chromosome 3 and 6. The (*alk)* gene on chromosome 6 that encodes soluble starch synthase *(SSIIa*) was responsible for the GT variation (Lanceras *et al.*, 2000; Li *et al.*, 2003; Umemeto and Terashima, 2002; Tian *et al.*, 2005; He *et al.*, 2006).

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MATERIALS AND METHODS

Part I: Molecular breeding of improved Sin-Thwe-Latt for submergence tolerance, bacterial leaf blight resistance, grain aroma and salt tolerance

1. Plant Materials

Yn3220-108-2-3-1 (STL108) was used as recurrent parent which is carrying *Saltol* QTL inherited from PK selected by MAS. It was one of the elite and promising lines of BC_3F_4 population derived from the cross between salinity sensitive HYV Sin-Thwe-Latt (IR53936-60-3-2-1) and salinity tolerant PK which line was validated for salt tolerant at EC 9 dsm^{-1} NaCl. The recurrent parent STL108 was intolerant to submergence, sensitive to bacterial leaf blight and non aroma.That breeding program was conducted under Mekong region project, collaborate research work between Rice Gene Discorvery Unit (RGDU), Kamphaeng Saen campus, Kasetsart University, Thaildand and Department of Agricultural Research (DAR),Yezin, Myanmar since 2004.

RGD07343-13-21-9 (RGD309) was used as donor parent. This line was an improved BC_4F_4 line of IR57514 by Rice Gene Discovery Unit (RGDU), derived from IR57514/KD571-77//IR57514. This line is carrying the submergence tolerance (*Sub1*), aroma (*badh2*) and BB resistant (*Xa21*) genes. IR57514 was developed by IRRI and had been identified as a wide-adapted to the rainfed lowlands of the Mekong region. Later, it was identified as submergence-tolerant variety that carries a *Sub1* locus derived from an ancestor cultivar FR13A. IR57514 had shown a high yielding performance with good agronomic characters across years and locations under rainfed lowland of the Mekong region (Jearakongman *et al.*, 1995; Romyen *et al*., 1998; Ouk *et al*., 2006).

In this study, Yn3220-108-2-3-1 (STL108) and RGD07343-13-21-9 (RGD309) were used as recipient and donor parents respectively to develop the

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backcross introgression population of the STL108. The two parents of panicle and seed morphological features are described in Figure 6.

Figure 6 Morphology of two parents plant , panicle, seed and grain

2. DNA markers for marker assited selection

In the foreground selection, three makers were used to select for fragrance, submergence tolerance and bacterial blight resistance in all breeding generations (Table 2). Indel marker R10783indel located in the region of *Sub1* gene on chromosome 9 was used to select for submergence tolerance (Toojinda *et al.*, 2003). PB7-8 is a gene specific marker for the *Xa21* on chromosome 11 (Chunwongse *et al.*, 1993). Aromarker is developed based on 8 bps deletion in the seventh exon of the *badh2* gene on chromosome 8 (Vanavichit, 2007). It can be used to distinguish between fragrance and non-fragrance.

For the background selection, SSR markers, RM1287 and RM3412, on a short arm of chromosome 1 for *Saltol* QTL region and *SKC1* region were used (Thomsom *et al.*, 2010; Lin *et al.*, 2004). Type and chromosomal location of molecular markers were presented in Table 2.

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Table 2 Type, chromosomal location and primer sequences of molecular markers for MAS

Gene/QTL	Marker	Type of marker	Chr.	Primer sequence (5' → 3')	Remark
Sub1 (Submergence)	R10783indel	Indel	9	F: CTGCTCCGACGACCTGATGG R: ATTAAATGGAACATTCGAGAAC	Siangliw *et al.* (2003)
badh2 (Aroma)	Aromarker	Indel	8	F: TGCTCCTTTGTCATCACACC R: TTTCCACCAAGTTCCAGTGA	Vanavichit *et al.* (2004)
Xa21	PB7-8	STS	11	F: AGA CGC GGA AGG GTG GTT CCC GGA R: AGA CGC GGT AAT CGA AAG ATG AAA	Chunwongs *et al.* (1993)
Saltol	RM1287	SSR	1	F: GTGAAGAAAGCATGGTAAATG R: CTCAGCTTGCTTGTGGTTA	www.gramene.org
Saltol	RM3412	SSR	1	F: AAAGCAGGTTTTCCTCCTCC R: CCCATGTGCAATGTGTCTTC	www.gramene.org

F = forward primer; R = reverse primer

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3. Development of introgression lines

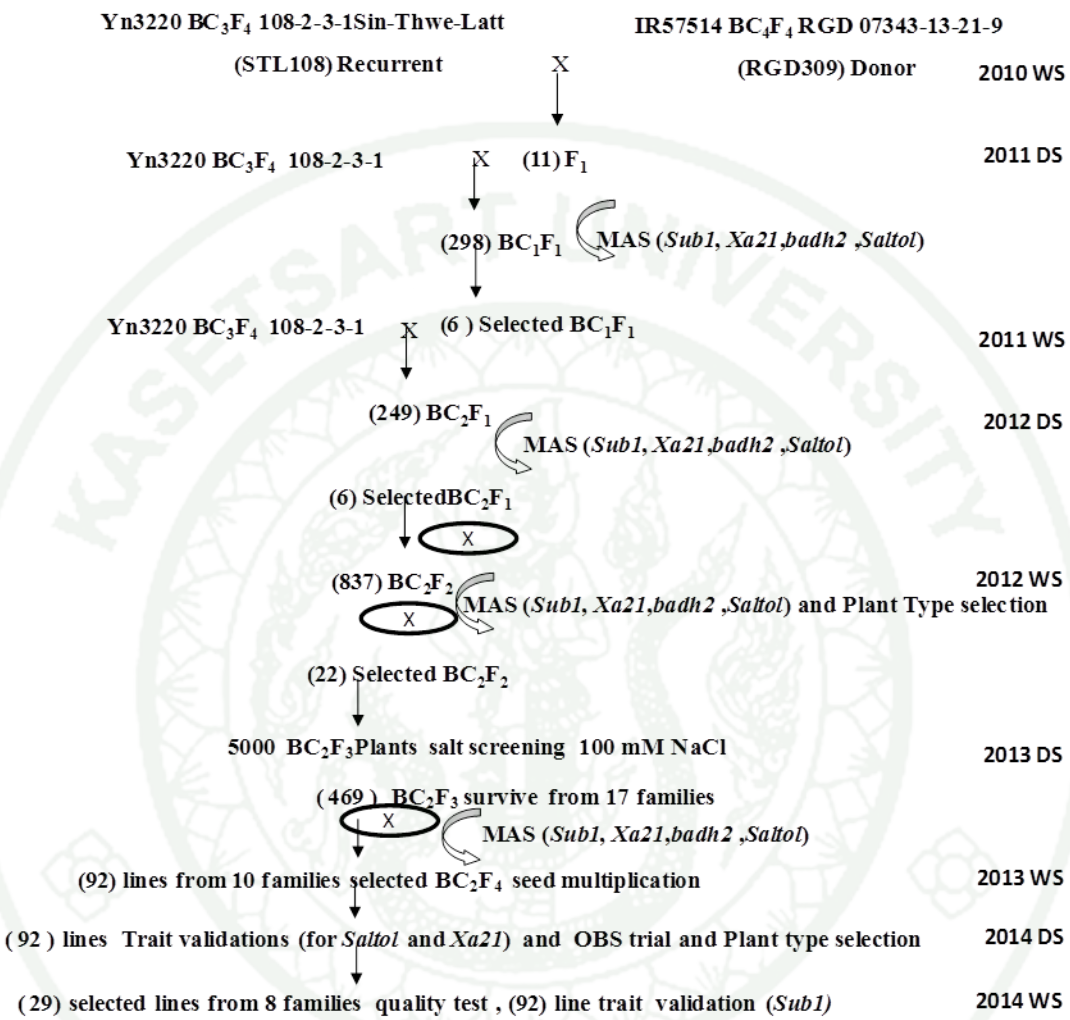

Figure 7 Schematic showing the development of STL108 introgression lines with *Sub1*, *Xa21*, *badh2* and *Saltol* by marker assisted and phenotypic selection in backcross breeding

As shown in Figure 7, to introgress the *Sub1*, *Xa21* and *badh2* genes from the RGD07343-13-21-9 (RGD309) into the Yn3220-108-2-3-1 (STL108), there was two cycles of backcrossing and generated until BC_2F_4 generation.

STL108 (recipient parent) was cross-pollinated with RGD309 (donor parent) in wet season (WS) 2010 to produce F_1 generation. The F_1 plants were checked for the

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cross-pollination by the PB7-8 marker. Eleven F_1 plants were selected and then backcrossed to the STL108 (using mixed pollen) to generate the BC_1F_1 generation in dry season (DS) 2011. 298 BC_1F_1 plants could be generated and molecular markers R10783 indel, PB7-8, Aromarker were used for foreground selection to identify the BC_1F_1 plants that carried the target alleles (heterozygous at the three markers and confirm with background markers RM1287 and RM3412 for *Saltol* QTL). Six BC_1F_1 plants were selected for second backcrossing to generate BC_2F_1 population in wet season 2011. After MAS was done, six plants were seclected out of 249 BC_2F_1 plants.

During 2012 DS, the selected plants were generated by selfing to generate BC_2F_2 population. MAS and plant type selection were done for the 837 selfed BC_2F_2 plants, among them 22 individual plants could be selected. In 2013DS, from those above selected lines, 5000 BC_2F_3 individual plants were screened in 100 mM NaCl at seedling stage and 469 survived plants were transplanted and done MAS. Among them, 92 lines from 10 families were selected as carrying the target genes. During 2014 DS, the selected lines carrying the target gens were grown as observation trial and the trait validation trials to screen salt tolerant and BB resistance and in 2014WS, submergence tolerant trait validation was done. Finally, based on the result of observation (OBS) trial, 29 lines from 8 families were selected. The selected lines were tested for grain and cooking quality tets as well.

4. DNA extraction and PCR amplification

The total genomic DNA of the parents and progenies were extracted from the young leaf tissue of all generation. In each sample, leaf sample of one-month-old seedling was collected and cut into small pieces and put in 96 well blocks. A total genomic DNA of two parents and individual progenies were isolated from 0.5 g of leaf tissue according to the DNA trap method developed by DNA Technology Laboratory, Kasetsart University, Kamphaeng Saen Campus, Thailand (http://dnatec.kps.ku.ac.th). The cut leaf sample were frozen with liquid nitrogen and ground by tissue striker for sample grinding into a fine powder and the powder was then added to 500 µl of extraction buffer and incubated at 65˚C for two hours. The sample was

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placed in ice for 5 minutes and added 100 µl of neutralizer and mixed well using vortex genie. The content was spun in a centrifuge at 4000 rpm for 20 min and then aqueous solution was transferred to a new 96 well-block. DNA was precipitated in 500 µl of trapping buffer and gently mixed and spun with 2200 rpm for one minute. The supernatant was removed and the pellet was washed twice with each 500 µl of washing buffer 1 and washing buffer 2, spun at 2200 rpm and 4000 rpm for one minute, respectively. The sample was dried at 65˚C for one hour, after that DNA was re-hydrated with 100 µl of elution buffer and incubated for 30 min at 65˚C. After finished centrifugation of sample for ten minutes at 4000 rpm, DNA was transferred into a new 0.2 ml tube to a final concentration of 50 - 100 ng/µl.

Genomic DNA was amplified by the polymerase chain reaction (PCR). The PCR reaction was performed in a 10 µl reaction mixture containing 2 µl of template DNA (50 ng), 1 µl of 10x PCR buffer, 0.8 µl of 25 mM $MgCl_2$ (final concentration 2 mM), 2 µl of 1 mM dNTPs (final concentration 0.2 µM), 0.4 µl of 5 µM forward and reverse primers (final concentration 0.2 µM), 0.5 µl of 1 unit of *Taq* DNA polymerase (final concentration 0.5 unit). The volume was completed to 10 µl with distilled water. Sample was covered with one drop of mineral oil. PCR reaction was initiated at 94˚C denatured temperature for 3 min followed by 35 cycles of 94˚C for 30 sec, 55˚C for 30 sec, 72˚C for 1 min and 30 sec and final 5 min incubation at 72˚C was allowed for completion of primer extension. The amplified product was electrophoresed on 4.5% denaturing silver-stained polyacrylamide gel for SSR markers.

The amplified PCR products of the PB7-8 marker was loaded into 1.2% agarose gel and separated by electrophoresis in 0.5X TBE buffer at 80 Volt, 500 mAmp for 1 hour. The PCR products were visualized using ethidium bromide staining. The amplified PCR products of the R10783indel, Aromarker, RM1287 and RM3412 were loaded into a 4.5% polyacrylamide gel electrophoresis (PAGE) and separated by electrophoresis in 1X TBE buffer at 50∨C and 60 Watt. The PCR products were visualized using silver staining. Polymorphisms in the DNA profiles were scored visually by comparison with two parents and a standard DNA ladder.

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5. Phenotypic evaluation as Trait validations and data recordings

5.1 Screening for salinity tolerance under soil-culture condition at seedling stage

Ninety-two lines of BC_2F_5 introgressed population, fix genotyping with target traits, were tested. PK, FL530, FL496 were used as salinity tolerant checks while IR29 and Sin Thwe Latt or IR53936 (STL) were used as susceptible checks. The experiment was done at screening house of RGDU, Kasetsart University, Kamphaeng Saen Campus. Seeds of BC_2F_5 (BILs) population and checks were aerobically germinated for 5 days in each petri-dish and sown directly in the 6 x 12 holes of plastic tray of 20 cm x 45 cm (width x length) size containing normal soil before adding salt. The tested plants were planted in 3 replications following a randomized complete block design (RCBD) compared with control condition. Six plants per line in each replication were planted. The transplanted seedlings in trays were grown in concrete pond (1 m x 1.8 m x 0.3 m: width x length x height) and which is equipped with an aquarium pump that allows the continuous flow of the medium in the whole system. Nutrient solutions used in screening were purchased from Bangsai Agricultural Center CO., Ltd (http://www. bangsaiagro.com/formula.asp). The pH of the solution was maintained at 5.7 by regular monitoring using a pH meter (Denver Instrument Ultra Basic pH meter).

Salinity stress of 100 mM (12 dS/m) was applied on 2 - 3 leaves (18 day-old-plant). Tolerance or susceptibility to stress was recorded after 16 days of salt treatment by following the method by Gregorio *et al.* (1997). At that time, tolerant and susceptible checks were showed different symptom. Salt injury was scored following to the Modified standard evaluation score (SES) of visual salt injury (Gregorio *et al.*, 1997) (Table 3).

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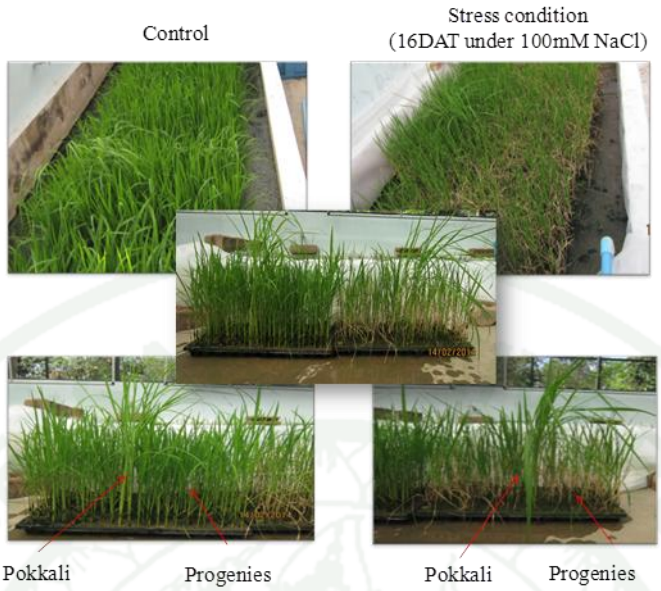

Figure 8 Comparison between control condition and 100mM NaCl stress condition showing the plant survival of standard tolerant check PK and some progenies

Table 3 Modified standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerant level
1	normal growth	highly tolerant
3	nearly normal growth; leaf tips or few leaves whitish and rolled	tolerant
5	growth severely retarded; most leaves rolled; only a few are elongating	moderately tolerant
7	complete cessation of growth; most leaves dry; some plants dying	susceptible
9	almost all plant dead or dying	highly susceptible

Data for SIS10 and SIS16 (injury score of 10 days and 16 days after salt stress), SvDAT (survival days after treatment), PPS (percent plant surviving), Na^+ and K^+ content and Na^+/K^+ ratio in shoot at 12 days after treatment, were collected.

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In order to observe the physiological characters of Na^+ and K^+ concentrations of 92 lines of BC_2F_5 introgressed population and parents, the leaf samples were collected, oven dried at 70˚C for 5 days and chopped. 0.2 g sample of flag leaf tissue were digested in 10 ml acetic acid (100 mM) at 90˚C for 2 hours. Extracted sodium and potassium ions were determined by using atomic absorption spectrophotometer (AA-680, Shimadzu, Japan) with unit of mg ion per g dry weight sample.

The collected data were subjected to statistical analysis using the CropStat software program version 7.2. Analysis of variance (ANOVA) was calculated based on a randomized complete block design (RCBD). Standard error of means was determined and least significant difference (LSD) was determined at a five percent probability level to make the comparison between the mean values of each line and standard checks. The STATGRAPHICS PLUS v.3 program was used for detecting the association between phenotype and genotype data by single marker analysis or simple regression analysis.

5.2 Screening for submergence tolerance (flash flooding for 10 days)

Ninety-two BC_2F_5 lines and seven check varieties: FR13A (origin of *Sub1*), original IR57514, donor parent RGD07343-13-21-9 (for tolerant checks), original STL, recipient parent STL108, KDML105 and RD6 (for intolerance checks), were assessed for their submergence tolerance. Two experiments were conducted for this study the first experiment was conducted under complete submergence in the outdoor lagoon located in RGDU Field, Kasetsart University, Kamphang Sean Campus, Thailand, during wet season of 2014. The second experiment was conducted under normal irrigation (control) in the experimental field located near by the outdoor lagoon. Both experiments were arranged in a randomized complete block design with two replications. Twenty-five days after germination, the seedlings of tested lines were transplanted into pond. The plants were grown in five-row plots with 25 plants per plots and 0.25 m between rows. Twenty days after transplanting, number of seedlings, tiller number per hill and plant heights were counted and measured for each plot in both experiment. In the experiment 1, lagoon was filled with water at a level of

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1 - 1.2 m depth. To impose severe submergence stress, seedlings were subjected to completely submerge. After 7 days of submergence, the plant height was recorded to know the shoot elongation and then the plants were continuously submerged for 10 days by keeping the water level at 1 - 1.2 m above leaf tip of the seedlings throughout the experimental period. The lagoon was drained out and seedlings were re-exposed to air for 7 days.

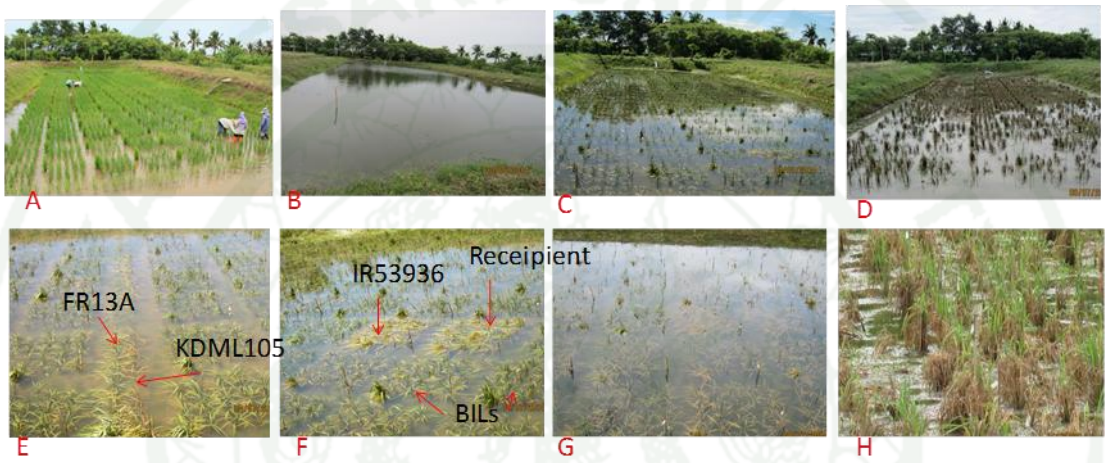

Figure 9 Submergence screening, during submerge and after submerge (A) 20 days after transplanted, (B) submerged uncovered for 10 days by keeping the water level at 1 - 1.2 m above leaf tip of the seedlings throughout the experimental period, (C) Plant height recorded at 7 days after submergence, (D) The lagoon was drained out (desubmergence) and seedlings were re-exposed to air (10 days submergenced), (E) comparing tolerant FR13A and susceptible KDML105 after 7 days of submergence, (F) Recipient, Sin STL and BILs after 7days of submergence, (G) 10 days after submergence and recorded for plant height, and (H) plant recovery after 7 days of desubmergence and recorded for percent of survived seedling.

Trait measurements including numbers of surviving seedling were taken 7 days after the water was drained from the submerging ponds. The percentage of surviving seedlings (PSS) was calculated as total number of surviving seedlings

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(counted 7 days after the water drained from the submerging ponds) was divided by total number of seedling counted before submergence than multiply by100.

$$\text{Percentage of surviving seedling (PSS)} = \frac{\text{Number of surviving seedling}}{\text{Total number of seedling}} \times 100$$

The percentage of seedling elongation (PSE) was used as a measure of the increment in shoots height during submergence and calculated as the average difference in shoot height before and after submergence. To compare the impact of submergence on shoot elongation, the extension in height in each individual line was set to be 100%.

$$\text{PSE} = \frac{\text{Shoot height after submergence - Shoot height before submergence}}{\text{Shoot height after submergence}} \times 100$$

5.3 Assessment of Bacterial blight (BB) resistance at tillering stage

Bacterial isolate: PSL2 isolate was collected from Phitsanulok Rice Research Center in 2011 and TXO152 was collected from Chinat Province in 2008 in Thailand.

Plant materials and preparation: Ninety two BC_2F_5 lines, two parents, Yn3220-108-2-3-1 (STL108), RGD07343-13-21-9 (RGD309), and original STL (IR53936) were used to evaluate for tillering stage resistance against to *Xoo* isolates compared with resistance and susceptible checks. While IR1188 was used as resistant checks, KDML105 and Jaohomnim were used as susceptible checks. Rice seeds were soaked in clean water for 24 hrs and then incubated at room temperature. The germinated seeds of each line were planted in plastic trays with 6 x 12 holes (hole size 5 x 5 x 4 cm) and kept in the greenhouse under good control to get good plant health.

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Xoo inoculation and disease scoring: Two *Xoo* isolates from lyophilized cultures were revived on peptone sucrose agar (PSA: peptone 5 g, sucrose 20 g and agar 20 g adjusted to 1 liter) and incubated at 28˚C for 72 hours. A 3-day-old culture of each isolate was used to prepare inoculum suspensions. For each isolate, the bacterial colonies on the PSA were suspended with sterilized distilled water and adjusted of concentrations approximately 10^9 cfu/ml prior to inoculation. The inoculation procedures were adapted from those described by Korinsak (2009a).

The rice plants were individually inoculated with each *Xoo* isolate at the tillering stage (60 days after sowing), following the clipping inoculation method as described by Kauffman *et al.* (1973). The inoculation was conducted in the morning, and the top two fully expanded leaves (a total of 10 leaves per rice line) were inoculated. The inoculated plants were kept in the greenhouse under high humidity to reduce the possible effects of high temperature on disease reactions. This condition also favors the entry of bacteria into infection courts in the presence of sufficient moisture on the leaf surface and maintains inoculation time consistency. The entire experiment was performed twice.

The disease was scored by measuring the lesion length (LL) of the inoculated leaves at 14 days after inoculation (DAI). The means of the LL were calculated for each line and used to identify associations between LL and genotypes (DNA markers). The resistance reaction was classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) if the lesion length was 0 – 3.0 cm, 3.1 – 6.0 cm, 6.1 – 9.0 cm and more than 9.0 cm, respectively (Yang *et al*., 2003; Zhang *et al*., 2006).

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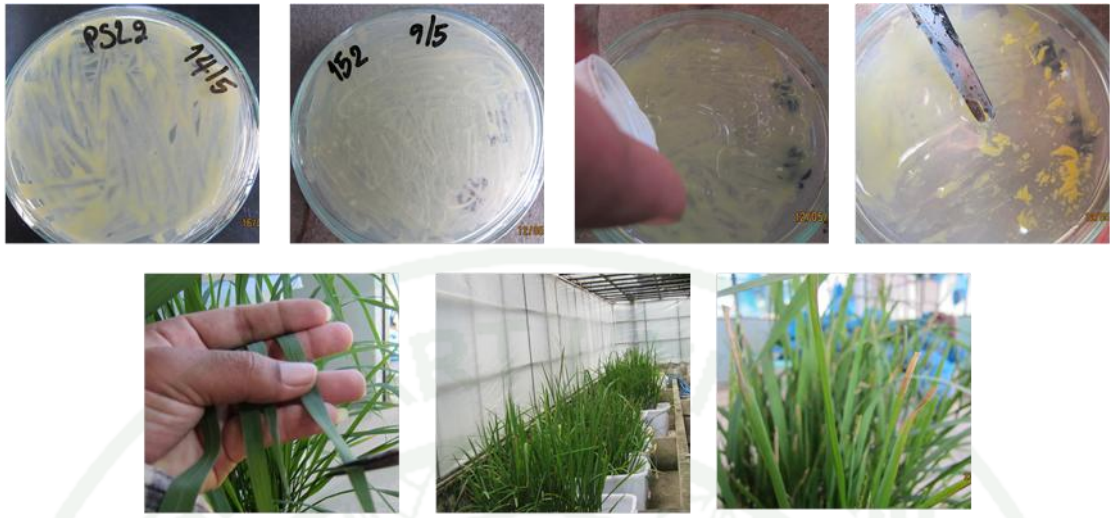

Figure 10 Inoculation of bacterial blight *Xanthomonas oryzae* pv. *oryzae* at tillering stage

6. Genome scan and evaluation of the introgressed traits on the rice genome of STL108 x RGD309

The selected 92 lines of BC_2F_5 population were used for genome scan with 135 SSR markers through twelve rice chromosomes. The total genomic DNA of the parents and progenies were extracted from the young leaf tissue followed by DNA trap kit (http://dnatec.kps.ku.ac.th). Genomic DNA was amplified by the polymerase chain reaction (PCR) and the profiles were described above. The amplified PCR products of the SSR marker was loaded into 4.5% polyacrylamide gel and separated by electrophoresis in 1X TBE buffer at 50˚C and 60 Watt. The PCR products were visualized using silver staining. Polymorphisms in the DNA profiles were scored visually by comparison with two parents and a standard DNA ladder.

The physical map was constructed based on the marker information from www.gramene.org. The genotypic result and trait validataion phenotypic result were detected to find out the putative QTLs by single marker analysis and multiple regression analysis with STATGRAPHIC Plus program.

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7. Evaluation on agronomic characteristics and morphological grain features of the BILs

The agronomic characteristics of the 92 BILs were evaluated comparing with parents in a replicated trial (3 replications) in which the tested varieties were arranged following a RCBD. The experiment was conducted by RGDU at the Kamphaeng Saen campus, Kasetsart University, Thailand, from March through July, dry season 2014. The seeds of BILs, STL, STL108 and RGD309 were sown in a seed bed nursery. Three-week-old seedlings were then manually transplanted into the rice field, with one seedling planted per hill. The plot size was 2.5 x 1.5 m^2, and each plot consisted of five rows with ten plants per row and a planting density of 25 cm between plants (within a row) and 25 cm between rows. Field management, including fertilizer application and weed and pest control, were done.

Grain quality including morphological features of the grain and cooking quality was evaluated using grain of selected lines' samples harvested from observation trial experimental field planted in 2014 dry season. Rice grains of the breeding lines and parents and checks were harvested and sun-dried naturally in a greenhouse. The dried grains were stored at room temperature for one month prior to the evaluation of grain quality traits. Grain samples of 200 g were taken from each replicate and combined. Grain samples were mechanically dehulled and polished by a mini polisher.

Recorded for the weight of the dehull grain weight and polished grain weigth to calculate the brown rice and milled rice percentage as follow (http:// uarpp. uark. edu/pdf_files/SOP/SOP-MILL). Brown rice yield (BRY) and milled rice are calculated as follows:

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BRY% = (Mass Brown Rice / Mass Rough Rice) x 100

Brown Rice = fraction of a rough rice sample remaining after hulls are removed
Rough Rice = all kernels in sample, prior to dehulling

MRY% = (Mass Milled Rice /Mass Rough Rice) x 100

Milled Rice = all kernels in a sample, including head rice and broken, after milling (removal of bran)
Rough Rice = all kernels in sample, prior to dehulling

Morphological features of rice grains include the appearance of the endosperm and the size and shape of the kernel. Ten seeds of milled rice kernel were used for the measurements of length and breadth using digital vernier caliper and the length/breadth ratios (L/B) were calculated. The appearance of the rice endosperms was determined by a visual assessment according to the procedure described by Tan *et al.* (2000). Collected data were shown as Table 4.

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Table 4 Description and unit of collected traits from DS2014 field experiment

Traits	Unit	Descriptions
Days to 50% flowering (DF50)	days	Number of days from planting to 50% of the plants within a plot with flowers
Plant Height (PH)	cm	Height of plant from soil level to the tip of panicle
Tillers number (TN)	count	Total number of tillers per hill
Panicles number (PN)	count	Total number of panicles per hill
Panicle Length (PNL)	cm	Length of panicle
Spikelets number (SN)	count	Total number of spikelets per panicle
Spikelets fertility (SF)	%	Percent of fertile spikelets per panicle
Grain Length (GL)	mm	Grain Length of unhusked rice
Grain width (GW)	mm	Grain width of unhusked rice
Grain size ratio GL/B		ratio of grain length and width/breadth
Kernel length (KL)	mm	Kernel length of polished rice
Kernel width (KW)	mm	Kernel width of polished rice
Kernel size ratio K L/B		ratio of kernel length and width/breadth
1000 GW	gram	Weight of 1000 seeds
Brown rice (BR)	%	Percentage of brown rice
Milled Rice (MR)%	%	Percentage of milled rice

8. Evaluation of cooking quality traits of BILs

8.1 Sensory test for fragrance

Evaluation for presence or absence of aroma in introgression lines and the parents was determined by sensory test method which was developed from Rice Gene Discovery Unit, Kasetsart University, Thailand (Wanchana *et al*., 2005). Five seeds of brown rice were placed into 1.5 ml centrifuge tube and added by 200 µl of distilled water and incubated at 65˚C for three hours with the lids on. The samples were

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allowed to cool and the lids were then opened one by one and the samples were smelled and scored for aroma by three panellists.

8.2 Amylose Content (AC)

Amylose content was measured using the procedure of Juliano (1985) with minor modification. 100 mg of rice powder was incubated at room temperature for overnight in a solution of 1 ml of 95% ethanol and 9 ml of 1 N sodium hydroxide to gelatinize the starch. After making up volume of the content to 100 ml with distilled water, 5 ml was taken into new conical flask. The sample was added with 1 ml of 1 N acetic acid solution and 2 ml of iodine reagent (0.2 g iodine and 2 g Potassium iodide in 100 ml water) and volume is made up to 100 ml with distilled water and mixed well. The absorbance was recorded at 620 nm using a spectro photo meter. The AC was estimated using a standard curve developed from known quantities of purified potato amylose from FlukaThailand (Figure 11). Rice varieties may be classified as high-, intermediate-, low-, very low and no- (glutinous or waxy) amylose classes with > 25%, 20 - 25%, 12 - 20%, 5 - 12% and 0 - 5% of the apparent amylose (Table 5) (Juliano *et al.*, 1981).

Figure 11 Standard curve and Amylose content testing

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Table 5 Classification of rice varieties based on proportion of amylose content

Varietal class	Amylose (%)	Texture
Waxy	0-5	sticky, glossy, firm
Very Low	5-12	moist, sticky, split
Low	12-20	moist, sticky, split
Intermediate	20-25	moist, tender, do not hard
High	25-33	dry, fluffy and hard

8.3 Gel Consistency (GC)

Gel consistency (GC) was measured by the length in a culture tube of cold gel according to the method of Cagampang *et al.* (1973). One hundred milligrams of rice powder was put in a 10 mm x 110 mm culture tube and wetted with 0.2 ml of 95% ethanol containing 0.025% thymol blue. Two milliliters of 0.2 N KOH was added. The sample was mixed using vortex Genie mixer. The test tube was covered with glass marble. The sample was cooked in a boiling water bath for 8 minutes, making sure that the tube content reach 2/3 the height of the tube. The test tube was removed from the water bath and let stand at room temperature for 5 minutes. The tube was cooled in an ice-water bath for 20 minutes and laid horizontally on a laboratory table lined with millimeter graphing paper. One hour later the total length of the gel was measured in millimeter as distance from the bottom of the tube to the front of the gel migration. The gel length thus obtained provides a measurement of the GC: the longer the distance, the softer the gel. The gel consistency value was evaluated by hard (26 - 40 mm), medium (41 - 60 mm) and soft (61 – 100 mm), short gel indicates hard GC and long gel represents soft GC. Gel consistency is classified as given below in Table 6 and Figure 12.

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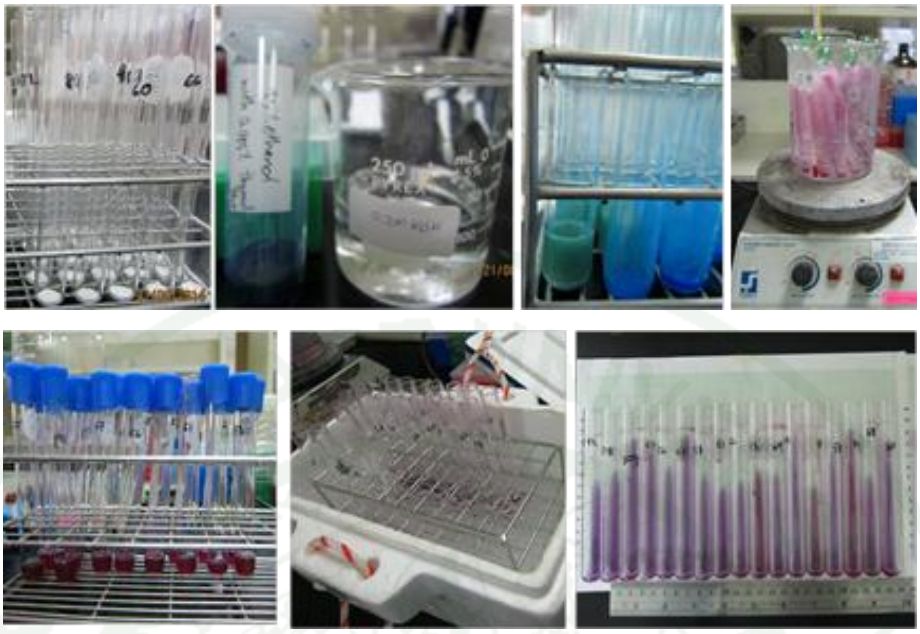

Figure 12 Schematic of gel consistency was measured by the length in a culture tube of cold gel according to the method of Cagampang *et al.* (1973)

Table 6 Classification of Gel Consistency

Category	**Consistency (mm)**
Soft	61-100
Medium	41-60
Medium hard	36-40
Hard	26-35

8.4 Gelatinization temperature (GT)

Gelatinization temperature (GT) was indirectly measured by evaluating the alkali spreading value (ASV) using the method of Little *et al.* (1958). Each sample was tested three times. Each time, six intact milled grains were put in a petridish, to which 10 ml of 1.7% KOH was added. The grains were carefully separated from each other using a forceps and incubated at 30˚C for 23 hours to allow spreading of the grains. The spreading value of the grains was scored on a numerical scale of 1 to 7 by

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visual assessment. 1 = grain unaffected; 2 = grain swollen; 3 = grain swollen, collar incomplete and narrow; 4 = grain swollen, collar complete and wide; 5 = grain split, collar complete and wide; 6 = grain dispersed, merging with collar and 7 = grain dispersed and disappeared completely. Alkali spreading value (ASV) corresponds to GT as follows; 1 - 2 high (74 - 79°C), 3 high-intermediate, 4 - 5 intermediate (70 - 74°C) and 6 - 7 low (55 - 69°C). A larger ASV represents more spreading in alkali, indicating a lower GT and a smaller ASV indicates a higher GT.

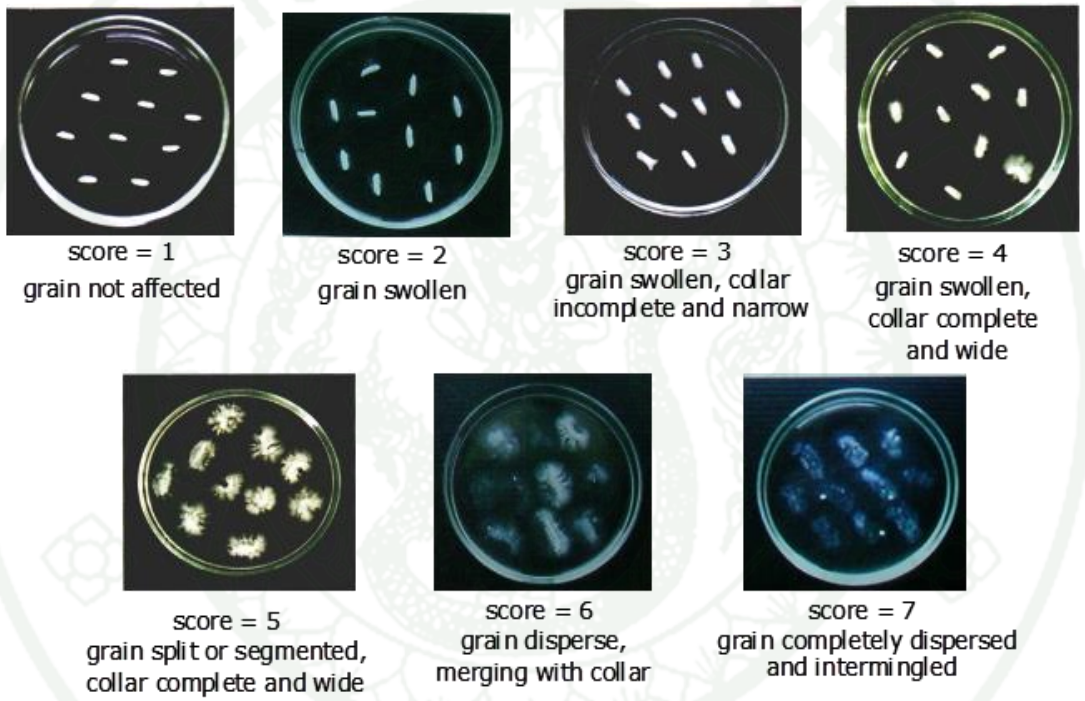

Figure 13 Alkali spreading scores for measuring gelatinization temperature by used 1.7% KOH (Little *et al.*, 1958)

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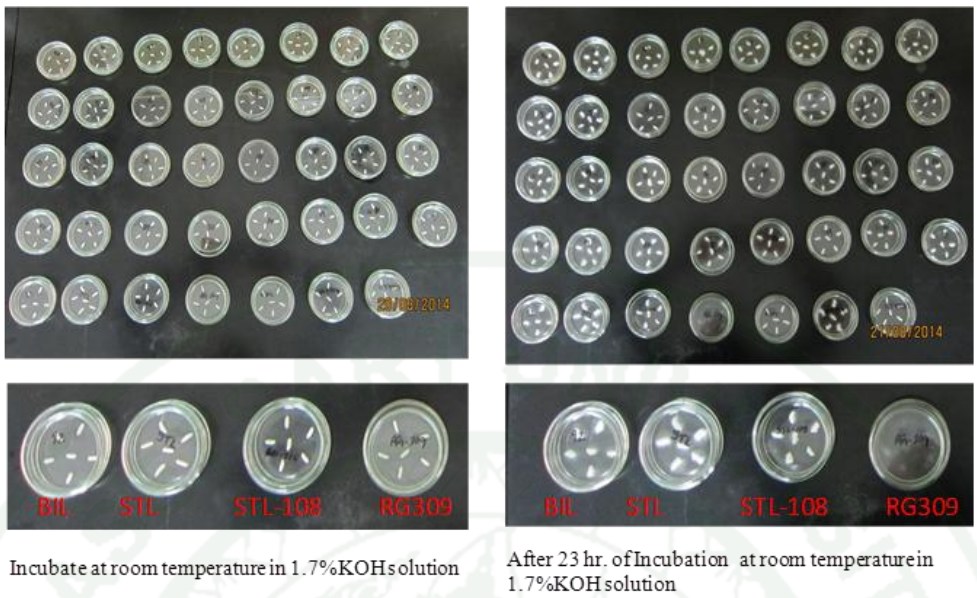

Incubate at room temperature in 1.7%KOH solution

After 23 hr. of Incubation at room temperature in 1.7%KOH solution

Figure 14 Gelatinization temperature (GT) of BILs and parents tested for alkali spreading values in 1.7% KOH for 23 hrs

Table 7 Alkali spreading scores for measuring gelatinization temperature (GT)

Gelatinization Temperature (^{o}C)	Range	Range of scores	Range of cooked time (minute)
< 65	Low	6 - 7	12 - 16
70 - 74	Intermediate	4 - 5	16 - 24
> 75	High	1 – 3	> 24

9. Statistical analysis

All traits in each experiment were subjected to statistical analysis with the CropStat 7.2 software program. An analysis of variance (ANOVA) was performed based on the RCBD. The LSD was determined at a 5% probability level to perform the comparison between the mean values of each entry and the parents. Grain quality performances, morphological features of all entries were compared with salt tolerant STL108 whereas the submergence and BB resistance reaction were compared with the RGD309 (donor parent).

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Part II: QTL mapping for salinity tolerance in 188 RILs derived from KDML105 x PK cross

1. Plant materials

188 F_6 progenies from cross KDML105 x Pokkali (RIL KDML105 x PK) were used. KDML105 is Thai jasmine rice with susceptible to salinity stress and where PK is standard salt tolerant landrace rice from India.

2. Place for experiment

Greenhouse experiment to screen the salinity tolerance of rice seedlings and reproductive stages under soil-culture were done at RGDU, Kasetsart University in Kamphaeng Saen campus in Nakorn Pathom province, Thailand. For Na^+ and K^+ determination for reproductive satge, flag leaf samples were digested at RGDU and ion determination by atomic absorption was done at Soil, Plant and Agricultural Material Testing and Research Unit of Central Laboratory and Greenhouse Complex of Kasetsart University.

3. Screening for salinity tolerance under Soil-culture condition for seedling and reproductive stages

3.1 Seedling stage screening

Seeds of F_6 generation of RIL KDML105 x PK, standard tolerant and susceptible checks were grown up in soil tray culture by the management described in PartI (5.1). Salinity stress of 150 mM (16 dS/m) was applied on 14 days old plants. Tolerance or susceptibility to stress was recorded 10, 16 and 21 DAT (days after treatment) of salt treatment by following Gergorio et al. (1997). At that time tolerant and susceptible checks were showing distinct salinity injury, the data of salinity injury score (SIS). Percent plant survival (PPS) and plant survival days after

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treatment (SvDAT) data were recorded at 21days after treatment. The survived plants were estimated and plus as 7 days more.

In this experiment, besides of the two parents (salt tolerant PK and susceptible KDML105), Nonabokra and IR29 were also used as standard tolerant and susceptible checks.

3.2 Reproductive stage screening and evaluation of Na^+/K^+ in flag leaf under salt stress

188 lines, the same set of (RIL KDML105 x PK) F_6 generation and standard tolerant and susceptible checks were aerobically germinated for 7 days in a petri-dish and seedlings were transplanted directly in PVC pipe (15cm x 5cm : height and wide) which is put in plastic to cover the base. The transplanted seedlings in PVC pipe were grown in concrete pond as described above in PartI (5.1). The plants were trimmed for just grown as main tiller. At the booting stage, the plants were moved into 100 mM NaCl salt added Nutrient solution (mentioned above). The tested plants were planted in 3 replications following a RCB design. Six plants per line in each replication were planted. In this experiment, besides of the two parents (PK and KDML105), Nonabokra, FL530 and FL496 were used as tolerant check while IR29 and RD6 were also used as standard tolerant and susceptible checks. The collected data were: SISPN, PNL, FSWT, FPNWT, DSWT, DPNWT, UFG, TGWT, TGN, FGP, FBIOM, DBIOM, $Na^+$and K^+ion content in flag leaf and Na^+/K^+ratio.

Panicle salt injury score was based on IRRI SES for PAcp score and modified as shown in Table 8.

In order to observe the physiological characters of Na^+ and K^+ concentrations of 188 F_6 population and parents were also recorded. At harvesting time, the flag leaf samples were collected and oven dried followed by the procedure expressed in PartI (5.1).

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Table 8 Salt injury visual score for panicle based on IRRI SES for PAcp score

Score	Observation	Tolerant level	
1	Normal	Highly tolerant	Excellent
3	Nearly normal; panicle tips or few spikelet whitish	Tolerant	Good
5	Under about 25% unfilled grain and good appearance of panicle and having a few white tip	Moderately tolerant	Fair
7	More than 75% unfilled grain and most spilkelets are severely injuried	Susceptible	Poor
9	Panicle is completely whitish or injured by salinity stress and drying	Highly susceptible	Unacceptable

4. Simple sequence repeats (SSR) genotyping

188 of F_6 population, mentioned above the cross KDML105 x PK, were extracted with DNA trap kit (http://dnatec.kps.ku.ac.th). One hundred and ninety rice microsatellite markers (SSR) on twelve rice chromosomes were used to do parental survey. Among them one hundred and twenty nine were polymorphic between parents on the whole rice genome. Information on the SSR markers, including the primer sequences and genomic locations, was obtained from the Gramene database (http://www.gramene.org/). DNA extraction, PCR amplification, and PAGE loading were the same procedure as described in Part I (4).

5. Statistical and QTL analysis

All traits in each experiment were carefully checked for outliers using scatter plot and linear regression in excel program and data were subjected to statistical analysis using the CropStat 7.2 software program. ANOVA was calculated based on a RCBD. Standard error of means was determined and LSD was determined at a 5%

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probability level to make the comparison between the mean values of each line and standard checks. The correlation among traits was analyzed using STATGRAPHICS PLUS v.3 for detecting significant correlations between the responsive traits which were relevant to salinity tolerance under seedling and reproductive stages. Linkage map was constructed by Joinmap 3.0 and MapChart 2.2 programs. QTLs were detected by simple interval mapping (SIM) and composite interval mapping (CIM) methods from Breeding Management System version 2.1.1 and multiple regression modules of the STATGRAPHICS v.3 programs. The presence of QTL was declared at probability value of less than 0.05. The proportion of the phenotypic variation explained by individual marker loci was determined by the R^2 values. The ordering of markers was compared to the physical distance from Gramene database (http://www.gramene. org/).

Part III: Screening of Myanmar germplasm for salt tolerance at seedling stage

1. Plant Materials

Total of 242 Myanmar Rice germplasms, from delta region (Ayeyawaddy division) costal region (Rakhine State) and middle dry area of Myanmar (Mandalay and Saging divisions), were used for screening. Those germplasms were provided by Seed Bank, Department of Agricultural Research Department (DAR), Yezin, Myanmar. In screening activities, PK, FL530 and FL496 were used as tolerant checks where as IR29 and KDML105 were used as susceptible checks.

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Figure 15 The salt affected area in Myanmar and the regions of germplasm source

2. Place for experiment

Greenhouse experiment to screen the salinity tolerance of rice seedlings under soil-culture was done at RGDU, Kasetsart University, Kamphaeng Saen Campus, Nakorn Pathom province, Thailand.

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3. Screening for salinity tolerance under soil-culture condition

Seeds of 242 gerplasms, tolerant and susceptible checks mentioned above were geminated, evaluated to salt stress and scored follow part I (5). The collected data were salt injury score (SIS), plant height (PH) and dry biomass weight comparing with normal (control) condition.

4. Statistical analysis

All traits in each experiment were calculated follow part I (9).

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RESULTS AND DISCUSSION

Part I: Molecular breeding of improved Sin-Thwe-Latt for submergence tolerance, bacterial leaf blight resistance, grain aroma and salt tolerance

1. Marker-assisted selection

Table 9 showed the result of marker assisted selection in this study. In wet season (WS) 2010 F_1 generation, eleven F_1 plants were selected out of twenty by PB7-8 marker. 298 BC_1F_1 plants could be generated in dry season (DS) 2011 and after doing MAS by using foreground markers (R10783indel, PB7-8, Aromarker) and additional markers (RM1287 and RM3412), six BC_1F_1 plants were selected for second backcrossing to generate BC_2F_1 population in WS 2011. After doing MAS selection with the same markers, six plants were selected out of 249 BC_2F_1 plants which were carrying heterozygous allele at the foreground markers and additional markers. During DS 2012 BC_2F_2 population were generated and done MAS and plant type selection for the 837 plants, among them 22 individual plants could be selected. In DS 2013, after doing screened in 100 mM NaCl at seedling stage of BC_2F_3 generation, 469 plants were survived out of 5000 individual. Those survived plants were transplanted and done MAS. Among them, 92 lines from 10 families were selected as carrying all the target QTL and genes (*Saltol*, *Sub1*, *Xa21* and *badh2).* During WS 2013, the selected 92 lines were multiplied for BC_2F_4 generation. During DS 2014 and WS 2014, based on the trait validations and Observation trial of BC_2F_5 population of the selected 92 lines (of 10 families), 29 lines (from 8 families) were selected by plant type selection and compared with trait validations results. These selected lines will be evaluated at salt affected and submergence areas in Myanmar in the future. The 29 selected lines were described as Table 10.

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Table 9 Selection on different generation of STL108 x RGD309

	Genera-tion	Select-ion	Foreground markers	Additional markers	Tested sample	Selected plants
1.	F_1	MAS	PB7-8		20	11 (Heterozygous)
2.	BC_1F_1	MAS	PB7-8 R10783indel Aromaker	RM1287 RM3412	298	6 Heterozygous)
3.	BC_2F_1	MAS	PB7-8 R10783indel Aromaker	RM1287 RM3412	249	6 (Heterozygous)
4.	BC_2F_2	MAS	PB7-8 R10783indel Aromarker	RM1287 RM3412	837	22 (Homo + Heterozygous)
5.	BC_2F_3	PS	100mM NaCl screening		5000	469
		MAS	PB7-8 R10783indel Aromarker	RM1287 RM3412	469	92 (Homozygous)
6.	BC_2F_4		Seed mulitplication		92	
7.	BC_2F_5	PS	Trait validations for Salt, Submergence, and BB		92	
			Genome Scan using 138 polymorphic SSR markers on 12 chromosome			
			Observation trial (Plant type selection)		92	29 from 8 families

MAS = Marker assisted selection; PS = Phenotypic selection

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Table 10 Marker genotypes of the twenty nine STL108 BILs carrying target genes

Sr.no	Pedigree	QTL/gene			
		Saltol	***Sub1***	***Xa21***	***badh2***
1	RGD11238-38-69-1	+	+	+	+
2	RGD11238-38-69-12	+	+	+	+
3	RGD11238-38-69-14	+	+	+	+
4	RGD11238-38-69-21	+	+	+	+
5	RGD11238-38-69-4	+	+	+	+
6	RGD11238-38-69-5	+	+	+	+
7	RGD11238-38-69-9	+	+	+	+
8	RGD11238-38-153-1	+	+	+	+
9	RGD11238-38-153-12	+	+	+	+
10	RGD11238-38-153-20	+	+	+	+
11	RGD11238-38-153-23	+	+	+	+
12	RGD11238-38-153-24	+	+	+	+
13	RGD11238-38-153-8	+	+	+	+
14	RGD11238-75-141-10	+	+	+	+
15	RGD11238-75-141-15	+	+	+	+
16	RGD11238-75-141-21	+	+	+	+
17	RGD11238-75-141-25	+	+	+	+
18	RGD11238-75-141-4	+	+	+	+
19	RGD11232-9-23-9	+	+	+	+
20	RGD11232-9-35-13	+	+	+	+
21	RGD11232-9-35-16	+	+	+	+
22	RGD11232-9-35-17	+	+	+	+
23	RGD11232-9-35-2	+	+	+	+
24	RGD11232-9-35-7	+	+	+	+
25	RGD11232-9-35-9	+	+	+	+
26	RGD11238-38-2-3	+	+	+	+
27	RGD11238-38-4-112	+	+	+	+
28	RGD11238-38-4-44	+	+	+	+
29	RGD11238-38-4-85	+	+	+	+
30	Yn3220-1-8-2-3-1(STL108)	+	-	-	-
31	RGD07343-13-21-9 (RGD309)	-	+	+	+

+ = presence of target gene

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2. Phenotypic evaluation as trait validations

2.1 Screening for salinity tolerance under soil-culture condition at seedling stage

The 92 lines of BIL (STL108 x RGD309) were validated for salt tolerance with 100 mM NaCl solution at seedling stage for 16days treatment comparing with tolerant check, PK and susceptible check, IR29. As shown in Table 11 and Figure 16, salt injury score 10 days after treatment (SIS10), the tolerant check PK was showing score 4.37 which was statistically lower than those of STL and susceptible check IR29 score as 6.06 and 6.4 respectively. The mean SIS10 value of progenies' was showing 5.52 and ranged 4.81 to 6.41. For SIS16, although the range of the score was 5.78 to 8.43 and the mean value was 7.06. Mean value of BILs was significantly higher than the tolerant check PK. It seems that the BILs were in the range of moderately tolerant to susceptible for 100 mM NaCl treatment after 16 days. The tolerant PK was at 4.88 while STL and IR29 were at significantly higher score as 8.25 and 7.96, respectively.

The average value of survival days after treatment (SvDAT) for BILs was 20.47 days in the range of 15.56 - 25.47 days. While the tolerant PK was assumed as 30 days surviving in 100 mM NaCl, the intolerant IR29 and STL were significantly less than PK as 22.76 and 17.74 days respectively. Mean percent for plant survival (PPS) of BILs was 73.04% which is not significantly different from tolerant check PK. The lowest plant survival percent of BILs line was 29.4 and the highest was full percent (100%) as PK. The background STL and intolerant check IR29 were only 38.03% and 33.18% respectively. Although the BILs are carrying the *Saltol* QTL, they are not tolerant as PK but some of them are better than the background STL as moderately salt tolerance.

The mean values of Na^+, K^+ content and Na^+/K^+ ratio in shoot were statistically significant among the tested lines at 12 days after salt treatment. While the Na^+ content of tolerant PK was 13.56 mg/g, the susceptible check IR29 and background STL were significantly higher than that of tolerant check as 33.18 and

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35.03 mg/g respectively and BILs were ranged 8.57 - 16.63 m/g. Contrarily between Na^+ ion and K^+ ion content in shoot of tolerant PK, K^+ value was shown as 38.28 mg/g that was significantly different from those of susceptible check IR29 (25.05 mg/g) and STL (25.75 mg/g). The mean value of BILs for K^+ content in shoot was 28.62 mg/g with the range of 22.24 to 33.67 mg/g. Higher Na^+/K^+ ratio of shoot was found as highly significant in susceptible varieties STL and IR29. For the mean value of Na^+/K^+ ratio, BILs (0.45) was statistically higer than that of tolerant PK (0.36) and significantly less than intolerant background STL (1.36) and IR29 (1.33). This indicates that the susceptible lines absorb salt and transport them immediately to the shoot which is exactly opposite reaction in the tolerant plants. It could be speculated that the tolerant varieties may block the translocation of salt to the shoot.

Based on the correlation coefficient between the tested traits, SIS scores are negatively correlated with SvDAT and PPS at $P < 0.01$ level. But SIS scores, SvDAT and PPS were not significantly correlated with Na^+, K^+ ion content and Na^+/K^+ traits (Table 12).

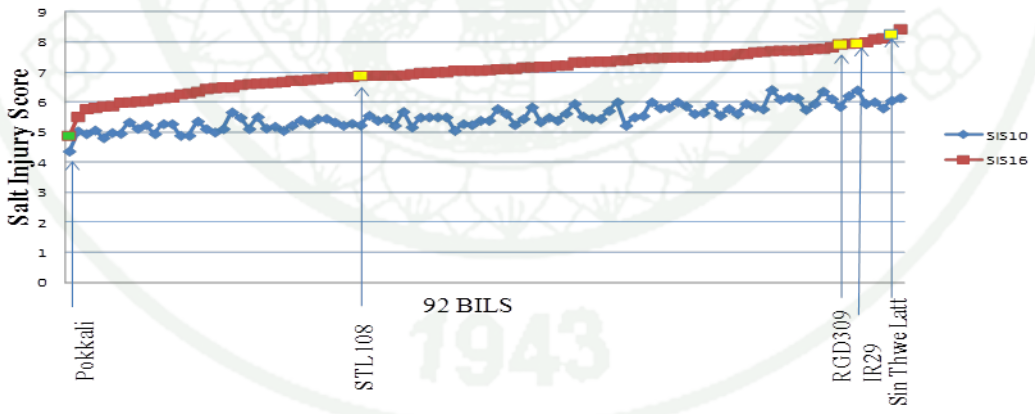

Figure 16 The SIS score for 10 and 16 days after salt treatment of BILs

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Table 11 Summary for salt tolerant trait validation under 100 mM NaCl at seedling stage

Materials		SIS10	SIS16	SvDAT	PPS	Na^+	K^+	Na^+/K^+
Ils	Mean	5.52^{ab}	7.06^{b}	20.47^{b}	73.03^{a}	13.02^{a}	28.62^{b}	0.45^{b}
	Range	4.81^{a}- 6.41^{b}	5.78^{a}- 8.43^{b}	15.56^{c}- 25.47^{b}	29.40^{b}- 100^{a}	8.57^{a}- 16.63^{b}	22.24^{c}- 33.67^{a}	0.33^{a}- 0.60^{c}
PK (T check)		4.37^{a}	4.88^{a}	30^{a}	100^{a}	13.56^{a}	38.28^{a}	0.36^{a}
STL		6.06^{b}	8.25^{b}	17.74^{b}	38.03^{b}	35.03^{c}	25.75^{bc}	1.36^{d}
IR29 (S check)		6.40^{b}	7.96^{b}	22.76^{b}	33.33^{b}	33.18^{c}	25.05^{bc}	1.33^{d}
	F test	**	**	**	**	**	**	**
	LSD0.05	0.92	1.19	5.65	29.44	4.5	5.13	0.12
	CV%	11.6	13.6	18.7	35.6	19.8	11.1	14.6

** = Significant at $P < 0.01$, In each column, means followed by the same letter are not significant different at the 5% level of LSD

SIS10, SIS16 = salt injury score at 10 days and 16 days after treatment;

SvDAT = survival days after treatment; PPS = Percent plant survive; Na^+ = Sodium content (mg/g) in shoot under 100 mM NaCl stress condition; K^+ = Potassium content (mg/g) in shoot under 100 mM NaCl stress condition; Na^+/K^+ = Sodium and Potassium ratio in shoot under 100 mM NaCl stress condition

Table 12 Correlation coefficient between the salt tolerant traits of seedling stage screening for STL background BILs

	SIS10	SIS16	SvDAT	PPS	K^+	Na^+	Na^+/K^+
SIS10	1						
SIS16	0.82**	1					
SvDAT	-0.70**	-0.85**	1				
PSS	-0.64**	-0.84**	0.68**	1			
K^+	-0.17^{ns}	-0.29**	0.30**	0.27**	1		
Na^+	-0.05^{ns}	-0.1^{ns}	0.19^{ns}	0.18^{ns}	0.69**	1	
Na^+/K^+	0.09^{ns}	0.16^{ns}	-0.04^{ns}	-0.05^{ns}	-0.06^{ns}	0.68**	1

** = Significant at $P < 0.01$; ns = non significant

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2.2 Screening for submergence tolerance (Flash flooding for 10 days)

92 lines of BILs (BC_2F_5), standard checks including FR13A, IR57514 (tolerant checks), KDML105, RD6, IR53936 (STL), (susceptible checks) and two parents were assessed for their responses to submergence stress for 10 days under 1 - 1.2 m above the plant height. One week after submergence, susceptible check KDML105 leaves were started to change yellow color but BILs plants were still green as FR13A. After 10 days of flooding, the experimental pond was stopped flooding and drained out because KDML105 and recipient parent STL108 were dead. The result indicated that submergence stress was distinctly limited the plant growth as shown in the significant difference of the BSTN, DSTN and PSE between normal and submerged experiments (Table 13).

2.2.1 Comparing percent seedling elongation (PSE) between stress and normal condition

As shown in Figure 17 and Table 13, under submergence condition, while the tolerant check lines (FR13A, IR57514 and donor RGD309) were showing lower PSE compared with normal condition, the intolerant checks (KDML105, STL, RD6, and recipient STL108) had almost the same PSE between two different conditions. Some of our BILs lines were clearly seen that they had lower PSE under stress condition than that of normal one. It is showing that the lower PSE lines were more tolerant than the higher PSE lines. Comparison of plant height was not much different before and after submergence. The plant height of FR13A was 66.3 cm and 68.3 cm before and after submergence respectively, (59.5 cm and 74.9 cm tall in normal condition) and PSE of FR13A was only 4.9% (20.6%.in the normal condition).

The susceptible checks, KDML105, RD6, STL and recipient STL108, were taller than before submergence and it seemed to be trying to escape from the submergence condition. Their PSE of normal and stress conditions were not different for all of the susceptible lines. Under stress condition, BILs showed that the

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mean plant heights of before and after submergence were not different, as 52.5 and 55.4 cm. The mean PSE of BILs was 8.5% which was almost the same as donor parent RGD309. In the normal condition, mean plant height of BILs was clearly increased from 45.6 cm to 55.5 cm and the PSE was 16.8%. Therefore the shoot elongation of BILs was suppressed to preserve carbohydrates under 10-days flash-flood condition, following the strategy of quiescent (Colmer and Voesenek, 2009). The figure 17 explained that the BILs were carrying *Sub1* gene and their shoot elongation were suppressed under submergence condition.

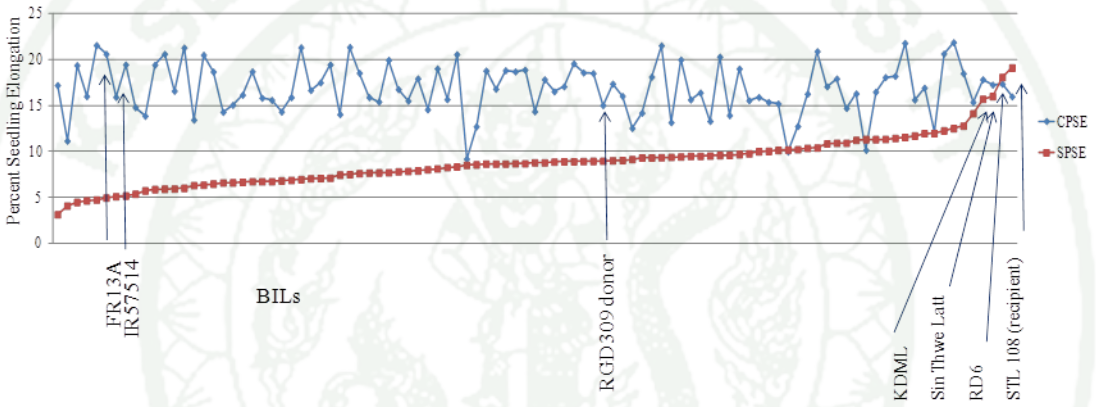

Figure 17 Comparison between percent seedling elongation (PSE) of submerged and normal condition (bule line = control; red line = submerged)

2.2.2 Comparing effective tiller numbers before and after submergence at stress and normal condition

Under stress condition, effective tiller numbers were decreased in all tested lines except the standard tolerant check FR13A, which of the effective tillers were increase from 5.7 to 7.4 tillers. Even one of the tolerant checks IR57514 and donor RGD 309 had also significantly decreased compare with FR13A. IR57514 was decreased as 9 to 4.1 tillers at before and after submergence while donor RGD309 was 8.5 to 3.1 tillers. For the susceptible check RD6, STL and recipient STL108 were decreased until 0(zero) tillers from 10.9, 9.5 and 11.2, respectively but KDML105 had decreased as 7.3 to 1.8 tillers at before and after submergence. The plants growing at

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normal conditions were clearly increasing tiller numbers in all tested lines (Table 15 and Figure 18).

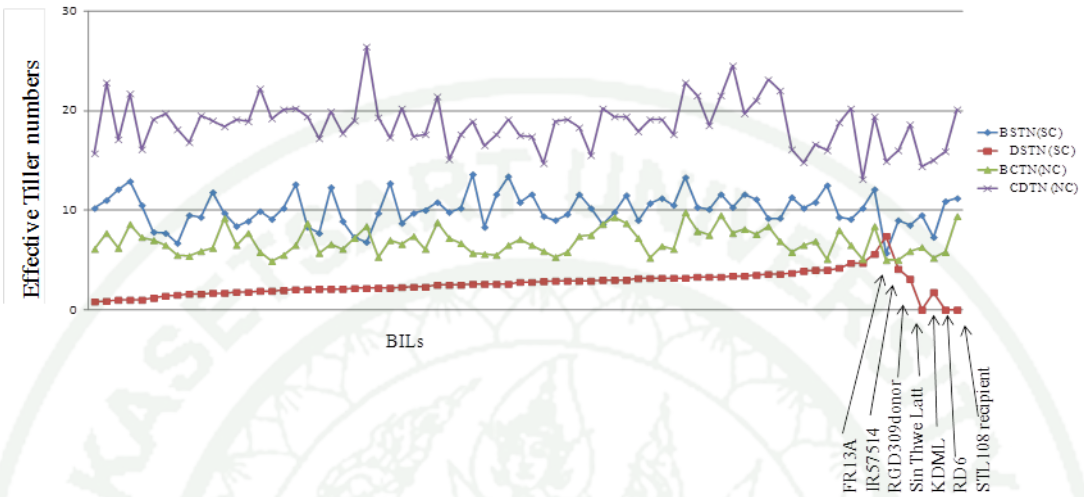

Figure 18 Comparison of effective tiller numbers of normal and subrmergence stress condition at beginning and de-submergence. Blue line BSTN (SC) = tiller number of before submergence (stress condition) Red line DSTN (SC) = tiller number of after desubmergence (stress condition); Green line BCTN (NC) = tiller number of before submergence (normal condition); Purple line CDTN (NC) = tiller number after desubmergence (normal condition)

2.2.3 Comparison between percent of survived seedling (PSS) and percent seedling elongation (PSE) at stress condition

After10 days of complete submergence stress, among the tested plants, all the traits were significantly different from each other except of plant height at 46 day-old. Under stress condition, the standard tolerant check FR13A showed 94% plant survival and the percent seedling elongation PSE was only 4.9%. The plant height of tolerant FR13A had not much different between before and after submergence. IR57514 and donor RGD309 had similar flow with FR13A.

Contrarily, the susceptible check KDML105 showed high PSE (15.7%) and only (4%) of low PSS and the other susceptible checks, RD6, STL and

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recipient STL108 were 18.1, 16 and 19.1% of PSE, respectively and only 0 % PSS at all. Their PSE percents were completely higher than that of tolerant FR13A and almost similar with PSE of normal condition. The mean value of PES for donor parent RGD309 was 8.9% and PSS was 72%. BILs were showing similar mean value of PSE with donor parent but the PSS was only 43.3% and the range of PES was 3.1 - 14.1% and PSS was 6.0 - 92%. At the normal condition, the PSE of tested plants was not significantly different.

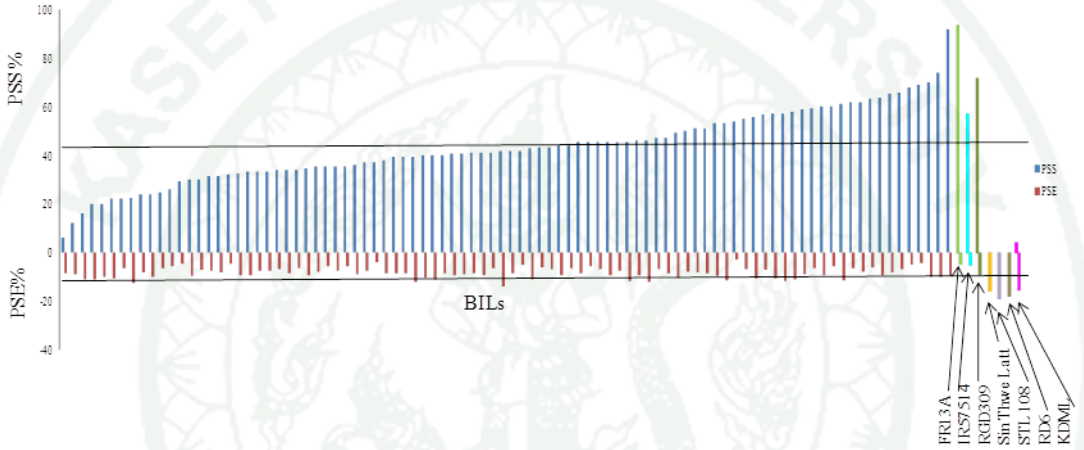

Figure 19 Comparison between PSS (blue line) and PSE (red line) of 92 BILs (STL108 x RG309 BC_2F_5) population and standard check under complete submerged for 10 days stress

The results of this experiment followed the strategy of flash flood tolerance at the rice seedling stage (Nagai *et al.*, 2010). Our BILs lines were found as quience plant growth and agree with Colmer and Voesenek (2009) who stated that quiescence strategy [i.e. the low-oxygen quiescence syndrome, in which shoot elongation is suppressed to preserve carbohydrates for a long period (10 - 14 days)] under flash-flood conditions. Submergence tolerant cultivars can restart their growth during de-submergence by using preserved carbohydrates.

Nagai *et al.* (2010) stated that flash flood tolerant rice stops growing so as not to consume energy while submerged. The plant restarts its growth

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using accumulated energy after the water recedes. Fukao and Bailey-Serres (2008) showed the mechanism by which *Sub1A* regulates plant growth. Slender rice-1 (SLR1) and SLR1 Like-1 (SLRL1) are repressors of GA signaling. The amount of SLR1 protein is elevated after submergence in submergence-tolerant rice (M202-*Sub1*), but not in submergence-intolerant rice (M202). Additionally, more SLRL1 protein accumulated in submergence-tolerant rice (M202-*Sub1*) than in submergence-intolerant rice (M202). These results suggest that the restriction of growth by submergence-tolerant rice is due to the accumulation of SLR1 and SLRL1 through *Sub1A* (Nagai *et al.*, 2010).

Under flash flooding, few characters were identified as playing a key role in submergence tolerance in rice, the most critical are: maintenance of high carbohydrate concentration, optimum rates of alcoholic fermentation and energy conservation by maintaining low elongation growth rates during submergence. Protective mechanisms as the up regulation of antioxidant system and low synthesis or sensitivity to ethylene during submergence were also found to be useful (Sarkar *et al*., 2006).

The flash-flood-tolerant East Indian rice cultivar FR13A shows restricted shoot elongation and reduced energy consumption under submergence (Setter and Laureles, 1996). The energy in FR13A plants are preserved during submergence, and upon de-submergence their growth can be restarted by using this energy (Fukao *et al.*, 2006). There is therefore a negative correlation between shoot elongation and survival rate under complete submergence (Setter and Laureles 1996).

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Table 13 Summary data for submergence tolerant screening (10 days under water 1.2 m depth) of STL108 x RGD309 BILs

Traits	Parents and checks												BILs						LSD (0.05)		F-test		CV (%)	
	STL		STL108		RGD309		KD		RD6		FR13A		IR57514		Mean		Range							
	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC	NC	SC
BSTN	6.3	9.5	9.4	11.2	5.9	8.5	5.2	7.3	5.8	10.9	5	5.7	5	9	6.65	9.9	4-9.8	5.2-13.6	2.64	4.7	**	ns	20.1	24.1
DSTN	14.4	0	20.1	0	18.6	3.1	15	1.8	15.9	0	14.9	7.4	16	4.1	19	2. 6	13.1-26.4	0.5-5.6	5.40	2.3	**	**	14.5	46.2
BSPH	55.2	58.1	46.9	53.8	53	55	61.8	63.9	64.1	70.4	59.5	66.3	47.8	61.3	45.6	52.5	38.9-55.5	45.1-59	9.5	6.3	ns	**	10.3	6
DSPH	66.7	63.8	55.9	64.1	62.3	58.8	75.2	70.8	77.8	78.2	74.9	68	59.3	60.6	55.5	55.4	47.3-68.4	47.9-63.1	17.0	6.7	**	**	10	6
PSE	17.3	16.0	16.	19.1	15.0	8.9	17.8	15.7	17.3	18.1	20.6	4.9	19. 5	5.2	16.8	8.5	9.2-21.9	3.1-14.1	7.5	6.0	ns	**	22.4	34.3
PSS		0		0		72		4		0		94		57.3		43.3		6-92		33.3		**		39

NC = normal condition; SC = submerged condition; BSTN = before submergence effective tiller number; DSTN = desubmergence effective tiller number; BSPH = plant height (before submergence); DSPH = plant height (desubmergence); PSE = percent of seedling elongation; PSS= percent of surviving seedlings; ** = significant at P < 0.01; ns = non-significant

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2.3 Assessment of bacterial blight resistance at tillering stage

In order to validate the efficiency of MAS, 92 BILs BC_2F_5 lines fixed as homozygous at the target allele of BB resistant gene *Xa21* and parents were evaluated with two Thailand BB isolates, TXO152 and PSL2, at tillering stage by artificial inoculation in May 2014. After 14 days of inoculation, the variation of resistance and susceptible checks' lesion length (LL) were clearly seen and measured for all tested lines. The LL of BILs, background variety STL (IR53936) and two parents were presented comparing with resistant and susceptible checks in Table 14, Figure 20 and Figure 21.

In this experiment, IR1188 was used as resistant check. LL on IR1188 was found as moderately resistance (MR) to TXO152 (4.8 cm) and resistant (R) to PSL2 (1.4 cm), respectively. The susceptible checks KDML105 and Jaohomnim were showing long LL on both isolates. KDML105 was susceptible reaction as LL 13.9 cm and 16.9 cm on each isolates and Jaohomnin was 14.5 cm and 17.8 cm of TXO152 and PSL2, respectively. RGD309, donor parent was showing resistance against as MR to both isolates: TXO152 (4.3 cm) and PSL2 (4.1 cm) at tillering stage. The recipient STL108 and background variety STL were showing the LL as susceptible to both isolates similarly to susceptible checks KDML105 and Jaohomnin. The recipient STL108 showed as susceptible (S) to both isolates with long LL (9.1 and 8.1 cm). The background STL was showing 12.7 cm and 12.8 cm against TXO152 and PSL2, respectively.

Mean of LL in BC_2F_5 population of STL108 x RG309 progenies' were 3.4 cm (MR) to TXO152 and 1.9 cm (R) to PSL2, at tillering stage screening. The ranges of their LL were 1.8 - 5.8 cm for TXO152 and 0.2- 5.4 cm for PSL2 isolate. The result of this screening was showing that the BILs were carrying the *Xa21* gene against to the Thai isolates at tillering stage (inoculated on 60 days old and evaluated after 14 days inoculation). Mazzola *et al.* (1994) stated that the studies of the BB resistance response conditioned by the resistance gene *Xa 21* indicate that *Xa21*-mediated resistance to *Xoo* is not expressed in the early stages of development. Singh

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et al. (2001) used MAS to improve BB resistance in indica rice cultivar PR106 with *xa5*, *xa13*and *Xa21*. They found that *Xa21* was the most effective followed by *xa5*, whereas *xa13* gene was the least effective against *Xoo*. Those BILs lines should be evaluated with Myanmar isolates in target area again.

Table 14 Resistance reaction of the standard checks, parents and BILs lines against to two Thai isolates at the tillering stage

Name	Gene	Remark	Lesion Length (mean±sd; cm)			
			TXO152		PSL2	
STL		Background	12.7 ± 3.7	S	12.8 ± 3.8	S
STL108	*Salol*	Recipient	9.3 ± 1.9	S	8.1 ± 2.7	MS
RGD309	*Sub/Xa21/badh2*	Donor	4.3 ± 1.7	MR	4.1 ± 3.3	MR
IR1188	*Xa21/qBB1/qBB8/ qBB11*	Resistant	4.8 ± 2.1	MR	1.4 ± 2.1	R
IR62266	*xa5*	Resistant	1.3 ± 0.6	R	0.8± 0.6	R
KDML105		Susceptible	13.9 ± 4.4	S	16.9 ± 6.8	S
Jaohomnin		Susceptible	14.5 ± 5.2	S	17.8 ± 6.2	S
92 BILsMean	*Saltol/Sub/Xa21/ badh2*		3.4 ± 1.3	MR	1.9 ± 1.3	R
Min			1.8 ± 0.5	R	0.2 ± 0.1	R
Max			5.8 ± 2.9	MR	5.4 ± 3.7	MR

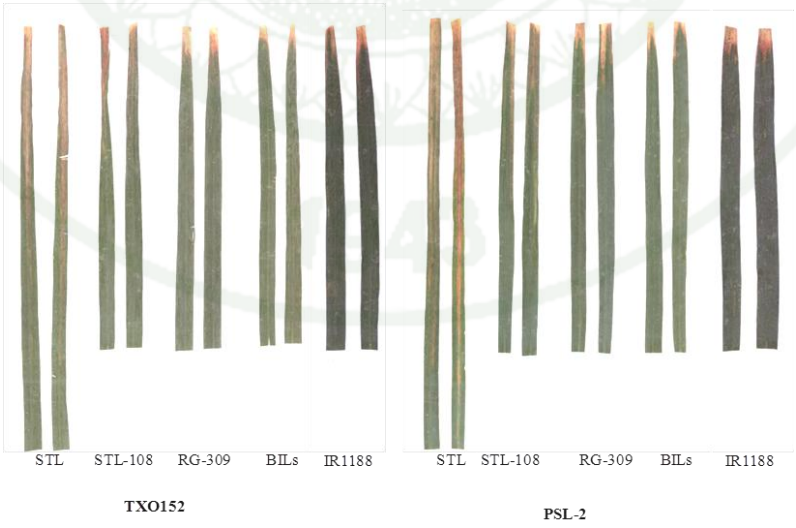

Figure 20 Resistance reaction of the tested lines against two Thai isolates at the tillering stage

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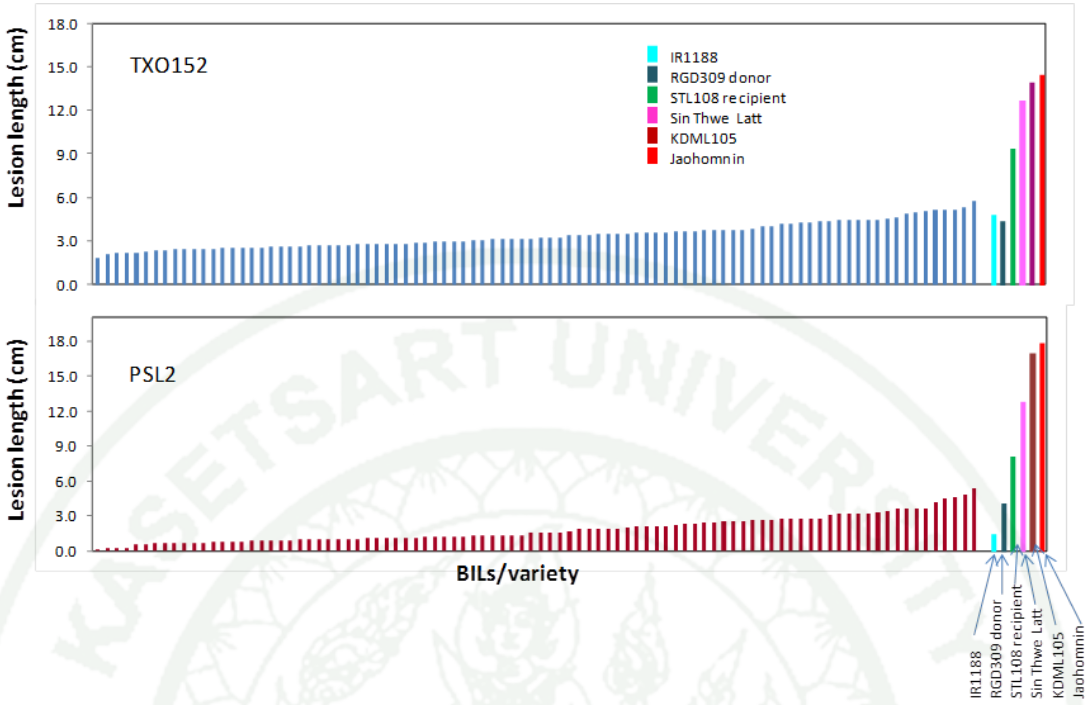

Figure 21 Mean LL of standard checks, parents and BILs lines against to two Thai *Xoo* isolates, TXO152 and PSL2 at tillering stage

2.4 The combined summary of all trait validations on final selected lines

All the tested 92 BILs lines were carrying the homozygous allele with donor parent for *Sub1*, *Xa21* and *Saltol* allele as PK. In the Table 15, summary for all the traits mean value of the final selected 29 lines which were selected based on their plant types in DS 2014 observation trail experiment, described with the mean value of trait validations for salt, submergence stress tolerant and BB reaction. By the marker assisted breeding progam, we could successfully introgress the target traits into BILs within 4 years into STL108.

Although the BILs were carrying the *Sub1, Xa21* and *Saltol,* homozygous allele with donor and recipient parents, (RGD309 and STL108), they showed variation in some trait validation experiments therefore we need to do genome scan and find out putative QTLs.

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Table 15 Mean values of final selected 29 BILs lines for target traits validation

Traits/QTL/Genes	ST (Saltol)		SUB (*Sub1*)				BLB (*Xa21*)			
							TXO152		PSL2	
Pedigree	SIS10	SIS16	PPS	SvDAT	PSE	PSS	(LL, cm)	Rx	(LL, cm)	Rx
RGD11238-38-69-1	4.96	5.99	100.00	22.41	7.43	56.92	3.7	MR	2.7	R
RGD11238-38-69-12	5.23	6.86	77.78	20.39	6.65	62.00	4.2	MR	2.4	R
RGD11238-38-69-14	5.12	6.03	91.67	24.06	11.54	53.28	3.4	MR	2.6	R
RGD11238-38-69-21	4.89	6.27	93.33	21.92	8.93	36.00	3.8	MR	1.6	R
RGD11238-38-69-4	4.94	5.78	100.00	24.89	8.65	34.00	3.8	MR	1.8	R
RGD11238-38-69-5	5.44	6.89	66.67	19.89	4.05	37.28	4.2	MR	2.5	R
RGD11238-38-69-9	5.28	6.17	86.67	23.06	8.89	40.00	4.4	MR	1.5	R
RGD11238-38-153-1	4.94	6.11	100.00	25.17	9.39	46.00	3.1	MR	3.1	MR
RGD11238-38-153-12	5.24	7.11	69.44	20.10	5.89	35.28	4.0	MR	2.1	R
RGD11238-38-153-20	5.52	7.33	50.00	20.06	8.10	50.00	3.8	MR	3.2	MR
RGD11238-38-153-23	5.34	7.18	62.74	16.31	10.90	56.00	4.9	MR	3.7	MR
RGD11238-38-153-24	5.33	6.00	100.00	25.06	9.56	44.00	4.4	MR	3.6	MR
RGD11238-38-153-8	5.49	6.58	82.18	19.89	11.97	45.28	4.6	MR	2.6	R
RGD11238-75-141-10	6.00	8.10	48.29	17.61	6.45	44.00	3.5	MR	2.5	R
RGD11238-75-141-15	6.21	7.94	30.00	17.89	4.70	29.28	4.4	MR	2.7	R
RGD11238-75-141-21	5.28	6.14	91.67	21.83	10.01	24.00	4.5	MR	0.8	R

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Table 15 (Continued)

Traits/QTL/Genes	ST (Saltol)				SUB (*Sub1*)		BLB (*Xa21*)			
							TXO152		PSL2	
Pedigree	**SIS10**	**SIS16**	**PPS**	**SvDAT**	**PSE**	**PSS**	**(LL, cm)**	**Rx**	**(LL, cm)**	**Rx**
RGD11238-75-141-25	5.50	6.98	63.33	20.89	9.31	40.92	3.2	MR	0.3	R
RGD11238-75-141-4	5.07	5.82	100.00	23.91	8.78	38.00	3.5	MR	2.1	R
RGD11232-9-23-9	5.51	6.62	88.89	21.77	6.94	55.28	5.1	MR	3.2	MR
RGD11232-9-35-13	5.22	6.83	72.22	22.28	9.46	63.67	5.1	MR	2.7	R
RGD11232-9-35-16	5.22	6.89	72.22	21.39	10.16	70.00	5.2	MR	4.5	MR
RGD11232-9-35-17	5.39	7.06	73.85	19.61	9.03	40.92	5.8	MR	4.6	MR
RGD11232-9-35-2	5.44	6.78	79.40	21.83	5.33	68.00	4.2	MR	5.4	MR
RGD11232-9-35-7	5.61	7.60	40.51	19.39	9.75	92.00	4.0	MR	3.1	MR
RGD11232-9-35-9	5.00	6.48	87.78	23.90	5.08	42.00	5.1	MR	3.2	MR
RGD11238-38-2-3	5.36	6.36	100.00	21.53	6.70	34.00	3.7	MR	1.0	R
RGD11238-38-4-112	4.81	5.87	100.00	25.47	12.50	46.00	3.6	MR	2.4	R
RGD11238-38-4-44	5.11	6.44	83.33	24.00	6.31	63.28	2.8	R	2.2	R
RGD11238-38-4-85	5.50	7.00	66.67	20.72	6.00	60.00	4.3	MR	2.0	R

SIS10, SIS16 = salt injury score at 10 days and 16 days after treatment;

SvDAT = survival days after treatment; PPS = percent plant survive; PSE = percent of seedling elongation; PSS = percent of surviving seedlings; TXO152, PSL2 = Thai *Xoo* isolates; MR = moderately resistance, R = resistance

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3. Genome scan and evaluation of the introgressed traits on the rice genome of STL108 x RGD309

92 lines of BC_2F_5 population were evaluated for genome scan with 135 SSR markers through 12 chromosomes. The physical map was constructed based on the marker information from www.gramene.org as shown in Figure 22. The putative QTLs were detected by single marker analysis and multiple regression analysis in STATGRAPHIC Plus program as described in Table 16.

For the salt tolerant trait of seedling stage, SIS10 was linked on long arm of chromosome 1 and 7 at RM315 and RM120 explaining 12.97% and 11.77%, respectively of the phenotypic variation (PVE). The total PVE of this trait was 17.05%. QTL on chromosome 1 was contributed by the STL108 allele while RGD309 contributed to the chromosome 7. The SIS16 was linked only on a long arm of chromosome 1 at RM8062 and RM6831 with PVE values of 9.4% and 12.49%, respectively. But the contributed alleles were different while RM 8062 was contributed with RGD309 and STL108 contributed to RM6831. The total PVE of SIS16 was 15.23%. Siangliw (2013) also reported that SIS-S and SIS-F were related with RM212 on a long arm of chromosome 1. He also reported that SIS score is easy to collect and more informative than the other traits and the correlation of this trait with other traits under soil-culture and hydroponic conditions support the evidance that SIS is a trait that represents other traits. When we do MAS for salt tolerant traits, we should selected not only on short arm of chromosome 1 for *Saltol* region but also for SIS score on a long arm of chromosome 1 designated as *qSt1b* region. Two markers were detected for PPS on chromosome 1 short arm and chromosome 7 at RM493 and RM336 with PVE 4.61% and 5.2%, respectively and both were contributed with STL108 allele. Total PVE was only 7.85%. Siangliw (2013) also found on a long arm chromosome 1 at RM212 and chromosome 4 at RM261 with KDML105 x FL496 and KDML105 x FL530 population.

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For SvDAT trait, 3 QTLs were found on chromosome 2, 6 and 8 at RM3355, RM30, and RM42 with 5.81, 8.92 and 4.89% of PVE respectively. The total PVE was 14.82%. The contributed allele of RM3355 was RGD309 while the rest two were contributed with STL108. It was the same result with Siangliw (2013) for QTLs related SDS-S.

Shoot Na^+ ion content trait was linked with chromosome 1 (short arm) and chromosome 11. The highest phenotypic variances explain 24.73% on chromosome 1 was at RM10701 contributing with STL108 and PVE 40.46% on chromosome 11 at RM206 was contributing with RGD309 respectively. Total PVE for this trait was 66.16 %. The QTLs related with shoot K^+ content were found on chromosomes 1 (short arm), 8 and 12 at RM10701, RM44 and RM19 which were explaining for phenotypic variation as 11.28, 18.22 and 15.47%, respectively contributed allele by STL108 for all loci. The total PVE of them was 30.65%. Na^+/K^+ in shoot trait was linked with chromosome 1 (short arm) and 11 at RM10701 and RM206 with 49.04% and 24.83% respectively while STL108 was contributing on chromosome 1, RGD309 was contributed on chromosome 11 and their total percentage of PVE was 74.67%. It can be concluded from the results that QTL in chromosome 1, *SKC1* region clearly control the behavior of ions particularly in the shoot at seedling stage salt tolerant. The same observation was found by Lin *et al.* (2004) that ion control in the shoot was controlled by *SKC1*. Thomson *et al.* (2010) on the other hand, reported that this region of chromosome 1 was also associated with ion accumulation but the QTL reported were found from both root and shoot tissues.

Although all the BILs were carrying *Sub1* gene on chromosome 9, phenotypic variation could be seen in trait validation under 10-days submergence. PSE trait was linked with chromosome 11 and 12 at RM206 and RM12 with PVE 11.2% and 7.83% respectively and both markers were contributing with tolerant parent RGD309 allele. The total PVE was found as 16.16%. Our result is in agreement with Toojinda *et al.* (2003) who mapped for DHL, RIL and F_2 populations for submergence tolerant traits. The relative shoot elongation was linked on chromosome 1, 8, 9, 10, 11, and 12. They

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also found the RM206 on chromosome 11, was linked with relative shoot elongation for F_2 population contributed with tolerant parent Jaohomnin. PSS was linked with chromosome 4 and 11 at RM8217 and RM21 with PVE of 21.52% and 10.2% respectively and RGD309 was contributed with both markers. Total PVE was 23.97%. Toojinda *et al.*, 2003 reported percent plant survival of F2 population derived from Jaohomnin x KDML105 were linked on chromosome 10 and 11 at RM 271, RM206 and RM209.

BILs carrying *Xa21*gene on chromosome 11 were detected that the reaction against to TXO152 was associated with chromosome 1 and 11 at RM 243, RM3523 and, RM206 with PVE of 22.94%, 45.79% and 32.57% respectively. Total PVE of this reaction was 70.78%. While RM243 was contributed with STL108, RM3523 and RM206 were contributed with resistant donor parent RGD309 allele. The LL of reaction for PSL2 isolate was related with chromosome 8 and 11 at RM44, RM206, and RM224 with PVE 35.24%, 23.66%, and 38.12 % respectively. Total PVE was 52.63%. BB resistant donor parent allele was contributed with all loci. Win *et.al.* (2012) also found the QTL of BB on chromosome 1, 8 and 11 for KDML105 backcross introgression lines in response to *Xoo* strains. They found the qBB1 on the chromosome 1 at 36.39 Mb. Tan *et al.* (2004) and Chen *et al.* (2009) found the *Xa29* and *xa33* on chromosome 1 upper region of 15.5 Mb and 17.4 Mb respectively.

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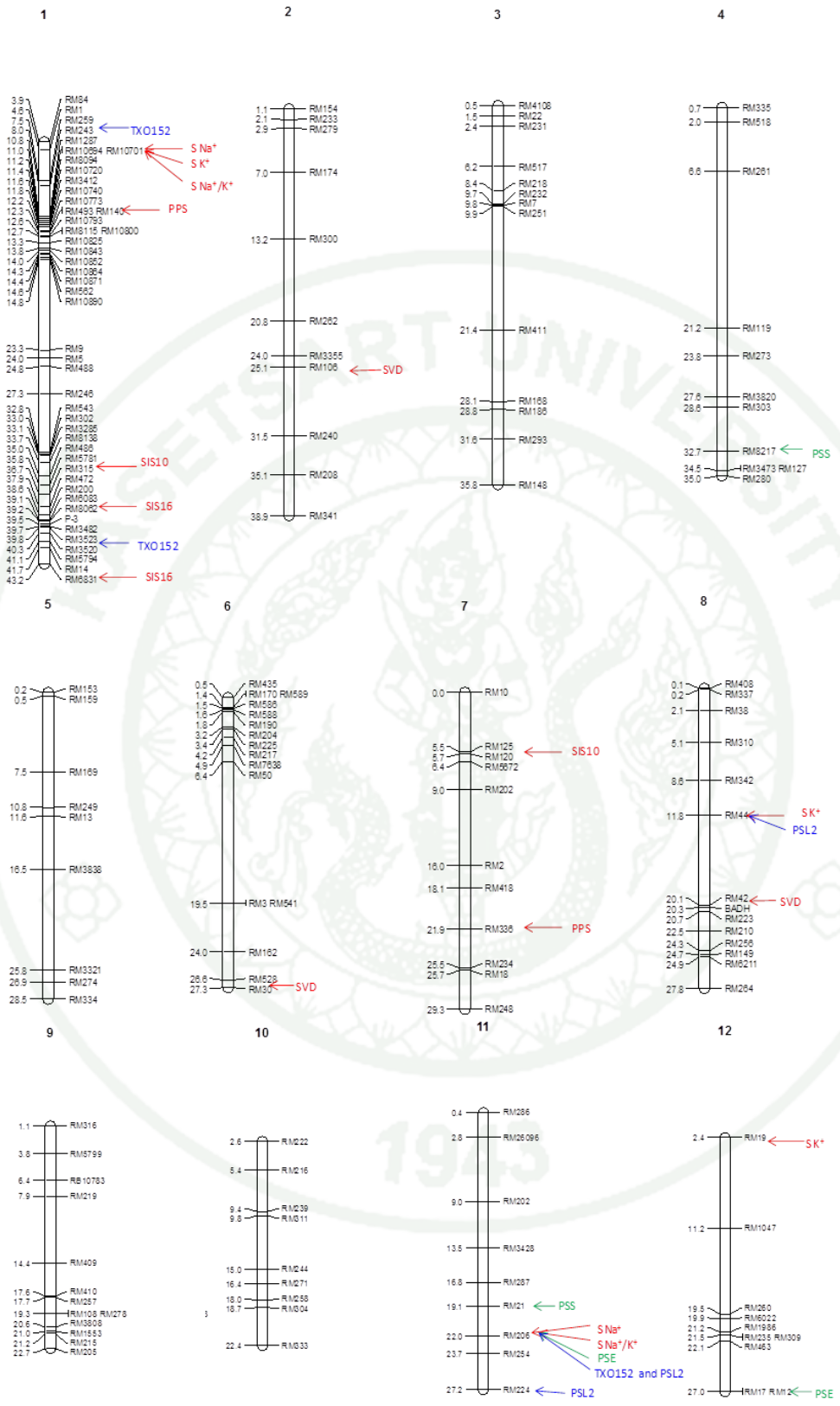

Figure 22 Physical map of rice genome STL108 x RGD309 cross

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Table 16 Single marker analysis ($P < 0.05$) of the putative QTLs related to the salt, submergence and BB stress condition which mapped of rice genome derived from the cross STL108 x RGD309

Trait[a]	Linked maeker	Chr	GP (Mb)[b]	Contribute allele[c]	QTL effect[d]	PVE[e] (%)	Total PVE[f] (%)
Salt tolerant							
SIS10	RM315	1	36.73	STL-108	-0.16	12.97	17.05
	RM120	7	5.74	RGD-309	0.16	11.77	
SIS16	RM8062	1	39.17	RGD-309	0.21	9.4	15.23
	RM6831	1	43.18	STL-108	-0.22	12.49	
PPS	RM493	1	12.28	STL-108	16.46	4.61	7.85
	RM336	7	21.87	STL-108	4.19	5.2	
SvDAT	RM3355	2	24.02	RGD-309	-0.98	5.81	14.82
	RM30	6	27.25	STL-108	0.98	8.92	
	RM42	8	20.09	STL-108	0.66	4.89	
S Na^+	RM10701	1	11.03	STL-108	-9.03	24.73	66.16
	RM206	11	22.01	RGD-309	6.81	40.46	
S K^+	RM10701	1	11.03	STL-108	4.45	11.28	30.65
	RM44	8	11.75	STL-108	2.75	18.22	
	RM19	12	2.43	STL-108	1.27	15.47	
S Na/K	RM10701	1	11.03	STL-108	-0.58	49.04	74.67
	RM206	11	22.01	RGD-309	0.24	24.83	
Submergence							
PSE	RM206	11	22.01	RGD-309	2.4	11.2	16.16
	RM12	12	22.01	RGD-309	1.22	7.83	
PSS	RM8217	4	32.66	RGD-309	-16.61	21.52	23.97
	RM21	11	19.14	RGD-309	-5.72	10.1	

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Table 16 (Continued)

Trait[a]	Linked maeker	Chr	GP (Mb)[b]	Contribute allele[c]	QTL effect[d]	PVE[e] (%)	Total PVE[f] (%)
Bacterial Blight							
TXO152	RM243	1	7.97	STL108	-0.76	22.94	70.78
	RM3523	1	39.82	RGD-309	0.77	45.79	
	RM206	11	22.01	RGD-309	2.96	32.57	
PSL2	RM44	8	11.75	RGD-309	1.89	35.24	52.63
	R206	11	22.01	RGD-309	3.11	23.66	
	RM224	11	27.17	RGD-309	0.82	38.12	

[a] = Trait names under different stress: salt stress, submergence and bacterial blight; [b] = Position (Mb) of physical map linked markers to QTL; [c] = Direction of allele contributed to the phenotypic effect: STL108 and RGD309 respectively; [d] = Additive effect of RGD309 allele; [e] = Percentage of phenotypic variance explained (PVE) by the QTL; [f] = Percentage of PVE by the total QTL

4. Evaluation on agronomic characteristics and morphological grain features of the BILs

Comparison of agronomic performance of introgression lines, background STL and two parents was presented in Table 17. The donor parent was photoperiod sensitivity so that the data of donor parent were collected in 2013 wet season. All measured traits of introgression lines were statistically significant difference from donor parent except SN, GB and KB. BILs lines were earlier DF50 than the original STL and same time with recurrent parent STL108. PH, TN and PN were not significant between two parents and progenies. SN of BILs was statistically similar with donor parent and STL. SF% was not significant with recurrent and STL. The morphological grain characters were collected from just only final selected 29 lines**.** GL and KL of the BILs were not significant with both parents, GB and KB was larger than both parents and STL. The mean ratio of GL/GB and KL/KB of BILs were less

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than parents. Mean value of 1000 GW of BILs was similar with recurrent parent. BR% and MR% were a bit less than recurrent parent and STL. In Table 18, the final selected 29 lines of physical characters were compared with both parents (STL108, RGD 309) and original STL.

Table 17 Comparison of agronomic characters and morphological grain features of STL, two parents and BILs cultivated in DS 2014 using analysis of variance

Trait	STL	STL108	RGD309	BILs	F test	5% LSD	CV (%)
DF (50%)	128.11^{a}	114.67^{b}	87^{c}	114.96^{b}	**	3.84	2.1
PH	106.11^{a}	90.78^{b}	88.00^{b}	81.84^{b}	**	11.44	8.6
TN	14.22^{a}	12.00^{a}	14.33^{a}	16.38^{a}	**	6.05	23
PN	14.22^{a}	11.89^{a}	13.67^{a}	16.33^{a}	**	5.95	22.7
PNL	31.31^{a}	26.71^{b}	24.37^{c}	26.67^{b}	**	1.48	3.4
SN	146.53^{b}	154.67^{a}	135.33^{b}	148.19^{b}	**	23.3	9.7
SF%	71.84^{b}	73.25^{b}	87.06^{a}	67.24^{b}	**	8.98	8.1
GL	10.48^{a}	8.97^{b}	8.82^{b}	9.33^{b}	**	0.52	4.5
GB	2.34^{b}	2.24^{b}	2.17^{c}	2.50^{a}	**	0.15	4.9
GL/B	4.48	4.00	4.07	3.74			
KL (mm)	7.69^{a}	6.89^{c}	7.11^{bc}	6.81^{c}	**	0.34	4
KB	2.17^{bc}	2.17^{bc}	2.10^{c}	2.21^{a}	**	0.1	3.8
K L/B	3.55	3.18	3.37	3.09			
TGW	28.31	23.49	22.87	23.86			
BR (%)	77.11	76.91	75.29	74.31			
MR (%)	69.24	68.71	66.42	66.51			

** = Significant at $P < 0.01$ level

In each trait, means followed by the same letter are not significant different at the 5% level of LSD

Traits' abbreviation is as shown in Table 4.

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Table 18 Physical grain characteristics of selected 29 BILs compared with parents

Pedigree	TGW (g)	BR (%)	MR (%)	GL	GB	G L/B	KL	KB	K L/B	Kernel color	Grain texture
RGD11238-38-69-1	23.98	75.87	68.5	9.2	2.4	3.83	6.97	2.19	3.18	W	T
RGD11238-38-69-12	23.42	75.66	68.69	9.09	2.51	3.61	6.66	2.44	2.73	W	T
RGD11238-38-69-14	24.38	77.09	69.19	9.96	2.5	3.99	7.18	2.13	3.38	W	T WB
RGD11238-38-69-21	23.71	75.56	67.74	9.3	2.53	3.68	7.24	2.47	2.93	W	TWB
RGD11238-38-69-4	23.71	73.82	66.53	9.52	2.54	3.75	6.96	2.34	2.97	W	T
RGD11238-38-69-5	24.06	75.46	67.75	9.66	2.38	4.05	7.3	2.26	3.23	W	T
RGD11238-38-69-9	23.15	75.99	67.22	9.52	2.56	3.71	6.95	2.21	3.15	W	T
RGD11238-38-153-1	24.4	71.72	64.85	9.91	2.49	3.97	6.92	2.14	3.24	W	T
RGD11238-38-153-12	22.92	75.48	67.69	9.69	2.61	3.71	6.95	1.98	3.51	W	T
RGD11238-38-153-20	26.34	75.83	69.03	9.95	2.56	3.89	7.32	2.32	3.16	W	T WB
RGD11238-38-153-23	23.31	76.9	68.14	9.38	2.47	3.79	7	2.17	3.22	W	T
RGD11238-38-153-24	24.96	72.63	64.74	9.83	2.46	4	7.1	2.19	3.24	W	T
RGD11238-38-153-8	23.22	74.75	67.59	9.07	2.5	3.64	7.14	2.22	3.22	W	T WB
RGD11238-75-141-10	23.14	70.42	59.19	9.29	2.42	3.84	6.88	2.13	3.23	R	T WB
RGD11238-75-141-15	23.12	72.34	63.45	9.48	2.57	3.69	6.67	2.25	2.96	R	T WB
RGD11238-75-141-21	22.44	72.6	64.67	9.04	2.55	3.55	6.53	2.2	2.97	R	T
RGD11238-75-141-25	23.71	71.18	61.93	9.58	2.48	3.87	6.81	2.17	3.14	R	T

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Table 18 (Continued)

Pedigree	TGW (g)	BR (%)	MR (%)	GL	GB	G L/B	KL	KB	K L/B	Kernel color	Grain texture
RGD11238-75-141-4	21.95	72.52	64.26	8.86	2.48	3.57	6.71	2.1	3.2	R	T
RGD11232-9-23-9	21.15	73.04	64.76	9.3	2.47	3.77	6.21	2.05	3.04	W	T WB
RGD11232-9-35-13	24.3	75.99	68.88	9.19	2.63	3.5	6.33	2.22	2.85	W	T WB
RGD11232-9-35-16	26.18	75.96	68.32	8.79	2.63	3.35	6.78	2.15	3.15	W	T WB
RGD11232-9-35-17	23.96	75.96	68.12	8.67	2.57	3.37	6.47	2.21	2.93	W	T WB
RGD11232-9-35-2	25.26	75.51	68.35	9.16	2.62	3.49	6.68	2.24	2.98	W	T
RGD11232-9-35-7	25.28	72.5	66.51	9.46	2.43	3.89	6.48	2.31	2.81	W	T WB
RGD11232-9-35-9	24.65	71.91	64.86	9.04	2.38	3.8	6.57	2.2	2.99	W	T WB
RGD11238-38-2-3	22.87	75.69	66.94	9.17	2.32	3.95	6.81	2.19	3.11	W	T
RGD11238-38-4-112	24.11	74.32	66.01	9.3	2.58	3.61	6.34	2.19	2.9	W	T
RGD11238-38-4-44	24.32	72.59	65.94	9.1	2.33	3.9	6.72	2.21	3.03	W	T
RGD11238-38-4-85	23.84	75.86	68.91	9.16	2.41	3.79	6.73	2.2	3.06	W	T
STL	28.307	77.11	69.235	10.48	2.34	4.48	7.69	2.17	3.55	W	T
STL108	23.485	76.905	68.705	8.97	2.24	4	6.89	2.17	3.18	W	T WB
RG309	22.8725	75.29	66.415	8.82	2.17	4.07	7.11	2.11	3.37	W	T

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Table 18 (Continued)

Pedigree	TGW (g)	BR (%)	MR (%)	GL	GB	G L/B	KL	KB	K L/B	Kernel color	Grain texture
F test				**	**		**	**			
BILs Mean	23.86	74.31	66.51	9.33	2.5	3.74	6.81	2.21	3.09		
BILs Range	21.15-26.34	70.42-77.09	59.19-69.19	8.67-9.96	2.32-2.63	3.35-4.05	6.21-7.32	1.98-2.47	2.73-3.51		
LSD (0.05 %)				0.52	0.15		0.34	0.1			
CV%				4.5	4.9		4	3.8			

** = Significant at $P < 0.01$ level

W = white, R = red, T = Translucent, TWB = translucent with white belly; TGW = 1,000 grain weight;

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5. Evaluation of cooking quality traits of BILs

Analysis of variance (ANOVA) of the chemical grain quality traits were presented in Table 19. Aroma was evaluated with numerical score 0 to 2 (0 = non fragrance; 1 = mild fragrance; 2 = strong fragrance). Significant differences between the parents were observed for fragrance, AC, GC and ASV. STL and STL108 were non-fragrance and donor RGD309 had strong fragrance (score 2). All BILs were carrying aroma gene from donor parent and almost were scored as mild fragrance (score 1) and some had strong fragrance like as donor.

Both parents were showing high AC (> 25%) and the mean AC value of BILs was indicated as 25.84% and the range from 23.32- 28.62%. Gel consistency measurement of both parents was found as soft (> 60 mm). The mean measurement of BILs was 117.78 mm and the range was 105 - 136 mm that's why all the breeding lines were soft gel consistency according to Cagampang *et al*. (1973). Between the two parents, ASV value was found that significantly different as high GT > 75°C (score 2.9) for STL108 and low GT < 65 °C (score 7.0) for RGD309 respectively. All the BILs were similar with recurrent parent STL108 and the mean value of ASV was found as high GT > 75°C (score 2.95) and the score was ranged as 2.7 - 3.1. The BILs were carrying the aroma gene with mild fragrance from donor parent and similar with recurrent parent as high in amylose content, high gelatinization temperature and soft of gel consistency.

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Table 19 Chemical characteristics of th parents and final selected BILs

	Pedigree	ASS	AC(%)	GC(mm)	ASV
1	RGD11238-38-69-1	1	25.75	117.0	3.0
2	RGD11238-38-69-12	1	25.24	113.0	2.8
3	RGD11238-38-69-14	1	25.61	116.0	3.1
4	RGD11238-38-69-21	1	25.98	122.5	3.0
5	RGD11238-38-69-4	1	26.34	114.0	2.9
6	RGD11238-38-69-5	1	25.53	105.0	3.0
7	RGD11238-38-69-9	1	26.93	129.0	3.1
8	RGD11238-38-153-1	1	25.64	125.5	3.0
9	RGD11238-38-153-12	1	25.28	132.0	2.9
10	RGD11238-38-153-20	1	27.21	110.0	2.8
11	RGD11238-38-153-23	1	26.51	119.5	3.0
12	RGD11238-38-153-24	1	26.23	107.0	3.0
13	RGD11238-38-153-8	1	25.19	112.5	2.8
14	RGD11238-75-141-10	1	25.69	136.0	2.9
15	RGD11238-75-141-15	1	26.46	117.0	2.8
16	RGD11238-75-141-21	2	25.06	108.5	3.0
17	RGD11238-75-141-25	1	27.38	113.0	3.0
18	RGD11238-75-141-4	1	24.27	123.5	3.0
19	RGD11232-9-23-9	2	23.99	133.5	2.8
20	RGD11232-9-35-13	2	26.56	116.5	2.9
21	RGD11232-9-35-16	1	26.38	122.5	3.0
22	RGD11232-9-35-17	1	23.32	111.5	2.8
23	RGD11232-9-35-2	1	26.01	111.5	3.1
24	RGD11232-9-35-7	1	25.51	117.5	3.0
25	RGD11232-9-35-9	1	26.80	117.5	3.0
26	RGD11238-38-2-3	1	24.92	117.0	3.0
27	RGD11238-38-4-112	2	28.62	117.5	3.0
28	RGD11238-38-4-44	1	24.93	118.5	2.7
29	RGD11238-38-4-85	1	25.98	111.0	3.0

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Table 19 (Continued)

Pedigree	ASS	AC(%)	GC(mm)	ASV
STL	0	26.34	110.0	2.8
STL108	0	26.85	117.5	2.9
RG309	2	30.20	89.5	7.0
F test		**	**	**
BILs Mean		25.84	117.78	2.95
BILs Range	1-2	23.32-28.62	105-136	2.7- 3.1
LSD(0.05)		2.33	15.14	0.25
CV (%)		4.4	6.4	4.0

** = Significant at $P < 0.01$ level Ass= Aroma sensory score,
AC(%)= Amylose content percent, GC(mm) Gel consistency,
ASV = Alkali spreading value

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Part II: QTL mapping for salinity tolerance in 188 RILs derived from KDML105 x PK cross

1. Screening for salinity tolerance under soil-culture condition for seedling and reproductive stages

1.1 Seedling stage salt screening with 150 mM NaCl

Screening under 150 mM NaCl solution, RILs lines were significantly different at $P < 0.01$ level for all tested traits. Salt injury score data collected at 10, 16 and 21 DAT of 150 mM NaCl solution are shown in Table 20 and Figure 23. The mean value of SIS score of the RILs were gradually increased during 21 days of treatment. Even the tolerant parent PK was showing moderately tolerance at 16 days after treatment , IR29 susceptible check and parent KDML105 were almost died at score around 8. The mean values of RILs were showing as moderately tolerant to susceptible range 4.58 - 8.42 and 5.17 - 9.00 at 16 and 21 DAT, respectively. While the tolerant parent PK was survived until the maximum value (constant estimated) at 28 DAT, the susceptible parent KDML105 and the susceptible chesks IR29 and RD6 were dead around 19 DAT. The survival days of RILs could be seen the range between 18 - 28 DAT.

PPS also could be seen clearly different between the tolerant and susceptible varieties as 97.2 percent to zero. The mean PPS values of RILs were found as 64.99% and ranged between 0 - 100%. All the traits at seedling stage screening were highly significantly correlated each other as shown in Table 21. As shown in Figure 23, the mean values of tested lines for SIS scores were normal distribution but SvDAT and PPS were negatively (left) skewed distribution. The SvDAT and PPS were showing nagetive correlation with SIS scores (Table 21 and Figure 24).

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Table 20 Trait mean values and phenotypic variation from ANOVA for salinity tolerance of KDML105 x PK RILs at seedling stage

Traits	POK	KD	IR29	RD6	NBK	RIL (KD x PK)		F test	LSD (0.05)	CV (%)
						Mean	Range			
SIS10 (score)	4.11	6.67	7.03	6.49	4.46	4.91	3.42-6.92	**	1.34	18.7
SIS16 (score)	5.08	8.25	8.28	8.13	4.88	6.14	4.58-8.42	**	1.34	16.9
SIS21 (score)	5.86	8.97	9.00	8.97	5.83	7.28	5.17-9.00	**	1.32	14.6
SVDAT (days)	28.0	19.50	19.17	19.36	28.00	25.83	18.33-28.00	**	4.48	12.3
PPS (%)	97.2	0.00	0.00	0.00	95.83	64.99	0 -100	**	34.26	58.3

** = Significant at $P < 0.01$

SIS10, SIS16, SIS21 = salt injury score at 10, 16 and 21 days after treatment, SvDAT = survival days after treatment; PPS = percent of plant survival; PK = Pokkali; KD = KDML105; NB = Nona Bokra

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Table 21 Correlation coefficients between the traits at seedling stage screening for salinity tolerant of KDML105 x PK RILs

	PPS	SIS10	SIS16	SIS21	SVDAT
PPS	1				
SIS10	-0.76**	1			
SIS16	-0.83**	0.89**	1		
SIS21	-0.88**	0.85**	0.94**	1	
SvDAT	0.79**	-0.85**	-0.90**	-0.85**	1

** = Significant at $P < 0.01$

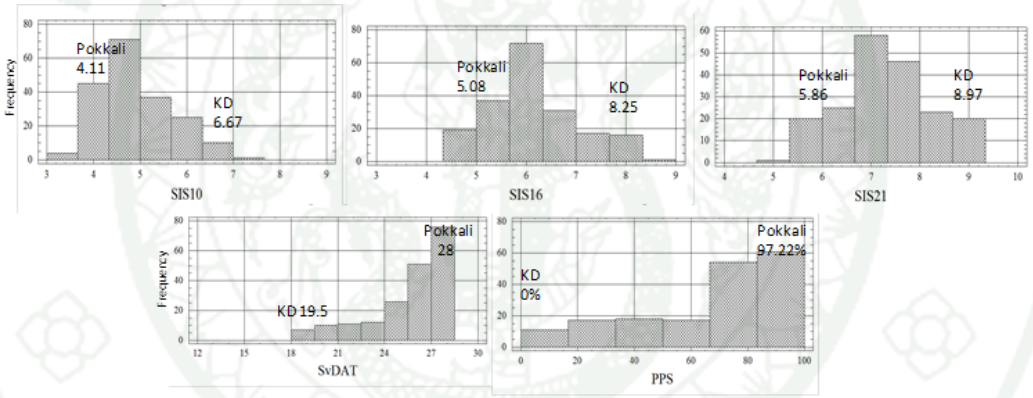

Figure 23 Histogram of Seedling stage characters of KDML105 x PK RILs. SIS10, SIS16, SIS21 = salt injury score at 10, 16 and 21 days after treatment, SvDAT = survival days after treatment, PPS = percent plant survival

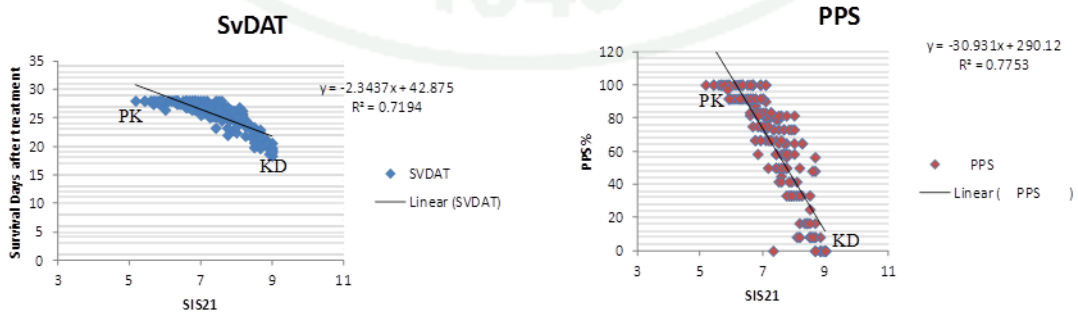

Figure 24 Scatter plots of 188 RILs and parents for SvDAT, PPS and SIS21 at seedling stage screening

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1.2 Rerpoductive stage screening and evaluation of Na^+/K^+ in flag leaf under 100 mM NaCl salt stress

As described in Table 22, all the traits can be seen significantly different among the tested lines. Whereas panicle salt injury scores SISPN of PK and tolerant checks were showing moderately tolerant appearance, the KDML105 and other susceptible checks were showing around score 7, susceptible appearance. The RILs were showing the SISPN range 5.18 - 8.19 and mean value was 7.11 which is significantly different from tolerant PK but not with intolerant KDML105.

Among the mean value of PK, KDML105 and RILs for the traits of PNL, FPNWt, and UFG were not statistically different from each other by comparing with LSD (0.05) value. While the mean value of FSWt, FRWt, DSWt, DRWt, TGWt, FGP, Na^+, K^+ and Na^+/K^+ were significantly different between tolerant PK and intolerant KDML105, mean values of RILs for FSWt and FRWt were siginificantly different from both parents and the values were between parents'. For the DSWt and DRWt values were not significant different between RILs and tolerant PK. For TGWt and FGP, the mean values of RILs were not significantly different from intolerant parent KDML105 but the mean value of RIL for TG number was significantly higher than both parents. Although FBIOM values of two parents were big different and RILs' value was between two parents. For the DBIOM weight, the RILs and tolerant parent PK were not significant but intolerant KDML105 was significantly less than those of tolerant PK and RILs. For DPNWt, the two parents were not significant but the RILs are significantly higher value than both parents.

The Na^+ ion content in flag leaf of PK was significantly less than intolerant parent KDML105 and RILs. K^+ ion content in flag leaf of PK and RILs were not statistically different but KDML105 was significantly different from them. The mean value of Na^+/K^+ ratio was significantly different between two parents and the RILs' mean value was statistically different from intolerant KDML105 but not with tolerant PK. The investigators found linear decreases in several yield components with increased salinity including the percent of sterile florets, tillers per

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plant and spikelets per panicle which translated into larger reductions in grain weight per plant at a given salinity (Grattan *et al.*, 2002; Zeng and Shannon, 2000). Na^+ ion and K^+ ion content in flag leaf traits means were also different among the tolerant and susceptible checks. Although Na^+ content in tolerant varieties, PK, FL530, FL496 and Nonabokra, had only 4 - 7 mg/g, the susceptible checks KDML105, RD6 and IR29 showed more than 10 mg/g. At that time RILs were ranged 2.10 - 23.06. In contrast, K^+ ion content in PK and tolerant varieties were higher than those of the susceptible varieties.

The current result agrees with the finding of Yeo *et al.* (1987); Yadav *et al.* (1996); Ochiai and Matoh (2002) and Calliste Jérémie Diédhiou (2006) who observed that in rice the rate of Na^+ uptake into shoots mediated by the intrusive apoplasticion transport is considerably high under salinity stress and mechanisms of salt tolerance: sodium, chloride and potassium homeostasis in two rice lines with different tolerance to salinity stress. Moreover, Munns and Tester (2008) explained that the osmotic and toxic effect of salt in soil and plants. The osmotic stress not only has an immediate effect on growth, but also has a greater effect on growth rates than the ionic stress. Ionic stress impacts on growth much later and with less effect than the osmotic stress, especially at low to moderate salinity levels. Only at high salinity levels, or in sensitive species that lack the ability to control Na^+ transport, does the ionic effect dominate the osmotic effect.

Na^+/K^+ ratio in flag leaf was higher in susceptible than tolerant parents. This indicates that in susceptible parents absorb salt and transport them immediately to the shoot which is exactly opposite reaction in the tolerant parents. It could be assumed that the tolerant varieties may block the translocation of salt to the shoot. Siangliw (2013) also stated that higher Na^+/K^+ ratio from shoot was found in susceptible varieties (KDML105 and IR29) under two conditions (hydroponics and soil-culture). For root, lower Na^+/K^+ ratio from hydroponic condition was found in susceptible check IR29 while susceptible parent (KDML105) was not high and the opposite was found from shoot obtained from the same treatment. On the other hand, under soil condition, shoot Na^+/K^+ ratio was high in susceptible and low in tolerant

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parents while Na^+/K^+ ratio in the roots was high in tolerant parent and low in susceptible parents. This indicate that in susceptible parents absorb salt and transport them immediately to the shoot which is exactly opposite reaction in the tolerant parents. It could be speculated that the tolerant varieties may block the translocation of salt to the shoot.

Among the traits, SISPN was negatively correlated with DPNWt, FGP, FPWt and positively correlative with UFGP at $P < 0.01$ significant level. DPNWt was positively correlated with DBIOM, FGP, FPNWt, TGN and negatively correlated with not only SISPN also with UFGP at $P < 0.01$ significant level. Na^+ and K^+ ion content in flag leaf value were not correlated with the other traits (Table 23).

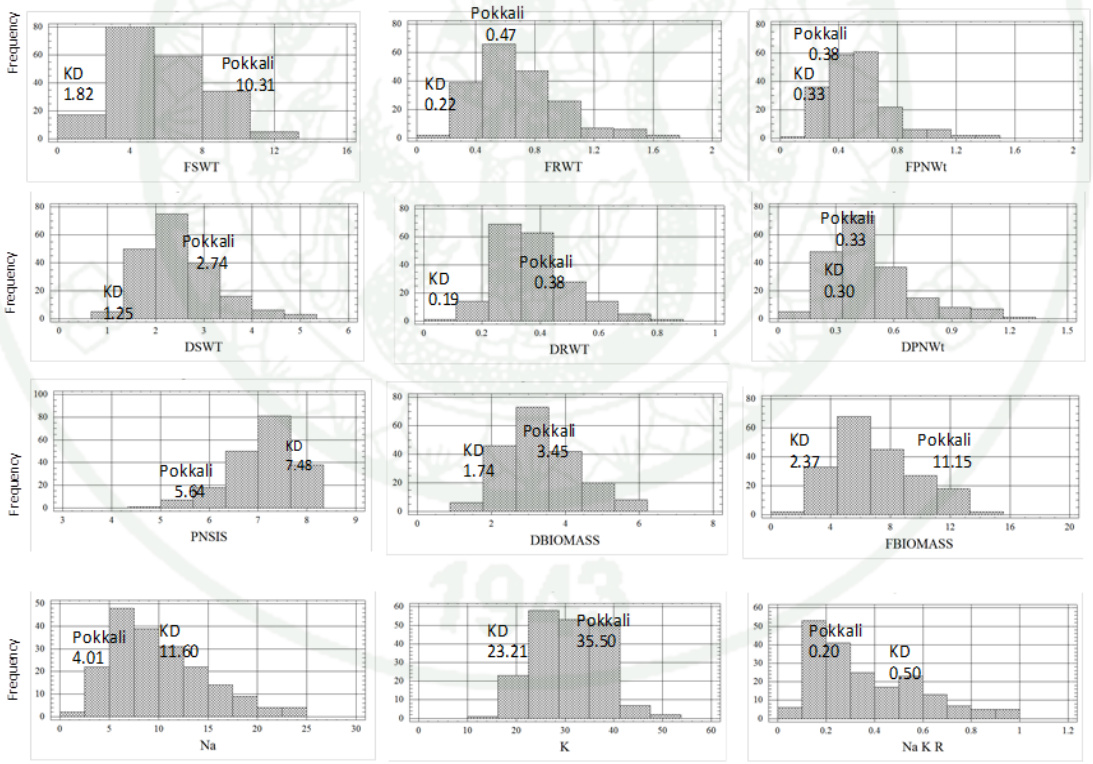

Figure 25 Histogram for the traits of reproductive stage salt tolerant screening for KDML105 x PK RILs at reproductive stage

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Table 22 Traits mean values and phenotypic variation from ANOVA for salinity tolerance of KDML105 x PK RILs under100 mM NaCl at reproductive stage

Traits	PK	FL530	FL496	NBK	KD	RD6	IR29	RIL KD x PK		F test	$LSD_{(0.05)}$	CV (%)
								Mean	Range			
SISPN(Score)	5.64	5.87	5.56	6.52	7.48	7.20	7.58	7.11	5.18-8.19	**	1.15	10.1
PNL(cm)	19.17	17.52	19.27	16.59	15.72	15.11	11.16	17.56	9.37-24.85	**	6.28	22.3
FGP(%)	34.25	27.09	42.15	31.18	23.10	18.14	12.94	25.08	0.76-48.92	**	8.36	20.7
UFG(%)	55.75	62.93	53.97	56.16	66.90	81.63	77.06	69.77	44.03-90.00	**	20.96	18.8
TGWT(g)	35.66	21.85	23.39	25.70	29.00	21.01	23.94	26.05	18.64-31.89	*	6.41	15.3
FSWT(g)	10.31	4.92	4.78	3.64	1.82	4.29	0.84	5.80	1.73-12.89	**	0.71	7.7
FRWT(g)	0.47	0.48	0.43	0.68	0.22	0.33	0.30	0.69	0.15-1.76	**	0.21	19.5
FPNWT(g)	0.38	0.53	0.41	0.78	0.33	0.28	0.22	0.53	0.14-1.45	**	0.21	24.8
DSWT(g)	2.74	2.50	1.87	1.74	1.25	2.02	0.80	2.45	1.15-4.75	**	0.19	4.9
DRWT(g)	0.38	0.38	0.30	0.33	0.19	0.26	0.21	0.38	0.10-0.78	**	0.15	24.5
DPNWT(g)	0.33	0.55	0.83	0.67	0.30	0.25	0.21	0.48	0.12-1.33	**	0.10	13.7
FBIOMASS	11.15	5.93	5.63	5.11	2.37	4.89	1.36	7.02	2.16-14.58			
DRYBIOMASS	3.45	3.43	2.99	2.74	1.74	2.53	1.21	3.30	1.52-5.78			
Na^+(mg/g)	4.01	5.13	6.93	5.64	11.60	10.38	10.91	10.13	2.10-23.06	**	5.37	33.3
K^+(mg/g)	35.50	35.11	48.35	29.21	23.21	24.25	29.37	30.73	15.63-50.23	**	8.84	17.9
Na^+/K^+	0.20	0.29	0.20	0.28	0.50	0.51	0.36	0.39	0.11-.99	**	0.29	50

** = Significant at $P < 0.01$; * = Significant at $P < 0.05$; KD = KDML105; PK = Pokkali; NBK = Nonabokra

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Table 23 Correlation coefficient between the traits of salinity tolerance screening for KDML105 x PK RILs under100 mM NaCl at reproductive stage

	DPNWT	DRWT	DBIOM	DSWT	FBIOM	FGP	FPNWT	FRWT	FSWT	K^+	Na^+	NA/KR	PNL	SISPN	TGWT	UFGP
DPNWT	1															
DRWT	0.4446**	1														
DBIOM	0.7018**	0.6531**	1													
DSWT	0.534**	0.5406**	0.9701**	1												
FBIOM	0.5977**	0.6251**	0.9019**	0.8792**	1											
FGP	0.6366*	0.1562*	0.2691**	0.1327 ns	0.1687*	1										
FPNWT	0.8846**	0.4758**	0.6172**	0.4537**	0.5581**	0.582**	1									
FRWT	0.3763**	0.7943**	0.5127**	0.4148**	0.5381**	0.1224 ns	0.4358**	1								
FSWT	0.5358**	0.5545**	0.8801**	0.8813**	0.9909**	0.1191 ns	0.4747**	0.438**	1							
K+	-0.0516 ns	0.0662 ns	0.0374 ns	0.0521 ns	-0.0155 ns	-0.0308 ns	-0.0693 ns	0.0718 ns	-0.0192 ns	1						
Na+	-0.113 ns	-0.1894**	-0.288**	-0.3053**	-0.2868**	0.0726 ns	-0.0493 ns	-0.1493*	-0.2948**	-0.4224**	1					
NAKR	-0.0528 ns	-0.135 ns	-0.1888**	-0.2046**	-0.1892**	0.0151 ns	-0.0297 ns	-0.1408*	-0.1898**	-0.5578**	0.7545**	1				
PNL	0.5347**	0.4666**	0.7494**	0.7283**	0.6542**	0.1245 ns	0.4576**	0.3818**	0.6363**	0.1079 ns	-0.2244**	-0.1193 ns	1			
SISPN	-0.6477**	-0.2765**	-0.4603**	-0.3552**	-0.3721**	-0.6904**	-0.5849**	-0.1865**	-0.336**	-0.0271 ns	0.0678 ns	0.0958 ns	-0.327**	1		
TGWT	-0.1237 ns	0.1042 ns	-0.0097 ns	0.0063 ns	0.0722 ns	-0.1581*	-0.0738 ns	0.1622*	0.0675 ns	0.1039 ns	-0.0537 ns	-0.0352 ns	0.0873 ns	0.0814 ns	1	
UFGP	-0.7095**	-0.2156**	-0.3893**	-0.2559**	-0.2765**	-0.8426**	-0.6404**	-0.1541**	-0.2292**	0.0546**	-0.0064 ns	0.0426 ns	-0.2464**	0.7645**	0.1892**	1

** Significant at $P < 0.01$; * = Significant at $P < 0.05$

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1.3 Identification of quantitative trait loci (QTL) for salinity tolerance in 188 RILs derived from KDML105 x PK cross

1.3.1 Linkage map and marker segregation

Among 190 SSR markers for parental survey, 129 loci were found as polymorphic between two parents. The percentage of polymorphism in two parents was 67.89 percent. The polymorphic SSR markers were distributed throughout 12 rice chromosomes was shown in Table 24.

Table 24 Parental survey genotyping markers information for KDML105 x PK RILs

Chromosome	Total survey marker (accession)	Polymorphic marker (accession)	Monomorphic marker (accession)	Not PCR amplification (accession)
1	78	38	25	15
2	11	6	2	3
3	14	13	1	0
4	8	7	1	0
5	10	10	0	0
6	11	10	1	0
7	8	7	1	0
8	12	9	3	0
9	13	9	2	2
10	8	6	1	1
11	9	8	1	0
12	8	6	1	1
Total	190	129	39	22

Linkage map was constructed and assigned on the 115 polymorphic markers by Joymap 3.0 version software with distances between markers computed using the Kosambi function. On chromosome1, it can be constructed as two linkage groups as chromosome 1a and 1b, so totally 13 linkage groups were consisted and

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covered with a genomic segment across 1239.512 cM and the average genetic distance of 10.78 cM between adjacent markers (Table 25 and Figure 26).

Table 25 Summary of linkage groups constructed markers and distance of each chromosome

Linkage group	Chromosome	No. of markers	Map distance (cM)
1a	1	21	50.617
1b	1	14	79.684
2	2	5	33.487
3	3	11	192.068
4	4	6	121.980
5	5	8	72.832
6	6	9	180.800
7	7	7	74.900
8	8	9	127.500
9	9	8	70.902
10	10	5	38.086
11	11	7	120.100
12	12	5	76.556
Total = 13		115	1239.512

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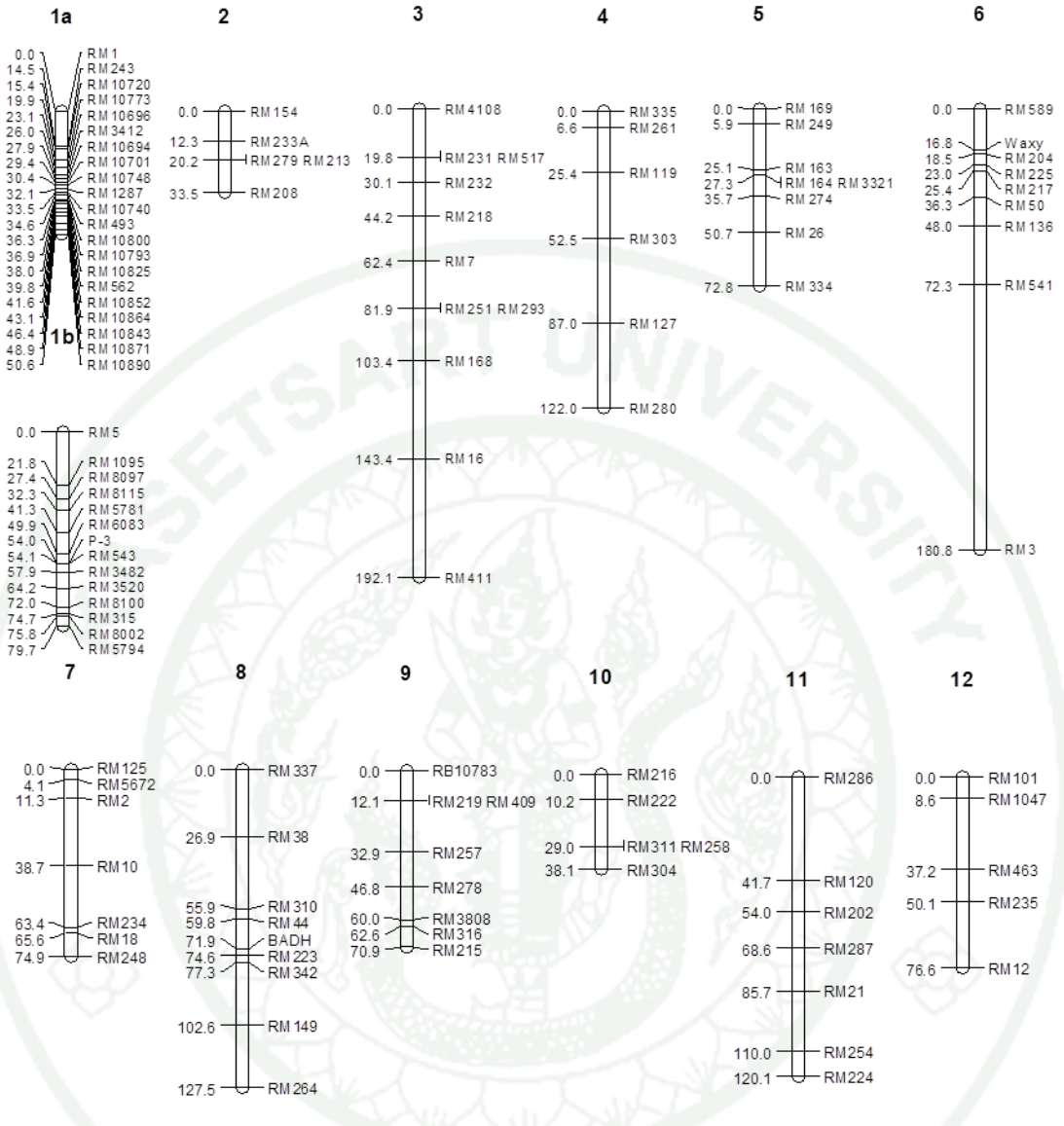

Figure 26 Linkage map for KDML105 x PK RILs

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1.3.2 General QTL analysis

QTL associated with salt tolerance were mapped in three linkage groups namely 1b, 6 and 11. A total 18 putative QTLs were found to be associated with the twelve traits studied using SIM and CIM, of which 11 were detected for seedling stage screening traits (SIS10, SIS16, SIS21, SvDAT and PPS) and seven for reproductive stage screening traits (N^+, K^+content, and Na^+/K^+ ratio in the flag leaf, dry and fresh biomass, dry and fresh shoot weight). The QTLs associated with all twelve salt tolerant traits in this study were found on chromosome 1 as linkage group 1a and 1b. The QTLs for the salt tolerant of seedling stage traits accounted for 9.33 - 18.12% of the phenotypic variations (R^2 or PVE) while the reproductive stage traits accounted for 10.58 - 63.42 of the phenotypic variations percent. Almost of the individual QTLs are accounted for more than 10% of the phenotypic variations. For the total QTL PVE (%) of seedling stage traits were ranged between 13.57 - 28.87 % while reproductive stage traits accounted for 10.58% to 63.42% (Table 26).

1.3.3 QTLs for seedling stage traits

All the QTLs associated with SIS scores (at 10, 16 and 21 days after treatment) traits were found on linkage group 1b and 6. The region of the SIS scores traits QTLs were not far from each other on chromosome 1b (45.6 to 64.2 cM). This result showed the confirmation for partI which also found that SIS scores were linked on chromosome 1 long arm. Moreover, on the chromosome 6, all SIS scores were associated with the same interval region RM217 and RM50 at 34.44 cM. Siangliw (2013) also found that SIS-S and SIS-F were related with RM212 on long arm chromosome 1.

While the QTLs associated with SvDAT were detected on chromosome 1b, and 11, the QTLs for PPS were found on chromosome 1b, 6 and 11 respectively. On the chromosome 1b, those of QTLs associated with SvDAT and PPS were found at the same position at 45.6 cM and the flanking markers were RM5781 and RM6083.

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As described above, the correlation coefficient (Table 21) of seedling stage traits phenotypic values, which were supported by this linkage and interval mapping analysis. Moreover, the similar result with Siangliw (2013) who described that on the long arm of chromosome 1 where *qSt1b* was located, QTL for phenotypic responses to salinity tolerance like SIS, SDS and PPS from soil culture technique were located. This finding was also agreement with Takehisa *et al.* (2004) who tested rice in paddy field treated with salt water. In this report, shoot length was determined as a measure of growth and coincided with the *qSt1b* region.

This study focuses on traits such as SIS, PPS and SvDAT which were final or the last stage symptoms for salinity tolerance at seedling stage. There were many QTLs related to this trait. Some research studied on other traits such as Na^+/K^+ ratio, osmoprotectant (proline, betaine, etc) macromolecules and detoxification. This means that *SKC1* gene controlling Na^+/K^+ ratio alone does not play the major role to salinity tolerance. This evidence was supported by our result wherein QTLs for SIS, PPS and SDS cannot be detected at the region of *SKC1* gene. Therefore, salinity tolerance in rice at seedling stage may be controlled by several genes which correspond to different mechanisms contributing to tolerance ability. However, based on this study, the QTLs related with SIS, PPS, and SDS which were interesting region to focus on for future studies in identifying genes controlling for those traits.

1.3.4 QTLs for reproductive stage traits

The QTLs associated with the Na^+, K^+ ion content and Na^+/K^+ ratio of flag leaf were found on chromosome 1a at the position of 33.54 cM and the LOD scores were 24.66, 16.72 and 31.361 respectively. The PVE were 53.38, 39.04 and 63.42%, respectively. That region is the *Saltol* region. The result of this study is agree with Koyama *et al.* (2001) who found the QTLs for shoot dry weight, ion uptake in the roots, high sodium and potassium uptake and Na^+/K^+ ratio. Chromosomes 1, 4, 6 and 9 were found associated with high Na^+, high K^+ and Na^+/K^+ ratio (Koyama *et al.*, 2001). But Lin *et al.*, (2004) found two QTLs with large effects for shoot Na^+

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concentration and shoot K^+ concentration that were located on chromosome 7. In the same study, root and shoot QTLs were not mapped in the same locations. Physiologically complex traits governed by few quantitative trait loci suits QTL analysis.

QTLs related to K^+ absorption, Na absorption, Na^+/K^+ ratio, shoot and root K^+ and Na^+ concentrations and traits such as survival days of seedlings, seedling vigor scoring and dry mass were mapped in chromosomes 1, 3, 4, 6, 7, 9, 10 and 12 (Zhang *et al.*, 1995; Prasad *et al.* 2000; Koyama *et al.*, 2001; Gregorio *et al.*, 2002; Lin *et al.*, 2004).

Siangliw (2013) supported to our study and who found that QTLs for salinity tolerance in rice at seedling stage under soil-culture condition was found on chromosome 1 while under hydroponic condition was found on chromosome 5 and 12. The major QTL under soil-culture and inland-saline field condition was located on chromosome 1. This QTL was designated as *qSt1b*. This can be used in maker-assisted selection (MAS) in breeding program for salinity tolerance at seedling stage in rice to select the plant showing tolerant ability but not for Na^+/K^+ ratio. This QTL was located in the different position from the *Saltol* region or *qSt1a* QTL which was positioned at 10 Mb on chromosome 1. Thomson *et al.* (2010) supported the importance of *qSt1b*. A QTL mapped on this region related to salinity tolerance at seedling stage was also identified. The QTL for Na^+/K^+ ratio was found located at the upper region of chromosome 1 (at *SKC1* region in *qSt1a*) while for seedling tolerance score or SIS, the location was at *qSt1b*. In this study, lines carrying PK allele at *qSt1b* showed salinity tolerance at seedling stage and it could be that this region indicates a different tolerance mechanism from the *SKC1* region identified by Ren *et al.* (2005).

In this study, on the long arm of chromosome 1(mapped as linkage group1b) QTLs for phenotypic responses to salinity tolerance like SIS, SvDAT and PPS of seedling stage were located. This finding was agreed with Siangliw (2013) and Takehisa *et al.* (2004). QTLs for dry and fresh Biomass and shoot weight of reproductive stage were also located on the long arm chromosome 1. Short arm of

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chromosome 1(*qSt1a*) controls the Na^+ and K^+ content and ratio in the flag leaf. That's why it could be assumed that the chromosome 1 is important for the salt tolerant related QTLs which are associated on not only *Saltol* region on short arm of chromosome 1 but also on long arm at *qSt1b*.

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Table 26 QTL analysis of salt tolerance in RILs derived from KDML105 x PK cross by simple and composite interval mapping (SIM and CIM) via Breeding Management version 2.1.1

Trait[a]	Linkage group	Marker intervals	Position (cM)[b]	LOD[c]	PVE (%)[d]	Add effect[e]	Favourable Allele[f]	Total PVE (%)[g]
Seedling stage screening								
SIS10	1b	RM5781-RM6083	45.60	4.32	12.53	0.25	PK	13.57
	6	RM217-RM50	34.44	4.65	15.06	0.28	PK	
SIS16	1b	RM3482-RM8100	64.2	5.32	11.67	0.28	PK	18.83
	6	RM217-RM50	34.44	4.29	13.43	0.31	PK	
SIS21	1b	RM3482-RM8100	64.20	6.08	13.03	0.032	PK	21.87
	6	RM217-RM50	34.44	5.77	18.12	0.38	PK	
SvDAT	1b	RM5781-RM6083	45.60	3.32	9.33	-0.73	PK	13.91
	11	RM120-RM202	49.90	4.23	13.27	-0.87	PK	
PPS	1b	RM5781-RM6083	45.60	6.65	18.50	-13.35	PK	28.82
	6	RM217-RM50	34.44	4.08	11.50	-10.52	PK	
	11	RM287-RM21	77.20	3.79	13.25	-11.3	PK	

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Table 26 (Continued)

Trait[a]	Linkage group	Marker intervals	Position (cM)[b]	LOD[c]	PVE (%)[d]	Add effect[e]	Favourable Allele[f]	Total PVE (%)[g]
Reproductive stage screening								
Na^+	1a	RM1287-RM493	33.54	24.66	53.38	3.55	PK	53.38
K^+	1a	RM1287-RM493	33.54	16.72	39.04	-4.29	PK	39.04
Na/K	1a	RM1287-RM493	33.54	31.36	63.42	0.18	PK	63.42
DBIOM	1b	RM3520-RM8100	68.08	3.76	10.58	-0.32	PK	10.58
FBIOM	1b	RM3482-RM8100	64.20	4.63	10.90	-0.89	PK	10.9
DSWt	1b	RM3520-RM8100	68.08	3.95	11.17	-0.25	PK	11.17
FSWt	1b	RM3520-RM8100	68.08	5.67	16.46	-0.99	PK	16.46

[a] = trait name under different condition of growth stage; [b] = position of linked markers to QTL; [c] = log-likeihood (LOD) scores;

[d] = percentage of phenotypic variance explained (PVE) by the QTL; [e] = additive effect of Pokkali allele;

[f] = favourable allele for target traits; [g] = percentage of PVE by the total QTL for each trait

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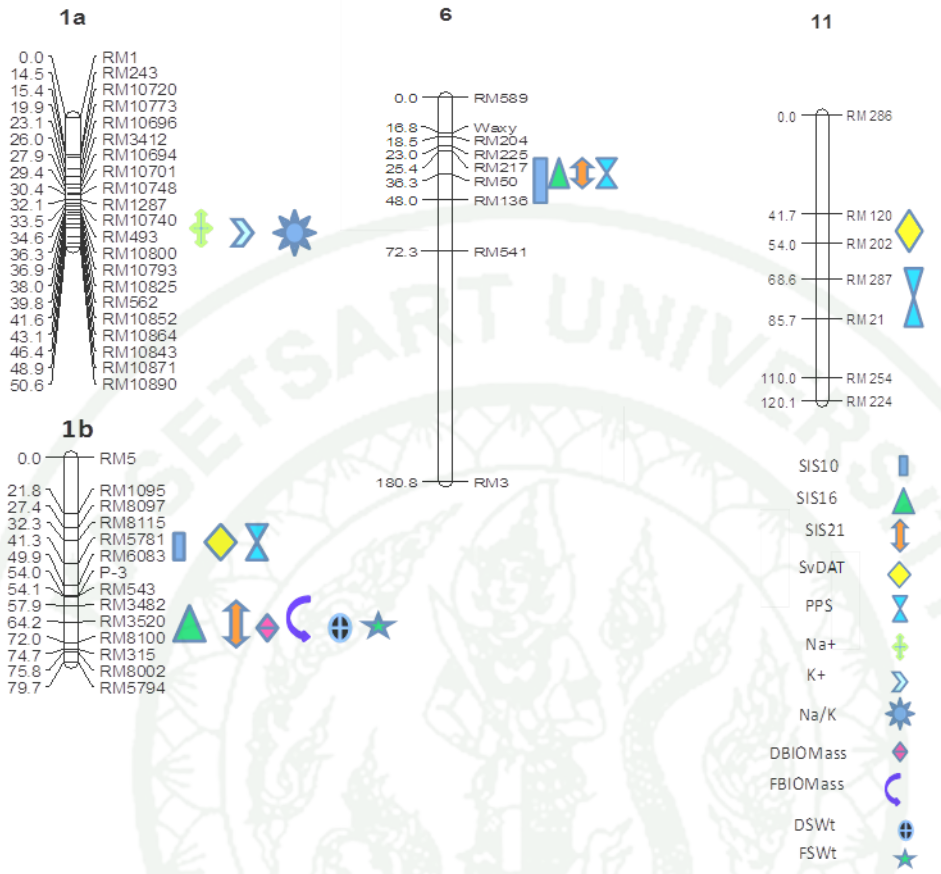

Figure 27 QTL mapping for salt tolerant related traits in 188 RILs of KDML105 x Pokkali population to salt (on chromosome 1, 6 and 11).

SIS10, SIS16, SIS21 = salt injury score on 10, 16, 21 days after treatment (seedling stage); SvDAT = survival days after treatment (seedling stage); PPS = percent plant survival (seedling stage); Na^+ = sodium ion content in flag leaf (reproductive stage); K^+ = potassium ion content in flag leaf (reproductive stage); Na^+/K^+ = sodium and potassium ion ratio in flag leaf (reproductive stage); DBIOMass = dry biomass weight (reproductive stage); FBIOMass = fresh biomass weight (reproductive stage); DSWt = dry shoot weight; FSWt = fresh shoot weight

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Part III: Screening of Myanmar Germplasm for salt tolerance at seedling stage

The 18-days old plants of 242 Myanmar germplasm were treated with 100 mM (12 dS/cm) NaCl solution for 16 days and salt injury was scored following by Gergorio *et al.* (1997) for visual condition. In this experiment, PK, FL530, and FL496 were used as tolerant checks while KDML105 and IR29 were used as susceptible checks. All the tested accessions were highly significant for all characters, plant height (PH), dry shoot weight (DSW), dry root weight (DRW), dry biomass (DBIOM) and dry shoot and root ratio (DSRR), of collected data at stress condition (Table 28). The list of of tested accession and collectd mean data value were described in Appendix Table 1.

Salt injury socore (SIS) at 16 days after stress treatment for Myanmar Germplam could be seen as normal distribution and it was ranged between 4 - 7.6237 (Figure 28 and Figure 29). According to the frequency of SIS16, the mostly tested varieties could be assumed that moderately tolerant (Gergorio *et al.*, 1997). While the tolerant check PK was showing SIS score 4, the susceptible check IR29 was scored at 7.6. Salt Injury Score at 16 days after treatment trait was statistically correlated with DBM, DRW, DSW and PH (Table 27).

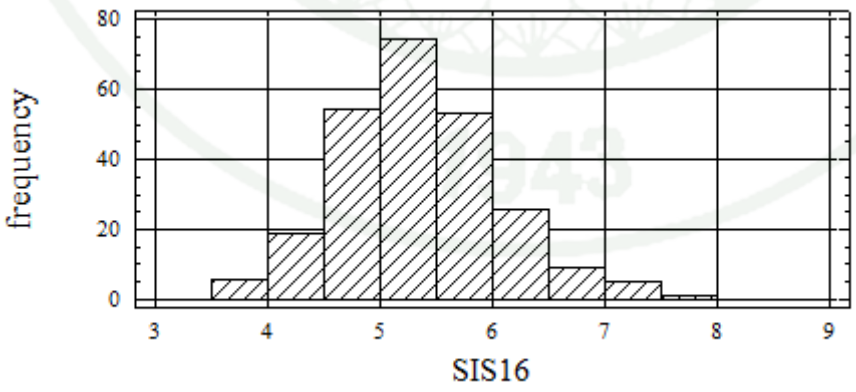

Figure 28 Histogram for salt injury score at 16 days after treatment of Myanmar germplasm

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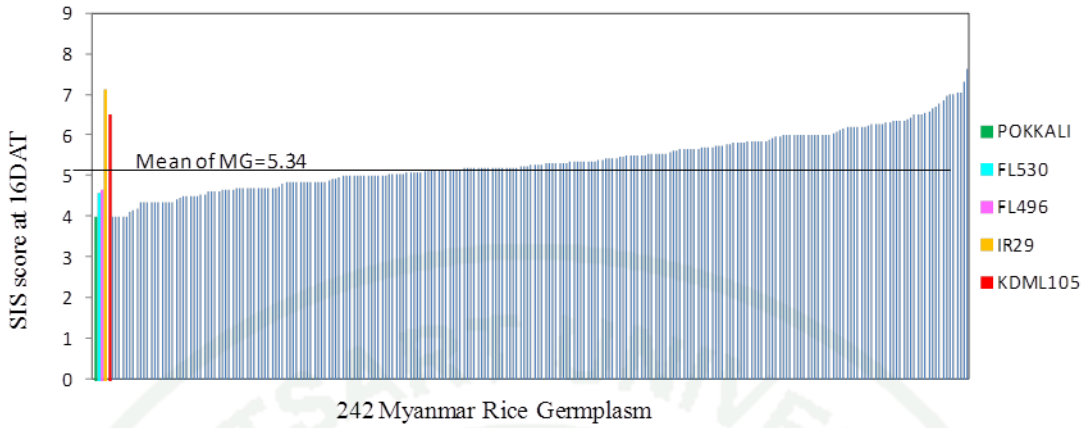

Figure 29 Mean value of Salt Injury Score at 16 days after treatment for tested lines

Table 27 Correlation coefficient for the salt tolerant traits of Myanmar Germplasm

	DBM	DRW	DSRR	DSW	PH	SIS
DBM	1					
DRW	0.82**	1				
DSRR	-0.05ns	-0.56**	1			
DSW	0.76**	0.56**	0.04ns	1		
PH	0.63**	0.38**	0.17*	0.48**	1	
SIS	-0.70**	-0.56**	0.01ns	-0.53**	-0.37**	1

** = Significant at $P < 0.01$

Myanmar germplasm were clusterd by NTSys program for UPGMA cluster based on SIS score. They could be divided as 4 cluster group at 0.06 coefficient level as shown in Figure 30a. Among the tested lines, 5 varieties namely, Blue Gyun Kyauk Kyi, Kauk Kyi Taung Pyan, Nga Kyein Thee, Nga Kywe Yin and Zaw Gyi Pyan (Mee Don) from Ayeyarwaddy Division and Rakhine state were found in the same cluster group with tolerant as the standared tolerant check PK (Figure 30b). Those varieties of PH, DSW, DRW, DBM and DSRR data were not significantly different from control (non salinity) condition analysised by student t test (Table 29).

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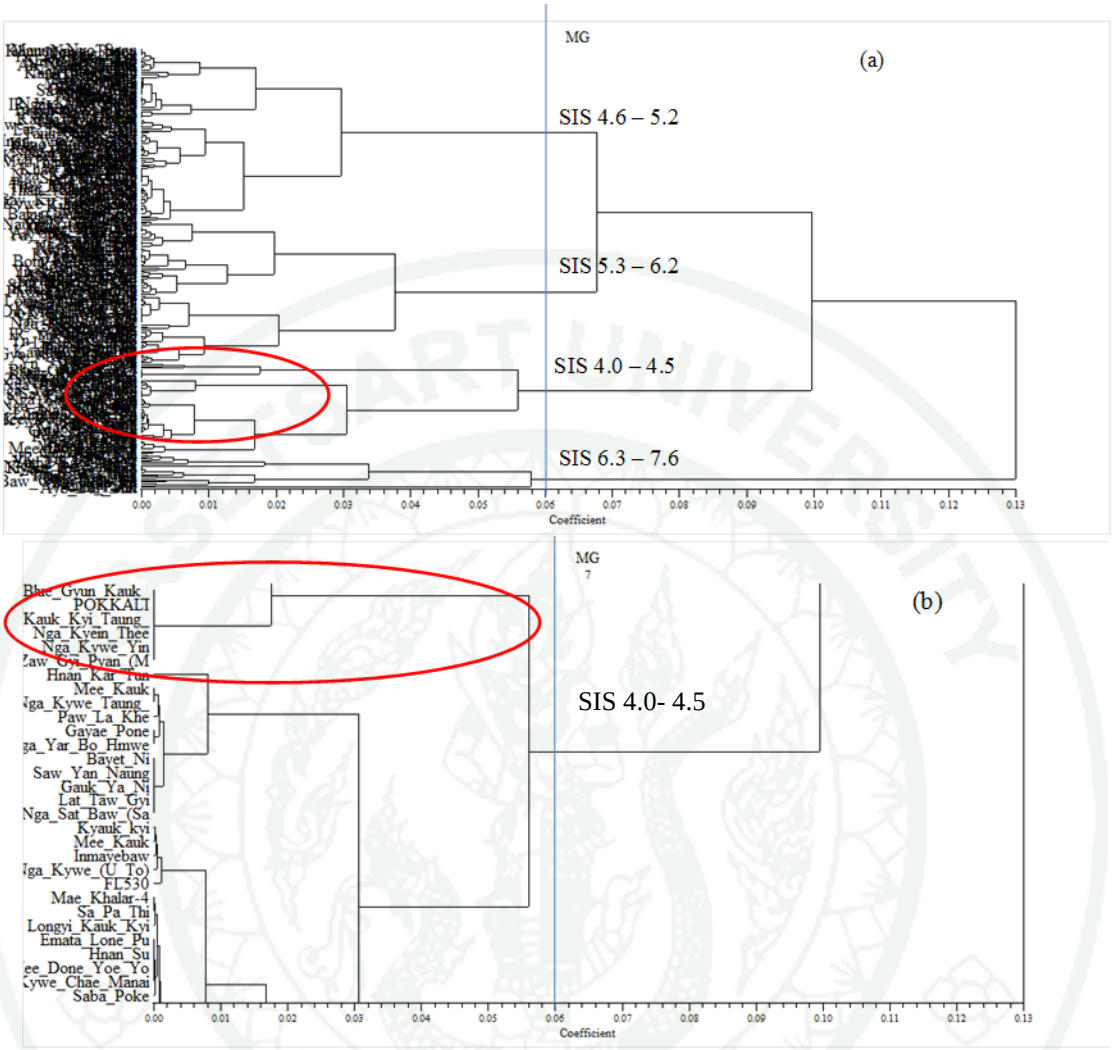

Figure 30 (a) Dendrogram of 242 Myanmar germplasm and 5 check varieties based on similarity of SIS score at seedling stage; (b) Five highest tolerant varieties (Gyun Kyauk Kyi, Kauk Kyi Taung Pyan, Nga Kyein Thee, Nga Kywe Yin and Zaw Gyi Pyan (Mee Don) grouped with Pokkali

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Table 28 Summary of salt tolerant screening for Myanmar germplasm at seedling stage under 100 mM NaCl stress

Germplasm	SIS16DAT	PH(cm)		DSW(g)		DRW (g)		DBM(g)		DSRR	
	S	S	C	S	C	S	C	S	C	S	C
Myanma Germplasm											
Mean	5.37	57.15	72.18	0.2641	0.3138	0.0480	0.0359	0.3088	0.3497	5.8932	10.5644
Range	4.0-7.6	36.89-69.08	38.33-93.00	0.1028-0 .8901	0.099-0.9171	0.0166-0.1078	0.0036-0.1650	0.1072-0.5256	0.1132-1.0271	2.7848-13.125	3.4918-52.5874
PK (T check)	4	70.55	80.33	0.4621	0.4860	0.0969	0.0854	0.5590	0.5714	4.8101	5.6920
IR29 (S check)	7.1	31.67	35.33	0.1113	0.1970	0.0232	0.0095	0.1345	0.2065	4.8788	20.7733
SE	±0.12	±0.10		±0.0221		±0.0069		±0.0260		±0.7864	
F test	**	**		**		**		**		**	
$LSD_{0.05}$	0.3517	2.79		0.0615		0.0191		0.0723		2.1849	
CV (%)	4.1	3.1		14.8		24.8		14.7		23.2	

** Significant at $P < 0.01$

S = salt stress condition, C = control condition; SIS16DAT = salt injury score at 16 days after treatment; PH = plant height; DSW (g) = dry shoot weight (gram); DRW (g) = dry root weight (gram); DBM (g) = dry biomass (gram); DSSR = dry shoot/root ratio

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Table 29 The best five salt tolerant Accessions of Myanmar germplasm under 100 mM NaCl at seedling stage

Germplasm			SIS	PH (cm)		DSW		DRW		DBM		DSRR	
Name	**Region**	**Acc.**	**S**	**S**	**C**	**S**	**C**	**S**	**C**	**S**	**C**	**S**	**C**
Blue Gyun Kauk Kyi	Ayeyarwaddy Divison,	2509	4	58.62	71.17	0.3865	0.397	0.0684	0.0376	0.4549	0.4346	6.0700	10.5492
Kauk Kyi Taung Pyan	Ayeyarwaddy Divison,	3049	4	62.09	79.17	0.3813	0.376	0.0714	0.0482	0.4527	0.4242	5.6680	7.8089
Nga Kyein Thee	Rakhine State	860	4	53.19	69.67	0.3099	0.3454	0.0737	0.0438	0.3836	0.3892	4.1971	7.8952
Nga Kywe Yin	Ayeyarwaddy Divison,	410	4	60.17	66.00	0.3968	0.3575	0.1078	0.0434	0.5046	0.4009	3.8408	8.2362
Zaw Gyi Pyan (Mee Don)	Ayeyarwaddy Divison,	1045	4	58.08	73.33	0.335	0.347	0.0796	0.0584	0.4147	0.4054	4.2373	5.9469
PK			4	70.56	80.33	0.4621	0.486	0.0969	0.0854	0.559	0.5714	4.8101	5.6920
IR29			7	31.67	35.33	0.1113	0.197	0.0232	0.0095	0.1345	0.2065	4.8788	20.7733
Mean				56.34	67.86	0.3404	0.3580	0.0744	0.0466	0.4149	0.4046	4.8146	9.5574
t Stat				-1.57		-0.3289		2.0926		0.1571		-2.3835	
P (T<=t) two-tail				0.15		0.7484		0.0583		0.878		0.0545	
T table value				2.2		2.201		2.1788		2.201		2.4469	
T test				ns		ns		ns		ns		ns	

SIS16DAT = salt injury score at 16 days after treatment; PH = plant height; DSW (g) = dry shoot weight (gram); DRW (g) = dry root weight (gram); DBIOM (g) = dry biomass weight (gram); DSSR = dry shoot/root ratio; ns = non-significant; Acc. = accession number

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Existence of genetic variability for salt tolerance within species is of paramount importance in crop improvement programme. Therefore choice of germplasm in breeding programme is most crucial as the success lies on it. Extensive germplasm collection provides a useful source of genetic diversity for the studied traits (http://www.knowledgebank.irri.org/).The elite salt-tolerant germplasm is the carrier of genetic analysis of the salt tolerance and the breed of variety with resistance to salt stress. Hu *et al.* (2012) disussed the breeders had launched to cultivate the salt tolerance rice variety since 1930s. For example, the high salt tolerance native variety PK, the salt tolerance commercial variety Kala Rata l-24 and hura Rata 4-10 were cultivated in India, the salt tolerance variety BRI, BR 203-26-2 and Sail were from Bengal. Since 1970, the International Rice Research Institute (IRRI) identified 10 salt tolerant varieties including PK, Getu and Nona Bokra from 9,000 rice varieties and pedigree, which provided the technical reserve for the breed of the salt tolerance variety.

In China the salt tolerance variety 80-85 was selected by the Academy of Agricultural Sciences in Jiangsu Province working with IRRI. The Chinese Academy of Agricultural Sciences selected 103 salt tolerance varieties including 27 indica, 76 japonica (the salt tolerance of some varieties were higher than PK) from the 2808 introduced varieties, among which the 81-210, Nonglin72 and American rice achieved large area in coastal area of Jiangsu province. Wu *et al.* (1989) found some salt-tolerant germplasm such as Jiucaiqing, Laohuangdao, Huangjingnuo and Hongmangxiangjingnuo from the native japonica in Taihu Lake Basin. China National Rice Research Institute has also screened out some salt tolerance varieties such as Mang Rice3, Hair Rice, Big Mang Rice and Sorghum rice.

From this experiment, we can find out some of the salt tolerant accessions from Ayeyarwaddy division and Rakhine state. Those tolerant accessions are already adapted in salt affected area in Myanmar and they are prospective for future breeding programs but should be learned more about genotypic and other agronomic characters.

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CONCLUSION

Increasing number of environmental stresses tolerant genes, mapping for the useful genes, and molecular marker assisted breeding using valuable genetic information are very wise and efficient strategies in rice improvement breeding programs. The main goal of our study is to develop rice variety STL for combining the stress tolerant and good quality by molecular breeding. Within four years, twenty nine introgression lines of STL background were successfully developed using MAS from BC_2F_5 population. The BILs are carrying the *Saltol*, *Sub1*, *Xa21* and *badh2* genes with same quality to recurrent parent STL108. Although the BILs are carrying the homozygous allele with partens for target QTL and genes, they were found as variation in trait validation experiments. Therefore we did genome scan for 92 BILs on 12 rice chromosomes with 135 SSR markers to find out the putative QTLs which were detected by single marker analysis on physical map. Salt tolerant related traits such as SIS, SvDAT, PPS are linked with long arm chromosome 1 and Na^+, K^+ ion content and Na^+/K^+ ratio in shoot were linked around *SKC1* region on short arm chromosome 1. Therefore the salt tolerant traits will be controlled by many genes and chromosome 1 is important for detection of Salt tolerant traits. Our finding was supported by the previous findings that related with salinity tolerant research works. The selected BILs will be carried to Myanmar and continuously grown in target areas: submergence and salinity prome areas in Myanmar to confirm this improved STL variety for its introgressed traits.

Then needed to confirm the result of putative QTLs from above part I, linkage map was constructed and assigned on 115 SSR markers distributed on 12 rice chromosome of 188 F_6 RILs derived from KDML105 x PK population based on seedling and reproductive stage salt stress experiments. There was some gaps between the markers on some chromosomes and should put more markers. The reproductive stage experiment had some weak points such as we could only study for main tiller due to the space of experimental site and it was very critical care to put into the stress at reproductive stage so we would like to suggest that the whole plant should be

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learned in the future study. From this study totally 18 putative QTLs were detected by simple and composite interval mapping (SIM and CIM) via Breeding Management version 2.1.1 program whereas 11 QTLs were related with seedling stage traits (such as SIS score at 10, 16, and 21 days after treatment, SvDAT, and PPS) and 7 for reproductive stage (such as N^+, K^+ content and Na^+/K^+ ratio in flag leaf, Dry and Fresh Biomass, Dry and Fresh shoot weight). All the traits were linked with chromosome 1. However, the QTLs of SIS, SvDAT and PPS for seedling stage were linked at a long arm of chromosome 1 *qSt1b*. N^+, K^+ content and Na^+/K^+ratio of flag leaf at reproductive stage were linked with *SKC1* region. The QTLs results could be confirmed to the finding of part I results. Therefore, it can be concluded that the salinity tolerance in rice may be controlled by several genes which correspond to different mechanisms contributing to tolerance ability. Moreover, we can say that the chormosome1 is important for salt tolerant related QTLs which are associated with not only *Saltol* and *SKC1* region on short arm of chromosome 1 but also on long arm at *qSt1b*.

A total of 242 Myanmar germplasm, from Department of Agricultural Research (DAR), Seed Bank,Yezin, Myanmar, were subjected to salinity evaluation at seedling stage under 100 mM NaCl for 16 days. Based on their salt injury score (SIS), 4 varieties from Ayeyarwaddy Division and 1 from Rakhine, totally 5 varieties were found as the same injury score with standard tolerant check PK. It is needed to learn more for the other salt tolerant related traits. The best five local varieties are prospective as they are already adapted in the salt affected area, nevertheless further studies should be continued to understand their genomic and other agronomic characters.

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APPENDIX

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Appendix Table 1. Mean value of Myanmar Germplasm salt screening

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
1	Blue Gyun Kauk Kyi	2509	AYWD	4.00	58.62	0.3865	0.0684	0.4549	6.0700
2	Kauk Kyi Taung Pyan	3049	AYWD	4.00	62.09	0.3813	0.0714	0.4527	5.6680
3	Nga Kyein Thee	860	RK	4.00	53.19	0.3099	0.0737	0.3836	4.1971
4	Nga Kywe Yin	410	AYWD	4.00	60.17	0.3968	0.1078	0.5046	3.8408
5	Zaw Gyi Pyan (Mee Don)	1045	AYWD	4.00	58.08	0.3350	0.0796	0.4147	4.2373
6	Kamar Kyi	6193	RK	4.11	61.28	0.3606	0.0726	0.4332	4.9698
7	Gauk Ya	2119	AYWD	4.15	56.11	0.3529	0.0589	0.4118	6.1041
8	Hnan Kye	419	AYWD	4.18	64.10	0.3075	0.0515	0.3590	5.9780
9	Bayet Ni	1973	AYWD	4.32	63.49	0.4258	0.0676	0.4934	6.4596
10	Saw Yan Naung	6034	RK	4.32	56.06	0.3364	0.0653	0.4017	5.2703
11	Gauk Ya Ni	6175	RK	4.32	59.67	0.3493	0.0622	0.4115	6.2933
12	Lat Taw Gyi	6180	RK	4.32	62.11	0.3259	0.0685	0.3945	4.7558
13	Nga Sat Baw (Saw Si)	6190	RK	4.32	64.89	0.3694	0.0829	0.4523	4.5286
14	Paw La Khe	556	AYWD	4.33	59.58	0.3363	0.0714	0.4077	4.7085
15	Mee Kauk	1209	AYWD	4.33	62.04	0.3018	0.0416	0.3434	8.7397
16	Nga Kywe Taung Pyan	1179	AYWD	4.33	58.36	0.3528	0.0379	0.3907	9.3179
17	Gayae Pone	1592	RK	4.34	65.00	0.3518	0.0772	0.4290	4.5566
18	Nga Yar Bo Hmwe Shay	6305	RK	4.34	62.87	0.4297	0.0959	0.5256	4.4876
19	Hnan Kar Tun	495	AYWD	4.40	59.92	0.2513	0.0418	0.2931	6.0475
20	Paw San	6323	RK	4.47	60.00	0.3102	0.0462	0.3564	6.6947
21	Gauk Ya Kyi	6195	RK	4.48	59.33	0.3596	0.0656	0.4252	5.4821
22	Aung Ze Ya	500	AYWD	4.50	62.62	0.3875	0.0694	0.4568	6.1252
23	Bauk Thar	6158	RK	4.50	58.72	0.3130	0.0557	0.3687	5.6085
24	Bahan Hmwe	1158	AYWD	4.50	61.50	0.3237	0.0580	0.3817	5.8177
25	Mae Khalar-3	1215	AYWD	4.50	62.68	0.3300	0.0554	0.3854	6.1272
26	Mee Done Hmwe	2117	AYWD	4.52	59.28	0.2587	0.0775	0.3362	3.3455
27	Ant Baw	464	RK	4.52	63.33	0.3315	0.0457	0.3772	7.2519
28	Nga Kywe (U To)	1105	AYWD	4.60	61.89	0.2914	0.0657	0.3572	4.4469
29	Kyauk kyi	499	AYWD	4.60	62.92	0.2999	0.0490	0.3489	6.2029

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
30	Mee Kauk	2152	RK	4.60	59.80	0.2937	0.0395	0.3332	7.4245
31	Inmayebaw		U	4.60	60.29	0.3535	0.0692	0.4227	5.1323
32	Pone inn yenet		U	4.65	62.60	0.3752	0.0701	0.4453	5.3310
33	Let Yone	797	RK	4.66	66.39	0.3346	0.0544	0.3890	7.0658
34	Det Kwa	2386	RK	4.66	60.44	0.3779	0.0438	0.4218	8.6237
35	Longyi Kauk Kyi	1842	AYWD	4.67	58.83	0.3000	0.0635	0.3636	4.8202
36	Mae Khalar-4	741	AYWD	4.67	51.33	0.2403	0.0516	0.2919	4.6333
37	Sa Pa Thi	6170	RK	4.67	55.69	0.3214	0.0679	0.3893	5.2804
38	Saba Poke	6134	RK	4.67	56.89	0.2558	0.0749	0.3307	3.4578
39	Emata Lone Pu	815	AYWD	4.67	54.37	0.2463	0.0467	0.2930	5.3285
40	Hnan Su	1618	AYWD	4.67	52.03	0.2099	0.0242	0.2341	8.8531
41	Mee Done Yoe Yoe	1953	AYWD	4.67	60.01	0.3045	0.0628	0.3673	5.0148
42	Kywe Chae Manaing	2462	AYWD	4.67	67.18	0.2735	0.0389	0.3124	7.2865
43	Lone Pu	1503	AYWD	4.68	57.17	0.2463	0.0463	0.2926	5.4065
44	Gauk Ya Chay	2131	AYWD	4.68	53.69	0.3233	0.0747	0.3980	4.4673
45	Ma Kye Mon	2438	AYWD	4.68	62.44	0.2856	0.0412	0.3269	6.9842
46	Nga gayar		U	4.68	60.44	0.3213	0.0522	0.3735	6.1517
47	Mae Khalar-2	1024	AYWD	4.68	43.49	0.2087	0.0399	0.2486	5.2089
48	Aungzeya		U	4.71	67.79	0.3828	0.0292	0.4120	13.1250
49	Shwe Hnya Oo	220	RK	4.81	58.27	0.2826	0.1051	0.3877	2.7848
50	Maung Nyo Sann	318	AYWD	4.82	58.00	0.2913	0.0450	0.3363	6.6901
51	Khun Naya Thee Hyat	2106	AYWD	4.82	55.90	0.2694	0.0443	0.3137	6.1619
52	Nyaung Aine	774	MDY	4.82	63.31	0.3033	0.0577	0.3609	5.3236
53	Lone Phyu	6040	RK	4.82	61.72	0.3200	0.0627	0.3827	5.1117
54	Yn 2995-6-2-2-3		U	4.82	47.39	0.2330	0.0566	0.2896	4.3388
55	Gon Ban	2507	AYWD	4.83	58.72	0.3315	0.0632	0.3947	5.2415
56	Hteik Pyaung	1582	AYWD	4.83	60.89	0.1585	0.0310	0.1895	5.1636
57	Nga Cheik	360	MDY	4.83	67.28	0.3147	0.0440	0.3587	7.2324
58	Ah Nauk Thama	731	RK	4.84	59.67	0.2695	0.0486	0.3181	5.5510
59	Ar BaungNi	6303	RK	4.84	51.00	0.2663	0.0830	0.3493	3.3370
60	Paw Tun	297	AYWD	4.84	56.56	0.3144	0.0550	0.3694	6.9808
61	Nga Kyee Ma	1853	RK	4.85	59.61	0.2674	0.0643	0.3317	4.1463
62	De Pu Zun	6197	RK	4.89	53.17	0.2728	0.0637	0.3365	4.2882

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
63	Tamasoe	6039	RK	4.91	57.08	0.2770	0.0582	0.3352	5.2401
64	Khaohlaing		U	4.91	58.24	0.3102	0.0591	0.3693	5.5988
65	Kauk Yar Shay	1988	RK	4.94	48.00	0.2305	0.0413	0.2718	5.9114
66	Kyauk Thwe	1520	AYWD	4.98	60.39	0.2728	0.0398	0.3127	7.0547
67	Khun Ni Shay	1852	RK	4.98	57.11	0.3068	0.0605	0.3673	5.0711
68	Kalai	1467	AYWD	5.00	64.83	0.3031	0.0362	0.3393	8.4224
69	Myaung Mya	2516	AYWD	5.00	43.00	0.1802	0.0249	0.2051	7.4045
70	Leik Kalay	1640	SG	5.00	62.48	0.3645	0.0420	0.4064	9.1750
71	Kauk Win	817	MDY	5.00	60.28	0.4079	0.0816	0.4895	5.1799
72	Ok Shit Pyu	1401	MDY	5.00	57.50	0.2431	0.0383	0.2814	6.5683
73	SawYan Aung	6302	RK	5.00	56.71	0.2388	0.0437	0.2825	5.5014
74	kauk Thwe	206	AYWD	5.00	58.06	0.2634	0.0514	0.3148	5.2220
75	Tadaung Po	217	AYWD	5.00	62.56	0.2689	0.0456	0.3145	6.1781
76	Ekarinkwa		U	5.00	58.67	0.2618	0.0490	0.3108	5.3264
77	Bay Kyaung	1106	AYWD	5.00	62.56	0.2547	0.0502	0.3049	6.5662
78	IR Yn 1047-56-534-UL20		U	5.01	37.61	0.1884	0.0518	0.2402	3.6315
79	Saba Ni	1395	MDY	5.02	52.77	0.2998	0.0545	0.3543	5.9048
80	Tu Maung	290	AYWD	5.02	65.11	0.3034	0.0500	0.3534	6.1174
81	Nga Kywe Phyu	348	AYWD	5.02	60.78	0.3080	0.0606	0.3686	5.2112
82	Meegauk		U	5.02	62.28	0.2692	0.0539	0.3231	4.9662
83	Let Ywe Zin Sein Tha Htay	2122	AYWD	5.03	60.22	0.2242	0.0296	0.2538	7.6958
84	Baw Kyan	3050	AYWD	5.07	60.22	0.2374	0.0233	0.2607	10.247
85	Paw San Hmwe		U	5.08	52.72	0.2772	0.0524	0.3295	5.3422
86	Kauk hnyin phyu		U	5.08	62.41	0.3431	0.0617	0.4048	5.7404
87	Lathar		U	5.08	60.61	0.3169	0.0676	0.3845	4.7062
88	Yn 3159-15-2		U	5.08	42.78	0.1964	0.0478	0.2442	4.4727
89	Khao san hwan		U	5.12	62.62	0.3203	0.0736	0.3939	4.6851
90	Watitun		U	5.15	45.22	0.3175	0.0798	0.3973	4.5334

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
91	Bahat Ni	481	AYWD	5.16	58.79	0.3001	0.0404	0.3405	7.4542
92	Gay Ku Kama Kyi	2515	AYWD	5.16	60.31	0.2934	0.0770	0.3705	3.8082
93	Kywe Chae Manaing	346	AYWD	5.16	58.06	0.1911	0.0319	0.2230	6.0577
94	Ywe Saba		U	5.16	64.56	0.3193	0.0783	0.3976	4.1124
95	Kamar Kyi	2384	AYWD	5.17	61.59	0.2845	0.0447	0.3292	6.3930
96	Than Taung Thama	480	RK	5.17	63.72	0.2869	0.0487	0.3357	5.9712
97	ZamBut	1600	RK	5.17	60.50	0.2813	0.0575	0.3388	4.9744
98	Mi khinta		U	5.17	65.06	0.3939	0.0821	0.4760	7.1328
99	Nat Pyi		U	5.17	55.56	0.2648	0.0595	0.3243	4.7365
100	Hnan Kar	334	AYWD	5.17	59.39	0.2689	0.0484	0.3173	5.8180
101	Khun War	1069	AYWD	5.18	58.12	0.2149	0.0433	0.2582	4.9580
102	Shwe Ni	1509	SG	5.18	62.30	0.2892	0.0690	0.3582	4.1910
103	Nga Kywe Dume	850	MDY	5.18	61.60	0.2724	0.0559	0.3283	4.8760
104	Zum Bute	6173	RK	5.18	61.83	0.3278	0.0382	0.3660	8.8238
105	SoeYan Aung	6176	RK	5.18	62.44	0.3030	0.0696	0.3726	4.3815
106	Ma Po Lay	285	AYWD	5.18	59.50	0.2016	0.0168	0.2184	12.014
107	Mayin		U	5.18	60.39	0.2724	0.0414	0.3138	6.5290
108	Paw san bay kyar		U	5.18	61.94	0.2843	0.0419	0.3262	6.8506
109	Kyauk Head	227	RK	5.19	61.00	0.3508	0.0772	0.4280	4.5456
110	Thee Dat Pya Pon	984	AYWD	5.19	62.33	0.3201	0.0593	0.3794	5.6119
111	Balu Gyun Nga Sein	1499	AYWD	5.20	58.80	0.2796	0.0400	0.3196	6.9946
112	Mai Saw	2286	SG	5.20	62.37	0.2676	0.0502	0.3178	5.3310
113	Bahat Ni	2454	AYWD	5.21	58.44	0.2666	0.0435	0.3101	6.3180
114	In Gyin Su	214	SG	5.21	58.78	0.2624	0.0360	0.2985	7.3663
115	Sann Put	707	RK	5.21	57.40	0.2102	0.0428	0.2530	4.9154
116	Kyaw Ze ya	1177	AYWD	5.21	40.55	0.2129	0.0303	0.2432	7.0200
117	Shwe Nan Gyi	1779	RK	5.22	56.22	0.2866	0.0632	0.3498	4.5365
118	Khun War	221	AYWD	5.23	58.34	0.2552	0.0479	0.3031	5.5037
119	Sit pwa phyu		U	5.25	63.21	0.2173	0.0309	0.2482	7.2690

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
120	Khao pha yaung		U	5.26	66.11	0.3881	0.0709	0.4590	5.5871
121	Naga New	109	RK	5.28	66.22	0.3021	0.0501	0.3522	6.1923
122	Khunayapo		U	5.28	57.72	0.3243	0.0461	0.3704	7.5321
123	MyoThant Kauk Ya	6157	RK	5.29	56.47	0.2207	0.0567	0.2774	3.8020
124	Hnankar		U	5.30	57.63	0.3169	0.0419	0.3588	7.5798
125	Kywe Chae Manaing	449	AYWD	5.31	56.87	0.2370	0.0347	0.2717	6.8330
126	Ma war phyu		U	5.32	62.60	0.2997	0.0578	0.3575	5.5005
127	Sut Phoe	2380	RK	5.32	61.00	0.2969	0.0450	0.3419	6.6012
128	Nga Yar Nga Se	365	AYWD	5.32	65.53	0.3319	0.0517	0.3836	6.4198
129	Nga kyee dume		U	5.32	54.72	0.2563	0.0435	0.2998	6.2351
130	Ban Pa Lay	583	RK	5.32	57.17	0.2041	0.0460	0.2501	4.4347
131	Poke Thwin Phyu	6169	RK	5.32	66.61	0.2719	0.0361	0.3080	7.5102
132	Hnan War Mee Don	2640	SG	5.34	66.39	0.3122	0.0731	0.3853	4.7581
133	Shwe At	1849	RK	5.34	60.71	0.3256	0.0660	0.3916	4.9472
134	Saba Net	163	MDY	5.34	64.41	0.2962	0.0511	0.3473	5.7960
135	Ton Kin	700	AYWD	5.35	59.50	0.1899	0.0229	0.2128	8.2941
136	Mya Sein	1891	AYWD	5.35	61.36	0.2384	0.0305	0.2689	7.8484
137	Khao mon montine		U	5.36	65.57	0.3133	0.0650	0.3783	4.8232
138	Let Ywe Zin Ma	267	AYWD	5.37	62.28	0.2852	0.0589	0.3441	4.8513
139	Mwe Swe Kauk Hnyin	1143	AYWD	5.40	60.23	0.2361	0.0513	0.2874	4.6495
140	Kauk Hmwe	2312	AYWD	5.40	59.20	0.2884	0.0402	0.3286	7.1731
141	Let Yone Gyi	869	AYWD	5.40	55.89	0.2912	0.0551	0.3463	5.4620
142	Khao mon		U	5.42	38.83	0.1665	0.0296	0.1961	5.6197
143	Sin new yin		U	5.42	41.56	0.2548	0.0398	0.2946	6.9688
144	Taung hteik pan		U	5.44	41.11	0.1982	0.0300	0.2282	6.7034
145	Saing Law	251	MDY	5.46	60.34	0.2324	0.0407	0.2731	5.8926
146	Sit Pwa	740	AYWD	5.48	58.56	0.2464	0.0483	0.2947	5.1055
147	Moke Seik Kyi	2511	AYWD	5.50	62.50	0.3100	0.0300	0.3400	10.329
148	Gyar Khun Ni	1923	SG	5.50	58.92	0.2371	0.0366	0.2736	6.6256

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
149	Thit Pin	2356	RK	5.50	59.39	0.3188	0.0548	0.3735	5.8196
150	As Shay Tha Ma	2624	RK	5.50	60.53	0.2892	0.0453	0.3345	6.3301
151	Yay Ma Naing Sit Pwa	953	AYWD	5.51	58.17	0.2679	0.0736	0.3414	3.7032
152	Pya Tha Let	820	RK	5.52	56.47	0.2436	0.0306	0.2742	7.9894
153	Nyaung aine		U	5.52	57.94	0.2584	0.0299	0.2883	8.8119
154	Laung Satt	2285	RK	5.53	64.67	0.2492	0.0398	0.2890	6.2000
155	LetYone Gyi	286	AYWD	5.53	57.18	0.2020	0.0305	0.2325	6.6959
156	MR 230		U	5.53	45.11	0.2641	0.0512	0.3153	5.1681
157	Hay Wun Dar	6098	RK	5.55	57.71	0.2668	0.0623	0.3291	4.2800
158	Yezin lone thwe		U	5.59	42.22	0.1682	0.0305	0.1986	5.6351
159	Kala Gyi	625	AYWD	5.60	59.78	0.2760	0.0427	0.3187	6.4655
160	Naung Tu Mee She	197	MDY	5.61	59.93	0.2670	0.0678	0.3348	3.9792
161	Bahat Ni	456	RK	5.64	59.00	0.2000	0.0303	0.2303	6.5639
162	Kauk Thwe Phyu	2268	SG	5.66	57.88	0.2368	0.0300	0.2668	7.8976
163	In Poke	6100	RK	5.66	58.56	0.2300	0.0301	0.2601	8.0301
164	Khun Ni Ma	2411	AYWD	5.66	61.57	0.2468	0.0424	0.2892	5.8247
165	Na Ma Tha Lay	2283	SG	5.67	49.94	0.1688	0.0388	0.2076	4.4999
166	SPR 86035-52-5-11		U	5.67	42.94	0.1859	0.0507	0.2366	3.7815
167	Let Ywe Zin	2320	SG	5.68	59.09	0.3125	0.0395	0.3520	8.6864
168	Phya Pon Thee Dat	622	AYWD	5.68	59.17	0.2505	0.0541	0.3046	4.6283
169	Lone Pu Gyi	228	AYWD	5.68	60.73	0.1960	0.0358	0.2318	5.9230
170	Shwe tasoke		U	5.70	67.34	0.3081	0.0546	0.3627	5.6370
171	Yn 2841-UL26		U	5.71	37.24	0.1325	0.0305	0.1630	4.3676
172	Deik Kyauk Kyi	1986	AYWD	5.73	56.96	0.2561	0.0424	0.2985	6.8367
173	Nat pyi hmwe		U	5.74	57.06	0.2065	0.0345	0.2410	6.0428

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
174	Khao kaing lan		U	5.75	69.08	0.3423	0.0534	0.3957	6.3848
175	Ta Yay Gyi	528	MDY	5.78	54.50	0.1906	0.0405	0.2311	4.7136
176	Kyee Kan Ma	2115	AYWD	5.82	54.75	0.2311	0.0336	0.2647	7.9034
177	Lu Pyo Gyi	6174	RK	5.82	59.22	0.2307	0.0452	0.2759	5.1720
178	Yebawlatt		U	5.82	57.06	0.2296	0.0505	0.2801	4.6165
179	Ynda nar toe		U	5.82	47.56	0.1982	0.0407	0.2388	5.0696
180	Bom Ma de Wa	482	SG	5.83	54.00	0.2044	0.0454	0.2498	4.5980
181	Pa chee phyu		U	5.83	59.10	0.1957	0.0372	0.2330	5.2599
182	Sein Gyi	385	AYWD	5.83	62.56	0.2484	0.0461	0.2945	5.8375
183	Naung htu		U	5.84	61.28	0.2966	0.0494	0.3461	6.0206
184	Mae Khalar-1	884	AYWD	5.84	45.71	0.1758	0.0386	0.2144	4.5479
185	Hnan Paw	490	RK	5.84	60.64	0.2311	0.0307	0.2618	7.5944
186	Yn 2991-2-1-1-3		U	5.89	44.11	0.2405	0.0258	0.2663	10.431
187	Shan Nyein		U	5.92	53.06	0.2200	0.0452	0.2652	4.9636
188	Mae Khalar-5	885	AYWD	5.96	56.56	0.2363	0.0298	0.2661	7.9934
189	Pyi Daw Aye	424	AYWD	5.97	55.39	0.2002	0.0409	0.2411	5.0497
190	Pakistan Hmwe	810	AYWD	5.98	61.06	0.2655	0.0371	0.3026	7.1395
191	Lone Thwe Hmwe	1175	AYWD	5.98	58.70	0.2378	0.0425	0.2803	6.8163
192	Yn-3106-10-3-1		U	5.98	42.90	0.1686	0.0299	0.1985	6.2194
193	Zine Din	644	RK	5.99	47.44	0.1403	0.0237	0.1640	6.1555
194	Kywet Thwa Gyi	1561	AYWD	6.00	59.41	0.3436	0.0598	0.4034	5.7265
195	Bo Daw Gyi	2111	AYWD	6.00	58.97	0.2588	0.0534	0.3122	5.0640
196	Du Me (Hnan Ka)	2123	AYWD	6.00	54.59	0.1970	0.0317	0.2288	6.5965
197	Kyauk San Gyi	2477	AYWD	6.00	55.82	0.1161	0.0166	0.1327	6.9758
198	Ekarin	382	RK	6.00	58.56	0.2670	0.0468	0.3138	5.7209
199	Ohn Ni Ma	576	RK	6.00	57.50	0.2006	0.0541	0.2547	3.7202
200	Ani Pyu Lar	1778	RK	6.00	57.01	0.1890	0.0291	0.2181	6.7073

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
201	Sa Gauk	299	AYWD	6.00	60.44	0.2362	0.0352	0.2714	6.7782
202	Nga Kyein Thee Shay	457	AYWD	6.00	56.98	0.2286	0.0300	0.2586	7.6183
203	Yn-2684-1-6-1-3-5		U	6.00	40.37	0.1376	0.0297	0.1673	4.6586
204	Shwe din gar		U	6.04	51.09	0.2054	0.0389	0.2443	5.2849
205	Sin thwe yin		U	6.09	40.61	0.1329	0.0305	0.1634	4.4825
206	Shan malay		U	6.10	61.71	0.2055	0.0359	0.2414	5.8087
207	Lin Ban Chaw	2316	AYWD	6.17	58.00	0.2068	0.0406	0.2474	5.1008
208	Pin Toe Sein	1458	SG	6.17	56.66	0.2928	0.0640	0.3568	5.1722
209	Tay Lay	523	SG	6.18	56.83	0.2459	0.0501	0.2960	4.9213
210	Pathein hmwe		U	6.18	60.06	0.3147	0.0514	0.3661	6.1665
211	Gyo Kyar Nauk Kone	6177	RK	6.20	53.72	0.2218	0.0709	0.2927	3.3426
212	Kaukhnyin Khunni		U	6.21	56.76	0.1834	0.0425	0.2260	4.3073
213	Yaza Hut	2291	RK	6.21	58.19	0.2107	0.0313	0.2420	7.3237
214	Ta Taung Po	612	AYWD	6.25	56.87	0.2590	0.0478	0.3069	5.8738
215	Ekarin		U	6.25	52.50	0.1699	0.0368	0.2067	5.2372
216	Siam Halas	940	AYWD	6.26	52.72	0.1880	0.0371	0.2251	5.3946
217	Thukha yin		U	6.26	38.11	0.1339	0.0298	0.1637	4.6242
218	Yn 3107-33-2-3		U	6.27	47.44	0.1857	0.0315	0.2172	5.8843
219	Naung Oke	1904	SG	6.32	58.61	0.1895	0.0427	0.2322	4.4478
220	IR Yn 1068-149-8-2		U	6.32	37.94	0.1625	0.0373	0.1998	4.5437
221	Ye Ma Naing	650	RK	6.33	55.40	0.1773	0.0447	0.2220	3.9976
222	Kywet thaw		U	6.33	53.62	0.1591	0.0241	0.1832	6.6856
223	Yn -2744-50-390-1-2-3		U	6.33	42.56	0.1596	0.0276	0.1872	6.1414
224	Na Ri The	2276	AYWD	6.34	61.40	0.2113	0.0345	0.2458	6.2787
225	Nga Kyuk	1908	SG	6.37	54.06	0.2103	0.0473	0.2576	4.4444
226	Pauk War Yin	513	AYWD	6.44	56.98	0.1964	0.0312	0.2276	6.2991
227	Kyone Pyaw Mae	924	AYWD	6.50	56.62	0.1893	0.0372	0.2265	5.0995
228	So Kalay Thee	600	RK	6.50	53.72	0.1625	0.0252	0.1876	6.6088
229	Lin Ban Chaw	2315	SG	6.51	58.06	0.2018	0.0387	0.2404	5.2019

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Appendix Table 1. (Continued)

	Name	Acc	Region	SIS	PH	DSWE	DRWE	DBME	DSR R
230	Bokehmwegyi		U	6.56	58.94	0.2354	0.0618	0.2972	4.5118
231	Ya Thae	567	SG	6.59	60.99	0.2477	0.0303	0.2780	8.9752
232	Nga Yar Po	1773	SG	6.66	40.94	0.8901	0.0182	0.1072	4.9031
233	Ohn Pauk	1914	SG	6.68	55.56	0.2302	0.0435	0.2737	5.2903
234	Mi Khin Ta	222	SG	6.78	57.80	0.1675	0.0390	0.2065	4.2774
235	Baw Gyun Nga Sein	2336	AYWD	6.83	57.07	0.1801	0.0293	0.2094	6.2013
236	Yatha Lay Lone Thwe	333	AYWD	6.96	49.71	0.1674	0.0265	0.1939	7.1677
237	Baw Gyi	537	AYWD	7.00	43.26	0.2065	0.0337	0.2401	6.8735
238	IR Yn 1068-7-1		U	7.01	37.83	0.1721	0.0343	0.2064	5.0991
239	Balarkyar	1290	AYWD	7.03	54.94	0.1743	0.0217	0.1960	8.1428
240	Yn 2997-2-4-1-1		U	7.06	36.89	0.1251	0.0223	0.1474	5.6567
241	Ni Ka Re San Pyu	2280	RK	7.29	57.50	0.1028	0.0212	0.1240	4.8486
242	Aye Yar Min	1252	AYWD	7.62	42.62	0.1137	0.0209	0.1346	6.1537

Acc=Accession, AYWD= Ayeyarwaddy Division (Delta region) , SG= Sagaing Division (Central Myanmar dry zone area) , MDY= Mandalay Division (Central Myanmar dry zone area)
RK= Rakhine Sate (Costal region) , U= Unknown region SIS= Salt Injury Score at 16Days after treatment, PH= Plant Height , DSW= Dry Shoot Weight, DRW=Dry Root Weight, DSR R= Dry Shoot and Root weight Ratio

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