

IDENTIFICATION AND CHARACTERISATION OF QUANTITATIVE TRAIT
LOCI LINKED WITH TOLERANCE TO SALINE-ALKALINE STRESS AT
GERMINATION STAGE IN *INDICA* RICE (*Oryza sativa* L.)

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Specially dedicated to my father Umar Hayat Khan Niazi
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ABSTRACT

As the direct seeding of rice is getting popular, the farmers have shifted from traditional transplantation method to direct seeding method. The problem with this method however is the direct exposure of seeds to abiotic stresses that affect crop establishment. Studies have not been conducted so far to improve the germination tolerance in saline-alkaline conditions. Therefore, the present study was conducted to perform the genomic analysis of tolerance to saline-alkaline stress at germination stage in rice. Parental varieties *Pokkali* and *Pak Basmati* were selected out of six rice varieties on the basis of their performance in saline-alkaline conditions. A mapping population (F_{2:3}) was constructed using *Pokkali* as tolerant and *Pak Basmati* as sensitive parent. Germination parameters such as final germination percentage (FGP), germination value (GV), germination energy (GE), germination velocity (GVe), speed of germination (SG), peak value (PV), germination capacity (GC), germination index (GI) and mean germination time (MGT) while growth parameters like germination vigour index (GVI), shoot length (SL), root length (RL) and total dry biomass (DBM) were recorded. Parental varieties were optimised for saline-alkaline stress limits. Phenotyping of F₃ progenies was conducted with the saline-alkaline limits (pH8/15 dS.m⁻¹) optimised in parental varieties. Correlation studies show that germination and growth parameters are positively correlated. It was also found that growth parameters were affected more than germination parameters. The genotypic data of 129 F₂ plants for 84 polymorphic markers was used to construct molecular linkage map, with an average interval size of 7.63cM with four gaps of \leq 40cM and the total length of 3435.5cM. Quantitative Trait Loci (QTL) Cartographer was used for genomic analysis using three mapping techniques i.e. simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM). Thirty-three QTLs (17 major and 16 minor) were identified using SIM. Forty QTLs (14 major and 26 minor) were identified by CIM. Sixty QTLs were identified using MIM technique. The parental varieties used in this study are potential candidates for abiotic stress studies. These findings would be beneficial in rice breeding programs to develop tolerant cultivars for the saline-alkaline environment through marker assisted selection.

ABSTRAK

Memandangkan pembenihan tabur terus padi semakin popular, petani telah beralih dari kaedah menanam secara tradisional ke kaedah tabur terus. Masalah dengan kaedah ini bagaimanapun adalah pendedahan benih secara terus kepada tekanan abiotik telah menjejaskan pertumbuhan tanaman. Sehingga kini kajian untuk meningkatkan toleransi percambahan dalam keadaan masin-alkali tidak pernah dijalankan. Oleh itu, kajian ini dijalankan untuk melaksanakan analisis genom toleransi kepada tekanan masin-alkali pada peringkat percambahan dalam padi. Jenis ibu bapa *Pokkali* dan *Pak Basmati* telah dipilih daripada enam jenis padi berdasarkan prestasi mereka dalam keadaan masin-alkali. Pemetaan Populasi ($F_{2:3}$) telah dibina menggunakan *Pokkali* sebagai toleran dan *Pak Basmati* sebagai induk yang sensitif. Percambahan parameter seperti peratus percambahan akhir (FGP), nilai percambahan (GV), percambahan tenaga (GE), halaju percambahan (GVe), kelajuan percambahan (SG), nilai puncak (PV), kapasiti percambahan (CA), indeks percambahan (GI) dan min masa percambahan (MGT) manakala parameter pertumbuhan seperti indeks percambahan tenaga (GVI), tempoh bertunas (SL), tempoh asal (RL) dan jumlah biojisim kering (DBM) telah direkodkan. Saluran induk telah dioptimumkan untuk had tekanan masin-alkali. Penelitian lapangan (*Phenotyping*) progeni F_3 telah dijalankan dengan had masin-alkali ($\text{pH}8 / 15 \text{ dS.m}^{-1}$) yang dioptimumkan dalam jenis ibu bapa. Kajian korelasi menunjukkan bahawa percambahan dan pertumbuhan parameter berkorelasi secara positif. Ia juga mendapati bahawa parameter pertumbuhan lebih terjejas daripada parameter percambahan. 129 data genotip tumbuhan F_2 untuk 84 penanda polimorf telah digunakan untuk membina peta rangkaian molekul, dengan saiz selang purata sebanyak 7.63cM dengan empat jurang sebanyak $\leq 40\text{cm}$ dan jumlah panjang sebanyak 3435.5cM. Ciri Kuantitatif Loci (QTL) telah digunakan untuk analisis genom menggunakan tiga teknik pemetaan iaitu Pemetaan Selang Mudah (SIM), Pemetaan Selang Komposit (CIM) dan Pemetaan Selang Berganda (MIM). Tiga puluh tiga QTLs (17 utama dan 16 kecil) telah dikenal pasti menggunakan SIM. Empat puluh QTLs (14 utama dan 26 kecil) telah dikenal pasti oleh CIM. Enam puluh QTLs telah dikenal pasti menggunakan teknik MIM. Jenis ibu bapa yang digunakan dalam kajian ini adalah calon berpotensi untuk kajian tekanan abiotik. Hasil kajian ini memberi manfaat dalam program pembenihan padi untuk membangunkan kultivar toleran untuk persekitaran masin-alkali melalui bantuan penanda pilihan.

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LIST OF ABBREVIATIONS

$\mu\text{mol.m}^{-2}.\text{s}^{-1}$	-	Micromole per meter square. per second
1N	-	One Normal
AFLP	-	Amplified Fragment Length Polymorphism
AFLPs	-	Amplified Fragment Length Polymorphisms
ANOVA	-	Analysis of Variance
APS	-	Ammonium persulfate
BC	-	Backcross
BC1	-	First Backcross Generation
BC ₁ F ₂	-	Second Backcross Generation after selfing
bp	-	Base pair
C	-	Codominant
Ca ²⁺	-	Calcium ion
Chr	-	Chromosome
Chr No	-	Chromosome Number
CIM	-	Composite Interval Mapping
Cl ⁻	-	Chloride anion
cm	-	Centimeter
cM	-	centiMorgan
CO ₃ ²⁻	-	Carbonate ion
CTAB	-	Cetyl Trimethyl Ammonium Bromide
D	-	Dominant
DBM	-	Dry Bio Mass
df	-	Degree of Freedom
DH	-	Double Haploids
DH	-	Double haploid

DHLs	-	Double Haploid Lines
DNA	-	Deoxyribonucleic Acid
dS.m ⁻¹	-	deciSiemens per meter
EC	-	Electric Conductivity
EC _e	-	Electrical Conductivity
EDTA	-	Ethylenediaminetetraacetic acid
E-QTLs	-	Epistatic Quantitative Trait Loci
ESP	-	Exchangeable Sodium Percentage
F ₁	-	First filial generation
F ₂	-	Second filial generation
F _{2:3}	-	Second filial generation advanced to third filial generation
F _{2:4}	-	Second filial generation advanced to fourth filial generation
F ₃	-	Third filial generation
FAO	-	Food and Agriculture Organization
FGP	-	Final Germination Percentage
Fwd	-	Forward
g	-	Gram
GC	-	Germination Capacity
GE	-	Germination Energy
GI	-	Germination Index
GV	-	Germination Value
GVe	-	Germination Velocity
GVI	-	Germination Vigour Index
ha	-	Hectare
HCl	-	Hydrogen Chloride
HCO ₃ ⁻	-	Bicarbonate ion
IM	-	Interval Mapping
IRRI	-	International Rice Research Institute
IRRI	-	International Rice Research Institute
K ⁺	-	Potassium ion
kb	-	kilo base

LED	-	Light emitting diode
LOD	-	Logarithm of Odd
LRT	-	Likelihood Ratio Test
MapQTL	-	Mapping Quantitative Trait Loci
MAS	-	Marker Assisted Selection
Mg ²⁺	-	Magnesium ion
MgCl ₂	-	Magnesium Chloride
mgkg ⁻¹	-	milligram per kilogram
MGT	-	Mean Germination Time
MIM	-	Multiple Interval Mapping
min	-	Minute
mM	-	milliMolar
mmol	-	Millimolar
MPa	-	Mega Pascal
M-QTLs	-	Main effect Quantitative Trait Loci
Na ⁺	-	Sodium ion
NaCl	-	Sodium chloride
NaCl	-	Sodium Chloride
NaOCl	-	Sodium Hypochlorite
ngμl ⁻¹	-	Nano gram per microliters
NO ₃ ⁻	-	Nitrate ion
OD	-	Optical Density
<i>p</i>	-	Significance Level
P ₁	-	Parent 1 used in crossing
P ₂	-	Parent 2 used in crossing
PAGE	-	Poly Acrylamide Gel Electrophoresis
PCR	-	Polymerase Chain Reaction
pH	-	Power of hydrogen
PIC	-	Polymorphism information content
PV	-	Peak Value
QTL	-	Quantitative Trait Loci
<i>r</i>	-	Pearson correlation
R ²	-	Phenotypic Variation

Rev	-	Reverse
RFLP	-	Restriction Fragment Length Polymorphism
RILs	-	Recombinant Inbred Lines
RL	-	Root Length
RM	-	Rice Marker
sec	-	Second
SG	-	Speed of Germination
SIM	-	Simple Interval Mapping
SL	-	Shoot Length
SNPs	-	Single Nucleotide Polymorphisms
SO ₄ ²⁻	-	Sulfate ion
SSRs	-	Simple Sequence Repeats
TBE	-	Tris Boric acid EDTA buffer
TE	-	Tris EDTA buffer
TEMED	-	Tetramethylethylenediamine
Tris-HCl	-	Tris hydrochloride
V or v	-	Version
WinQTLCart	-	Windows Quantitative Trait Loci Cartographer
χ^2	-	Chi Square
μL	-	Microliters

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world. It is essential source of staple food for more than 2.7 billion people all around the globe, most of them living in developing countries (FAO, 2016). It is cultivated on one-tenth of the arable land of the planet earth. By the year 2025, a total increase of 21% would be needed to meet the food requirements compared to the needs in year 2000 (Bhowmik *et al.*, 2007). Second only to wheat, rice production contributes to almost 20% of the total cereal crop production of the world (Acquaah, 2007). Rice is second biggest crop produced all over the world with a total of 740.95 million tons produced from 163.1 million hectares at an average of 4.54 tons of rice per hectare (FAO, 2010). In addition to its importance as one of the biggest sources of food, rice is rich in genetic diversity as there are thousands of varieties and accessions all over the world (Ammar *et al.*, 2007). There are two main methods being practised for rice cultivation. Traditional transplanting system (TPR), an important plant establishment technique where pre-germinated seedlings are transferred to the fields (Farooq *et al.*, 2007). The seedlings are provided ideal growth conditions before being transplanted. The second method is direct seeding of rice (DSR) which entails broadcasting the seeds directly through hands or machines (Farooq *et al.*, 2011). Both these methods have their own pros and cons.

TPR requires labour and time, and it is economically expensive as well. Handling the pre-germinated seeds is difficult (Farooq *et al.*, 2007). While DSR has a major drawback that seeds are directly exposed to abiotic stresses (Farooq *et al.*,

2011). Cultivation areas are shrinking because of rapid urbanization and industrialization (Jiang and Li, 2016). Therefore, utilisation of less productive or saline-sodic marginal soils would be a suitable option to bring under cultivation by improving the crops tolerance to saline-alkalinity. Ever increasing population demands higher amounts of rice and other food crops. This can be achieved by producing high yielding varieties, by expanding the total area presently under cultivation or by following the latest management practices to increase the overall rice production. However increased population and natural disasters causing a direct threat to the food security (Gardi *et al.*, 2015; Gardner, 1996; Nath *et al.*, 2015) hence expanding the cultivated areas is becoming hard. There are variety of abiotic and biotic stresses affecting the food production adversely, in general and rice production in particular. Only in Asia these stresses cause a total of 23% of production decrease compared to its full potential (Hossain, 1997). Salinity is second biggest abiotic stress condition after drought, in rice cultivating areas. Thirty percent of total rice grown area all over the world is severely affected by high salinity levels. These levels adversely affect the normal growth of rice plants.

A total of 1.5×10^9 ha land area is cultivated all over the world out of which 23% area is saline and another 37% are sodic in nature (Shi and Wang, 2005). The cations present in the saline and sodic soils are Na^+ , Ca^{2+} , Mg^{2+} and K^+ and anions Cl^- , SO_4^{2-} , HCO_3^- , CO_3^{2-} and NO_3^- are present. Studies have shown that alkali stress results mainly from certain levels of sodium bicarbonate and sodium carbonate (Shi and Yin, 1993). Quite a lot of literature is available on salt stress (Munns and Tester, 2008) while a little attention was paid to the saline-alkaline stress. Saline-sodic soils occur within the boundaries of 75 countries and the area under saline-alkalinity is gradually increasing (Qadir *et al.*, 2007). Sodic and saline-sodic soils are grouped together because of sharing same characteristics and the management practices (Qadir *et al.*, 2007). Rice seeds are affected by these abiotic stresses when broadcasted directly through DSR. It was observed that alkalinity was more damaging than salinity at germination stage (Guo *et al.*, 2010). Plants accumulate more Na^+ at the expense of K^+ . They also start accumulating soluble osmolytes which are compounds affecting osmosis. Severe inhibition in wheat root and shoot under saline-alkaline conditions has also been reported (Guo *et al.*, 2010). Grain

security is becoming a major concern and urbanization is one of the factors behind food crisis. Effective land use planning is critical for food security but soil erosion, land degradation and climatic changes are causing a rapid increase in marginal lands (Kang *et al.*, 2013). Scherr, (1999) has emphasized on the reduced annual yields and role of soil quality in overall crop production and also emphasized on the need to improve the soil quality. We have marginal soils i.e. the soils with problems but we do not have the varieties that could grow there because we do not have much knowledge or data about tolerance to abiotic stress. Because of these factors, the crops are becoming low yielding. Already cultivated area is shrinking because of growing salinity, sodicity and drought (Valipour, 2014).

A prudent measure to address the salinity and sodicity issue is to develop tolerant varieties. The recent developments in the field of quantitative genetics have greatly influenced the study of complex quantitative traits and this has made it possible to dissect the complex quantitative traits or polygenes for certain traits to Mendelian factors. Identification of loci controlling genetic variations in segregating populations has become possible with the use of molecular markers and QTL analysis technology. Characterisation of these loci according to their position on genetic map, their mode of action, phenotypic and pleiotropic effects and epistatic interactions with other QTLs have also become possible (Dufey *et al.*, 2015; Ogawa *et al.*, 2016; Zhao *et al.*, 2016). Several studies have been conducted for salinity tolerance in rice at different stages (Heenan *et al.*, 1988b; Kumar *et al.*, 2015; Rahman *et al.*, 2016), however, 37% of the total cultivated land is sodic as well, and it is urgently needed to see and dissect combined effects of salinity and sodicity. Rice is considered as the moderately salinity sensitive cereal crop plant which can tolerate saline levels of 4-8 dS.m⁻¹ (Sairam and Tyagi, 2004).

The genetic variation that a rice plant possesses makes it suitable candidate for quantitative trait studies. This variation helps not only in understanding the mechanisms behind biotic and abiotic stress studies but also to develop new varieties with improved abiotic and biotic stress tolerance. There have been efforts to develop salt tolerant rice varieties (Flowers, 2004; Khan *et al.*, 2016; Lutts *et al.*, 1995). Genetic variability for salinity tolerance has been reported among a large number of

economically important crops including rice (Flowers, 2004). This genetic information about salt tolerance is essential for developing salt tolerant rice cultivars. This information can later be used in marker assisted selection (MAS) and plant breeding studies to exploit the identified genomic regions known as quantitative trait loci (QTLs).

Saline soils have high pH levels (alkaline) and affect the uptake of nutrients and limit the germination. Studies to improve tolerance to saline-alkaline stress in rice at germination stage has not been conducted yet. QTL identification is another way to speed up breeding tolerant rice varieties through marker assisted selection. Present study focused on genetic dissection of saline-alkaline tolerance mechanisms.

Farmers follow two methods for rice cultivation; transplantation and direct seeding of rice (Singh, 2013). Transplantation is done both manually and mechanically where seedlings are provided with the ideal growth conditions and are transferred to the field when seedlings are 3 to 4 weeks old (Farooq *et al.*, 2007) depending on type of rice varieties. Therefore, it is considered expensive because of labour (Coelli *et al.*, 2002) and water use (Bhushan *et al.*, 2007). Reduced tillage and direct seeding on flat land and raised beds can result in significant water savings (Gupta *et al.*, 2003). Thus, direct seeding is an efficient and economical rice planting option. Plants grown by direct seeding get mature 7-10 days earlier than those transplanted (Farooq *et al.*, 2011).

1.2 Problem Statement

Rapid urbanization and industrialization resulted in labour shortage for the rice transplantation from nurseries to the irrigated paddy fields. It motivated the farmers to shift from transplantation method to direct seeding of rice. Direct seeding of rice is also suitable for the farmers because they can obtain maximum plant density and productivity with lesser labours and low economic inputs. Direct seeded rice reduces the crop water requirements, soil organic-matter turnover, nutrient

relations, carbon sequestrating, weed biota and greenhouse-gas emissions (Farooq *et al.*, 2011). Therefore, direct seeding is more popular among farming community. Majority of the saline soils are alkaline in nature (Vega-Jarquín *et al.*, 2003). When direct seeding method is used where the soils are affected with salinity or alkalinity the seed is exposed directly to the stresses. These abiotic stresses inhibit the seed germination adversely affecting the crop establishment (Vinocur and Altman, 2005). There are two ways to address this problem. One of them is to do the chemical amendments to improve saline-alkaline soil which is not durable and often not environmental friendly (Hai *et al.*, 2010). The second way is to develop the plant saline-alkaline tolerance genetically at germination stage.

Rice is reported sensitive to salinity at different growth stages (Al-Karaki, 2001). The genetic studies revealed the quantitative trait loci (QTLs) linked with tolerance at these growth stages (Koyama *et al.*, 2001; Cheng *et al.*, 2008). However, direct seeding is a new rice culture method and the studies regarding tolerance to saline-alkaline stress at germination stage in rice has not been conducted yet. Present study as shown in Figure 1.1 focused on identification of QTLs linked with tolerance to saline-alkaline stress using $F_{2:3}$ segregating population.

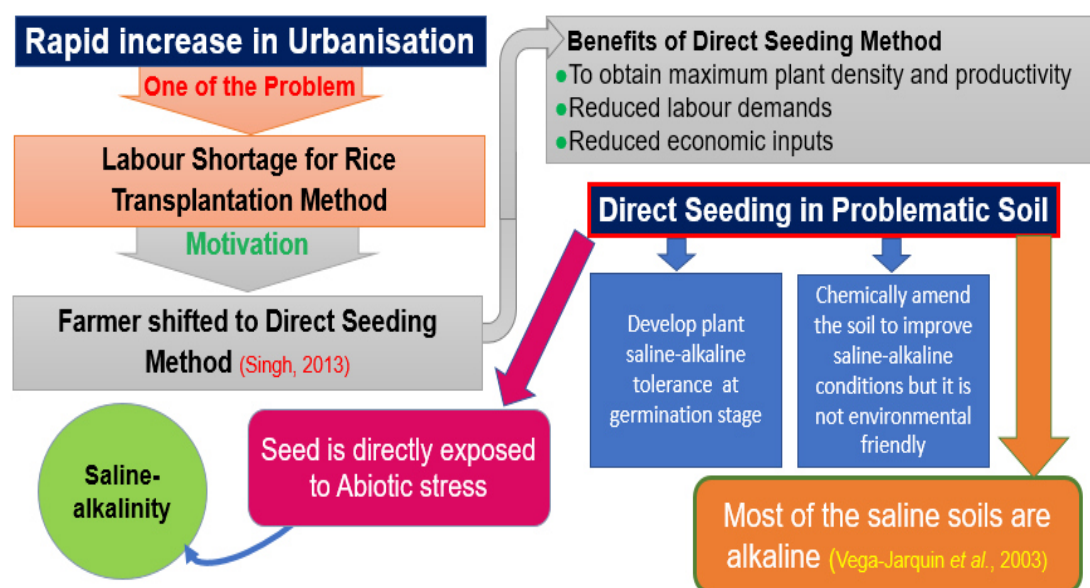


Figure 1.1 Illustration of problem statement

1.3 Research Objectives

- i. To develop a mapping population to dissect the genetic background for tolerance to saline-alkaline stress at germination stage in *Indica* rice.
- ii. To investigate the tolerance potential in F₃ progenies against the stress limits optimized in parental lines.
- iii. To construct a microsatellite molecular linkage map based on F₂ microsatellite data using MapMaker.
- iv. To identify the microsatellite loci associated with seed germination tolerance to saline-alkalinity based on molecular linkage map and F₃ progenies stress tolerance data using QTL cartographer.

1.4 Research Scope

A preliminary study was conducted to select tolerant and sensitive rice varieties under saline-alkaline conditions to develop mapping population. *Pokkali* and *Pak Basmati* were selected as tolerant and sensitive varieties, respectively. These varieties were crossed and F₂ population was developed after confirming the pedigree of F₁ seedlings. F₂ (129) population was used to get marker data by using 84 microsatellite markers. Marker data was used to construct molecular linkage map by MapMaker. F₃ progenies (129) were phenotyped under an optimised stress level. Potential QTLs were identified linked to germination traits under saline-alkaline conditions by three methods simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM) using QTL cartographer.

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