FINE MAPPING AND PYRAMIDING OF QTL'S FOR ROOT TRAITS IN RIL POPULATION OF RICE (*Oryza sativa* L.)

Ph.D. THESIS

By

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FINE MAPPING AND PYRAMIDING OF QTL'S FOR ROOT TRAITS IN RIL POPULATION OF RICE (*Oryza sativa* L.)

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CERTIFICATE - I

This is to certify that the thesis entitled **"Fine mapping and pyramiding of QTL'S for root traits in RIL population of rice (*Oryza sativa* L.)"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Agriculture** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), is a record of the bonafide research work carried out by **Hemant Sahu** under my/our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date: 20-7-2016

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CERTIFICATE - II

This is to certify that the thesis entitled "*Fine mapping and pyramiding* **of QTL's for root traits in RIL population of rice (*Oryza sativa* L.)**" submitted by **Hemant Sahu** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfillment of the requirements for the degree of "**Doctor of Philosophy in Agriculture**" in the Department of **Genetics and Plant Breeding** has been approved by the external examiner and Student's Advisory Committee after oral examination.

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LIST OF NOTATIONS/SYMBOLS

%	Per cent
°C	Degree Celsius
µl	Microlitre
bp	Base pairs
cm	Centimeter
cM	Centimorgan
d.f.	Degree of freedom
et al.	And Others
g	Gram
H_2O	Water
ha	Hectare
HCl	Hydrochloric Acid
HI	Harvest Index
hrs	Hours
i.e.	That is
KCl	Potassium chloride
m	Meter
M	Molar
mb	Megabase Pairs (106 bp)
$MgCl_2$	Magnesium Chloride
min	Minutes
ml	Milliliter
NaCl	Sodium Chloride
ng	Nanogram
rpm	Rotations Per Minute
U	Units

LIST OF ABBREVIATION

100SW	100 Seed Weight
Approx.	Approximately
ARD	Average Root Diameter
BY	Biological yield
Chr	Chromosome
cm	Centimeter
dATP	Deoxy adenosine 5’ triphosphate
dCTP	Deoxy cytidine 5’ triphosphate
dGTP	Deoxy guanosine 5’ triphosphate
DNA	Deoxyribo nucleic acid
dNTPs	Deoxynucleotide triphosphates
DTF	Days to 50% Flowering
dTTP	Deoxy thymidine 5’ triphosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
EtOH	Ethanol
EtBr	Ethidium Bromide
FLL	Flag Leaf Length
FLW	Flag Leaf Width
GY	Grain Yield
HI	Harvest Index
hrs	Hours
LLI	Length of Last Internode
LRD	Lateral Root Development
MAS	Marker Assisted Selection
mb	Megabase Pairs (106 bp)
NILs	Near Isogenic Lines

PCR	Polymerase Chain Reaction
PH	Plant Height
PL	Panicle Length
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RIL	Recombinant Inbred Line
RL	Root Length
RA	Root Angle
RE	Root Elongation
RV	Root Volume
SDS	Sodium Dodecyl Sulphate
SBM	Shoot Biomass
SH	Seedling Height
SLL	Second Leaf Length
SLW	Second Leaf Width
SPF	Spikelet Fertility
SSR	Simple Sequence Repeats
TBE	Tris Boric Acid EDTA buffer
TEMED	N,N,N',N'-Tetram Ethylethylene Di Amine
TSD	Terminal Stage Drought

THESIS ABSTRACT

a)	Title of Thesis	:	Fine mapping and pyramiding of QTL's for root traits in RIL population of rice (*Oryza sativa* L.)
b)	Full Name of Student	:	Hemant Sahu
c)	Major Subject	:	Genetics and Plant Breeding
d)	Name and Address of the major Advisor	:	Dr. Ritu R. Saxena
e)	Degree to be Awarded	:	Ph. D. in Agriculture

Ritu Rani Saxena. 20/7/16
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Date: 20/7/16

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ABSTRACT

Rice is the world's most important food crop and a primary food source for about half of the world's population. Rice growing areas consist of the tropics, subtropics, semi arid tropics, and temperate regions of the world. The predominantly rice growing areas in Asia (130 million hectares) are often threatened by severe abiotic stresses, the most common being drought. Drought is one of the most important and highly unpredictable abiotic stresses causing drastic reductions in yield under rainfed rice environments. To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. The complex nature of drought tolerance, genotype x environment interaction, lack of understanding of inheritance of drought tolerance, poor understanding of physiological basis of yield under water limited conditions, inefficient phenotypic selection and difficulty of effective drought tolerance screening methodology complicate the development of drought tolerance varieties. Identification of QTLs and molecular markers linked to root traits and drought tolerance can substantially improve selection efficiency. Therefore, the present study was undertaken to carry out the genetic analysis coupled with identification of QTLs for traits related to drought tolerance.

Seventy-one lines of BC_1F_7 and BC_1F_8 generation of cross between Swarna Sub-1 and IR-86918-305-B were evaluated in RCBD design with two replications each under five different stress and non-stress conditions along with rhizotrons for root studies during wet season 2014 and wet season 2015, at Research Cum Instructional Farm of CoA, IGKV, Raipur, to generate the phenotypic data. The cross, Swarna Sub-1 X IR-86918-305-B of BC_1F_7 and BC_1F_8 generation was taken for the study. The genetic parameters studied namely, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance, association, path along with stability analysis. The same population was further used for fine mapping of QTLs for root traits and grain yield. For expression analysis again six lines from the same cross were taken. Of these, three were tolerant (line # 50, 53 and 55) to water stress and three were susceptible (line # 33, 39 and 59). For pyramiding of QTLs for root traits, initially, a RIL population was developed using Danteshwari (released variety) and a land race, Dagad Deshi (deep rooted drought tolerant), from which a set of 122 RILs were selected. Out of 122 RILs, a set of 38 RILs were selected on the basis of leaf rolling and vegetative stage drought screening which clearly classify into stress tolerant and stress susceptible. Therefore, out of 38 RILs, 2 tolerant lines were taken for crossing programme to develop R X R cross. From this R X R cross, F_2 and F_3 segregating generation was made. Six progeny lines from above said cross was taken to generate the genotypic data. The DNA from each line was isolated, quantified, diluted and PCR amplified using 21 SSR markers (for fine mapping) and 32 SSR and HvSSR based markers (for gene pyramiding study) for developing genotypic data of populations at Marker Assisted Selection Laboratory, Dr R. H. Richharia Research Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur.

The analysis of variance showed significant variation among the population for grain yield and its contributing traits under different conditions. High Drought intensity index (DII) was recorded for the mean yield performance of the population, indicating substantial reduction in grain yield under stress condition compare to irrigated condition. Grain yield under irrigated, rainfed and terminal stage drought (TSD) condition showed moderate to high heritability. Thus, direct selection of lines under drought condition could be a better option. Plant height was the only trait to have high heritability accompanied with high genetic advance; it indicates that most likely the heritability is due to additive gene effects and selection will be effective. Root traits showed significant negative correlation with grain yield under irrigated condition. Shoot biomass, root pulling resistance, second leaf width, biological yield, harvest index, spikelet fertility, root length, root volume and average root

diameter showed positive direct effect on grain yield under all conditions. These may be used as reliable selection criteria for improving grain yield. About 39-49 % of genotypes in the study expressed low DSI values (< 1) for yield. The line number 15 and 16 had high value of mean grain yield, near unit regression (bi=1) and deviation is non-significant from zero (S^2di). Hence, these lines were found to be stable and suitable for all environments.

The phenotypic and genotypic data of seventy-one lines of cross between Swarna Sub-1 and IR-86918-305-B was statistically analyzed for single marker analysis for estimating association between marker and trait. Seventy eight SSR primers were screened for detecting parental polymorphism, out of which 21 showed polymorphisms. Fourteen QTLs have been identified for trait grain yield, three QTLs for root length, seven QTLs for root volume and three QTLs for average root diameter. Markers present in chromosome # 5, 6 and 9 were associated with root traits *i.e.,* root length, root volume and average root diameter. Above all the five lines *viz.*, 25, 27, 28, 29, and line # 31, carry the genes for qDTY 12.1 as well as for root traits. Selection of these lines will be extremely beneficial from farmer's point of view

Six root specific genes were taken for study. They were expressed in all condition (Control, Moderate and Stress), while some genes showed higher expression in resistance lines and lower expression in susceptible lines and vice-versa. In qPCR experiment, there was strong expression of OsPIN3t gene in all the six selected lines under control, moderate and stress conditions with respect to house keeping gene tubulin. The quantitative expression result obtained with primer for OsPIN3t gene is opposite to that obtained with primers for genes DRO1 and OsIAA23.

Gene pyramiding is a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. On the basis of gene pyramiding study, we conclude that progeny number 6 was pyramided for root traits (*viz.,* root length, root volume and average root diameter). Progeny number 1, 2 and 3 were pyramided for root traits and grain yield.

अ)	शोध का शीर्षक	:	धान (ओराइजा सटाइवा) की पुनः संयोजित अंतः प्रजनित पंक्तियों की आबादी में जड लक्षणों के क्यु. टी. एल. का उत्कृष्ट मानचित्रण एवं जीन संकलन
ब)	छात्र का पूरा नाम	:	हेमन्त साहू
स)	प्रमुख विषय	:	आनुवंशिकी एवं पादप प्रजनन
द)	प्रमुख सलाहकार का नाम एवं पता	:	डॉ ऋतु आर. सक्सेना
इ)	डिग्री से सम्मानित किया जाना है	:	पी. एच. डी. (कृषि)

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सारांश

धान विश्व की सबसे महत्वपूर्ण खादय फसल है एवं विश्व की आधी जनसंख्या के लिए प्राथमिक खादय स्त्रोत है । विश्व की उष्ण कटिबंधिय, उपोष्ण कटिबंधिय, अर्द्ध शुष्क उष्ण कटिबंधिय एवं शीतोष्ण क्षेत्र में धान का उत्पादन किया जाता है। एशिया में धान उत्पादन क्षेत्र सामान्यत अजैविक तनाव से ग्रसित होते हैं । जिसमें सूखे की समस्या सामान्य है । सूखा महत्वपूर्ण एवं अत्यधिक अप्रत्याशित अजैविक तनाव है । जो कि वर्षा आधारित परिवेश में धान के उत्पादन में अत्यधिक कमी करता है । विश्व के विस्तृत क्षेत्रों में जहां वर्षा सीमित अथवा अस्थिर है, वहां फसल उत्पादकता में वृद्धि के लिए फसल प्रदर्शन में सुधार को मुख्य लक्ष्य बनाकर पादप प्रतिकिया की किया विधी को समझना आवश्यक है। सूखा सहनशीलता की जटिल प्रकृति, जीन प्रारूप एवं परिवेश की अंतंकिया, सूखा सहनशीलता की वंशागति की अल्प समझ, जल सीमित दशाओं में उपज की कार्यकी आधार की समझ में कमी, अप्रभावी लक्षण प्रारूप का चयन एवं प्रभावी सूखा सहनशीलता की जांच कियाविधी की कठिनता, सूखा सहनशील किस्मों की विकास की जटिलता को बढ़ाती है। जड़ों के लक्ष्ण एवं सूखा सहनशीलता से संबंधित क्यु. टी. एल. और आण्विक चिन्हक की पहचान से चयन क्षमता में सुधार किया जा सकता है । उपरोक्त बिन्दुओं को ध्यान में रखते हुए वर्तमान अध्ययन, सूखा सहनशीलता के लिए अनुवांशिक विश्लेषण के साथ क्यु. टी. एल. की पहचान करने हेतु किया गया ।

स्वर्णा सब–1 X आई.आर.–86918–305–B संकरण के BC_1F_7 व BC_1F_8 पीढ़ी के इकहत्तर पंक्तियों का लक्षण प्रारूपिक आंकड़े विकसित करने हेतु उन्हें अनुसंधान सह अनुदेशात्मक प्रक्षेत्र कृषि

महाविद्यालय ई.गां.कृ.वि.,रायपुर में यादृक्षिक खंड अभिकल्पना में दो पुनरावृत्ति के साथ लगाया गया । वर्षा ऋतु 2014 व वर्षा ऋतु 2015 में जड़ों के अध्ययन हेतु राइजोट्रान के साथ, प्रत्येक पुनरावृत्ति को पांच अलग–अलग तनाव व तनावरहित दशाओं में मूल्यांकन किया गया । स्वर्णा सब–1 X आई. आर. –86918–305–B संकरण के BC_1F_7 व BC_1F_8 पीढ़ी के आनुवांशिक मानक जैसे लक्षण प्रारूप भिन्नता गुणांक जीन प्रारूप भिन्नता गुणांक, वंशागति, आनुवांशिक उन्नति, सहसंबंधता के साथ स्थिरता विश्लेषण के अध्ययन हेतु लिया गया। इन्ही समष्टियों को जड़ों के लक्षण एवं अनाज उपज से संबंधित क्यु. टी. एल. के उत्कृष्ट (महीन)मानचित्रण में उपयोग किया गया। इसी संकरण से छः पंक्तियों को अभिव्यक्ति विश्लेषण के अध्ययन हेतु प्रयोग किया गया । जल अभाव के लिए इनमें से तीन पंक्तियां (50, 53 व 55) सहनशील एवं तीन पंक्तियां संवेदनशील थे।जड़ लक्षणों के क्यु. टी. एल. के संकलन हेतु 2003 में दंतेश्वरी एवं दगड़ देशी का उपयोग कर एक पुनः संयोजित अंतः प्रजनित पंक्तियों की आबादी विकसित की गई । इनमें से 38 पुनः संयोजित अंतः प्रजनित पंक्तियों के एक वर्ग का चयन पर्णचक्रण एवं कार्यिक अवस्था में सूखा परीक्षण के आधार पर किया गया। जो तनाव सहनशीलता एवं तनाव संवेदनशीलता को स्पष्ट रूप से वर्गीकृत करता है। अतः सहनशील X सहनशील संकरण विकसित करने के लिए अड़तीस (38) पुनः संयोजित अंतः प्रजनित पंक्तियों में से दो सहनशील पंक्तियों को लिया गया। इस सहनशील X सहनशील संकरण से F_2 व F_3 विसंयोजी पीढ़ी का निर्माण किया गया । उपरोक्त संकरण से छःसंतति पंक्तियों को जीन प्रारूप आंकड़े विकसित करने के लिए प्रयुक्त किया गया। ई.गां.कृ.वि.,रायपुर के आर एच रिछारिया प्रयागशाला मे प्रत्येक पंक्ति का डी.एन.ए. विलगन कर इन्हें इक्कीस एस.एस.आर.मार्कर (उत्कृष्ट मानचित्रण) व उन्तीस एस.एस.आर. एवं एच.वी.एस.एस. आर. आधारित मार्कर (संकलन अध्ययन हेतु) का उपयोग कर जीन प्रारूप आंकड़ा विकसित किया गया।

विचरण विश्लेषण में विभिन्न दशाओं के अन्तर्गत अनाज पैदावार एवं अनाज उपज योगदान लक्षणों के लिए समष्टियों के औसत प्रदर्शन के आधार पर उच्च, सूखा तीब्रता सूचकांक दर्ज किया गया। जो यह दर्शाता है कि अनाज पैदावार सिंचित परिस्थितियों की तुलना में तनाव ग्रस्त स्थिति में काफी कम होती है। अनाज का उपज, सिंचित, वर्षा आधारित एवं सूखे की अंतिम अवस्थाओं में अनाज की उपजता के लिए वंशागतित्व मध्यम से उच्च पाई गई, अतः सूखें की स्थिति में इनका प्रत्यक्ष चयन करना एक बेहतर विकल्प हो सकता है।

वंशागतित्व एवं अनुवांशिक प्रगति केवल पौधें की उंचाई के लिए उच्च पाई गई है, यह इंगित करता है कि वंशागतित्व योगशील जीन क्रिया के कारण होता है। अतः इस स्थिति में चयन करना प्रभावी होगा। सिंचित दशाओं में जड़ों के लक्षण एवं अनाज उपज के मध्य नकारात्मक सह संबंध पाया गया। सभी दशाओं में पौधे का भार,पत्तियों की चौड़ाई, जैविक उपज,फसल सूचकांक,अन्न की बालियों की प्रजनन क्षमता, जड़ की लम्बाई, जड़ों का आयतन एवं औसत जड़ ब्यास मापदण्ड अनाज उपज के उपर सीधा प्रभाव प्रदर्शित करता है। इन मापदण्डों का उपयोग अनाज की पैदावार के सुधार के लिए

किया जा सकता है। उपज के लिए अध्ययन में करीब 39 – 49 प्रतिशत जीन प्रारूपों का सूखा संवेदनशील गुणांक का मान कम आया । पंक्ति क्रमांक 15 व 16 की औसत अनाज उपज प्रतिगमन गुंणांक इकाई के समीप एवं विचलन निम्न पाया गया। अतः ये पंक्तियां स्थिर हैं और सभी परिवेशों के लिए उपयुक्त हैं।

स्वर्णा सब–1 X आई.आर.–86918–305–B संकरण के इकहत्तर पंक्तियों के लक्षण प्रारूप आंकड़ो का सांख्यिकी विश्लेषण, एकल मार्कर विश्लेषण द्वारा मार्कर व लक्षण के बीच संबंध का आकंलन करने हेतु किया गया। जनक बहुरूपता का पता लगाने के लिए 78 एस.एस.आर. मार्कर द्वारा जांच किया गया। जिनमें से 21 मार्करों में बहुरूपता दिखाया। इन 21 मार्करों को जीन प्रारूप आंकड़े विकसित करने हेतु प्रयोग किया गया। अनाज उपज लक्षण के लिए चौदह क्यु. टी. एल. जड़ लक्षण के लिए तीन, जड़ के आयतन के लिए सात एवं औसत जड़ के ब्यास के लिए तीन क्यु. टी. एल. की पहचान की गई। गुणसूत्र संख्या 5, 6,एवं 9 में उपस्थित मार्कर जड़ लक्षणों अर्थात जड़ की लम्बाई, आयतन और औसत ब्यास के साथ संबंधित पाये गये इकहत्तर पंक्तियों में से पंक्ति संख्या 25, 27, 28, 29 एवं 31 में उपज हेतु क्यु. टी. एल. qDTY12.1 एवं जड़ों के क्यु. टी. एल. पाये गये हैं। इन पंक्तियों का चयन किसानों के लिए बहुत ही लाभदायी होगा।

अभिव्यक्ति अध्ययन के लिए सात जड़ विशिष्ट जीन लिया गया। सभी जीन सभी परिस्थितियों में (उच्च तनाव, मध्यम तनाव एवं नियंत्रित अवस्था में अभिव्यक्ति प्रदर्शित करती पाई गई। कुछ प्रतिरोधी एंव संवेदनशील पंक्तियों में जीन की अभिव्यक्ति उच्च एवं निम्न और इनके पूरक पाई गई। qPCR प्रयोग के अंतर्गत नियंत्रित माध्यम एवं उच्च तनाव दशाओं में सभी छः पंक्तियों में OsPIN3t जीन हाउसकीपिंग जीन की तुलना मे मजबूत अभिव्यक्ति प्रदर्शित करती है। मात्रात्मक अभिव्यक्ति में OsPIN3t जीनक` प्राइमर से प्राप्त परिणाम DRO1 एवं OsIAA23 जीन के प्राइमर के विपरीत पाई गई।

जीन संकलन का उद्देश्य कई माता–पिता के वांछनीय जीन को एक ही संतति में लाना है । जीन संकलन के अध्ययन के आधार पर हम यह निष्कर्ष निकाल सकते हैं कि संतति संख्या 6 जड़ों के विभिन्न लक्षणों हेत क्यु. टी. एल संकलित हुए हैं । संतति संख्या 1, 2 एवं 3 जड़ों के लक्षण एवं अनाज उपज हेतु संकलन प्रदर्शित करता है।

CHAPTER I
INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important wetland food crop, grown in all parts of the world and a primary food source for about half of the world's population. Rice is a member of the family Poaceae originated from South-East Asia, where more than 90 % of world's rice is produced and consumed (Li and Xu, 2007) thus, rice is immensely important to food security of Asia. The two cultivated species of rice are *O. sativa* (Asian rice, grown worldwide) and *O. glaberrima* (African red rice, grown on a limited scale in West Africa) (Khush, 2000).

The global production of rice has been estimated to be at the level of 720.7 million tones and the area under rice cultivation is estimated at 156 million hectares (FAOSTAT, 2013). India ranks 1st in area (43.97 million ha) and 2nd in production (105.30 million tones) after China in 2011-12 (Anonymous, 2012a). Chhattisgarh (C.G.) the south east central state is also called the "Rice bowl of India". Total area of rice in C.G. is 3.79 million ha, production is 6.03 million tones and productivity is 1682 kg/ha in 2011 (Anonymous, 2012b).

In India rice is grown under four ecosystems: irrigated, rainfed lowland, rainfed upland and flood prone. More than half of the rice area (55 %) is rainfed and distribution wise 80 % of the rainfed rice areas are in eastern India, making its cultivation vulnerable to vagaries of monsoon. Drought is one of the most important and highly unpredictable abiotic stresses causing drastic reductions in yield under rainfed rice environments, affecting 20 % of the total rice growing area in Asia (Pandey and Bhandari, 2008). Even though, the region receives good rainfall, yield losses caused by drought every year reach 2.9 million tones annually (Widawsky and O'Toole, 1990). During drought year 2002-03 reduction of grain yield was 17.36 million tones as compare to normal year in India (Subbiah *et al*., 2008).

Drought stress is a major constraint to the productivity of rice in upland ecosystem (Nguyen *et al.,* 1997). Recent climate change estimates predict the

water deficit to further deteriorate in the years to come (Wassmann *et al*., 2009) and the intensity and frequency of drought are predicted to become worse (Bates *et al*., 2008). The annual reduction in rice yields due to drought averages 18 million tones globally (O'Toole, 2004). In Asia alone, it is estimated that a total of 20 million ha of rice field (16 million ha in upland and 13 million ha in lowland) are drought prone. In the eastern states of India *viz*., Jharkhand, Orissa and C.G. alone, total rice production losses due to severe droughts (about 1 year in 5) are averages 40 %, valued at $ 650 million (Pandey *et al*., 2005). In these areas, drought risk reduces productivity even in non drought years, because farmers avoid purchasing inputs when they fear crop loss, becoming mired in cycle of low productivity, poverty and food insecurity (Pandey *et al*., 2005).

Since, drought is stage specific phenomenon and is developmentally regulated, selection and breeding is difficult. Tolerance at one stage of plant development may be poorly correlated with tolerance at other developmental stages. An approach to minimize agricultural losses incurred by drought stress is to develop, via genetic means, plant cultivars that can escape or withstand periods of drought. Such development should have a lasting economic impact on agriculture worldwide. Accumulating evidence suggests that, plant response to drought tolerance is controlled by more than one gene (Zhang *et al*., 2001) and is highly influenced by environmental variation.

Roots are the principal plant organ for nutrient and water uptake. Root systems form one of the important components of drought resistance. Rice plant with a deep root system is therefore beneficial in avoiding water stress by absorbing water from deep soil layers (Clark *et al.*, 2002). Root characteristics such as thickness, depth of rooting, and root length density have been associated with drought avoidance in rice (Ekanayake *et al.*, 1985; O'Toole and Chang, 1979; Yoshida and Hasegawa, 1982). Root penetration ability (RPA) through compacted soil layers or hardpans has been recognized as an important breeding objective for improvement of drought resistance in rice (Hanson *et al.*, 1990).

Yield is a polygenic trait affected by different factors that exhibit low heritability (Xiong, 1992). A number of morphological, physiological and

phenological traits have been reported to improve the performance of rice challenged by drought. Adaptive mechanisms of plants in response to drought have been reported by several scientists (Fukai and Cooper, 1995; Nguyen *et al*., 1997; Chopra and Sinha, 1998). Direct selection for yield under stress in managed stress environments (MSEs) (Venuprasad *et al.*, 2007) and target environments (TEs) (Kumar *et al.,* 2008; Yadav *et al*., 2013) is considered a promising approach to improve drought tolerance in rice

Progress is being made in developing drought tolerant rice germplasm through conventional breeding (physiological dissection) and the use of molecular tools. Identification of quantitative trait loci (QTLs) conferring improved drought resistance may facilitate breeding progress (Bernier *et al.*, 2009a). The advent of saturated molecular maps promised rapid progress towards the improvement of crops for genetically complex traits like drought resistance *via* analysis of quantitative trait loci (QTLs) (Price *et al.,* 2002a).

Benefit of MAS is the sharp reduction of required population size because many lines can be discarded in earlier breeding generations after MAS. Successful applications of marker assisted back crossing (MABC) and marker assisted gene pyramiding (MAGP) for improving yield or yield component traits by using well characterized major QTLs are recently being reported (Steele *et al*., 2007; Swamy and Kumar, 2011; Gregorio et al, 2013; Uga *et al*., 2013). For quantitative traits such as yield, a single QTL rarely accounts for a significantly large portion of the trait variation. Several QTLs with additive effects need to be stacked to achieve significant improvement. Gene pyramiding is a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. MAGP has gained popularity in the last decades (Ye and Smith, 2010).

Recently, a large effect of *qtl12.1, DTY 1.1, DTY 2.1, qRL 6.1, Dro1* etc. has been identified which has increased effect (yield) with increase of drought intensity. The identification and isolation of genes associated with drought tolerance is of major importance in order to better understand this trait and increase the efficiency in developing drought tolerant varieties (Tuberosa and Selvi 2006, Lattife *et al*., 2007 and Sreenivasulu *et al*., 2007). Roots are one of the primary

sites for stress signal perception in which a signaling mechanism initiates a cascade of gene expression responses to drought. These transcriptional changes can result in successful adaptations leading to stress tolerance by regulating gene expression and signal transduction in the stress response (regulatory proteins) or directly protecting the plant against environmental stress (functional proteins) (Perin *et. al.*, 2007). Several functional genomics studies of rice have been performed using different approaches such as macro and microarray (Kawasaki *et al.*, 2001 and Rabbani *et al.*, 2003), RT-qPCR, SAGE (*Serial Analysis of Gene Expression*), MPSS (*Massive Parallel Signature Sequencing*) and more recently oligoarray using the transcriptome of rice to evaluate responses to abiotic stresses (Yamaguchi and Shinozaki, 2006).

Thus, this study aims at transfer of drought tolerant QTL, *qDTY 1.1*, in well adopted Swarna-S*ub1* variety of rice and fine mapping of these QTLs in the BC_1F_7 population obtained from a cross Swarna *Sub1* and IR 86918-305-B (serving as the donor of QTL, *qDTY 1.1*).

In this study an attempt has been made to fine map the QTLs involved in the control of root traits and grain yield under water stress and non-stress conditions. An attempt was also taken to study the expression pattern of genes related to root under control, moderate and stress condition. Gene pyramiding study was also taken to pyramid the genes from different lines. Keeping all these things in mind, the major objectives of study were as follow:

1. Fine mapping of QTL for root traits
2. Understanding the association of root traits with yield and yield contributing traits
3. Gene expression study in rice
4. Pyramiding of QTL for root traits in rice

CHAPTER II
REVIEW OF LITRATURE

In this chapter, an attempt has been made to review the available literatures on genetic mechanism and identification of QTLs for yield and different yield contributing traits in rice. The available literatures have been classified into following headings:

2.1 Impact of drought on yield and yield contributing characters

2.2 Correlation of traits related to drought tolerance

2.3 Mechanism of drought tolerance

2.4 Heritability of traits related to drought tolerance

2.5 Drought susceptibility index

2.6 Mapping populations

2.7 Molecular markers

2.7.1 SSRs (Simple sequence repeats)

2.8 Genetic map using microsatellite

2.9 Development and concept of QTL analysis

2.10 Fine mapping analysis

2.11 Development and concept of gene expression

2.12 Development of concept of gene pyramiding

2.1 Impact of drought on yield and yield contributing characters

Puckridge and O'Toole (1980) showed that biomass production of rice is a function of water use. The shortage of water in the soil suppresses the leaf expansion, tillering and photosynthetic rate along with leaf area due to senescence. All these factors are responsible for reduction in dry matter accumulation.

Row and Venkateswarlu (1983) studied the yield component of rice varieties *viz.*, Rasi and IR-20 under moisture condition. The reproductive and ripening phase were vulnerable and crucial for moisture stress, which resulted in permanent damage to growth and yield.

Jeena and Mani (1990) studied root characters and grain yield on some upland rice varieties and indicated that high root length density and root weight are important for selecting drought tolerant genotypes.

Krishnayya and Murty (1991) exposed five upland rice varieties to soil moisture stress of 25% field capacity at seedling stage. With increase in soil moisture stress, there was a decrease in relative water content and water potential of leaf. The cultivar which maintained high relative water content and positive turgor, in spite of reduced leaf water potential during stress also had optimum photosynthesis and solute accumulation.

Wopereis *et al.* (1996) reported that water stress during flower induction and inflorescence development leads to delay in flowering (anthesis) or even to complete inhibition as apical morphogenesis is sensitive to water deficit.

Jongdee *et al.* (1997) reported that late season drought contributed to yield loss between 13% and 35%.

Nguyen *et al.* (1997) suggested that four major physiological components of drought resistance in rice are root characteristic, osmotic adjustment, cuticular transpiration, and water use efficiency. A thick and deep root system is considered a favorable component allowing rice crops to maintain their water status under conditions in which there is water available at deep soil layers.

Yadav *et al.* (1997) revealed that effective use of the absorbed water is very important traits for enhancing biomass and yield.

Drought is generally avoided in irrigated rice production systems, but it is a consistent feature across much of the 63.5 million hectares of rainfed rice sown annually, most of which is in tropical Asia, Africa, and Latin America (Narciso and Hossain 2002).

Pantuwan *et al.* (2002) reported that the drought stress developed prior to flowering generally delayed the flowering of genotypes and such a delay was associated with drought susceptibility in rice.

Deming *et al.* (2004) found that drought stress during reproductive stages showed severe drought stress in panicle development stage caused great yield loss by reducing spikelets per panicle in upland rice.

In the eastern states of India *viz*., Jharkhand, Orissa and C.G. alone, total rice production losses in severe droughts (about 1 year in 5) averages 40%, valued at $ 650 million (Pandey *et al.*, 2005).

The amount of accumulated dry matter in green leaves is different from one cultivar to another cultivar. In addition, the amounts of assimilate transferring from stems and leaves to filling grains will increase parallel with drought stress. Drought stress is effective on stabilizing of yield in those cultivars which have late leaf senescence during the period of grain filling (Kumar *et al.*, 2006).

Yield reductions, as a result of drought stress, are mainly from a reduction in tiller numbers (Jongdee *et al.*, 2006), small leaf area growth and stomatal closure (Boonjung, 1993) and reduced spikelet fertility (Liu *et al.*, 2006).

Deshmukh *et al.* (2007) observed that in rice, water stress during flowering reduced the harvest index by as much as 60 %, largely as a result of a reduction in seed s*et.* Panicles in stress plants fail to full exert (emerge) from the flag leaf sheath, flowering was delayed and the percentage of spikelet that open at anthesis were reduced. The failure of panicle exertion alone accounts for approximately 25 % to 30 % of spikelet sterility because the un-exerted spikelet not completed anthesis and shed pollen, even when development was otherwise normal.

Drought stress during vegetative growth, flowering and terminal period of rice cultivation, can interrupt floret initiation (which cause spikelet sterility) and grain filling, respectively (Botwright Acuna *et al.*, 2008).

Shrivastava and Verulkar (2009) reported that delay in flowering under drought conditions was related to low water status and was an indicator of drought susceptibility. Delay in flowering was also associated with higher spikelet sterility.

Fu *et al.* (2010) reported significant positive correlations of grain yield per plant with spikelets per panicle, 1000 seeds weight, number of panicles per plant and percentage seed set; whereas number of panicles per plant had significant negative correlations with spikelets per panicle, seeds per panicle and 1000 seeds weight.

Gomez *et al.* (2010) reported significant positive correlation between plant height and biomass under stress. Significant negative correlations were also

noticed between water stress indicators such as leaf rolling and leaf drying with biomass under stress.

Abarshahr *et. al.* (2011) observed that during the late drought stress, the capacity of assimilate transmission into seeds increases and this is a useful physiological phenomenon under drought stress conditions. Exertion of drought stress at vegetative stage leads to increase of dry matter accumulation in sinks.

Mohankumar *et. al.* (2011) reported that below ground traits roots traits has a direct relevance in improving grain yield under aerobic condition. Root traits such as root volume and root dry biomass were positively and significantly correlated with grain yield. Leaf characters such as total leaf area (TLA) also correlated significantly with grain yield.

Under drought, both structural growth (sink) and assimilation (source) processes are down regulated, resulting in changed source-sink relations that may depend on environment and genotype. Plant passes from a carbon (C) source to sink limited situation as the reduction of organ growth and development (i.e. sink activity) appears to happen earlier than C starvation under water deficit conditions (Muller et *al.* 2011).

Moosavi *et. al.* (2015) found that grain yield per plant showed significant correlation with panicle number, harvest index, dry weight and panicle length these traits can be used as indicators for indirect selection of grain yield.

Combined genetic and physiological analysis of reproductive-growth traits and their effects on yield and yield components under drought stress is important for dissecting the biological bases of drought resistance and for rice yield improvement in water-limited environments.

2.2 Correlation of traits related to drought tolerance

Shahid *et al.* (1994) reported positive correlation between root length, root dry weight, shoot dry weight, stomata frequency and drought tolerance, whereas negative correlation existed between shoot length and stomata size.

Garrity and O'Toole (1994) reported that grain yield, relative grain yield and spikelet fertility were significantly and negatively related to the number of days after the beginning of the stress period that a cultivar flowered. Percentage spikelet fertility was highly correlated with grain yield under reproductive stage

water stress. Spikelet fertility was the most practical character by which to score cultivar performance.

Jongdee *et al.* (1997) found that only biological yield and harvest index were found to be significantly associated with grain yield under drought treatments.

Babu *et al.* (2003) reported positive correlation of biomass under stress with yield, per cent spikelet fertility, number of seeds per panicle, harvest index and relative yield. On the other hand, leaf rolling scores had negative correlation with yield and harvest index.

Kumar *et al.* (2004) observed that in rice there is a negative association between delay in flowering and grain yield, relative water content and post flowering dry matter production under rainfed condition. A direct relationship was observed between delay in flowering and sterility. The flowering delay under drought stress condition is governed by leaf water status.

Lafitte *et al.* (2004a) found negative correlation between plant height and yield. Tall lines yield poorly in rainfed experiment but no significant association was observed between yield and height under control condition.

Lanceras *et al.* **(**2004) used double haploid mapping population to measure correlation between traits and grain yield at different water levels and reported correlation between grain yield and biological yield ranges 0.70 to 0.54, indicated that genetic improvement in the grain yield would likely be accompanied by improvement of biological yield. Further they found that as drought stress, increases correlation between grain yield and harvest index increase dramatically, indicating that harvest index is also a primary determinant of grain yield. Positive correlation was also found between total spikelets number and grain yield under stress condition. Negative correlation was found between grain yield and days to flowering after initiation of irrigation gradient.

Rajeshwari and Nandrajan (2004) observed positive correlation between numbers of filled grains per panicle and grain yield.

Singh *et al.* (2004) reported significant and positive correlation between grain yield per plant and yield contributing traits, effective flag leaf breadth and

total seeds per panicle. A negative correlation was found between grain yield and plant height.

Verulkar *et al.* (2004) reported positive correlation of spikelet fertility, harvest index and biological yield with yield under stress.

Under well-watered conditions, Lanceras *et al.* (2004) observed a high genetic association for biological yield, harvest index, days to flowering after initiation of irrigation gradient, percent spikelet fertility, total spikelet number, plant height, and grain yield. Grain yield in all irrigation treatments showed negative correlations with PSS indicating that drought stress occurring during the reproductive stage increased the PSS and consequently decreased the grain yield.

Gomez *et al.* (2005) observed that root thickness and root weight were positively correlated with root length; root thickness was significantly and positively correlated with root weight and biological yield; and plant height was positively correlated with grain number per panicle. Leaf area per plant showed the highest positive direct effect on root weight followed by biological yield and root thickness. Selection based on biological yield may help to identify drought-tolerant types.

Lafitte *et al.* (2006) showed that, there is a negative and significant correlation between plant heights and final yield under drought stress conditions. The significant and positive correlation was observed between flag leaf width and paddy yield while under conditions of optimum irrigation regime, number of spikelet per panicle had significant and positive correlation with paddy yield that shows the importance of flag leaf at grain filling stage under water deficit stress.

Gomez *et al.* (2006) reported positive and significant correlation of panicle length and number of productive tillers with grain yield under stress. They also observed positive correlation of root traits with plant height under stress. Similar relation was also reported earlier in rice by Venuprasad *et al.* (2002).

Umadevi *et al.* (2009) reported that grain yield per plant was positively and significantly associated with days to 50 % flowering, number of productive tillers per plant, number of secondary branches per panicle and straw yield.

Fu *et al.* (2010) reported significant positive correlations of grain yield per plant with spikelets per panicle, 1000 seeds weight, and number of panicles per plant and percentage seed s*et.* The number of seeds per panicle also had strong positive correlations with percentage seed set, but the number of panicles per plant had significant negative correlations with spikelets per panicle, seeds per panicle and 1000 seeds weight. In addition to this, there was a significant negative correlation between panicles per plant and percentage seed s*et.*

Nandan *et al.* (2010) revealed strong positive association of yield with days to 50 % flowering, plant height, number of grains per panicle, number of spikelets per panicle and spikelet fertility. The result of path analysis indicated that the number of grains per panicle had maximum direct effect on grain yield per plant followed by kernel length after cooking, days to 50 % flowering, hulling percentage, plant height, harvest index and kernel breadth after cooking. He also reported Negative correlation of 1000 grain weight with grain yield.

Gomez *et al.* (2010) reported significant positive correlation between plant height and biomass under stress. Significant negative correlations were noticed for water stress indicators such as leaf rolling and leaf drying and positive correlations for plant production traits with biomass have been reported in rice. It was also reported by Babu *et al.* (2003), Gomez *et al.* (2006) and Srinivasan *et al.* (2008).

Abarshahr *et al. (2011)* reported significant correlation among paddy yield with plant height, panicle number per plant, flag leaf width, number of filled grains per panicle, number of spikelet per panicle and 1000-grain weight ($p < 0.01$), panicle length, brown grain length a day to complete maturity and chlorophyll index ($p < 0.05$) under optimum . correlations under drought stress conditions showed that, paddy yield had significant and positive correlation with plant height, panicle length, number of panicles per plant, flag leaf width, days to 50% flowering, number of filled grains per panicle, number of spikelet per panicle, 1000-grain weight ($p < 0.01$), flag leaf length, brown grain width, panicle fertility

percentage and chlorophyll index ($p < 0.05$) while correlation between paddy yield and plant height or paddy yield and days to 50% flowering were negative

Akthar *et al.* (2011) found heritability to be highest for number of grains per panicle, days to maturity, plant height and paddy yield while lowest for number of tillers per plant. Paddy yield had strong genetic correlation with number of grains panicle-1, days to maturity and 1000-grain weight.

Ekka *et. al.* (2011) conducted association analysis studies and revealed that grain yield per plant had positive significant correlation with leaf width, days to 50% flowering, plant height, panicle length, number of filled grains per panicle, 100 seed weight and paddy (grain) length.

Haider *et al.* (2012) reported that root length, root shoot ratio, thousand seeds weight, seeds per panicle, spikelet fertility and drought response index showed positive and significant association with yield per plant under drought stress at genotypic level; whereas, leaf drying had significantly negative correlation with yield.

Gopikannan and Ganesh (2013) reported significant positive association of grain yield per plant with number of productive tillers per plant, panicle length, spikelet fertility percentage, number of filled grains per panicle, proline content, total chlorophyll content and chlorophyll stability index.

Shrivastava *et al.* (2014) reported that the traits viz., harvest index, biological yield per plant, flag leaf width, 1000 seed weight, plant height, flag leaf length, panicle length, decorticated seed width, seed width and seed length had high heritability accompanied with high genetic advance.

Kumar *et al.* (2014) studied correlation and path analysis and revealed that the traits such as panicles weight per plant, number of spikelets per panicle, panicle index, number of leaves per plant, plant height, days to heading, average panicle weight, flag leaf width, grain length and hulling % exhibited significant positive correlation with grain yield per plant as well as positive direct effects on grain yield per plant.

Shellammal *et al.* (2014) found that Plot yield under stress was significantly and positively correlated with harvest index and 1000-grain weight, but negatively associated with leaf rolling score and days to 50% flowering.

2.3 Heritability of traits related to drought tolerance

Babu *et al.* (2003) reported high heritability (broad sense) of leaf rolling, grain yield, plant height, spikelet fertility, harvest index and days to heading while, low to moderate for relative water content, plant height, grain yield, biomass, spikelet fertility, seeds per panicle and 1000 seeds weight under stress.

Lanceras *et al.* (2004) computed broad sense heritability (h^2) across five water regimes using 154 double haploid mapping population derived from a cross between two rice cultivars, CT9993-510 to 1-M and IR62266-42 to 6-2. They reported that panicle number and plant height had highest h^2, 0.61 and 0.73, respectively; harvest index and spikelet fertility had moderate h^2, 0.46 and 0.45, respectively, while grain yield and biological yield had the lowest h^2, 0.32 and 0.31, respectively.

Singh *et al.* (2004) observed high heritability of traits like grain yield and yield contributing character, suggesting that the importance of these traits as criteria for improving overall yield under stress. Moderate heritability was also observed for panicle length while low heritability for leaf breadth and test weight.

Karim *et al.* (2007) reported high heritability with high genetic advance in percent of mean (GAPM) was observed for 1000-grain weight followed by spikelet sterility (%) and number of filled grains per panicle

Kumar *et al.* (2008) reported heritability in broad sense for grain yield, harvest index, 50 % flowering, leaf rolling and leaf drying was 0.48, 0.45, 0.47, 0.20 and 0.42, respectively for IR55419 × IR64 and 0.45, 0.38, 0.73, 0.26 and 0.30, respectively for IR55419 × Way Rarem, derived progenies.

Srinivasan *et al.* (2008) reported heritability in broad sense of leaf rolling, days to flowering, plant height, panicle length, 1000 seeds weight, grain yield, biomass yield and harvest index was 0.63, 0.64, 0.48, 0.54, 0.63, 0.46, 0.51 and 0.27, respectively in doubled haploid (DH) line population from the cross CT9993-5-10-1-M × IR62266-42-6-2.

Venuprasad *et al.* (2009b) reported heritability in broad sense under lowland stress was 0.73 in 2006 and 0.55 in 2007 for grain yield.

Bernier *et al.* (2009a) reported heritability 0.75, 0.49, 0.54, 0.94 and 0.44 for grain yield, biomass yield, harvest index, days to 50 % flowering and plant height, respectively.

Umadevi *et al.* (2009) estimated high heritability in broad sense of days to 50 % flowering, plant height (cm), panicle length (cm), number of secondary branches/ panicle, leaf length (cm), leaf width (cm), seed length (mm), seed breadth (mm), seed L/ B ratio, straw yield (g), 100 seeds weight (g) and grain yield per plant (g).

High heritability with high genetic advance as percent of mean was reported by Nandan *et al.* (2010) for number of effective tillers per plant, panicle weight, number of grains per panicle, number of spikelets per panicle, 1000 grain weight, kernel length before cooking, length breadth ratio, water uptake ratio and grain yield per plant.

Verulkar *et al.* (2010) reported broad sense heritability of 50 % flowering (0.92 for control, 0.78 for moderate and 0.83 for severe drought), plant height (0.96 for control, 0.81 for moderate and 0.83 for severe drought), grain yield (0.55 for control, 0.52 for moderate and 0.73 for severe drought) and harvest index (0.58 for control, 0.56 for moderate and 0.78 for severe drought).

Seyoum *et al.* (2012) reported high heritability for plant height, followed by 50 % flowering, 1000 seeds weight, panicle length, and spikelets per panicle, which indicated high heritable proportion of variation under rainfed upland rice.

Kiani (2013) reported high heritability coupled with high genetic advance found for total seeds per panicle, filled seeds per panicle, tiller number and grain yield make these traits suitable for selection.

Dama *et al.* (2014) observed high heritability coupled with high genetic advance and high GCV were observed for number of leaves per plant, number of tillers per plant, number of productive tillers per plant, flag leaf width, panicles weight per plant, average panicle weight, number of spikelets per panicle, number of filled spike lets per panicle, spikelet fertility per cent, spikelet density, grain yield per plant, biological yield per plant and harvest index.

Shellammal *et al.* (2014) reported that the plot yield, 1000-grain weight, panicle exsertion and canopy air temperature difference exhibited high heritability

under the control conditions, whereas spikelet sterility and single plant yield exhibited high heritability under the moderate stress conditions. Traits such as days to 50% flowering, plant height and osmotic potential showed high heritability under the severe stress conditions.

Khatun *et al.* (2015) reported highest heritability for photosynthetic rate, transpiration rate, number of filled grains/panicle and yields/plant (g).

2.4 Mechanism of drought tolerance

Rice, like other crops can potentially resist drought stress using three different strategies: drought escape, drought avoidance and drought tolerance. A proper timing of life cycle, resulting in the completion of most sensitive developmental stages while water is abundant, is consider to be a drought escape strategy (Price *et al.*, 2002b). This mechanism involves rapid phonological development (early flowering and early maturity), developmental plasticity (variation in growth period depending on extend of water-deficit) and remobilization of pre-anthesis assimilates to grain (Turner, 1979).

Drought avoidance is the ability of the plant to maintain relatively high tissue water potential despite a shortage of moisture. Mechanisms for improving water uptake, storing in plant cell and reducing water losses confer drought avoidance. Drought avoidance was performed by maintenance of turgor through increased rooting depth, efficient root system and increased hydraulic conductance and by reduction of water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding and reduced evaporation surface (leaf area) (Turner, 1979; Passioura, 2002). Numbers of worker have emphasized the importance of traits responsible for avoidance mechanism of drought tolerance.

Drought affects rice at morphological, physiological and molecular levels such as delayed flowering, reduced dry matter accumulation and partitioning and decreased photosynthetic capacity as a result of stomatal closure, metabolic limitations and oxidative damage to chloroplasts. Small-statured rice plants with reduced leaf area and short growth duration are better able to tolerate drought stress, although the mechanisms are not yet fully understood. Increased water uptake by developing larger and deeper root systems and the accumulation of

osmolytes and osmoprotectants are other important mechanisms for drought resistance (Farooq *et al.*, 2009). Mechanism such as osmotic adjustment where by a plant maintains cell turgor pressure under reduced soil water potential, increase in elasticity in cell and decrease in cell size and desiccation tolerances by protoplast are categorized as drought tolerance mechanisms (Sullivan and Ross, 1978).

O'Toole and Datta (1986) suggested that increase root depth and root density in rice increase the capacity to extract available soil water and may be responsible for increase drought avoidance in some rice genotypes.

Kobata *et al.* (1994) reported that constitutive traits (*i.e.* under no drought stress: *e.g.*, rooting depth, root thickness, branching angle and root distribution) influenced the expression of both induced (hardpan penetration and osmotic adjustment) and secondary traits such as maintenance of plant water status, canopy temperature, leaf rolling score and leaf death score. These secondary traits may then reduced spikelet fertility, yield components and ultimately yield.

Umayal *et al.* (2001) observed that drought tolerant *indica* land race in southern India had thicker roots with wider xylem vessels than susceptible cultivars.

Hacke *et al.*, (2001) and Stiller *et al.* (2003) revealed that the wider xylem vessels may decrease chances of cavitations, which would reduce xylem water conductivity. Cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells.

A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Wahid and Rasul, 2005).

However, Passioura (2006) reported that the contribution of root depth to drought avoidance may be site specific, as the subsoil is often inhospitable to root growth due to unfavorable pH or nutrient content.

Zeid and Shedeed, (2006) found that germination potential, hypocotyls length, and shoot and root fresh and dry weights were reduced by polyethylene glycol-induced water deficit, while the root length was increased.

Root development and plasticity have been identified as a key trait in plant adaptation to drought as they determine plant access to soil water. For instance, longer primary root and/or larger xylem diameters in deep roots and/or larger lateral root system are desirable root traits which help plants adapt better to drought by acquiring water from lower soil layers or foraging subsoil surface moisture (Manavalan *et al.,* 2009; Comas *et al.,* 2013).

Mapping quantitative trait loci (QTLs) for root traits and their use in marker assisted breeding (MAB) is an alternate approach in selecting for root traits that are difficult to phenotype (Coudert *et al.,* 2010).

In general, to cope with drought, a number of adaptive mechanisms are activated in plants, through various signal transduction pathways which lead to the activation of various molecular, biochemical, and physiological responses (Hadiarto and Tran, 2011; Ha *et al.,* 2012; Hossain *et al.,* 2013;Deshmukh *et al.*, 2014; Karan and Subudhi, 2014; Khan *et al.,* 2014).

The drought susceptibility index (DSI) is a measure, based on yield *per se* under stress and non-stress conditions. The drought susceptibility indexes (DSIGY) were calculated by determining the changes in grain yield (GY) under two soil moisture levels (irrigated and drought) and confirmed that they are good indicators of drought tolerance in plants (Grzesiak *et al.*, 2013).

Rice plant with a deep root system is therefore beneficial in avoiding water stress by absorbing water from deep soil layers (Clark *et al.,* 2002). Root characteristics such as thickness, depth of rooting, and root length density have been associated with drought avoidance in rice (Ekanayake *et al.,* 1985; O'Toole and Chang 1979; Yoshida and Hasegawa 1982). Root penetration ability (RPA) through compacted soil layers or hardpans has been recognized as an important breeding objective for improvement of drought resistance in rice (Hanson *et al.,* 1990). Root morphology, anatomy and rooting pattern directly affect the amount of water available to a crop.

Drought resistance in rice can be improved through an improved root system. There exists genotypic variation in the ability of roots to penetrate compacted soil layers and simulated compacted soil layers (O'Toole 1982; Yu *et al.*, 1995). Developing cultivars with high root-penetration ability has been recognized as an important breeding objective for drought resistance improvement in rice (Hanson *et al.*, 1990), and will have great impact to boost rice production and sustain yield stability in rainfed lowland areas. However, incorporation of selection criteria into breeding programs has been difficult due to lack of reliable and efficient screening techniques and the laborious, time-consuming nature of measuring root traits such as root-penetration ability and root thickness (Garrity *et al.*, 1986; Ingram *et al.*, 1994; O'Toole 1989). Molecular genetic approaches may be one solution to improve drought tolerance in rice (Nguyen and Joshi 1994). Mapping genes controlling root-penetration ability and other important root traits could facilitate the development of rice cultivars better adapted to water deficit environments. Progress has been made in locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers (Champoux *et al.*, 1995).

2.5 Drought susceptibility index (DSI)

The DSI for yield was calculated to characterize the relative tolerance of genotypes. The use of DSI can help to distinguish suitable variety for drought from phenology and yield potenti*al.* Larger DSI values indicate greater drought susceptibility (Chauhan *et al.*, 2007). Lower DSI mean values (DSI<1) observed for yield indicated that lines is relative tolerant to stress. Ouk *et al.* (2006) concluded that DSI under drought condition would allow breeders to identify resistant line with high drought tolerance. The presence of large variation in the DSI values for certain genotypes might be due to timing, intensity and stage of stress and genetic diversity among lines (Clarke *et al.*, 1984). The use of DSI is likely to be most beneficial in selecting parents for development of drought tolerant populations, particularly when yield potential vary greatly among the tested genotypes (Mall *et al.*, 2012).

Rebello *et al.* (2008) estimated drought susceptibility index based on yield as 0.73 for Prata Ligeiro (tolerant) and 1.57 for IRAT20 (susceptible).

Babu *et al.* (2011) reported low DSI values of yield under stress for varieties MAS26, SEL 128, 25P25, PHB71 and MAS25, which indicated drought tolerance.

Mall *et al.,* (2012) reported consistently low DSI value for Lalsar, CR 143-2-2, RR 383-2, RR 440-167-2-13, Thara and Vandana.

Prakash *et.al.,* (2015) reported "RM 202" and "RM 258" showed significant linkage with DSI at 5% level of significance in F_6 population derived from a cross between Mahamaya × Budda cultivar. The data of five SSR markers were used to identify QTLs for drought susceptibility index. Two QTLs were detected for DSI on chromosome 10 and 11 on rice genome. These closely linked SSR markers with drought tolerance will facilitate early selection of drought tolerant lines and shorten breeding period.

2.6 Mapping populations

Several types of populations, such as F_2 or F_3 progeny, backcross, doubled haploid lines (DHLs), near isogenic lines (NILs) and recombinant inbred (RI) lines, have been used for molecular genetic mappings (Knapp, 1991). DH lines and RI lines (Burr and Burr, 1991) have several advantages over other kinds of populations as they are genotypically true-breeding or homozygous, stable and permanent and well suited to QTL analysis. The advantage of DHLs is that the homozygosity is achieved in a single step (but all genotypes/ species are not equally amenable to anther culture), whereas RILs take a longer time to produce, *i.e*., 7-10 generations of inbreeding. As RILs undergo multiple rounds of meiosis before homozygosity is reached, there is a greater chance for the linked genes to be recombined, providing an opportunity for accurate detection of QTLs. Each individual RIL or DHL can produce hundreds of homozygous seeds in a single generation so that unchanging genotypes can be evaluated repeatedly over years, locations, replications and for multiple traits (Burr and Burr, 1991; Mc Couch and Doerge, 1995). The ability to replicate experiments using fixed genotypes offers an elegant way of discriminating environmental effects from genetic factors in the expression of a phenotype. RIL populations have increasingly been used in many crops, including rice (Xiao *et al.,* 1996; Champoux *et al.,* 1995 and Nair *et al.,* 1995) for QTL mapping.

Zhang *et al.* (2001) made comprehensive study of mapping the drought resistance components (osmotic adjustment and root traits) in doubled-haploid rice (*Oryza sativa* L.) population of 154 lines.

Different mapping populations were used for drought resistance like RILs of IRAT109 × Zhenshan97B by Liu *et al.* (2010), RILs of Bala × Azucena by Gomez *et al.* (2006), RILs of Apo × Swarna by Venuprasad *et al.* (2009b), RILs of IRAT109 × Yuefu by Liu *et al.* (2008); CO39 × Moroberekan (Lilley *et al.* 1996); Vandana × IR64 (Venuprasad *et al.* 2007); Vandana × IR72 (Venuprasad *et al.* 2009a); RILs of Akihikari × IRAT109 (Kato *et al.*, 2008); Apo × IR64 (Venuprasad *et al.*, 2009a); Azucena × Kalinga3 (Steele *et al.*, 2007); ZS97B × IRAT109 for QTL *qPND4* (Liu *et al.*, 2008); RILs of Zhensha97B × IRAT109 for coleoptiles length (CL) and drought resistance index (Hu *et al.*, 2007); Apo × IR72 and P124 × IR64 for maximum root length (Toorchi *et al.*, 2002); Guichao2 × Dongxiang QTLs associated with drought tolerance, *qSDT2-1* and *qSDT2-2*, which were located on chromosome 2 and 12 (Zhang *et al.*, 2006).

Subashri *et al.*, (2009) developed a subset mapping population of 93 near flowering recombinant inbred lines with uniform phenology was constituted for genetic analysis of reproductive stage drought resistance. The population was phenotyped for 22 physio-morphological traits under two contrasting water regimes imposed at reproductive stage.

Gomez *et al.*, (2010) used a subset of 250 recombinant inbred lines (RILs) of F8 generation derived from two *indica* rice lines (IR20 and Nootripathu) with contrasting drought-resistance traits were used to map the QTLs for morpho-physiological and plant production traits under drought stress.

Kanagaraj *et al.*, (2010) identified markers linked to drought resistance using 23 recombinant inbred (RI) lines of IR20 × Nootripathu, two *indica* ecotypes with extreme drought response.

Srividhya *et al.*, (2011) developed a recombinant inbred population derived from the cross between a semi-dwarf variety IR64 and landrace INRC10192 for mapping putative QTLs relating to five seedling traits viz., shoot length, maximum root length, shoot dry weight, root dry weight and root to shoot dry weight ratio.

Bhoopathi *et al.* (2012) evaluated near isogenic lines (NILs) carrying large-effect QTL (*qtl12.1*) influence on grain yield under upland drought stress conditions.

Marathi *et al.* (2012) used Pusa1266 × Jaya based 310 RILs for mapping QTLs of yield and yield contributing traits in rice.

Suji *et al.* (2012) developed NILs by introgression of three root QTLs from CT9993, an upland japonica into IR20, a lowland indica cultivar through MAB. Considerable variation in drought response and grain yield under rainfed condition in traits in target populations of environment (TPE) was observed among the NILs.

Singh *et al.* (2013) used RILs mapping populations were developed from a cross between NPT II (IR 68552-100-1-2-2) and HBC 19 (pureline selection from Taraori basmati) for genetic study of fragrance gene by microsatellite marker.

Wang *et al.* (2013) performed quantitative trait locus (QTL) analyses using 215 recombinant inbred lines derived from a cross between Xieqingzao B (XB), a maintainer line with short roots and R9308, a restorer line with long roots.

Selvi *et.al.* (2015) developed a set of twenty-nine near-isogenic lines with Root QTL introgressions of IR64 (*indica*, high yielding) with QTL introgressions from Azucena (*japonica*, drought tolerant) controlling root morphology.

Dixit *et al.* (2015) used BC_2F_3- mapping population derived from parents Moroberekan and Swarna for traits related to drought tolerance, yield potential, lodging resistance, and adaptation to dry direct seeding.

Nie *et al* (2015) developed a population of 105 advanced BILs (backcross introgression lines) derived from a cross between Zhenshan97B and IRAT109 in Zhenshan97B background for Screening of candidate genes and fine mapping of drought tolerance quantitative trait.

Prince et *al.* (2015) developed three hundred and ninety-seven RILs from a cross between IR20 and Nootripathu for mapping Yield QTLs under Drought Stress.

2.7 Molecular markers

Molecular markers help to track the genetic loci controlling drought-resistance traits without having to measure the phenotype, thus reducing the need for extensive field testing over space and time (Nguyen *et al.* 1997).

There are many types of DNA markers that are produced by a wide variety of techniques (Mohan *et al.,* 1997) yet only a subset has been widely used for whole genome mapping projects. RFLPs, RAPDs, AFLP simple sequence repeat (SSR) and more recently SNP are the most popular molecular markers used in genome mapping.

The advent of molecular markers has revolutionized the genetic analysis of complex traits such as drought resistance in crop plants (Boopathi *et al.,* 2011).

DNA markers are employed to construct the genetic map of organism, to map the genes governing qualitative and quantitative traits, to exercise indirect selection for various agronomic traits and to isolate the gene based on map position.

2.7.1 SSRs (Simple sequence repeats)

Simple sequence repeats (SSRs), also known as microsatellites, are present in the genome of all eukaryotes. These are ideal DNA markers for genetic mapping and population studies because of their abundance. These are tandemly arranged repeats of mono, di, tri, tetra and penta nucleotides with different lengths of repeat motifs (A, T, AT, GA, AGG, AAAC etc.). These repeats are widely distributed throughout plant and animal genomes that display high levels of genetic variation based on differences in the number of tandemly repeating units at a locus.

In rice more than 2500 microsatellite markers have been developed and used to construct genetic map (McCouch *et al.,* 2002).

Singh *et al.* (2009) developed 436 HvSSR markers which are evenly distributed in the rice genome.

SSRs are easier to use than Restriction Fragment Length Polymorphism (RFLP), owing to the smaller amount of DNA required, higher polymorphism and the ability to automate assays. SSR markers can easily be exchanged between researchers because each locus is defined by the primer sequences. SSR assays are more robust than Randomly Amplified Polymorphic DNA (RAPD) and more transferable than Amplified Fragment Length Polymorphism (AFLP). SSRs are now replacing RFLPs in genetic mapping of crop plants. A combination of SSRs with AFLPs is used to produce detailed genetic maps. The co-dominant nature of SSRs is also an advantage for genetic mapping.

Srividhya *et al.* (2011) used 412 rice microsatellite primers to screen the parents, 113 found to be polymorphic and they are used these polymorphic marker for Construction of Linkage Map and QTL Mapping.

Bhoopathi *et al.* (2012) used 34 SSR markers mapped in the vicinity of RM28048 and RM511 were selected for BSA analysis.

Karmakar *et al.* (2012) studied landraces were genotyped with 22 reported SSR markers mapped within osmotic stress tolerance linked QTL loci from four different chromosomes.

Suji *et al.* (2012) used flanking SSR markers, RM252–RM348 and RM348–RM280 for basal root thickness and root pulling force QTLs on chromosome 4 and RM257–RM242 for penetrated root thickness QTL on chromosome 9 and these were used in foreground selection of backcross progenies.

The SSR markers have been used widely for rice germplasm evaluation, interpreting population structure and genetic dissection of quantitative trait loci (QTLs) for important agronomic traits (Akkaya *et al.*, 1992; Yang *et al.*, 1994; McCouch *et al.*, 1997, 2002; Cho *et al.*, 2000 and Singh *et al.*, 2013).

Wang *et al.* (2013) prepared linkage map for the RILs, which comprised 198 simple sequence repeat (SSR) markers, spanned 1814.5 cM, with an average spacing of 9.2 cM between adjacent markers.

The five SSR markers viz., RM202, RM212, RM258, RM264 and RM279 were found polymorphic among the 66 SSR markers screened between parents (Mahamaya × Budda) by Prakash *et al.*, (2015). The data of five SSR markers were used to identify QTLs for drought susceptibility index.

A total of 96 pairs of InDel and SSR markers were designed and used by Nie *et al.* (2015), of which 52 pairs expressed polymorphism in both parents. And of these 52, 20 pairs were used in mapping of drought tolerance quantitative trait under drought stress.

2.8 Genetic map using microsatellites

Microsatellite also known as simple sequence repeats (SSRs) that are tandem arrays of short nucleotide repeat to from 1 to 5 bases per unit. The genus *Oryza* contains two cultivated species and more than 20 wild species. Their genome constitution is AA, BB, CC, DD, EE and FF, as well as some unidentified

genomes (Vaughan, 1989). The entire chloroplast genome of *Oryza sativa japonica*, cv. Nipponbare was sequenced by Hiratsuka *et al.,* (1989) and 12 SSR were identified in that sequence by Provan *et al.* (1997).

The average density of one SSR marker every 4 cM on the IR64 × Azucena double haploid map (Guiderdoni *et al.,* 1992 and Huang *et al.,* 1994) has been developed. As more genomic sequence information become available for rice, additionally polymorphic microsatellite markers can be easily designed to saturate the genetic map at predicted densities of one SSR per 20-50 kb and in regions of particular interest on where overall polymorphism is not a limiting factor, higher density coverage (up to one SSR per 3-10 kb) is likely.

Microsatellites have been extensively exploited for genome mapping and for a wide range of population and evolutionary studies in human (Bowcock *et al.*, 1994), mouse (Dietrich *et al.,* 1996), *Drosophilla* (Goldstein and Clark, 1995; Schlotterer *et al.*, 1997 and Schug *et al.,* 1998), *Arabidopsis* (Innan *et al.*, 1997) rice (Yang *et al.*, 1994) and other animal and plant species (Jarne and Lagoda, 1996 and Powell *et al.*, 1996).

In rice (*Oryza sativa*) early studies (reviewed by McCouch *et al.,* 1997) demonstrated that microsatellite markers are distributed relatively uniformly throughout the genome and detect a high level of allelic diversity in cultivated varieties and distantly related species. A map consisting of 121 microsatellite loci and providing genome wide coverage in rice has been published (Chen *et al.*, 1997). There are estimated 5700-10000 microsatellite sequences with different di, tri and tetra nucleotide repeats in rice genome that can be potentially used construct a genetic map based solely on microsatellite markers. The distributions of SSR sequences showed that regions of the rice genome that were richer in expressed genes also tend to be richer in SSR sequences (McCouch *et al.*, 1997).

Successful development of many informative, SSLP markers based on GT/ CA SSR motif for maize (Chin *et al.,* 1996; Taramino and Tingey, 1996), barley (Liu *et al.,* 1996) and wheat (Roder *et al.,* 1998) suggests that it can be a valuable source of microsatellite markers in some other plant species in addition to the most frequently exploited GA motif.

A genetic linkage map was constructed using a total of 213 microsatellite markers (SSRs) derived from the Cornell SSR linkage map (McCouch *et al.*, 2002).

The higher density of SSR markers was found on chromosome #1, #3 and #5 (with 5.6, 5.8 and 5.6 SSRs per Mb, respectively) and the lower density was observed on chromosomes #8, #9, #10, #11 and #12 (McCouch *et al.*, 2002).

The 436 HvSSR markers were evenly distributed in the rice genome, except that some regions of the chromosomes 4, 8 and 11 are poorly represented (Singh *et al.*, 2009).

2.8.1 Genome mapping

The developments in field of molecular biology have opened up avenues for directed improvement of crop plants, which were unthinkable till recently through conventional means. Botstein *et al.,* (1980) published the first landmark paper giving a conceptual framework for construction of saturated genome maps employing DNA based genetic marker in human. Mapping and sequencing would help to explain gene function, gene regulation and their expression. High-resolution linkage maps are being developed for many crops. Construction of detailed genetic map for the crop of interest will make available a precise but vast amount of information that plant breeders can use to identify, manipulate and complement traits to their maximum advantage.

2.8.1.1 Molecular map

The advent of saturated molecular maps promised rapid progress towards the improvement of crops for genetic complex traits like drought resistance *via*. analysis of QTLs (Price *et al.*, 2002b).

The first restriction fragment length polymorphism (RFLP) map of rice was constructed in the 1980s at Cornell University (McCouch *et al.,* 1988).

Maps are constructed based on the linkage and crossing over between the genes or markers in a genome. The tendency of two or more genes to stay together during inheritance is known as linkage. Linkage is the consequence of the concerned genes being located on the same chromosome (Shanmugasundaram, 2001).

For the construction of linkage maps with molecular markers, parents are chosen that show the maximum of polymorphic loci in order to ensure the mapping of as many as possible (Nagarajan, 2001). In such studies, it is desirable, to include only those genes which shows less than 20 % preferably, 10 % or less recombination with each other to avoid confusion due to double and triple cross over. If the crossing over between the genes in test crosses, were more than 20 % the linkage maps would not be very reliable (Shanmugasundaram, 2001).

During the past two decades, DNA markers have been successfully used for screening plant genomes for quantitative trait loci (QTLs) controlling complex traits, including tolerance to abiotic stresses (Ribaut *et al.*, 1997; Zhang *et al.*, 2001). The developments of molecular genetic linkage map for many species provide the foundation and tools for QTL mapping, marker-assisted breeding, physical mapping and map based cloning of gene.

Genome coverage provided by these simple sequence length polymorphisms (SSLPs) is sufficient for genotype identification, gene and quantitative trait locus (QTL) analysis, screening of large insert libraries and marker-assisted selection in breeding (McCouch *et al.*, 1997). SNP, RFLPs, RAPDs, simple sequence repeats (SSR) and AFLPs are most popular molecular markers used in genome mapping.

Temnykh *et al.* (2000) developed and evaluated some 188 new microsatellite markers for allelic diversity. The new simple sequence length polymorphisms (SSLPs) were incorporated into the existing map previously containing 124 SSR loci. The 312 microsatellite markers reported provide whole-genome coverage with an average density of one SSLP per 6 cM.

Mu *et al.* (2003) produced a molecular linkage map for drought resistance with 94 RFLP markers and 71 SSR markers covering 1535.1 cM by using double haploid (DH) population of IRAT109 × Yuefu.

A genetic linkage map was constructed by Gomez *et al.* (2010) using 101 polymorphic PCR-based markers distributed over the 12 chromosomes covering a total length of 1529 cM in 17 linkage groups with an average distance of 15.1 cM this study involve 250 RILs, F8 generation of IR20 × Nootripathu.

The main-effect QTLs associated with panicle number, tiller number, leaf water potential, canopy temperature, stem base width, and thousand kernel weight were mapped in the 30-kb region RIO02002-RM02002 narrowed down from the target region RM561-RM341 on chromosome 2 via molecular marker by Nie et *al.,* (2012) using the advanced BILs (Backcrross inbred Lines) population.

Mishra *et al.* (2013) constructed genetic linkage map based on 106 polymorphic SSR markers in backcross inbred line (BIL) population.

2.9 Development and concept of QTL analysis

Quantitative trait (QT) is a term of central importance in the field of biology and agriculture. As the term indicates, QT refers to characters that can be measured on a quantitative scale (Arunachalam, 2001). Many important characters are a consequence of the joint action of several genes. Such characters are often referred to as polygenic or quantitative. The genetic loci for such characters have been referred to as quantitative trait loci (QTLs). Several characters of plant species, including traits of agronomic importance are inherited quantitatively. Yield, maturity date and drought tolerance are examples of such characters. Although, the principles of QTL analysis were first outlined and successfully applied in the early 1920s to map a QTL for seed size which tightly linked to a gene controlling seed pigmentation (Sax, 1923). However a wide scale application of QTL analysis was not possible at that time due to the paucity of genetic markers available. The systematic identification and characterization of QTLs was finally made possible more than 50 years later, following the introduction of the first class of molecular markers (restriction fragment length polymorphism, RFLP) suitable for an adequately detailed genome wide survey (Botstein *et al.*, 1980). The time consuming process of map construction was considerably shortened with the introduction of the PCR based microsatellite (SSR, simple sequence repeat) markers, particularly suited for mapping purposes due to their high level of polymorphism (Taramino and Tingey, 1996). From the mid 1990s, the addition of AFLPs (amplified fragment length polymorphism (Vos *et al.,* 1995) has provided an unprecedented level of map saturation (Vuylsteke *et al.,* 1999).

QTLs are marvelous genetic entities demonstrating great utility in genetic understanding of complex traits and promise greater impact in crop improvement

endeavors they have provided clear view of the genetics of agronomically important and several other traits too. The distinction and clarity between monogenic and polygenic traits is blurred with the increase in power and precision of methods to detect and map QTLs (Shashidhar *et al*., 2005).

In principle, once QTLs have been identified, introgression of the favorable alleles and their pyramiding into elite germplasm (*e.g*., parental lines, populations etc.) becomes possible through marker-assisted selection (MAS) (Ribaut and Hoisington, 1998; Stuber *et al.,* 1999). However, only a few successful applications of MAS for the improvement of quantitative traits have been described to date (Hu *et al.,* 1997 and Ribaut *et al.,* 2000) due mainly to weak association (in terms of genetic distance) between markers and target QTLs and/ or the high costs of MAS (Stuber *et al.,* 1999 and Salvi *et al.,* 2001). Certainly, a more rosy picture for MAS emerges considering single-gene traits such as disease resistance (Witcombe and Hash, 2000).

DNA markers have been most useful in dissecting complex agricultural traits like QTLs controlling drought, disease, salinity, yield traits, cold tolerance, maturity etc. Most of these traits have not only been mapped and QTL identified, their effect quantified, the alleles detected, some QTLs have been cloned and characterized. In few cases the most probable genes underlying the QTL have been identified. This is the most useful area where markers could make an impact on plant breeding applications with precision. There is now enough information available on QTLs, which would help us to select the superior alleles of different genes controlling the complex traits *via*. markers (Hittalmani *et al.,* 2003).

There are several statistical methods developed to analyze mapping data to search systematically for major QTLs in experimental organisms. The traditional method known as single marker analysis or point analysis is used to detect association between a QTL and a marker. Thoday (1961) and Soller and Brody (1976) described the idea of using single genetic markers one at a time to systematically characterize and map individual polygene's controlling the quantitative trait. For detecting a QTL near a marker, phenotypic means for the progeny of each marker class are compared (Edwards *et al.,* 1987). The difference between the means provides an estimate of the phenotypic effect of substituting a

'B' allele for an 'A' allele at the QTL. A one-way analysis of variance (*i.e*., linear regression) can be performed to test whether the inferred phenotypic effect is significantly different from zero. A significant value indicates that a QTL is located in the vicinity of the marker. The additive effect associated with the marker locus can be estimated by linear regression of marker genotype means on genotypes.

Stuber *et al.* (1992) and Lander and Botstein (1989) proposed a method known as interval mapping using the likelihood approach (LOD score) which has been in use widely now-a-days. In this method, instead of using one marker at a time, sets of linked markers are analyzed simultaneously with regard to their effects on the quantitative traits. They developed a computer program known as Mapmaker/ QTL (Lincoln *et al.* 1992) for implementing interval mapping, which is being used commonly (Redona and Mackill, 1996; Xiao *et al.* 1996). Typically, a LOD score threshold value of between 2 and 3, depending on the density of genetic markers, chromosome length and trait distributions, is required for declaring the significance of a putative QTL (Doerge and Rebai, 1996). Kruglyak and Lander (1995) recommended that the dense-map threshold always be used, regardless of the actual density of the map, in order to minimize the false positive rate. Churchill and Doerge (1994) and Doerge and Churchill (1996) proposed some methods of estimating empirical threshold values for declaring significant QTL effects in a genome or at any point within a genome by the application of a permutation test. These methods can also be used for detecting the presence of minor QTLs effects.

Stuber *et al.* (1992) and Bubeck *et al.* (1993) compared the one-at-a-time analysis method with the maximum likelihood-based interval mapping method. Both analytical methods showed virtually the same results in detecting QTL because maximum likelihood estimates reduce least squares estimate when data are normally distributed. Jansen and Stam (1994) proposed a method for multiple linear regression of a quantitative phenotype (putative QTLs) on genotype (marker).

There are three widely used methods for detecting QTLs, single marker analysis, simple interval mapping and composite interval mapping (Tanksley,

1993). Single marker approach, sometime referred to as the single factor analysis of variance (SF-ANOVA) or simple point analysis, is the simplest method for detecting QTLs associated with single markers. SF-ANOVA is done for each marker locus independent of information from other loci. F-test provides evidence whether differences between marker locus genotype classes are significant or not. The statistical methods used for single-marker analysis include t-test, analysis of variance (ANOVA) and linear regression. Linear regression is most commonly used because the coefficient of determination (R2) from the marker explains the phenotypic variation arising from QTL linked to the marker. This method does not require a linkage map and can be performed with basic statistical software programs. However, the major disadvantage with this method is that the further a QTL is from a marker, the less likely it will be detected. This is because recombination may occur between the marker and the QTL. This causes the magnitude of the effect of a QTL to be underestimated (Tanksley, 1993). Q-Gene and MapManager QTX are commonly used computer programmes to perform single-marker analysis (Nelson, 1997)

Different software was developed for QTL analysis *viz*., MAPMAKER/ QTL (Lincoln *et al.,* 1992), QTL cartographer (Basten *et al.,* 1997), Map Manager QT (Manly and Elliott, 1991), MQTL (Tinker and Mather, 1995), QTL Network (Yang *et al.*, 2007) and QTL IciMapping (Li *et al.*, 2007).

2.9.1 Identification of quantitative trait loci for grain yield and traits related to drought tolerance

Molecular approaches to drought tolerance have been widely applied to rice, beginning with QTL analysis. QTLs have been identified for many traits that are associated with drought response such as root characters, membrane stability, osmotic adjustment and morphological and physiological traits where tolerance is measured as yield under drought.

QTLs were identified in RIL population of a cross between Co39 and Moroberekan for tiller, root number, thickness and dry weight in rice (Champoux *et al.,* 1995). QTLs for root morphology and root distribution in DHL mapping population of a cross between IR64 and Azucena in rice were detected (Yadav *et al.,* 1997). On the same DHL population of IR64 X Azucena, (Hemamalini *et al.,*

2000) identified 15 QTLs for morphological and physiological traits related to drought resistance in rice. Venuprasad *et al*., (2001) worked on IR64 X Azucena DH mapping population of rice in three diverse environments and detected QTLs for ten traits at a threshold. The QTLs were spread across six chromosomes 1, 3, 4, 5, 6, and 7. Three QTLs for grain yield were detected one each on chromosome 3, 4 and 5. QTLs for root thickness and root penetration index in rice were detected (Zhang *et al*., 2001). Price *et al.* 2002 also reported 24 QTLs for various root growth traits in RIL population of a cross between Azucena and Bala in rice for drought tolerance. QTLs for yield, biomass, osmotic adjustment and roots in rice reported (Chandra Babu *et al.*, 2003). Lafitte *et al*. (2004) identified 31 QTLs for yield and its components under rice drought on working with RIL population of Bala X Azucena. QTLs for yield; yield components, panicle sterility etc in rice was identified (Jonaliza *et al.*, 2004) in RIL population of a cross between CT9993 and IR62266. Xu *et al.* (2005) identified 36 QTLs in introgression indica lines of rice for yield and its components under drought.

Many QTLs have been reported in rice for traits that are putatively associated with performance under drought, such as root system morphology (Champoux *et al.*, 1995; Yue *et al*., 2006b; Courtois *et al*., 2009; Obara *et al*., 2010 and Uga *et al*., 2011), osmotic adjustment (Robin *et al*., 2003), leaf membrane stability (Tripathy *et al.*, 2000) and visual symptoms of leaf stress such as rolling and drying (Courtois *et al.*, 2000 and Deokar *et al*., 2007). But it is still unclear whether these secondary traits significantly contribute to grain yield under drought stress.

Hemamalini *et al.* (2000) worked on the DHL population of IR64 × Azucena and identified 15 QTLs for morphological and physiological traits related to drought resistance in rice.

Venuprasad *et al.* (2001) worked on IR64 × Azucena DH mapping population of rice in three diverse environments and detected QTLs for ten traits. The QTLs were spread across six chromosomes 1, 3, 4, 5, 6 and 7. Three QTLs for grain yield were detected one each on chromosome 3, 4 and 5.

A QTL for penetrated root thickness was mapped on chromosome 9 in CT9993/IR62266 DH lines (Zhang et *al.*, 2001).

Hittalmani *et al.* (2002) detected a total of 34 quantitative trait loci (QTLs) for 11 traits across three locations; one QTL was identified for grain yield per plant.

Price *et al.* (2002c) reported 24 QTLs for various root growth traits in RIL population of a cross between Azucena and Bala in rice for drought tolerance.

Babu *et al.* (2003) reported 47 QTLs for leaf rolling and drying scores, days to heading (days after sowing), plant height, grain yield, biomass, spikelet fertility, seeds per panicle, harvest index and relative yield by interval mapping *via.* QTL Mapper version 1.0 in a doubled-haploid rice population of 154 lines from CT9993 and IR62266 in different trials.

Hittalmani *et al.* (2003) reported a genomic region of 7.9 cM (from 135.8 to 143.7 cM) on chromosome 1 was associated with drought resistance traits such as leaf rolling, number of spikelet, heading date and harvest index in IR64 × Azucena rice DH lines. Similar result was found by Kanagaraj *et al.,* (2010) by using 23 recombinant inbred (RI) lines of IR20 × Nootripathu.

QTLs for root traits have been mapped in CT9993/IR62266 rice DH lines (Zhang et *al.*, 2001; Nguyen et *al.*, 2004).

Lafitte *et al.,* (2004b) identified 31 QTLs for yield and its components under rice drought on working with RIL population of Bala × Azucena.

Lanceras *et al.* (2004) used RIL population of a cross between CT9993 and IR62266, and identified 77 QTLs for yield; yield components, panicle sterility etc. in rice.

Verulker *et al,* (2004) identified of 143 QTLs for various traits in rice.

Ashikari *et al.* (2005) identified a major QTL for grain number (*Gn1*) and a QTL for plant height (*Ph1*) by using the progeny from the cross between a japonica rice, Koshihikari, and an indica rice, Habataki.

Xiao *et al.* (2005) reported identification of 2, 4, 4, 1, 2, 2 and 3 QTLs for grain yield, 100 kernel weight, kernel number per ear, cob weight per ear, kernel weight per ear, ear weight and ear number per plant respectively under well-watered regime and 1, 5, 2, 6, 1, 3 and 2 QTLs respectively under water-stressed condition.

Xu *et al.* (2005) identified 36 QTLs in introgression *indica* lines of rice for yield and its components under drought.

QTLs for grain yield and its component traits (e.g., spikelet fertility, spikelet number per panicle, 1,000-grain weight, and panicle number) were identified by Zou *et al.* (2005).

Yue *et al.* (2006a) reported QTLs *nlr2*, *nlr3* and *nlr8* for leaf rolling on chromosome 2, 3 and 8 by using RI population derived from a cross of Zhenshan97 and IRAT109.

Yue *et al.* (2006b) reported 39 QTLs for productivity, spikelet fertility, leaf rolling, biomass, harvest index and grain weight with RIL population of *indica* lowland × tropical *japonica* upland.

Gomez *et al.* (2006) identified 24 QTLs for various physio-morphological and plant production traits under drought stress. The number of QTLs per trait under stress was: 5 for leaf rolling, 4 for leaf drying, 3 for days to 50 % flowering, 5 for plant height, 2 for number of productive tillers, 1 for panicle length, 3 for grain yield and 1 for straw yield.

Bernier *et al.* (2007) used population of 436 random F3 derived line from a cross between the upland rice cultivar Vandana and Way Rarem, and reported *qtl12.1* on chromosome 12 with large effect on grain yield under stress. They also reported that under stress also increases harvest index, biomass yield and plant height while reducing the number of days to flowering.

Kumar *et al.* (2007) reported QTL on chromosome 1 near *sd1* that explained 32 % of the genetic variation for yield under stress, but only 4 % under non-stress. They also found their effect consistant across years.

Panicle length–associated QTLs, panicle number–associated QTLs, and spikelet number per panicle–associated QTLs were mapped in RM17169-RM17183, RM5320-RM17355, and RM5320- RM17355, respectively, within the target region RM273- RM255 on chromosome 4 via molecular marker by Liu (2007).

Lin *et al.* (2007) reported the association of 5 QTLs with yield and other QTLs *dis4.1, lr8.1, lr10.1, lr12.1, y6.1, y6.2, y1.1* and *y8.1* affecting agronomically

important traits in rice for drought, found in F2 mapping population of Taichung189 × Milyang23.

Deokar *et al.* (2007) identified 11 QTLs for leaf rolling and relative water content.

Cui *et al.* (2008) reported 34 QTLs for plant height, maximum root length etc. by using Zhenshan97 × Minghui63 population.

A total of 19 putative QTLs associated with drought tolerance were detected by Srividya *et al.* (2011). Chromosomes 1 and 8 harbor most of the QTLs. Seven QTLs were found associated with root shoot ratio. Of them, five namely *qrs*1.1, *qrs*1.2, *qrs*2.1, *qrs*8.1 and *qrs*8.2 were detected under the stress condition.

qSOR1, a major rice QTL involved in soil-surface rooting in paddy fields was studied (Uga *et al.,* 2012) and QTLs for root morphology of a rice population adapted to rainy lowland conditions were analyzed (Kamoshita *et al.,* 2002a). Some QTLs associated with root traits that increase yield in upland rice have been investigated (Steele et *al.*, 2013). A major QTL, Dro1, involved in deep rooting of rice under upland field conditions, was detected on chromosome 9 (Uga *et al.,* 2011). A distinct QTL-qREP-6 involved in root elongation induced by phosphorus deficiency was detected on the long arm of chromosome 6 (Shimizu *et al.,* 2008). Brt4, a major QTL conferring basal root thickness, was located to chromosome 4 (Liu *et al.,* 2008).

The region RM1-RM495 on chromosome 1, studied by Srividya *et al.* (2011) found to harbor five QTLs for shoot and root related traits which has been reported by many earlier workers. Li *et al.* (2005) have reported QTLs for root thickness, root number and root dry weight, while length of mesocotyl by Redona and Mack-ill (1996) in the same region. Further, Xu *et al.* (2004) and Price *et al.,* (2000) have reported QTLs for PH and root number, respectively in the same region. Cui *et al.* (2008) have identified a QTLs cluster in the same region for plant height and shoot fresh weight under well watered conditions.

Wang *et al.* (2013) identified a QTL (associated with root length were mapped on chromosomes 7), named qRL7, was located between markers RM3859 and RM214 on chromosome 7 and explained 18.14–18.36% of the total phenotypic variance evaluated across two years.

Three main-effect QTLs related to panicle length, panicle number, and spikelet number per panicle were identified in the target interval of RM273-RM255 on chromosome 4 (Nie *et al.,* 2015). The panicle length-related QTL had two loci located in the neighboring intervals of RM17308-RM17305 and RM17349- RM17190, which explained 18.80% and 20.42%, respectively, of the phenotypic variation, while the panicle number-related QTL was identified in the interval of RM1354-RM17308, explaining 11.47% of the phenotypic variation. As far as the spikelet number per panicle-related QTL was concerned, it was found to be located in the interval of RM17308-RM17305, which explained 28.08% of the phenotypic variation.

Prince *et al.* (2015) studied three QTL regions on chromosome 1 (RM8085), chromosome 4 (I12S), and chromosome 6 (RM6836) harbor significant additive QTLs for various physiological and yield traits under drought stress. The similar chromosomal region on 4 and 6 were found to harbor QTLs for canopy temperature and leaf drying under drought stress conditions.

Ren *et al.* (2016) detected four quantitative trait loci (QTLs) for the BRR on chromosomes 1, 8, 9, and 10, respectively. In addition, three QTLs for appearance traits, including grain weight and grain length/width ratio, were detected on chromosomes 6, 9 and 10, respectively.

Studies on Quantitative Trait Loci (QTLs) mapping and Linkage Disequilibrium (LD) mapping have provided needed information on how gene combinations might work under specific environments against particular genetic backgrounds.

2.10 Fine mapping concept

Obtaining a fine map with a distance of 1-2 cM between markers is necessary to better define QTL positions and thus to facilitate the use of the map-based cloning to isolate genes linked to the trait of interest (Ronin *et al.,* 2003, Mott 2006). Different methods are available for fine mapping and include the selection of certain genotypes, the increase of the mapping population size or of the number of markers (Melchinger 1998, Ronin *et al.,* 2003, Vales *et al.,* 2005, Xu *et al.,* 2005). A large population reduces the impact of incorrect genotyping scoring when screening the population with polymorphic markers and is a way to

accumulate more recombinants in the interval of interest (Ronin *et al*., 2003). The development of highly polymorphic DNA molecular markers which are easy to use and transferable between populations and/or species has facilitated the creation of saturated maps. Molecular markers are specific fragments of DNA that show a variation of the DNA sequence among the different individuals of a population (Jones *et al.*, 1997). Restriction Fragment Length Polymorphism (RFLP) markers were first developed for use in the production of genetic maps in humans (Botstein et *al.* 1980), and were quickly adapted to plants (Helentjaris *et al.,* 1985). Over the last three decades new generations of markers have been introduced (Collard *et al.,* 2005). Compared to morphological or isozyme markers, DNA molecular markers are abundant throughout the genome, completely independent of environmental conditions, can be detected at any stage of development of the plant and do not disturb the physiology of the organism (Mohan et *al.* 1997, Jones *et al.*, 1997). They are tools in various fields and are nowadays widely used in crop improvement, for cultivar identification, parental analysis, synteny mapping, genome mapping and tagging of agronomically important genes (Joshi *et al.*, 1999, Saha *et al.*, 2005).

With molecular markers, the fine mapping can be focused on a specific region of interest and the varieties of markers available give the possibility to select the most useful for the study. The markers can be selected in function of the population, the species, the amount of DNA available and rely on the objectives of the study. SSR markers are efficient tools widely used for their high degree of polymorphism, abundance, distribution along the genome and ease of use (Powell *et al.*, 1996, Kuleung *et al.*, 2004, Saha *et al.*, 2006). But detailed knowledge of DNA sequences are required for their development and few SSRs are available for species with low genomic sequence resources. For these species another approach, which consists of the use of the synteny between species to transfer markers between species or to develop additional markers.

In recent years more attention has started to be given to mapping of QTLs for grain yield under managed stress environments (Zhang *et al.,* 1999; Lafitte *et al*., 2002; Babu *et al*., 2003; Lanceras *et al.,* 2004; Subashri *et al*., 2009;

Venuprasad *et al*., 2009a; Bernier *et al.*, 2007, 2009b; Liu *et al*., 2010; Gomez *et al*., 2010; Vikram *et al*., 2011 and Yadaw *et al.*, 2013).

Lang and Buu (2008) mapped drought recovery score (DRS) genes between RM201, RM328 and that flanked 0.4cM and 13.8 cM respectively on chromosome 9.

Wan et al. (2008) were narrowed down grain width QTL, *gw-5* to a 49.7-kb genomic region with high recombination frequencies on chromosome 5 using 6781 BC_4F_2 individuals and 10 newly developed simple sequence repeat markers.

The three yield-associated meta-QTLs identified on chromosome 1, based on a genome-wide analysis, the region RM543–RM212 spans a small genetic distance of 0.27 kb and makes it suitable for use in MAB and pyramiding of QTLs for yield and drought tolerance in rice (Swamy et *al.*, 2011).

Salunke *et al.* (2011) mapped RM8085 in the middle of the QTL, linked to leaf rolling and leaf drying region on chromosome 1 previously identified in recombinant inbred lines and reducing the QTL interval from 7.9 to 3.8 cM. Further, they reported that the region, RM212-RM302-RM8085-RM3825 on chromosome 1, harbours large effect QTLs for drought-resistance traits across several genetic backgrounds in rice.

Two QTLs for shoot dry weight under stress condition have been mapped at the interval RM106-RM5897 on chromosome 2 by Srividhya *et al.* (2011). They also reported two QTLs for mean root length in The vicinity of RM570- RM251 on chromosome 3. The region, RM38-RM331 on chromosome 8 was observed to have effects on root dry weight.

Suji *et al.* (2012) fine mapped three root QTLs on chromosome 4 and 9 linked to basal root thickness, root pulling and penetrated root thickness. The selected QTLs had relatively small confidence interval (5.2–9.7 cM; 1.1–3.1 Mb). The flanking SSR markers, RM252–RM348 and RM348–RM280 for basal root thickness and root pulling force QTLs on chromosome 4 and RM257–RM242 for penetrated root thickness QTL on chromosome 9.

Wang *et al.* (2013) performed fine mapping of qRL7 using eight BC_3F_3 recombinant lines mapped the QTL to between markers InDel-11 and InDel-17, which delimit a 657.35 kb interval in the reference cultivar Nipponbare.

Nei *et al.* (2015) reported a main-effect QTL associated with both panicle length and spikelet number per panicle and it was fine mapped in the target interval RM17308-RM17305 on chromosome 4. Another QTL associated with panicle length was mapped in the target region RM17349- RM17190. The QTL controlling panicle number was located in the region RM1354-RM17308.

Prince *et al.* (2015) identified major QTLs, were mined and found to possess 248 genes in an interval of 1.61Mbp (chromosome 1; RM8085–RM3825), 350 genes in an interval of 2.4Mbp (Chromosome 4; RM5424–RM3042) (Additional file 5: Table S5), two genes (Chromosome 4; RM6909) and 1 gene (Chromosome 6; RM6836). In the chromosome 1 QTL region, 17 genes were highly expressed in drought stress conditions on the flag leaf, leaf, panicle, and root tissues.

Ren *et al.* (2016) reported four quantitative trait loci (QTLs) for the brown rice rate (BRR) on chromosomes 1, 8, 9, and 10, respectively. Chromosome segment substitution lines (CSSLs) were established at the qBRR-10 locus. qBRR-10 was narrowed to a 39.5 kb region on chromosome 10.

2.11 Development and concept of gene expression

Drought tolerance is a complex trait and involves mechanisms that act in isolation or combined to avoid or tolerate periods of water deficit. It is expected that genotypes responding differently to drought stress show differences in gene expression, and that a portion of the differences is related to drought tolerance. Therefore, the analysis of the genes found exclusively in the tolerant genotype is of interest to identify genes associated with water usage efficiency.

Roots are one of the primary sites responsive to restrictive conditions of water availability and, as a result, synthesize chemical signals for a rapid response of the plant to drought stress (Wilkinson and Davis, 2002). This occurs since the response in leaves must be stimulated rapidly to avoid irreversible damage to the photosynthetic machinery. The response of roots to water limiting conditions seems to be crucial to trigger drought tolerance mechanisms, since roots are one of the primary sites for stress signal perception in which a signaling mechanism initiates a cascade of gene expression responses to drought. These transcriptional changes can result in successful adaptations leading to stress tolerance by

regulating gene expression and signal transduction in the stress response (regulatory proteins) or directly protecting the plant against environmental stress (functional proteins) (Perlin *et. al.*, 2007).

Several functional genomics studies of rice have been performed using different approaches such as macro and microarray (Kawasaki *et.al.* 2007 and Rabbani *et. al.,* 2003), RT-qPCR, SAGE (*Serial Analysis of Gene Expression*), MPSS (*Massive Parallel Signature Sequencing*) and more recently oligoarray using the transcriptome of rice to evaluate responses to abiotic stresses (Shinozaki and Shinozaki, 2006). Proteome analyses have also been increasingly employed to complement genomic studies (Salekdeh *et al.,* 2002 and Agrawal *et al.,* 2006) however in a lower rate. Although numerous genes and proteins, which potentially contribute to drought tolerance in rice, have been reported (Fu *et al.,* 2007 and Wu *et al.,* 2006)

SRT6 is restricted specifically to the development of primary roots. Its expression is phase-specific, and greatly reduced primary root length and diameter (Yao *et al.,* 2003).

Rabello *et al.* (2008) performed proteome analysis of roots from stressed plants was performed and 22 proteins putatively associated to drought tolerance were identified by mass spectrometry. Genes exclusively expressed in the tolerant genotype were related to maintenance of turgor and cell integrity.

Moumeni *et al.* (2011) performed Global gene expression analysis and they reported that about 55% of genes differentially expressed in roots of rice in response to drought stress treatments. The number of differentially expressed genes (DEGs) increased in NILs as the level of water deficits, increased from mild to severe condition, suggesting that more genes were affected by increasing drought stress. Gene ontology (GO) test and biological pathway analysis indicated that activated genes in the drought tolerant lines were mostly involved in secondary metabolism, amino acid metabolism, response to stimulus, defense response, transcription and signal transduction, and down-regulated genes were involved in photosynthesis and cell wall growth.

The first comprehensive transcriptome study of the rice root system was published in 2012 (Takehisa *et al.,* 2012). They identified genes, specifically

expressed in root caps, in the initiation or developmental zone of lateral roots, and those involved in water and nutrient transport. Data are available in the Rice X Pro data base (Sato *et al.*, 2013).

The root transcriptomes of the super-hybrid rice variety Xieyou 9308 and its parents were analyzed at tillering and heading stages by Zhau *et al.* (2013). They reported a total of 829 and 4186 transcripts that were differentially expressed between the hybrid and its parents (DG_{HP}) were identified at tillering and heading stages, respectively. Out of the DG_{HP}, 66.59% were down-regulated at the tillering stage and 64.41% were up-regulated at the heading stage. Several significant DG_{HP} that could be mapped to quantitative trait loci (QTLs) for yield and root traits are also involved in carbohydrate metabolism and plant hormone signal transduction pathways.

Basu and Roychoudhury (2014) chosen the candidate genes for expression studies were *HKT-1, SOS-3, NHX-1, SAPK5, SAPK7, NAC-1, Rab16A, OSBZ8, DREBP2, CRT/DREBP, WRKY24, a*nd *WRKY71*, along with the candidate proteins OSBZ8, SAMDC, and GST. Gene expression profile revealed considerable differences between the salt-sensitive and salt-tolerant rice varieties, as the expression in the latter was higher at the constitutive level.

Mai *et al.*, (2014) reported *DRO1 gene* expression in the IR64 background increases the angle between roots and the horizontal axis, inducing deeper rooting.

In rice lines over-expressing *OsRPK1*, which encodes an LEUCINE RICH REPEAT RECEPTOR-LIKE KINASE (LRR RLK), the expression levels of different *OsPIN* genes are down regulated, polar auxin transport is perturbed and the length of the seminal root is reduced, as is the number of crown roots and the density of lateral roots (Zou *et al*., 2014).

Drouge *et al.* (2014) Microarray data were confirmed by analyzing expression levels of 14 representative genes using reverse transcription quantitative polymerase chain reaction (RT-qPCR). This includes seven up-regulated genes and seven down-regulated genes belonging to seven of the 15 categories described in Figure 1. These results show that the array data are in accordance with the RT-qPCR data ($R^2 = 0.83; P_{value} = 2.7.10^{-10}$).

Rice responded by down-regulating many processes, which are mainly involved in inhibiting growth and development, was reported by Minh-Thu *et al.* (2013). Phytohormones (ABA, Cytokinin, Brassinosteroid) and protective molecules were induced to answer to multiple stresses. Leaf and root tissues shared some common gene expression during stress, with the purpose of enhancing protective systems. Roots up-regulated fewer genes and focused on inducing antioxidants and enhancing photosynthesis.

2.12 Development and concept of gene pyramiding

For quantitative traits such as yield, a single QTL rarely accounts for a significantly large portion of the trait variation. Several QTLs with additive effects need to be stacked to achieve significant improvement. Gene pyramiding is a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. The end product of a gene pyramiding program is a genotype with all of the target genes. Marker assisted gene pyramiding has gained popularity in the last decades (Ye and Smith, 2010). As foreground selection in the context of MABC, markers are used to ensure the presence of the target QTLs. With the recombination frequencies among genes and markers, the frequencies of various satisfactory and desirable genotypes in a population can be predicted according to simple genetic principles. The minimum population size to ensure the presence of the desired genotypes can be calculated using a simple statistical computation. In principle, pyramiding multiple genes is achieved by crossing parental lines with complementary desirable genes and then selecting the desired recombinants among the progeny populations (Allard, 1999). Given that breeding is time-consuming, breeders aim to combine as many desirable alleles as possible in a single breeding cycle (from crossing to the generation of near-homozygous breeding lines). Once the number of genes to be assembled is known, gene pyramiding then aims to obtain near-homozygous breeding lines that are fully homozygous for the desirable alleles of the target genes by using the minimum number of generations, as well as the lowest genotyping and phenotyping costs.

Ashikari *et al.* (2005) pyramided lines for major QTL for grain number (*Gn1*) and a QTL for plant height (*Ph1*) under the Koshihikari genetic background. Pyramiding line carrying *Gn1* and *Ph1* (*sd1*) was selected from the progenies by

using MAS. The pyramided line showed increased grain production (23%) and reduced plant height (20%) compared with Koshihikari.

Ando *et al*. (2008) developed a rice line with two major QTLs, *qSBN1* (for the secondary branch number on chromosome 1) and *qPBN6* (for the primary branch number on chromosome 6).

Swamy and Kumar (2011) successfully developed pyramided lines containing different combinations of the four major drought QTLs (*qDTY2.2*, *qDTY4.1*, *qDTY9.1*, and *qDTY10.1*). Introgressed lines with three and two QTLs in an IR64 background show yield advantages under drought.

Wang *et al.* (2012) developed NILs containing one or more target genes via transfer of 93-11 alleles at *qHD8*, *qHD7*, and *qHD6.1*, and the *GS3* gene for grain size, into Zhenshan 97. The pyramid line, NIL (*qHD8* + *GS3*), shows higher yield potential, longer grains, and a more suitable heading date than Zhenshan 97.

Zong *et al*. (2012) developed pyramided rice lines with eight yield-related QTLs in the background of 93-11. The pyramided lines show increased panicle and spikelet size.

Pinta *et al.* (2013) pyramided blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection.

Suh *et al.* (2013) transferred three resistance genes (Xa4 + xa5 + Xa21) from an indica donor (IRBB57), using a marker-assisted backcrossing (MAB) breeding strategy, into a BB-susceptible elite japonica rice cultivar, Mangeumbyeo, which is high yielding with good grain quality.

Selvi *et al.* (2015) developed two QTL and three QTL pyramid lines for roots and evaluated under drought, aerobic and in different locations to study the performance.

Pradhan *et al.* (2015) pyramided three major BB resistance genes (*Xa21*, *xa13* and *xa5*) into Jalmagna variety, exhibited high level of resistance and are expected to provide durable resistance under deep water situation

Shamsudin *et al.* (2016) pyramided three drought yield QTLs, $qDTY_{2.2}$, $qDTY_{3.1,}$ and $qDTY_{12.1}$ with consistent effect on grain yield under reproductive stage drought stress through marker assisted breeding with the objective of

improving the grain yield of the elite Malaysian rice cultivar MR219 under reproductive stage drought stress.

CHAPTER III
MATERIALS AND METHODS

The present study entitled "**Fine mapping and pyramiding of QTL's for root traits in RIL population of rice (*Oryza sativa* L.)"** was conducted at the Research cum Instructional Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), (210° 16' N and 810° 36' E at altitude of 289.6 meter above sea level), during wet season 2014 and 2015 to generate the phenotypic data under managed field conditions and Marker Assisted Lab, R. H. Richharia Lab, IGKV, Raipur to generate the genotypic data using microsatellite SSR marker.

3.1 Phenotyping of mapping population and development of crosses

3.1.1 Materials

The material in present study includes recombinant inbred lines (RILs) population of a cross between Swarna Sub-1 and IR-86918-305-B. A experiment was conducted during 2010 in which the RIL population of 271 lines of above said cross were evaluated under water stress (absolute rainfed condition) and irrigated condition (control) and based on performance under these two conditions along with important traits like grain yield, leaf rolling, plant height and root pulling resistance, highest and lowest genotypes for grain yield, 71 lines were selected. Selected 71 RILs grown in BC_1F_7 (2014) and BC_1F_8 (2015) generations were subsequently used for this study. The mapping population of recombinant inbred lines (RILs) was developed by using modified single seed descent (SSD) method.

This cross viz., Swarna Sub-1 X IR-86918-305-B of BC_1F_7/BC_1F_8 generation was taken for the study of genetic parameters namely, phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), heritability, genetic advance, association, path along with stability the same population was used for fine mapping of QTLs for grain yield and root traits. For expression analysis, again the lines from the same cross were taken. Of these, six lines of which, three were tolerant (50, 53 and 55) to water stress and three were susceptible (33, 39 and 59). Lines were selected based on leaf rolling and

vegetative stage drought screening (Gana, 2011). For this analysis, RT-qPCR and RT-PCR technique were used. Seven root gene specific primers were taken (table 3.10). The linkage map was constructed with QTL Cartographer 2.5. Graphical genotyping of these molecular data was done using GGT 2.0 (Van Berloo, 1999). The phenotypic and genotypic data was analyzed using QTL cartographer 2.5 (Single Marker Analysis) (Wang *et al*., 2005).

Table 3.1 Characteristic features of parents

Parent	**Reaction to water stress under field**	**Salient Features**
Swarna Sub 1	Susceptible	High yielding, dwarf, high tillering, late maturity (143 days), Medium slender grain
IR 86918- 305-B	Tolerant	Strong culm, tall, shy tillering, broad leaves, bold seeded, early maturity (100 days)

Table 3.2: Selected lines of Swarna Sub-1 X IR-86918-305-B (3 tolerant and 3 susceptible lines) under irrigated, moderate stress and severe stress condition for expression analysis

	Fully Irrigated	**Moderate stress**	**Severe Stress**
Resistant lines	Line #50	Line #50	Line #50
	Line #53	Line #53	Line #53
	Line #55	Line #55	Line #55
Susceptible lines	Line #33	Line #33	Line #33
	Line #39	Line #39	Line #39
	Line #59	Line #59	Line #59

Pyramiding of QTLs for root traits: initially, a RIL population was developed using Danteshwari (released variety) and a land race, Dagad Deshi (deep rooted drought tolerant) during 2003. A set of 122 RILs were selected. For pyramiding of QTLs for root traits, out of 122 RILs a set of 38 RILs were selected on the basis of leaf rolling which clearly classify into stress tolerant and stress susceptible. Therefore, out of 38 RILs, two tolerant lines were taken for crossing program to develop R X R cross. From this R X R cross, F_2 and F_3 segregating

generation was made. Six lines from above said cross was taken to generate the genotypic data. Further, graphical genotyping was prepared by the software (GGT 2.0) based on the above lines.

3.2 Methods

3.2.1 Field studies

The Recombinant Inbred lines (RILs) derived from a cross between Swarna Sub-1 and IR-86918-B-305 were evaluated in the field during wet season 2014 and wet season 2015 at Research cum Instructional Farm of College of Agriculture, IGKV, Raipur. The field trials for BC_1F_7 generation were conducted under irrigated, rainfed and reproductive or terminal stage drought condition (TSD), whereas BC_1F_8 generation were conducted under irrigated and terminal stage drought (TSD). The experiments were conducted in sandy or clay loam inceptisols, with soil pH ranging from 6.8 to 7.4 and organic carbon of 0.32-0.34 %. The screening for drought tolerance was conducted under rainout shelter condition. The rainfed and terminal stage drought condition, fields selected for the study were upland in topology with good drainage and percolation rate. All the genotypes were replicated twice in RCBD design. All normal packages of practices (except water management) were followed to raise a good crop. The detailed information about field conditions is given in Table 3.3.

3.2.2 Observations recorded under field conditions

The observations, for physiological traits contributing for yield, were recorded at plant specific stage during their maximum tillering stage, vegetative stage, maturation stage and some important post-harvest observations, were also recorded according to SES (IRRI 2002). The five plants were selected from each line and observations were recorded for all physiological traits.

3.2.2.1 Seedling height (cm)

Seedling height was measured in centimeter (cm), after 30 days under transplanted as well as direct seeded condition from soil surface to tip of the upper leaf.

3.2.2.2 Seedling shoot biomass (g)

At vegetative stage, the shoot portion of the five plants was taken out and at the same time weighed using the weighing balance separately for each plant and line.

Table 3.3 General information about the field conditions

Conditions	Date of sowing	Date of trans-planting	Spacing (cm)		No of row/ line	Length of row (m)	Number of replication
Kharif season -2014							
I (T)	18 June	16 July	15	15	4		2
RF (Ds)	20 June	-	15	-	5	3	2
TSD (Ds)	26 June	-	15		3	2.0	2
Kharif season -2015							
I (T)	19 June	7 July	20	20	5	2.2	2
TSD (T)	17 July	18 August	15	15	3	2.4	2

Where, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds= Direct seeded, T= Transplant

3.2.2.3 Root pulling resistance (kg)

Root pulling resistance was measured as kilogram (kg) of force required to lift a plant vertically from the soil. The root pulling pressure was taken at maximum tillering stage. Two observations were taken from each line.

3.2.2.4 Number of tillers

Total number of tiller of five selected plants was recorded by counting number of tillers present in each hill of plant at maximum tillering stage and averaged it.

3.2.2.5 Leaf rolling

A 1-9 scale was used for recording leaf rolling which was prepared on the basis of number of leaves rolled, shape of rolled leaf (shallow, V, U and O) and type of leaf rolled (mature, youngest, both and all).

3.2.2.6 Days to 50 % flowering

The time taken (days) from sowing to flowering period of half of the plants from each line were recorded on plot basis by visual means.

3.2.2.7 Plant height (cm)

Plant height of individual five selected plants was measured in centimeter (cm) from soil surface to tip of the tallest panicle at maturity using standard scaling method and averaged it.

3.2.2.8 Panicle length (cm)

Panicle length of five selected plants was measured in centimeters (cm) from base to tip of panicle using standard scaling method.

3.2.2.9 Flag leaf length (cm)

Flag leaf length of selected plants was recorded by measuring the length (cm) from node to tip of flag leaf and averaged it.

3.2.2.10 Flag leaf width (cm)

Flag leaf width (cm) was recorded by measuring the leaf width at the central part of the flag leaf.

3.2.2.11 Second leaf length (cm)

Second leaf (after flag leaf) length of 5 randomly selected plants were recorded by measuring the length (cm) from nod to tip of second leaf and averaged it.

3.2.2.12 Second leaf width (cm)

Second leaf width (cm) was recorded by measuring the leaf width at the central part of the leaf.

3.2.2.13 Biological yield (gm^{-2})

The biological yield was recorded as actual weight in gram of total biomass of shoot portion per plot (one meter sq.).

3.2.2.14 Grain yield (gm^{-2})

The actual yield of grain in gram per plot was recorded and later converted to g/ m2 for further analysis.

3.2.2.15 Harvest index (%)

Harvest index was worked out by using the formula as given below:

$$\text{HI (\%)} = \frac{\text{Economic yield (grain yield)/}}{\text{Biological yield (bundle weight)}} \text{ X } 100$$

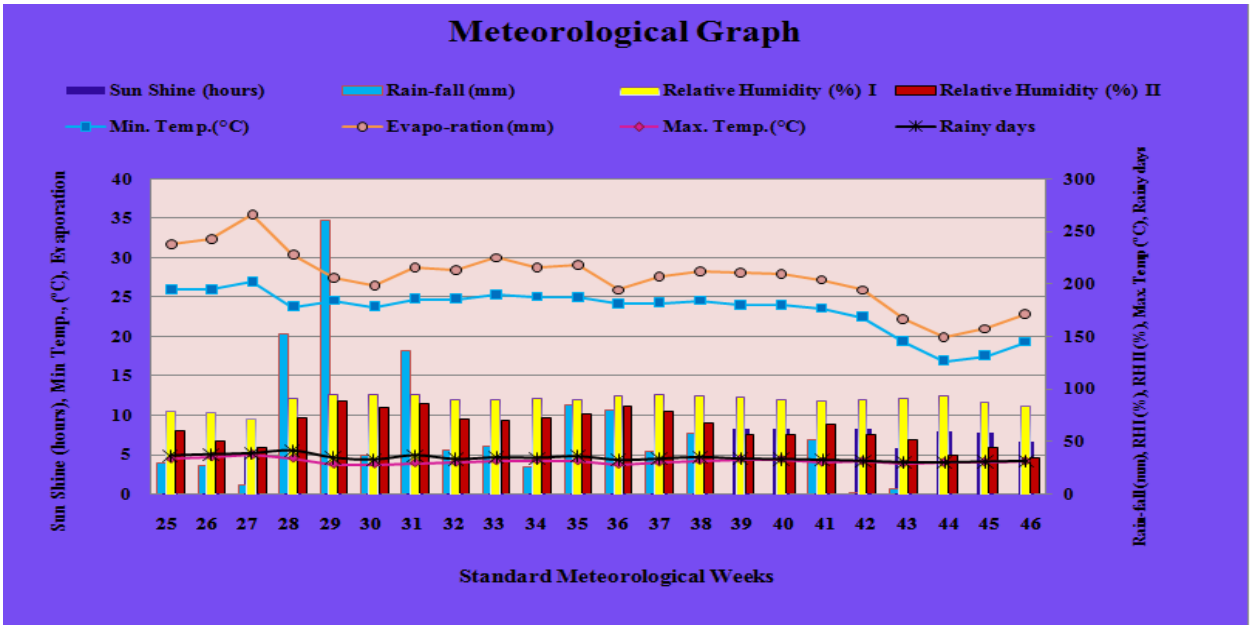

Fig 3.1(a) Maximum, minimum temperature and rainfall (vertical bars) during the field evaluation of RI lines (wet season 2014)

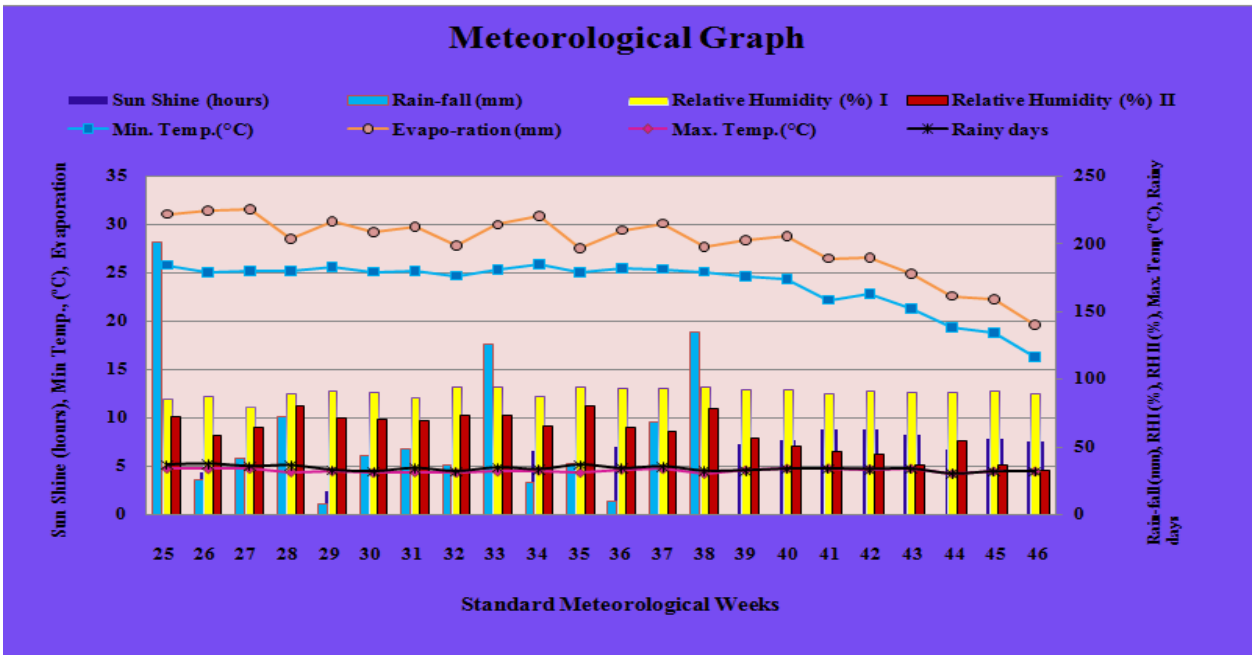

Fig.3.1(b) Maximum, minimum temperature and rainfall (vertical bars) during the field evaluation of mapping population (Swarna Sub-1 X IR-86918-305-B) (wet season 2015)

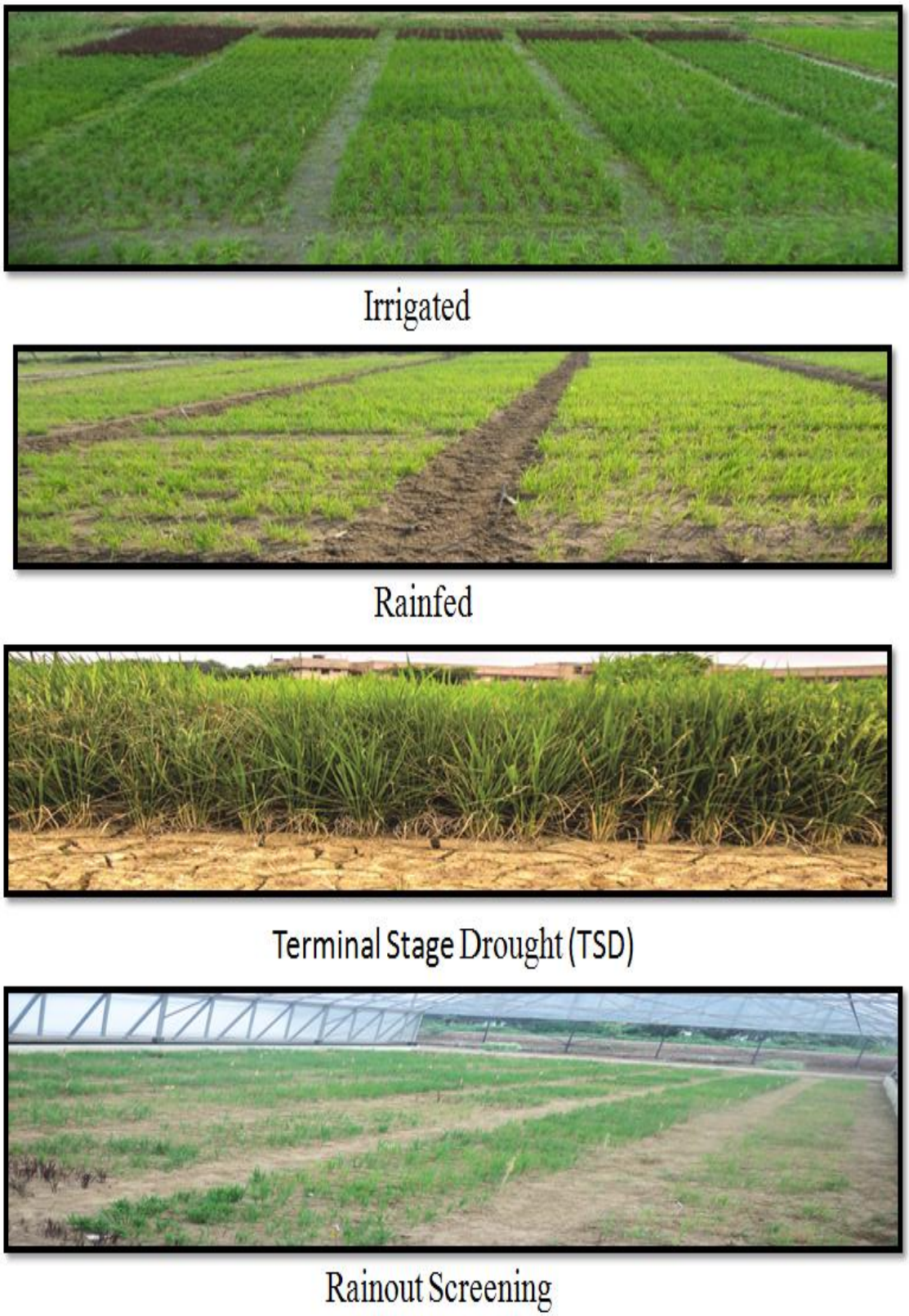

Fig. 3.2 Field view of Experiments under Irrigated, Terminal Stage Drought (TSD), Rainfed condition and Rainout screening

Fig 3.3: Recording root pulling resistance observation in the field

3.2.2.16 Spikelet fertility (%)

Spikelet fertility was worked out by using the formula as given below:

$$\text{Spikelet fertility (\%)} = \frac{\text{Total number of filled spikelets}}{\text{Total number of spikelets}} \times 100$$

3.2.2.17 Spikelet sterility (%)

Spikelet sterility was worked out by using the formula as given below:

$$\text{Spikelet sterility (\%)} = \frac{\text{Total number of unfilled spikelets}}{\text{Total number of spikelets}} \text{ X } 100$$

3.2.2.18 100 seeds weight (g)

100 seeds weight was recorded for each line in grams (g).

3.2.3 Root studies under soil-filled glass rhizotron

A set of selected lines based on phenotypic performance were grown for 45 days in thin rhizotron made up of transparent glass plates filled with a mixture of cocopeat (60%), sand (20%) and field soil (20%). In order to increase the number of roots growing along the rhizotron surface, rhizotrons were stored at an angle of ~15°. After 45 days rhizotrons were open and plants were taken out. Root were separated from the shoot and cleaned from the substrate (Plate2). Finer soil particles still attached to the root were removed using a small painting brush. Plant roots were stored in a 25% ethanol until the scanning procedure.

The scanning was performed with a custom flatbed scanner by placing a transparent acrylic made flat container over the surface of the scanner using Winrhizo software. Once the fragment was positioned, its lateral roots were carefully untangled (with a painting brush if needed). The scan was done with a 600 dpi resolution. The same procedure was repeated for every fragment of the root system. The final outputs of the scanning were therefore saved as an image and Microsoft excel data files for different root parameters. Detailed procedure is outlined below.

3.2.3.1 Materials used

- Plates of 4 mm thick clear glass cut to 450 × 300 mm.
- Soil sieved using a coarse sieve (approximately 5 mm mesh) to remove stones and large clumps.
- Duct tape, two straight 15 mm thick and 400 mm long wood, 15 mm thick plastic ring.

3.2.3.2 Methods:

Two clean glass plates were taken. One was placed on a work surface with two of the four edges slightly overhanging. Two lengths of 15mm thick wood were placed on top of the first plate, a 15mm thick plastic ring was placed at the top and the bottom of the glass as spacers and then the second sheet of glass was placed over the top.

- Duct tapes were used to join the two sheets of glass together at the overhanging edges. The sheets were turned so that the remaining long edge was overhanging, and that was then sealed with duct tape. Three of the four sides were therefore completely sealed with duct tape.
- The empty rhizotrons was set vertical, a single strip of duct tape was wound right around the rhizotrons at the top and bottom, and the two lengths of wood were removed. The two plastic ring prevented glass from coming together and empty rhizotrons was stacked.
- One at the top ring was removed at a later date during the soil-filling process. The plastic ring at the bottom remained in the rhizotron. The empty rhizotrons was stood upon a soft support such as expanded polystyrene sheet and sieved soil was then encouraged into the rhizotrons. When the rhizotrons was nearly full, the upper ring was removed. When full, the rhizotron was lifted and then gently dropped onto the support, caused the soil level drop by 10-15 cm due to packing of the soil. The rhizotrons was refilled, gently dropped once more and refilled to within 5 mm of the top for a final time.
- A small drainage hole was made at each side at the bottom using a sharpened pencil.
- Rhizotrons were placed in stacks of eight and was leaned at an angle of 15^0 to encourage roots to grow on the lower face. The exposed face of the first stack was backed with an insulation sheet to reduce heat exchange and prevent light penetration.
- Three seeds were sown in each rhizotron and thinned to two when they have emerged. Proper watering was done up to 60 days.

- At the end, the rhizotrons was photographed with a high-resolution digital camera.
- At the end of the experiment, shoots were removed in a single day and dried to assess shoot dry weight.
- Roots were washed and then scanned for analysis using software WinRhizo, and dried to assess root dry weight.

3.2.3.3 Root washing

3.2.3.3.1 Materials required for root washing

Large plastic containers, 50 ml Tarson tube, metal forceps, small plastic container, markers for labelling and 25/50% ethanol.

3.2.3.3.2 Procedure for root washing

Tarson tubes for root samples were prepared first, with the exact sample name. For root scanning the roots were washed with tap water two times.

Roots were preserved in ethanol solution (25%) in 50 ml Tarson tubes for root scanning. The procedure was conducted cautiously to prevent supplementary root damage and losses. Debris and dead roots were removed from vital roots.

3.2.3.4 Root scanning of rice lines

Root studies were followed according the root scanning protocol given by International Rice Research Institute (IRRI), Philippines. After 45 days, samples were collected for root scanning. Protocol used for root scanning (www.irri.org) under three steps (a) Root studies under soil filled glass rhizotrons (b) root washing and (c) root scanning.

3.2.3.4.1 Materials used for root scanning

Plastic forceps, Water, Plexiglas trays (Acrylic trays) for WinRhizoReg 2009 scanner (clean with no scratches), computer, Win RhizoReg 2009 USB key.

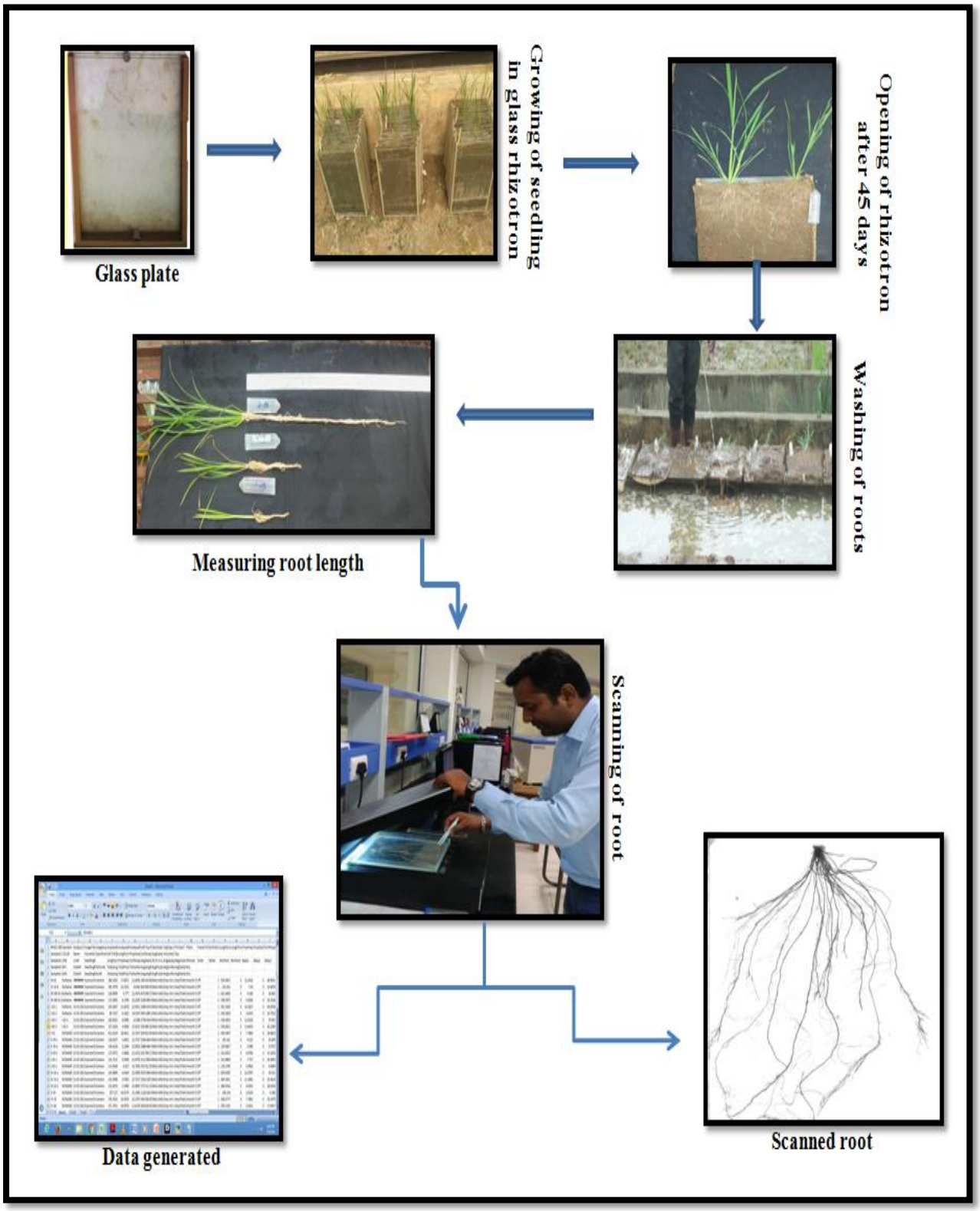

Fig 3.4: Root studies under glass rhizotrons and scanning of Root

3.2.3.4.2 Procedure

3.2.3.4.2.1 Root scanning of selected rice lines

The stored roots of each line in two replicated were used for root scanning. The roots of three plants from each line were used for root scanning which given the detailed information about all root parameters including root length, root volume, root diameter, root tips, number of forks etc. The root scanning was done by using root scanner machine Epson Perfection V700/ V750, 3.81 Version, WinRhizoReg 2009, the data was recorded automatically in the computer for different root parameters including root length, average root diameter, root volume, number of tips, forks, surface area etc.

(a) Preparing roots for scanning

The acrylic trays were first washed with water and dried completely, the tray was filled with clear clean water and the roots were placed in tray. Roots were then floated in water in acrylic trays on the scanner, this allows the roots to be arranged so as to reduce overlap and crossing of roots. Plastic forceps was used tool for arranging the roots in specific manner, this is delicate work; good lighting and steady hands are helpful.

b) Scanning roots

For best results, WinRhizoReg 2009 with an approved scanner used for root scanning, which allows the roots to be light from above and below while the roots were being scanned. This is an important feature (called "Dual Scan" in Regent's documentation), which reduces shadows on the root image. The Regent Positioning System allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Generally roots scanned at 600 dpi in 10x15 cm trays. Root length analysis was carried out with grayscale images.

c) The right threshold value is important

Analysis results can be sensitive to the threshold parameters used. WinRhizo can automatically set these; one you may manually tweak them from time to time. The color traces on the root indicate where roots have been detected.

d) Analyzing Scanned Images

The image was analyzed by selecting the region of interest, and it is analyzed. When scanned images are analyzed, the software uses thresholding to determine what is root and what not root is. A few second later, the analysis was complete and roots found by WinRHIZO were identified by colored line in image. The colors used for drawing them are coded according to root diameter. Portions of the image can be excluded from analysis if necessary, and there are basic editing tools if minor image editing is required.

e) Save the measurement data

The last step of the analysis was data saving WinRHIZO knows when data was easily recordable by many programs including spreadsheet style like Excel. Image and their analysis was also save to file for later validation, reanalysis, or for visualization in other software programs.

3.2.3.5 Observations recorded under soil filled Glass rizhotron

The observations for physiological traits contributing for root traits were recorded at plant specific stage from each rhizotron plate. Two replicated plants from each line were taken for recording the observation such as root length, fresh/dry weight of root and shoot.

Table 3.4 Observations recorded after Root scanning using WinRhizo software

S.N.	Trait	Stage and Observation
1	Root length (cm)	45 DAS
2	Root volume (WinRhizo)	45 DAS
3	Average diameter (mm)	45 DAS

3.2.3.5.1 Root length:

Root length was measured in centimeters (cm) from base to tip of root using standard scaling method.

3.2.3.5.2 Root Volume :

It is the area occupied by roots which is analyzed by WinRhizo software after scanning of root in root scanner.

3.2.3.5.3 Root Diameter:

Average diameter of all type of roots (primary, secondary, tertiary) present in a single plant; which was analyzed by WinRhizo software after scanning of root in root scanner.

3.3 Molecular studies

A set of 71 RILs along with the parents (Swarna Sub-1 and IR-86918- 305-B) were used for molecular studies.

3.3.1: Genomic DNA isolation

DNA was isolated by modified CTAB method of DNA extraction for Rice

- The leaf bits were cut in a 2 ml Eppendorf tube.
- Added 700 µl CTAB Buffer in this. Add bits.
- Grinded the leaf & add some more CTAB (300 µl)
- Keep it in water bath for 20 min @ 65°C.
- Add 700 µl of Chloroform: Isoamylalcohol (24:1).
- Vortex the sample.
- Centrifuge it for 5 min @ 14000 rpm.
- Transferred upper clear layer in new 1.5 ml Eppendorf tube.
- Add 700 µl of Chloroform: Isoamylalcohol (24:1).
- Vortex the sample.
- Centrifuge it for 5 min @ 14000 rpm.
- Transferred upper clear layer in new 1.5 ml eppendorf tube.
- Add 70 µl ($1/10^{th}$ of supernatant) of 3 M Sodium Acetate and 400 µl of chilled isopropanol in this & kept it for incubation at 4°C for 2 hours or -20°C for overnight.
- Centrifuged @14000 rpm for 20 min and discard the supernatant.
- Then wash the pellet with 70% ethanol (50 µl).
- Centrifuge it for 3 min @14000 rpm.
- Air dry the pellet.
- Add 100 µl of TE Buffer and dissolved

3.3.2 Quantification of DNA

The DNA samples were quantified on Nano Drop Spectrophotoscopy (NANODROP 2000c). After quantification, the DNA was diluted with nuclease, protease free water at the final concentration of approximately 50 ηg/µl. This was further used for PCR analysis.

3.3.3 PCR amplification SSR primers

2 µl of diluted template DNA of each genotype was dispensed at the bottom of 96 well PCR plates (AXYGEN/TARSON). Separately cocktails were prepared in an eppendoff tube as described in table 3.5. 18µl of cocktail was added in each tube.

Table 3.5: PCR mix for one reaction (Volume 20 µl)

Reagent	**Stock concentration**	**Volume (µl)**
Sterile and nanopure H_2O	-	11.9
PCR buffer with 15 mM $MgCl_2$	10 X	2.0
dNTPs (Mix)	1 mM	2.0
Primer (reverse and forward)	5 ρM	2.0
Taq polymerase	5 U /µl	0.1
DNA template	40 ηg/µl	2.0
	Total	**20.0**

The mixture was carried out for 34 cycles in 96 well PCR, thermal cycler (Applied Biosystems Life technologies, USA). Temperature profile for PCR is presented in table 3.6.

After the PCR reaction was completed, 3 µl of 6 X loading dye was added to 20 µl PCR amplicons and 7.5 µl (PCR product with dye) was loaded on 5 % PAGE in a vertical electrophoresis system (CBS scientific) along with 50 bp ladder (Genei/Merech Bioscience Pvt. Ltd.). Electrophoresis was done for 1 hour at 160 volts. Gels were stained using EtBr solution then visualized and photographed by using Gel Doc Unit, BIO-RADgeldocXR$^+$.

3.3.3.1 Detection of parental polymorphism using highly variable simple sequence repeats SSR primers

For detecting the parental polymorphism was detected by using seventy eight SSR primers taken (ILS/Sigma Aldrich) were taken.

Table 3.6: Temperature profile used for PCR amplification using microsatellite markers

Temperature (°C)	Duration (min.)	Cycles	Activity
94	4	1	Denaturation
94	0.45	↑	Denaturation
55	0.30	34	Annealing
72	0.45	↓	Extension
72	7	1	Final Extension
4	24 hrs	1	Storage

3.3.6 Development of genotypic data of population

The primer exhibited polymorphism between parents was further used for PCR amplification on all of the 71 RILs of rice. Genotypic data were generated with a set of 21 polymorphic primers. The primers used for this purpose are listed in Appendix C.

3.3.7 Scoring of data

The banding pattern of population developed by each SSR primers was scored separately (table 3.7).

Table 3.7: Scoring SSR banding pattern in population

S. No.	Code	Type of band
1.	A	Swarna Sub-1 (female)
2.	B	IR-86918-305-b (male)
3.	-	Not Amplified

3.4 Temporal expression analysis of putative candidate genes for root traits

To identify the expression level of selected candidate genes on chromosome # 9 related to root traits, semi-quantitative RT-PCR/qPCR analysis was carried out in three different water stress conditions namely, irrigated,

moderate stress, sever stress. The experimental material consisted of 6 selected lines as tabulated in table 3.2

3.4.1 Collection of root tissue and isolation of RNA

Root samples of 12 selected genotypes were collected in two replications under three treatment conditions i.e. irrigated (control), moderate stress, severe stress. After collection, samples were kept in a -80°C freezer for total RNA extraction. The total RNA was extracted using Powerplant RNA isolation Kit as follows:

Table 3.8: Reagents present in RNA isolation kit

S. No.	Components	Amount
1	Solution D1 (DNase I Buffer)	2.5 ml
2	Solution D2 (DNase Wash Buffer)	25 ml
3	Solution D3 (Wash Buffer II)	2 x 28 ml
4	2 ml Collection Tubes	50

3.4.3 Important Notes before Starting

1. Solution PR1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to use. Solution PR1 should be used while still warm.
2. Prepared solution PR1/βME (594 ul PR1 + 6 ul βME) by adding β-mercaptoethanol (βME) to Solution PR1.

For each prep 600 µl of PR1/βME was required.

3.5.4 Protocol

- Placed up to 50 mg of plant samples cut into smaller pieces into the 2 ml PowerPlant® RNA Bead Tube provided.
- Added 600 µl of solution PR1/βME to the PowerPlant® RNA Bead Tube and homogenized the tissue on MO-BIO TissueLyzer® instrument.
- Centrifuged at 13,000 rpm for 2 minutes at room temperature and transfered all the supernatant (500 to 600 µl of lysate) to a clean 2 ml collection Tube .

- Added 150 µl of solution PR2 and vortexed briefly to mix. Incubated at 4°C for 5 minutes.
- Centrifuged the tubes at 13,000 rpm for 2 minutes.
- Avoiding the pellet, transfered the supernatant (not more than 650 µl) to a clean 2 ml collection tube.
- Added 650 µl of solution PR3 and 650 µl of solution PR4. Vortexed briefly to mix.
- Loaded 650 µl of supernatant onto the Spin Filter and centrifuge at 13,000 rpmfor 1 minute. Discarded the flow through and placed the Spin Filter back into the 2 ml Collection Tube. Repeated until all the supernatant was loaded onto the Spin Filter. A total of three loads for each sample processed was required
- Added 600 µl of Solution PR5 to the Spin Filter and centrifuge at 13,000 rpmfor 1 minute.
- Discarded the Spin Filter.

The RNA is now ready for downstream applications. The RNA in the tube can be stored at -80°C until ready for use.

3.4.1.1 Quantification and estimation of purity of RNA

The isolated RNA was quantified using Nano®drop spectrophotometer ND1000 (Thermo SCIENTIFIC *NANODROP* 2000c). Two microlilter of RNA was placed over tip of Nano®drops to record absorbance at 230nm. The absorpation ration (A_{230}/ A_{260}) and (A_{230}/ A_{280}) was recorded for each sample to estimate samples quantity and purity of RNA. The absorbance ratio (A_{230}/ A_{260}) for pure RNA was more than 1.8.

3.4.1.2 cDNA Synthesis

cDNA was synthesized from isolated and quantified RNA using Thermo Scientific Verso™cDNA Synthesis Kit as per manufacturer's instructions. The kit consisted of following components and is suitable for 1pg to 1µg of RNA. The stepwise procedure as described in Thermo Scientific Verso™cDNA Synthesis Kit was followed. The reaction was set in ice all the time.

3.4.1.2.1 The stepwise protocol for cDNA synthesis areas follow:

- After thawing the template RNA on ice required amount was dispensed in each tube.
- Each component as primer solutions, 5x cDNA synthesis buffer, dNTP mix and water (PCR grade) were ice thawed and vortexed briefly to settle the contents.
- Master Mix (except Verso Enzyme Mix and template RNA) was prepared on ice (Table 3.6).
- The master mix was mixed by vortexing gently for not more than 5 sec.
- RNA dependent DNA polymerase (Verso Enzyme Mix) was added to master mix and mixed by pipetting.
- Master Mix was distributed in equal amount to each tube. (Master Mix = 20µl – amount of template RNA).
- The 20µl reaction volume were then incubated in PCR at 42ºC for 30 min. followed by 95 ºC for 2 min.
- Stored at -20 ºC till further use.

Table 3.9: cDNA synthesis (reverse-transcription) reaction component

Components	**Final Concentration**	**Volume/Reaction**
Master mix		
5x cDNA synthesis buffer	1x	4 µl
dNTP Mix	500 µM each	2 µl
RNA primer		1 µl
RT Enhancer		1 µl
Verso Enzyme Mix		1 µl
Water (PCR grade)		Variable
Template RNA	1µg	Variable
Total volume	**20 µl**	

3.4.1.3. Designing of primers for root related candidate genes for expression analysis

The sequence of 7 putative candidate genes was downloaded from TIGR rice database in FASTA format (http://rice.plantbiology.msu.edu). Exonic sequences were used for the designing of primers using Batchprimer3 software (http://www.probes.usda.gov/batchprimer3/).Primer designing was done by putting the nucleotide sequences of exon in FASTA format with desired specifications for GC content, annealing temperature, Tm values, primer length and length of amplified fragments with other parameters as default setting (Table 3.10). The details of primer sequences for 7 putative candidate genes are given in Table 3.11.

3.4.2 Semi Quantitative RT-PCR

Semi quantitative reverse transcriptase PCR was performed using cDNA synthesized previous step in 20 µl of reaction with gene specific primers (Table 3.12, 3.13). The PCR product was electrophoresed on 1.5 % agarose gel at 100 V and visualized by gel documentation system (Bio-Rad). The expression was analyzed by comparing the relative fluorescent intensities of cDNA amplicons under gel documentation system. Alpha tubulin was used as an house keeping gene (internal control) for normalization of the results. The semi quantitative RT-PCR amplicons were digitized using GelQuant.NET Analyzer software (www.biochemlabssolutiond.com) and the relative expression of putative candidate genes were expressed in terms of fold change for each plant sample with respect to corresponding control samples.

3.4.3 Relative quantification of candidate gene expression - qPCR analysis

In quantitative qPCR, a specific or non-specific detection chemistry allows the quantification of the amplified product. The amount detected at a certain point of the run is directly related to the initial amount of target in the sample. Relative quantitation of a target against an internal standard is particularly useful for gene expression measurements. Relative quantification is the most widely used technique. Gene expression levels are calculated by the ratio between the amount of target gene and an endogenous reference gene, which is present in all samples.

Table: 3.10 Primers used for expression analysis of candidate root traits related genes underlying consensus genomic region.

Sr no	Gene	Locus ID	Reference	Chr	Function	Left	Right
1	DRO1	LOC_Os09g26840	Yi et al. (2002)	9	RA	CAACACCACCAGCATGAACA	GACTCCATGTAGTCGGCGTA
2	GLR3.1	LOC_Os04g49570	Li et al. (2006b)	4	RE	CAACACCACCAGCATGAACA	GACTCCATGTAGTCGGCGTA
3	Os GLU3	LOC_Os04g41970	Zhang et al.(2012a)	4	RE	AGGCCTTCTACAGCATCCTC	TTCATGCTGGTGGTGTTGTG
4	OsAGAP	LOC_Os02g10480	Zhuang et al. (2005)	2	RE	TATGCCTCAAGTGTTCCGGT	TTTTCTCGCTCTTCCTGGGT
5	OsIAA23	LOC_Os06g39590	Ni et al. (2011)	6	LRD	CAAAGCTCGTCGACTTGGTC	CAGACAGATCAATTGCGGCA
6	OsPIN3t	LOC_Os01g45550	Zhang et al.(2012)	1	RE	TGACGGGAGAATGTAGACCG	GCCTGAGCGAGCTAGAACTA
7	OsRR2	LOC_Os02g35180	Zhao et al. (2009)	2	RE	CGCATGATTTGAGTGGGCAT	ACCCAGAGAGAAAGAGAGCG

****RA= Root Angle, RE= Root elongation and LRD= lateral root development**

Table: 3.11 Specifications required for gene specific primer designing

Criteria	Optimum	Range
Length of target sequence to be amplified	100-300bp	100-300bp
Tm	60^0C	$57\text{-}65^0$C
GC content	55%	50-60%
Length of primer	20bp	18-20bp

Table 3.12: PCR components and their quantity used for semi-quantitative PCR

Components	Concentration	Quantity
cDNA	1,000 ng/µl	2.0 µl
buffer	10X	2.0 µl
dNTP mix	2mM	2.0 µl
Primer Forward	10µM	1.0 µl
Primer Reverse	10µM	1.0 µl
Taq polymerase	1U/ µl	1.0 µl
Milli Q water	-	11.0 µl
Total		**20.0 µl**

In the present investigation, qPCR was performed with selected gene based primers with actin and tubulin as internal controls or housekeeping genes. qPCR was carried out in Applied Biosystem's Quantstudio as follows. qPCR was performed using SYBR green qPCR mix (Applied Biosystems and Thermo Fisher make) using approximately 700-1000 ng total cDNA in a 20 ul reaction mixture containing final composition of 1X qPCR mix and 0.5-0.8 uM of each forward and reverse primers. Reaction was set as per manufacturers instructions. Blank was always set for each primer during every PCR setup.

Table 3.13 Temperature profiles used for Semi quantitative PCR

Steps	Temperature (^{0}C)	Duration	Cycles	Activity
1	94	4 min	1	Initial Denaturation
2	94	30 sec	↑	Denaturation
3	variable(56-63ºC)	45 sec	35 ↓	Annealing
4	72	1 min		Extension
5	72	7 min	1	Final extension
6	4	For ever	1	Store

The Passive Reference (ROX™ dye) is a dye molecule included in the Power SYBR® Green PCR Master Mix that does not participate in the PCR amplification. On Applied Biosystems Real-Time PCR systems, the Passive Reference provides an internal reference to which the SYBR® Green dye/dsDNA complex signal can be normalized during data analysis. Normalization is necessary to correct for well-to-well fluorescent fluctuations.

Quantitative PCR software uses the exponential phase of PCR for quantification. PCR is initially an exponential process but eventually reaches a plateau phase, when one of the reagents becomes limited. Reactions can plateau at different levels even if they have the same starting concentration of target. During the exponential phase, the amount of target is assumed to be doubling every cycle and no bias is expected due to limiting reagents. Analysis takes the Ct (cycle number) value, at the point when the signal is detected above the background and the amplification is in exponential phase. The more abundant the template sample, the quicker this point is reached, thus giving earlier Ct values. Delta delta Ct (ΔΔCt) methodis the simplest one for quantitative estimation, as it is a direct comparison of Ct values between the target gene and the reference gene.

RQ = Relative quantification = 2-ΔΔCt

Where, DeltaDelta Ct = ΔCt sample1 – ΔCt calibrator

Delta Ct = Ct gene test – Ct endogenous control

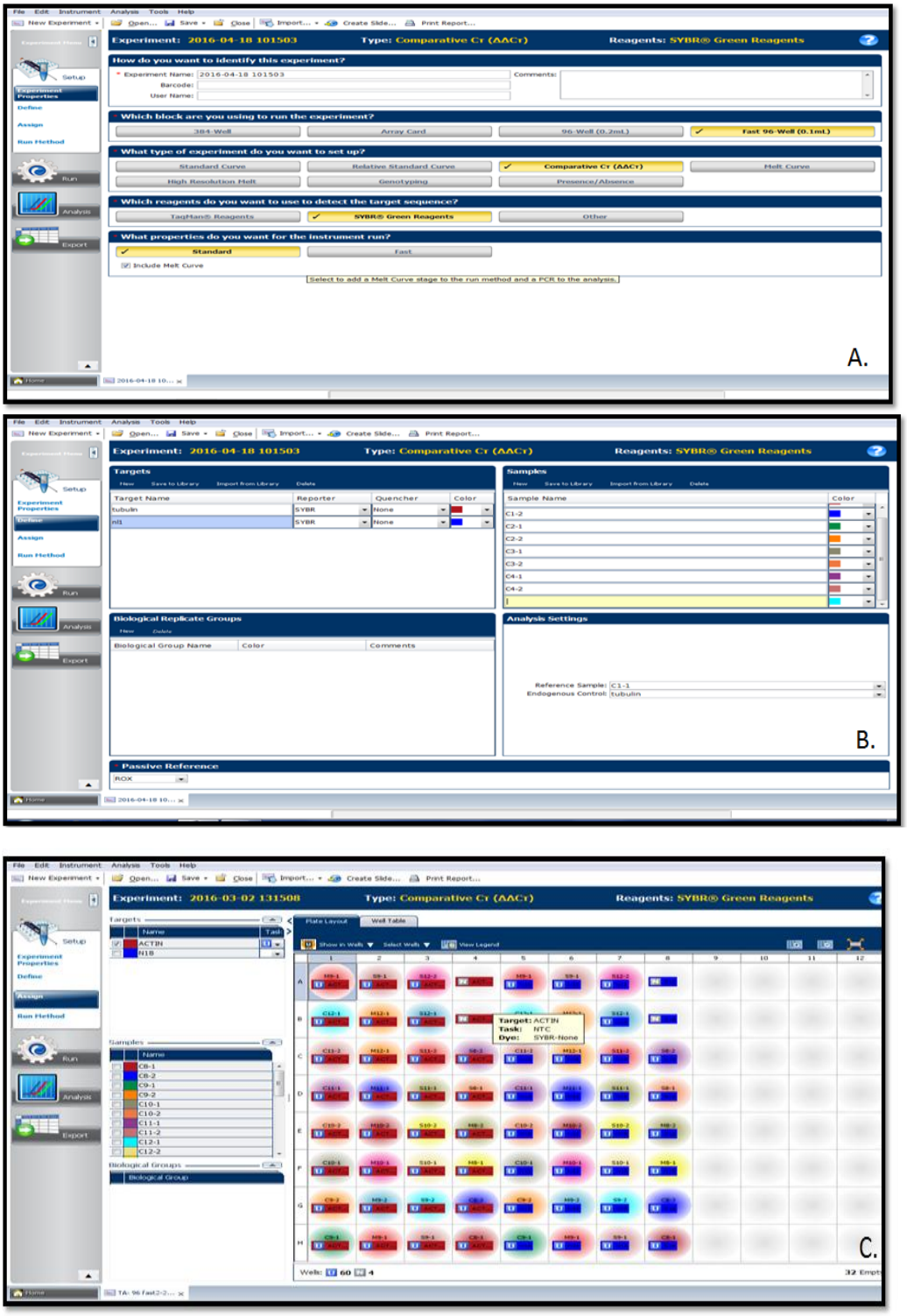

Fig3.5a: Setting up the Applied Biosystem instrument for qPCR. A Setting of Quant Studio, B and C: Assigning of house keeping gene and root specific gene

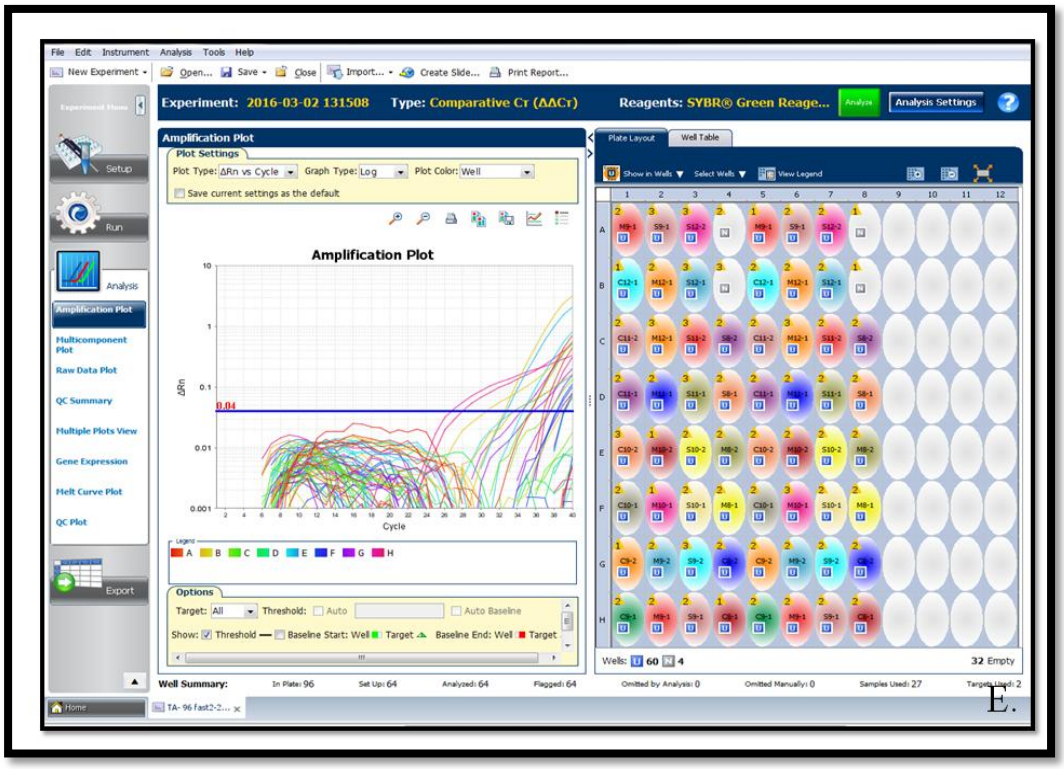

Fig:3.5b Setting up the Applied Biosystem instrument for qPCR. D. qPCR Temperature profile, E. qPCR result

3.5 Statistical analysis

The data recorded on all the traits related to yield and yield contributing characters under all the seasons were statistically analyzed as followed.

3.5.1 Analysis of variance

The mean data of each replication was used for analysis of variance using RCBD design. The replicated data were subjected to variance analysis and test of significance as per the method of Fisher (1935).

3.5.2 Parameters of variation

3.5.2.1 Mean

Mean is the average value of observation of genotypes of a series. It represents the standard average value over fluctuation in the environment. Mean was calculated by the following formula:

$$\overline{X} = \Sigma X_i / n$$

Where,

ΣXi = Summation of all the observations

n = Total number of observations

3.5.2.2 Range

Range is the difference between the highest and the lowest value of a series of observations and thus, provides the information about the extent of variability present in the genotypes.

Range = Highest value - Lowest value

Standard error of mean is calculated as follows

$$SE_m = \sqrt{\frac{MSe}{r}}$$

3.5.2.3 Coefficient of variation (%)

$$CV = \left(\frac{\sqrt{MS_e}}{\overline{X}} \right) \times 100$$

Where, $\overline{X}$ general mean of character

The standard error of the difference between any two treatments is expressed by the following formula:

$$SE_d = \sqrt{\frac{2MSe}{r}}$$

Where, MS_e = error mean square

r = number of replications.

The significance of difference among the treatments means was tested by F-test at 5 and 1% level of significance. Whenever the F value was found to be significant, critical difference was calculated to test the significance of difference between any two treatment means as follows:

C.D. (at 5%) = SE_d × t-value (5%) at error d.f.

3.5.2.3 Estimation of Variability

Different coefficients of variances were computed following Burton (1952).

3.5.2.3.1 Phenotypic coefficient of variation (PCV%)

$$PCV\ (\%) = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

3.5.2.3.2 Genotypic coefficient of variation (GCV%)

$$GCV\ (\%) = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

3.5.2.4 Heritability (broad sense)

It is the ratio of genotypic variance to the phenotypic variance (total variance). Heritability for the present study was calculated in a broad sense by adopting the formula as suggested by Hanson *et al*., (1956):

$$h2(bs)\ \% = \frac{\sigma 2g}{\sigma 2P} \times 100$$

Where,

h^2 (bs) = heritability in broad sense

$\sigma^2 g$ = Genotypic variance

σ^2_P = Phenotypic variance

The estimates of heritability broad sense were classified as low, moderate and high according to Robinson (1966):

< 50 %	–	**Low**
50-70 %	–	**Moderate**
> 70 %	–	**High**

3.5.2.5. Estimation of Genetic Advance as percentage of mean

Improvement in the mean genotypic value of progeny of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection.

Genetic advance = $h^2_{(bs)} \times \sigma_p \times K$

$h^2_{(bs)}$ = heritability in broad sense

σ_p = phenotypic standard deviation

K = selection differential at 5% selection intensity

The value of K (at 5% selection intensity) = 2.06 (Allard, 1960)

Genetic advance expressed as per cent of population mean was calculated as follows:

$$\text{Genetic advance as \% of mean} = \frac{\text{Genetic Advance}}{\text{General population mean}} \text{ x } 100$$

3.5.3 Drought susceptibility index (DSI)

The drought susceptibility index was calculated for each RIL using the formula suggested by Fischer and Maurer (1978):

$$\text{DSI} = \frac{1 - (\text{Ys/Yc})}{1 - (\text{meanYs/meanYc})}$$

Where,

Ys = Yield under drought condition (stress environment) (stress yield)

Yc = Yield under irrigation (Stress free environment) (potential yield)

meanYs = mean yield of all genotypes under drought condition

meanYc = mean yield of all genotypes under irrigated condition

3.5.3.1 Drought intensity index (DII)

The Drought Intensity Index was calculated for each condition using the following formula given by Ramirez-Vallejo and Kelly (1998):

$$\mathrm{DII} = 1 - \frac{\mathrm{YS}}{\mathrm{YI}}$$

Where

YS= Under stress condition over all mean yield

YI= Under irrigated condition overall mean yield

3.5.4 Character association

3.5.4.1 Correlation coefficient

Correlation coefficients were computed to determine the association among the characters studied. The correlation coefficients at phenotypic and genotypic levels were estimated from the analysis of variance and covariance. Correlation coefficient (r) was calculated for grain yield and yield contributing characters by using the standard procedure given by Searle (1961).

Test of significance for correlation coefficient

For testing the significance of *r*, the *t*-value was calculated as follows

$$t = \frac{r}{\sqrt{1-r^2}}\sqrt{n-2}$$

Where *r* is the estimate obtained from *n* pairs and the ratio is distributed in sampling as *t* with *(n-2)* degrees of freedom. The significance for correlation coefficient was tested by comparing observed value of correlation coefficient with tabulated values for n-2 degree of freedom. If the observed value is more than the table value, the correlation coefficient is considered to be significant.

3.5.4.2 Path coefficient analysis

The phenotypic and genotypic correlation coefficient were further partitioned into direct and indirect effect with the help of path coefficient analysis as suggested by Wright, 1921 and elaborated by Dewely and Lu, 1959. The path coefficient was estimated by solving following sets of simultaneous equations indicating the basic relationship between correlation and path coefficients.

$$r_{iy} = P_{iy} + r_{i1}P_{1y} + r_{i2}P_{2y} + \cdots\cdots\cdots\cdots + r_{i(i-1)}P_{iy}$$

i = 1, 2, 3……n

Where,

i is the no. of independent characters (causes); r_{1y} to r_{iy} denotes coefficient of correlation between independent variables (1…..i) with dependent character y; r_{i1} to $r_{i(i-1)}$ denotes coefficient of correlation among all possible combination of caused factors (independent variables); P_{1y} to P_{iy} denotes the direct effects of independent variables (1….i) on the dependent variable y.

3.6 Stability analysis

Analyzed the data of two years over four environments (locations) by using stability model proposed by Eberhart and Russell (1966). The model involves the estimation of mean, regression coefficient and deviation from regression, which are defined by a mathematical formula.

$$Yij = \mu i + \beta i\, Ij + Sij$$

Where,

Yij = Mean of the i_{th} genotype at the j_{th} environment

μi = Mean of i_{th} genotype over all environments

βi = Regression coefficient that measures the response of i_{th} genotype to varying environments

Ij = Environmental index obtained by subtracting the grand mean from the mean of all genotypes at the j_{th} environment

Sij = Deviation from regression of the i_{th} genotype at the j_{th} environment

3.7 QTL analysis

Single Marker Analysis was performed with the help of QTL Cartographer 2.5. Graphical genotyping of molecular data was done using GGT 2.0 (Van Berloo, 1999).

3.8 Reagents and solutions

3.8.1 Reagents for PCR

a. Primers: Highly variable microsatellite markers from ILS, USA

b. dNTPs: (dATP/dCTP/dGTP/dTTP)

1 mM stock of dNTP (GeNei) was used.

c. PCR buffer (10 X): From Genei Mereck Bioscience Pvt Ltd along with $MgCl_2$

d. *Taq* polymerase

1 unit/µl, 1000 U *taq* (GeNei) was used for PCR.

3.8.2 Stock solutions

Table: 3.14 Preparation of DNA extraction buffer

	For 200 ml	For 400 ml	For 1000 ml
100 mM Tris HCl	2.42 g	4.84 g	12.1 g
1.4 M NaCl	16.36 g	32.72 g	81.8 g
20 mM EDTA	1.49 g	2.98 g	7.45 g
CTAB	6 g	12 g	30 g
Volume make up by DDW			
Autoclave 20 min			

b. TE buffer

1 M Tris-HCl (pH-8) 1 ml

0.5 M EDTA 0.2 ml

Final volume was adjusted to 100 ml and autoclaved

c. EDTA (0.5M, pH-8)

186.12 g of EDTA was dissolved in 700 ml of distilled water. NaOH pallets were added for proper dissolving of EDTA. The pH was set

to 8 using NaOH/HCl. Final volume was adjusted to 1000 ml with distilled water and sterilized by autoclaving.

d. 4 M NaCl

35.04 g of NaCl was dissolved in 100 ml of distilled water. Final volume was adjusted to 150 ml and sterilized by autoclaving.

e. 1 M Tris HCl (pH 8.0 at 25°C)

31.52 g of Tris Cl/Trizma base was dissolved in 100 ml of distilled water. The pH was set to 8.0 using concentrated HCl. The final volume was adjusted to 200 ml with distilled water and sterilized by autoclaving.

i. Absolute alcohol (pre chilled)

j. 70 % Ethanol

70 ml of absolute ethanol was taken and volume makeup 100 ml with distilled water.

3.8.3 Solutions for electrophoresis

a. 10 X TBE buffer

Tris base	104 g
Boric Acid	55 g
EDTA (0.5 M)	40 ml
Distilled water	500 ml

Final volume was adjusted to 1 litre with distilled water.

b. Tank buffer (1 X TBE)

100 ml of 10 X TBE + 900 ml of distilled water.

c. 5 % Acrylamide gel solution (1000 ml)

Acrylamide	47.5 g
Bis-Acrylamide	2.5 g
10 X TBE	100 ml

Final volume was adjusted to 1 liter with distilled water.

Acrylamide and bis-acrylamide were weighed and dissolved one by one in 500 ml distilled water then added to 100 ml of 10 X TBE and the volume was made upto 1000 ml by adding autoclaved double distilled

water. The solution was sterilized by passing through 0.22 micron and stored in amber colour bottle at 4^0 C.

d. 10 % Ammonium persulphate (APS)

Ammonium persulphate 1.0 g

Distilled water 10 ml

e. Ethidium bromide

Ethidium bromide powder 10 mg

Water 1 ml

f. Gel staining solution

Ethidium bromide 10 µl

Distilled water 200 ml

g. 6 X loading dye

Bromophenol blue 0.25 g

Glycerol 40 ml

Final volume was adjusted to 100 ml with distilled water.

d. 50 bp DNA ladder (GeNei)

Step up 50 bp ladder (500 µg/ml) 0.1 ml

Gel loading buffer (6 X) 0.2 ml

Water (Nuclease free) 0.4 ml

1.5 µl are used at the time of sample loading on gel.

CHAPTER IV
RESULTS AND DISCUSSION

The present investigation entitled **"Fine mapping and pyramiding of QTLs for root traits in RIL population of rice (*Oryza sativa* L.)"** was carried out. Drought is one of the most serious worldwide problem in agriculture, and rice is the largest water consumer crop (Luo and Zhang, 2001; Gowda *et al.*, 2011 and Luo *et al.*, 2011), the development of drought tolerant varieties is largely based on the quick and precise screening of germplasm and breeding materials in water limited environments, mapping and cloning of DT (drought tolerance) genes, incorporating the drought tolerant genes of high yield, good quality and DT using MAS (Marker Assisted Selection) (Teng *et al.*, 2002; Steele *et al.*, 2007; Cairns *et al.*, 2009; Bernier *et al.*, 2009)

In this study our attempt was to estimate the genetic variability parameters along with association, path coefficient and stability analysis. In this study, a population of 71 BC_1F_7 (wet season 2014) and BC_1F_8 (wet season 2015), derived from a cross between Swarna Sub-1 (female) and IR-86918-305-B (male), were used, under three water regime conditions viz., irrigated (I) as control, rainfed (RF) and terminal stage drought (TSD) as water stress in 2014 and two conditions namely, irrigated (I) and terminal stage drought (TSD) condition in 2015.

4.1.1 General observations

During wet season 2014 total 1249 mm rainfall was received in crop period. No breaks in the rainfall were observed during crop growth, rain fall was not observed after last week of October 2014. During 2015 total 1082 mm rainfall was received where rain stopped after third week of September 2015.

Within 71 RILs, line number 53, 7 and 42 had highest grain yield under irrigated condition, line number 8, 9 and 13 had highest grain yield under rainfed condition, while line number 30, 42 and 29 had highest grain yield under terminal stage drought (TSD) condition during wet season 2014. Line number 53, 46 and 52 had highest grain yield under irrigated condition and line number 27, 30 and 28 had highest grain yield under terminal stage drought (TSD) condition during wet

season 2015. This indicates that genotypes perform differentially under different methods of establishment. The area under direct seeding is likely to be increase considering the constraints of labor availability and water shortage, therefore evaluating the lines under direct seeding is important. On overall basis only three lines # 7, 52 and 53 had highest yield under irrigated conditions in both the years, i.e. performed well under managed condition. Under TSD condition line # 29 and 30 perform well despite of stress condition.

4.2 Variability

4.2.1 Analysis of variance

The population was evaluated under wet season 2014 and 2015 for various phenological, agronomical and physiological traits. The experiments were conducted with 2 replication in RCBD design. The mean data of individual lines of the population were statistically analyzed to generate overall mean and other genetic parameters. The results of RCBD analysis under different conditions of wet season 2014 and 2015 is presented in table 4.1.

4.2.2 Mean and variability parameters

The population was evaluated under different water stresses as well as non-stress conditions during wet season 2014 and 2015. Drought intensity index (DII) under rainfed 0.81 and TSD condition 0.83 during wet season 2014 and 0.85 under TSD during wet season 2015, showed good severity of stress imposed (Table 4.1). Such high level of stress could be imposed because of field on higher topology with light soils, good drainage which loses its soil moisture rapidly to allow the development of severe drought stress in the field. To make sure that most of the lines were subjected to drought stress at the early stage of panicle initiation (when the growth of rice is most sensitive to soil moisture) sowing and transplanting of TSD condition was delayed by 25 days so as to coincide the terminal stage drought of the crop with the dry spell after the with-drawl of monsoon. The crop was irrigated till 4 weeks after transplanting and thereafter the paddy field was drained. This situation led to exposure of the breeding material to water stress. Experiments were conducted under irrigated, rainfed and terminal stage drought condition each with direct seeded and transplanted.

Table: 4.1 Mean, range and other genetic parameters of grain yield and yield related traits.

Traits	Parameter	Wet Season 2014			Wet Season 2015	
		I	RF	TSD	I	TSD
Seedling height (cm)	Mean	35.62	35.51	34.62	35.61	34.44
	Range	22-50	25.78-46.00	34.00-57.50	26.35-49.70	24.23-47.78
	TMSS	102.72**	68.02**	78.67**	75.23**	79.04**
	h^2(%)	72.68	92.89	85.80	80.10	87.44
	GA (% mean)	32.42	32.00	25.78	29.98	26.32
	GCV	18.46	16.12	13.51	16.25	13.66
	PCV	21.65	16.73	14.58	18.15	14.61
Root Pulling Resistance (Kg)	Mean	23.07	-	-	24.86	-
	Range	15.25-29	-	-	16.50-34.50	-
	TMSS	11.65**	-	-	19.10**	-
	h^2	47.60	-	-	49.13	-
	GA (% mean	13.03	-	-	14.57	-
	GCV	9.17	-	-	10.09	-
	PCV	12.29	-	-	14.40	-
Shoot Biomass (gm)	Mean	13.77	12.95	13.40	13.28	13.50
	Range	8.63-18.67	8.76-17.94	8.36-16.98	8.58-18.24	8.87-15.62
	TMSS	13.88**	10.13**	12.81**	12.05**	12.43**
	h^2	45.81	73.40	58.92	54.29	85.47
	GA (% mean	19.38	28.22	25.07	23.53	33.78
	GCV	13.89	15.99	15.86	15.50	17.74
	PCV	20.54	18.66	20.66	21.04	19.19
Days to 50% Flowering	Mean	94.92	93.20	93.38	94.90	92.2
	Range	74.50-110.50	73.50-108.00	74.50-107.00	74-105	73.50-103.00
	TMSS	94.41**	134.74**	127.46**	102.89**	134.76**
	h^2	98.66	96.99	97.50	94.26	96.99
	GA (% mean	14.92	17.73	17.28	14.89	17.73
	GCV	7.29	8.74	8.50	7.45	8.74
	PCV	7.33	8.88	8.60	7.67	8.88

* and ** are significant at 5% and 1% probability level, respectively, TMSS= Treatment Sum of Square, h^2 =heritability, GCV and PCV = Genotypic and Phenotypic Coefficient of variation, I=Irrigated , RF= Rainfed, TSD= Terminal Stage Drought, - denoted as data not recorded

Traits	Parameter	Wet Season 2014			Wet Season 2015	
		I	RF	TSD	I	TSD
Tillers Number	Mean	14.62	-	14.84	15.09	14.65
	Range	9.95-21.40	-	9.75-20.75	10.10-21.50	9.16-20.22
	TMSS	15.51**	-	14.59**	14.61**	14.39**
	h^2	65.47	-	77.64	43.38	61.39
	GA (% mean	28.32	-	30.89	18.91	25.77
	GCV	16.94	-	17.02	13.93	15.97
	PCV	20.93	-	19.31	21.15	20.38
Plant Height (cm)	Mean	105.39	100.42	88.27	104.09	86.67
	Range	83.80-159.85	76.10-152.50	64.75-130.25	80.80-161.50	63.80-130.99
	TMSS	755.76**	661.07**	374.11**	785.04**	382.11**
	h^2	97.33	76.70	96.56	95.01	97.10
	GA (% mean	37.23	30.43	31.09	37.96	32.14
	GCV	18.32	16.87	15.36	18.71	15.83
	PCV	18.57	19.26	15.63	19.00	16.07
Panicle length (cm)	Mean	25.43	23.46	22.33	23.09	21.80
	Range	21.50-27.90	18.50-31.60	17.00-26.75	21.15-26.20	16.00-27.10
	TMSS	5.37**	9.50*	11.49**	5.29**	11.39**
	h^2	44.92	62.10	77.23	47.14	78.60
	GA (% mean	5.87	2.73	18.15	7.64	18.76
	GCV	4.82	4.07	10.02	5.40	10.27
	PCV	8.16	12.49	11.41	7.87	11.59
Flag Leaf Length (cm)	Mean	29.98	28.53	25.72	29.74	25.73
	Range	23.10-29.70	21.30-38.00	18.88-33.91	22.50-39.30	18.03-34.35
	TMSS	25.58**	18.03**	20.52**	27.49**	21.63**
	h^2	66.55	47.31	80.53	72.79	77.99
	GA (% mean	17.92	11.95	21.74	20.12	21.77
	GCV	10.66	8.44	11.76	11.45	11.97
	PCV	13.07	12.27	13.11	13.42	13.55

*** and ** are significant at 5% and 1% probability level, respectively, TMSS= Treatment Sum of Square, h^2 =heritability, GCV and PCV = Genotypic and Phenotypic Coefficient of variation, I=Irrigated , RF= Rainfed, TSD= Terminal Stage Drought, - denoted as data not recorded**

Traits	Parameter	Wet Season 2014			Wet Season 2015	
		I	RF	TSD	I	TSD
Flag Leaf Width (cm)	Mean	1.42	1.26	1.21	1.38	1.32
	Range	1.04-1.78	0.99-2.65	1.00-1.55	1.12-1.75	0.95-1.65
	TMSS	0.05**	0.09*	0.03*	0.04**	0.04**
	h^2	65.75	66.86	22.56	59.43	80.09
	GA (% mean	16.13	3.26	5.99	13.83	17.49
	GCV	9.66	6.04	6.12	8.71	9.49
	PCV	11.01	23.07	12.89	11.29	10.60
Second Leaf Length (cm)	Mean	41.52	39.68	34.68	40.47	34.40
	Range	33.00-58.60	30.25-53.75	26.00-45.55	33.10-57.80	25.61-44.94
	TMSS	68.13**	56.96**	43.72**	65.36**	46.91**
	h^2	72.40	79.44	79.75	73.60	84.43
	GA (% mean	22.54	23.24	23.36	22.44	25.50
	GCV	12.88	12.66	12.70	12.70	13.47
	PCV	15.14	14.20	14.22	14.80	14.66
Second Leaf Width (cm)	Mean	1.18	1.08	1.10	1.21	1.09
	Range	0.93-1.44	0.75-1.33	0.75-1.35	0.8-1.54	0.70-1.30
	TMSS	0.028**	0.03**	0.03**	0.025**	0.03**
	h^2	48.15	56.08	34.84	44.44	39.53
	GA (% mean	11.56	14.79	9.89	9.92	10.23
	GCV	8.09	9.59	8.14	7.22	7.90
	PCV	11.66	12.81	13.78	10.83	12.56
Length of Last Internode (cm)	Mean	33.81	30.99	28.67	31.41	28.33
	Range	26.80-43.10	22.90-29.30	23.75-34.50	27.65-41.80	23.65-34.25
	TMSS	17.02**	17.78**	13.22**	16.26**	15.09**
	h^2	60.81	46.22	39.07	66.96	73.12
	GA (% mean	12.05	10.71	8.66	12.88	15.70
	GCV	8.50	7.65	6.72	7.64	8.91
	PCV	9.62	11.25	10.76	9.34	10.42

*** and ** are significant at 5% and 1% probability level, respectively, TMSS= Treatment Sum of Square, h^2 =heritability, GCV and PCV = Genotypic and Phenotypic Coefficient of variation, I=Irrigated , RF= Rainfed, TSD= Terminal Stage Drought, - denoted as data not recorded**

Traits	Parameter	Wet Season 2014			Wet Season 2015	
		I	**RF**	**TSD**	**I**	**TSD**
Biological Yield (gm)	Mean	1631	649.90	502.82	1102.19	964.46
	Range	871.18-2319.79	419.11-1039-56	340.17-749.17	645-1479.32	335.42-1556.25
	TMSS	207279**	35414**	12675.28**	88402.26**	73557.49**
	h^2	84.98	58.26	67.63	68.56	65.40
	GA (% mean	35.92	27.64	24.09	29.34	29.46
	GCV	19.92	17.58	14.22	17.20	17.68
	PCV	20.15	23.03	17.29	20.78	21.87
Grain Yield (gm)	Mean	693.69	128.24	114.94	458.63	66.10
	Range	375.69-930.56	29.67-237.56	59.17-181.83	273.64-638.18	2.43-244.45
	TMSS	45690.89**	5722**	1775.68**	16837.74**	7.14**
	h^2	67.84	61.71	77.47	52.49	98.96
	GA (% mean	33.07	58.97	43.92	24.78	36.88
	GCV	19.55	36.44	24.22	16.60	41.92
	PCV	23.82	46.38	27.52	22.91	42.40
	DII	-	**0.81**	**0.83**	-	**0.85**
Harvest Index (%)	Mean	42.69	20.83	23.30	40.87	8.61
	Range	26.15-54.31	0.81-39.04	9.49-36.51	21.26-62.61	0.27-26.35
	TMSS	70.14**	193.24**	71.49**	85.04**	83.10**
	h^2	35.04	84.09	67.01	38.89	87.13
	GA (% mean	12.28	85.20	38.85	12.25	70.33
	GCV	10.03	45.10	23.04	10.70	43.75
	PCV	16.86	49.19	28.14	19.25	45.12
100 Seed Weight (gm)	Mean	2.16	1.99	-	2.28	1.91
	Range	1.71-2.71	1.44-1.62	-	1.72-2.92	1.30-2.76
	TMSS	0.102**	0.16**	-	0.11**	0.22**
	h^2	72.26	83.63	-	59.63	69.90
	GA (% mean	16.75	25.60	-	14.13	26.97
	GCV	9.56	13.59	-	8.89	15.66
	PCV	11.25	14.86	-	11.51	18.73

*** and ** are significant at 5% and 1% probability level, respectively, TMSS= Treatment Sum of Square, h^2 =heritability, GCV and PCV = Genotypic and Phenotypic Coefficient of variation, I=Irrigated , RF= Rainfed, TSD= Terminal Stage Drought, - denoted as data not recorded**

Traits	Parameter	Wet Season 2014			Wet Season 2015	
		I	RF	TSD	I	TSD
Spikelet Fertility (%)	Mean	81.56	78.03	-	80.45	39.20
	Range	63.48-94.33	51.29-92.71	-	62.85-90.71	5.83-79.51
	TMSS	104.31**	205.58**	-	86.78**	699.91**
	h^2	55.52	48.63	-	36.38	39.97
	GA (% mean	16.19	24.42	-	7.43	43.78
	GCV	8.50	12.60	-	5.98	46.96
	PCV	9.19	13.37	-	9.92	48.45
Root Length (cm)	Mean	42.69	-	-	45.47	-
	Range	12.00-58.02	-	-	12-57.50	-
	TMSS	132.93**	-	-	124.01**	-
	h^2	79.13	-	-	73.44	-
	GA (% mean	32.89	-	-	30.12	-
	GCV	17.95	-	-	17.06	-
	PCV	20.18	-	-	19.91	-
Root Volume (cm^3)	Mean	0.504	-	-	0.56	-
	Range	0.10-1.12	-	-	0.19-1.20	-
	TMSS	0.109**	-	-	0.125**	-
	h^2	67.31	-	-	59.50	-
	GA (% mean	70.06	-	-	60.70	-
	GCV	41.45	-	-	38.20	-
	PCV	50.522	-	-	49.52	-
Average Root Diameter (mm)	Mean	0.23	-	-	0.27	-
	Range	0.17-0.32	-	-	0.21-0.35	-
	TMSS	0.003**	-	-	0.002**	-
	h^2	45.14	-	-	26.70	-
	GA (% mean	16.99	-	-	2.04	-
	GCV	12.28	-	-	13.82	-
	PCV	18.27	-	-	14.79	-

*** and ** are significant at 5% and 1% probability level, respectively, TMSS= Treatment Sum of Square, h^2 =heritability, GCV and PCV = Genotypic and Phenotypic Coefficient of variation, I=Irrigated , RF= Rainfed, TSD= Terminal Stage Drought, '-' denoted as data not recorded**

Irrigated transplanted condition was similar to farmers having irrigation facility. Rainfed condition trials were an effort to simulate the farmer's field conditions where no irrigation facility is available and farmers totally dependent on rainfall. Direct seeded condition matched with small farmers having less resource for transplanting. Terminal stage drought condition where sowing was late, which was similar with farmers sowing late due to many reasons *i.e.*, availability of seeds, irrigation facility etc. Our study was under combination of irrigated, rainfed and TSD with direct seeded and transplanted, so these conditions mainly represent the targeted population in different environment.

Observations were recorded on plant basis or plot basis at an appropriate stage. Replicated data of each trait was employed on RCBD design for testing significance of treatment. Treatment showed significant difference in analysis of variance, indicating differences from each other for different traits under study. Significant variation was noticed among the population for grain yield and yield contributing traits under different conditions. This was expected as the two parents involved in the development of population differed markedly for most of the morphological and physiological traits, including reaction to water stress and particularly yield under water stress. These results were in agreement with the results of Singh *et al*. (1984) and Manickavelu *et al*. (2006), as they have also reported genetic variability.

Root pulling resistance is also a trait that is highly correlated with root length, thickness, branching number, and dry mass in rice (Price *et al.*, 1989). Root pulling resistance is recommended as an indirect screen to select genotypes that achieve drought tolerance *via* producing a large root system (Ekanayake *et al.*, 1985; Lafitte *et al.*, 2006). Maximum root pulling resistance was recorded 29 kg with mean of 21.65 kg, whereas, minimum root pulling resistance 15.25 was reported during wet season 2014. Whereas, during wet season 2015, 34.50 kg maximum root pulling resistance was reported with mean of 24.86 kg and minimum root pulling resistance was 16.50 kg. The high root pulling resistance observed in seedlings indicates, substantially superior root growth and genotypes with higher resistance are adoptable to drought prone areas. Line number 1 and 2 perform highest average root pulling resistance (30.25 kg and 29.75 kg,

respectively), over the year (table 4.2). Line number 30 and 47 exhibited lowest root pulling resistance (15.875 kg and 18.25 kg, respectively) over the year.

Table: 4.2 Average root pulling resistance of lines in wet season 2014 and 2015

High root pulling resistance					Low root pulling resistance				
Line	**RPR (kg)**	**RL**	**RV**	**ARD**	**Line**	**RPR (kg)**	**RL**	**RV**	**ARD**
1	30.25	43.25	0.67	0.25	**30**	15.88	42.75	0.43	0.24
2	29.75	46.75	0.70	0.28	**47**	18.25	41.25	0.37	0.22
54	28.53	40.75	0.71	0.28	**27**	18.63	33.75	0.77	0.23
24	27.63	26.25	0.34	0.23	**31**	19.25	12.00	0.20	0.28
23	27.38	41.38	0.65	0.26	**9**	20.25	43.00	0.39	0.30
33	27.38	57.75	0.83	0.27	**26**	20.40	24.25	0.25	0.22
41	27.25	51.50	1.13	0.24	**34**	20.50	47.50	0.41	0.20
52	27.25	49.50	0.97	0.27	**35**	20.51	35.75	0.49	0.22
69	26.43	51.33	0.86	0.25	**10**	20.63	41.5	0.435	0.259
71	26.29	51.50	0.64	0.27	**42**	21.00	44.00	0.25	0.25

Seedling height under irrigated condition ranged from 22-50 cm with a mean of 35.62 cm, under rainfed condition it was ranged from 27.78 to 46 cm with mean of 35.51 cm. Under TSD condition it ranged from 34-57.50 with mean of 34.62 cm during wet season 2014. Whereas during wet season 2015, under irrigated condition it ranged from 26.35 to 49.70 cm with the mean of 35.61 cm, while in TSD condition it ranged from 24.23 to 47.38 cm with mean of 34.44 cm.

Days to 50% flowering under irrigated condition ranged from 74.50 to 110.50 days with a mean of 94.92 days, under rainfed condition it ranged from 73.50 to 108 days with mean of 93.20 days. Under TSD condition it ranged from 74.50 to 107 days with mean of 93.38 days during wet season 2014. Whereas during wet season 2015, under irrigated condition it ranged from 74 to 105 days with the mean of 94.90 days, while in TSD condition it was ranged from 73.50 to 103 days with mean of 92.2 days. The delay in flowering under rainfed and TSD condition was due to low temperature during seedling and early vegetative phase, otherwise under same temperature condition it was reported to increased days to 50 % flowering under drought condition (Kamoshita *et al.*, 2008). Ndjiondjop *et al.* (2012 b) observed flowering and maturity were consistently delayed across genotype and tolerance levels. Shrivastava and Verulkar (2009) reported that delay

in flowering under drought conditions was related to low water status and was an indicator of drought susceptibility. Pantuwan *et al.* (2002) reported that drought stress developed prior to flowering generally delayed the flowering of genotypes and such a delay was associated with drought susceptibility in rice.

Shoot biomass under irrigated condition ranged from 8.63 to 18.67 g with a mean of 13.77 g, under rainfed condition it ranged from 8.76 to 17.94 g with mean of 12.95 g. Under TSD condition it ranged from 8.36 to 16.98 g with mean of 13.40 g during wet season 2014. Whereas during wet season 2015, under irrigated condition it ranged from 8.58 to 18.24 g with the mean of 13.28 g, while in TSD condition it ranged from 8.87 to 15.62 g with mean of 13.50 g.

Tiller number ranged from 9.95 to 21.40 with mean of 14.62 under irrigated condition while under TSD condition, mean tiller number was 14.84 and it ranged from 9.75 to 20.75 during wet season 2014. Under irrigated condition 2015, it ranges from 10.10 to 21.50 with mean of 15.09 and under TSD, it ranged from 9.16 to 20.22, with mean of 14.65.

Mean plant height, 105.39 cm was reported under irrigated condition, it ranged from 83.80 to 159.85 cm, under rainfed condition it ranged from 76.10 to 152.50 cm with mean of 100.42 cm and under TSD condition, it ranges from 64.75 to 130.25 cm with mean of 88.27 cm during wet season 2014. During wet season 2015, under irrigated condition, plant height ranged from 80.80 to 161.50 cm with mean of 104.09 cm and under TSD condition it ranged from 63.80 to 130.99 cm with mean of 86.67 cm. Plant height under irrigated condition was higher than the stress condition, this was expected as the plant had inadequate water during vegetative stage.

Mean panicle length 25.43 cm was reported under irrigated condition during wet season 2014 and it ranged from 21.50 to 27.90 cm, under rainfed condition it ranged from 18.5 to 31.6 cm with mean panicle length of 24.46 cm and under TSD condition mean panicle length was 22.33 cm which ranged from 17 to 26.75 cm. Under irrigated condition it ranged from 21.15 to 26.20 cm with average panicle length of 23.09 cm and under TSD condition mean panicle length was 21.80 cm, it ranged from 16 to 27.10 cm during wet season 2015. Average values of panicle length for the 2 years under different conditions differed significantly

for the different water levels, this indicate that panicle development was affected by water deficit. The influence of late-stage water deficit caused varied decrease in panicle length (Liu *et al.*, 2010).

Flag leaf length under irrigated condition ranged from 23.10 to 29.70 cm with a mean of 29.98 cm, under rainfed condition it was ranged from 21.30 to 38 cm with mean of 28.53 cm. Under TSD condition it ranged from 18.88 to 33.91 cm with mean of 25.72 cm during wet season 2014. Whereas during wet season 2015, under irrigated condition it ranged from 22.50 to 39.30 cm with the mean of 29.74 cm, while in TSD condition it was ranged from 18.03 to 34.35 cm with mean of 25.73 cm.

Flag leaf width under irrigated condition ranged from 1.04 to 1.78 cm with a mean of 1.42 cm, under rainfed condition it was ranged from 0.99 to 2.65 cm with mean of 1.26 cm. Under TSD condition it ranged from 1 to 1.55 cm with mean of 1.21 cm during wet season 2014. Whereas during wet season 2015, under irrigated condition it ranged from 1.12 to 1.75 cm with the mean of 1.38 cm, while in TSD condition it was ranged from 0.95 to 1.65 cm with mean of 1.32 cm.

Mean second leaf length 41.52 cm was reported under irrigated condition during wet season 2014 and it ranged from 33 to 58.60 cm, under rainfed condition it ranged from 30.25 to 53.75 cm with mean panicle length of 39.68 cm and under TSD condition mean panicle length was 34.68 cm which ranged from 26 to 45.55 cm. Under irrigated condition it ranged from 3.10 to 57.80 cm with average panicle length of 40.47 cm and under TSD condition mean panicle length was 34.40 cm, it ranged from 25.61 to 44.94 cm during wet season 2015.

Length of last internode under irrigated condition ranged from 26.80 to 43.10 cm with a mean of 33.81 cm, under rainfed condition, it ranged from 22.90 to 29.30 cm with mean of 30.99 cm. Under TSD condition, it ranged from 23.75 to 34.50 cm with mean of 28.67 cm during wet season 2014. Whereas during wet season 2015, under irrigated condition, it ranged from 27.65 to 41.80 cm with the mean of 31.41, while in TSD condition, it was ranged from 23.65 to 34.25 cm with mean of 28.33 cm.

Biological yield of RIL population under irrigated transplanted condition ranged from 871.18 to 2319.79 gm^{-2} with population mean of 1631 gm^{-2}, mean

biological yield under rainfed condition was recorded 649 gm^{-2} and it ranged from 419.11 to 1039.56 gm^{-2}, under TSD condition it ranged from 340.17 to 749.17 gm^{-2} with a mean of 502.82 gm^{-2} during wet season 2014, whereas during wet season 2015, under irrigated condition it ranged from 645.00 to 1479.32 g with a mean of 1102.19 gm^{-2} g and under TSD transplanted condition it ranged from 335.42 to 1556.25 gm^{-2} with a mean of 964.46 gm^{-2}. Mean biological yield under irrigated condition was higher than the rainfed and TSD conditions. Higher biomass production under irrigated condition was due to higher crop growth duration. Similar biological yield was also reported by Bernier *et al*., 2007.

Harvest index (HI %) under irrigated condition ranged from 26.16 to 54.31 % with a mean of 42.69%, under rainfed condition 20.83% mean HI was reported which ranged from 0.81 to 39.04 %. Under TSD condition HI ranged from 9.49 to 36.51% with mean of 23.30 % during wet season 2014. Whereas during wet season 2015, under irrigated condition the HI ranged from 21.26 to 62.61 %with the mean of 40.87 %, while during TSD it was ranged from 0.27 to 26.35% with mean of 8.61%.

100 Seed weight under irrigated transplanted condition ranged from 1.71 to 2.71 g with the population mean of 2.16, mean of 100 seed weight under rainfed condition was recorded 1.99 g and it ranged from 1.44 to 2.64 g during wet season 2014, whereas during wet season 2015, under irrigated condition it ranged from 1.72 to 2.92 g with a mean of 2.28 g and under TSD transplanted condition it ranged from 1.30 to 2.76 g with a mean of 1.91 g.

Spikelet fertility was calculated for different season and under irrigated condition it ranged from 63.48 to 94.33 % with mean of 81.56 % during wet season 2014, under rainfed condition, it ranged from 51.29 to 92.71 % with mean of 78.03%. During Wet Season 2015, under irrigated condition 80.45 % mean spikelet fertility was reported, which ranged from 62.85 to 90.71 %, whereas under TSD condition in same year, it ranged from 5.83 to 79.51 % with mean of 39.20 %. The specificity of significance of variation for spikelet fertility under drought stress showed the higher level of tolerance to drought

Analysis of means showed that during wet season 2014, under irrigated condition, root length was ranged from 12 to 58.02 cm. The root length was found

to be 42.69 cm. whereas; during wet season 2015 average root length 45.47 cm was observed. It ranges from 12.00 to 57.50 cm.

Analysis of means showed that during wet season 2014, under irrigated condition, root volume was ranged from 0.10 to 1.12 mm. The root volume mean was found to be 0.504 mm. whereas; during wet season 2015 average root volume 0.56 mm was observed and it ranges from 0.19 to 1.20 mm.

Average root diameter under irrigated condition during wet season 2014 ranged from 0.17 to 0.32 mm with population mean of 0.23mm. Whereas; during wet season 2015, under irrigated condition it ranged from 0.21 to 0.35 mm with a mean of 0.27 mm.

Root traits leading to better water and nutrient uptake, tolerance to mild to moderate drought (Dixit *et al.,* 2015). Increased root thickness improves drought resistance as the roots are capable of increasing root length density and water uptake by producing more and larger root branches. Lines with greater root characteristics considered as outstanding ones for improving drought tolerance (Ganapathy *et al.,* 2010).

Grain yield under irrigated transplanted condition ranged from 375.69 to 930.56 gm^{-2} with population mean of 693.69 gm^{-2}, under rainfed condition it ranged from 29.67 to 237.56 gm^{-2} with a mean of 128.24 gm^{-2}, under TSD condition it ranged from 59.17 to 181.83 gm^{-2} with a mean of 114.94 gm^{-2} during wet season 2014. Under irrigated transplanted condition it ranged from 273.64 to 638.18 gm^{-2} with a mean of 458.63 gm^{-2}, whereas under TSD transplanted condition it ranged from 2.43 to 244.45 gm^{-2} with a mean of 66.10 gm^{-2} during wet season 2015. Range of grain yield under different condition was very high, which indicates marked differences among the population for grain yield under stress condition, which also indirectly indicates the lines have ability to cope with water stress.

The mean reduction in grain yield of population due to TSD and rainfed over irrigated condition was 83.43 % (TSD) and 81.51 % (RF) during 2014 and 85.59 % (TSD) during 2015, which indicated very high stress levels. Yield reduction of more than 60 % under stress was also reported by Kumar *et al*., 2008; Verulkar *et al*., 2010, Kumar *et al*., 2012 and Verma (2013). This high level of

stress could be imposed because of light textured soil, higher topology of field and proper drainage. Such high stress levels were desirable because a high percentage reduction of yield is necessary to remove the effect of yield potential and clearly identify lines that are suitable for drought resistance (Babu *et al*., 2003 and Lafitte *et al*., 2006). Similar results of reduction in yield were also reported by Lanceras *et al.* (2004). Reduction in average yield in rainfed areas was also reported by Mall *et al*. (2011).

4.2.2.1 Drought susceptibility index (DSI)

Fischer and Maurer (1978) proposed a drought susceptibility index (DSI) in terms of minimal depression of yield in dry environments as compared with favorable environments. This index, however considers neither the confounding effects of flowering time on yield, nor the effects of yield potential on grain yield under drought stress (Arraudeau, 1989).

Drought susceptibility index (DSI) was calculated using the relative grain yield of each line under stress condition compared to grain yield under irrigated conditions, also considering the overall mean under stress and non-stress conditions (table 4.3). Differences for DSI among genotypes were analyzed for yield under stress condition in comparison of irrigated condition. About 39-49 per cent of genotypes in the study expressed low DSI values (< 1) for yield. The DSI values for yield varied from 0.16 to 1.18 under all the stress condition. Line number 2, 16, 17, 18, 20, 21, 25, 26, 27, 28, 29, 30, 31, 32, 36, and 42 showed low value of DSI under all sets of stress conditions. Line number 1, 3, 5, 6, 7, 12, 22, 24, 34, 41, 46, 47, 48, 49, 50, 51, 52, 53, 54, 56, 57, 61, 68, 69, 70 and 71 showed high value of DSI under all sets of stress conditions. These low DSI value indicated that grain yield is relative resistant to stress and use to distinguishable variety for drought stress from phenology and yield potential (Babu *et al*., 2011). However higher DSI (>1) values showed that these genotypes are relatively prone to drought stress. The promising lines number 27, 29 and 31 with low DSI recorded more than 160 g/m^2 under stress condition. The results indicated that the above said genotypes produced higher yield (>160 g/m2) under drought condition attributed to their specific adaptation and other drought tolerant traits such as root traits. Similar result was also reported by Chauhan *et al*. (2007), Ouk

et al. (2006), Babu *et al*. (2011) and Mall *et al*. (2012) using DSI as selection of genotypes under stress condition.

Table: 4.3 DSI value under different stress conditions

Lines	DSI RF-I	DSI TSD-I4	DSI TSD-15	Lines	DSI RF-I	DSI TSD-I4	DSI TSD-15
1	1.01	1.04	1.03	**37**	1.04	0.91	0.83
2	0.93	0.95	0.95	**38**	1.15	1.11	0.93
3	1.06	1.03	1.02	**39**	1.05	0.95	0.90
4	1.00	1.00	0.97	**40**	1.03	0.97	0.95
5	1.03	1.03	1.09	**41**	1.10	1.02	1.07
6	1.02	1.06	1.07	**42**	0.87	0.83	0.72
7	1.12	1.10	1.15	**43**	1.05	1.03	0.93
8	0.89	1.04	1.06	**44**	0.90	0.88	1.14
9	0.88	1.02	1.08	**45**	1.13	0.99	1.15
10	0.93	1.05	1.08	**46**	1.14	1.07	1.15
11	0.95	1.01	1.06	**47**	1.05	1.09	1.15
12	1.01	1.06	1.15	**48**	1.07	1.09	1.15
13	0.90	1.05	1.08	**49**	1.18	1.04	1.14
14	0.91	0.98	1.03	**50**	1.11	1.09	1.15
15	0.95	0.95	1.03	**51**	1.09	1.10	1.15
16	0.99	0.99	0.82	**52**	1.05	1.12	1.15
17	0.89	0.90	0.76	**53**	1.11	1.08	1.15
18	0.80	0.87	0.83	**54**	1.12	1.02	1.15
19	1.06	0.99	0.89	**55**	0.98	1.10	0.97
20	0.96	0.92	0.84	**56**	1.13	1.08	1.15
21	0.78	0.93	0.76	**57**	1.17	1.00	1.07
22	1.16	1.10	1.04	**58**	0.95	1.01	1.01
23	0.87	0.99	1.01	**59**	0.98	1.01	0.93
24	1.14	1.05	1.14	**60**	0.92	0.95	1.03
25	0.82	0.88	0.39	**61**	1.01	1.03	1.01
26	0.81	0.81	0.52	**62**	0.97	1.00	1.14
27	0.69	0.75	0.16	**63**	0.96	1.05	0.94
28	0.80	0.85	0.37	**64**	0.97	1.03	0.93
29	0.71	0.68	0.25	**65**	0.94	0.95	1.00
30	0.75	0.74	0.41	**66**	1.01	0.97	1.15
31	0.69	0.77	0.63	**67**	1.15	1.07	0.95
32	0.97	0.99	0.97	**68**	1.06	1.04	1.15
33	1.03	0.96	1.05	**69**	1.15	1.07	1.16
34	1.05	1.01	1.04	**70**	1.11	1.06	1.15
35	0.92	0.84	1.00	**71**	1.18	1.07	1.14
36	0.98	0.95	0.80				

*RF= Rainfed, TSD = Terminal stage drough

4.2.3 Vaiability studies

4.2.3.1 Phenotypic and genotypic co-efficient of variance (PCV and GCV)

The estimated values of phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) for all the characters, under all five conditions during both the season i.e. wet season 2014 and 2015 (Table 4.1). It clearly indicates that the apparent variation is not due to genotype but also due to the influence of environment. Comparative study of coefficient of variation on various characters revealed relatively high contribution of genotypic variation in determining the total phenotypic variation for most of the traits.

Seedling height followed by shoot biomass, tiller numbers exhibited moderate values for GCV for all conditions in both the years *i.e.* 2014 and 2015 as well.

Traits namely, root pulling resistance and 100 seed weight exhibited moderate values of PCV under irrigated condition of 2014 and 2015.

Likewise root length and average root diameter possessed moderate PCV along with GCV in irrigated condition over both the years.

Character viz., plant height followed by second leaf length, biological yield and grain yield exhibited moderate significant values of PCV coupled with GCV.

Ahmed *et al.,* (2016) reported high GCV and PCV for seedling height, 1000 seed weight and plant height. Devi *et al.,* (2016) reported moderate PCV and GCV for the traits plant height and effective tillers.

Thus considering the values of PCV and GCV coupled with h^2 and genetic advance, plant height is the single trait to have high values for the above said genetic parameters in 2014 and 2015.

4.2.3.2 Heritability

Heritability is the heritable portion of the phenotypic variance. It is a good index of the transmission of characters from parents to offspring (Falconer, 1981). Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone. However, it is not necessary that a character showing high heritability will also exhibit high genetic advance (Johnson *et al.,* 1955)

Seedling height showed high heritability 72.68%, 92.89% and 85.80 % under irrigated, rainfed and TSD condition respectively, during wet season 2014. During wet season 2015, also it exhibit high heritability under irrigated (80.10%) and TSD (87.44%) condition.

Root pulling resistance exhibited moderate heritability under irrigated condition 47.60% and 49.13% during wet season 2014 and wet season 2015, respectively. Ekanayake *et al.,* (1985) reported a low heritability (39 to 47%) for root pulling resistance in rice.

Days to 50% flowering showed high heritability 98.66%, 96.99% and 97.50 % under irrigated, rainfed and TSD condition respectively, during wet season 2014. During wet season 2015 also it exhibit high heritability under irrigated (94.26%) and TSD (96.99%) condition.

Number of tillers showed moderate (65.47%) and high (77.64%) heritability under irrigated and TSD condition, respectively during wet season 2014. It showed moderate heritability under TSD (61.39%) and irrigated (43.38%), respectively during wet season 2015.

Plant height showed high heritability 97.33%, 95.96% and 76.70% under irrigated, TSD and rainfed conditions, respectively during wet season 2014. During wet season 2015, it showed high heritability under irrigated (95.04%) and TSD (97.10%).

Panicle length exhibited moderate heritability under irrigated (44.92%) and rainfed (62.10%) condition during 2014 wet season and irrigated condition (47.14%) during wet season 2015. It showed high heritability under TSD conditions, viz. 77.23% and 78.60% during wet season 2014 and 2015, respectively. Singh *et al.* (2004) and Lanceras *et al.* (2004) observed moderate heritability for panicle length.

Flag leaf length showed high heritability 80.53 % under TSD condition, during wet season 2014, it also exhibit moderate heritability during irrigated (65.55%) and rainfed conditions (47.31%). During wet season 2015 also it exhibit high heritability under irrigated (72.79%) and TSD (77.99%) condition.

Length of last internode showed high heritability 73.12% under irrigated condition during wet season 2015. Whereas it exhibit moderate heritability

60.81%, 46.22% and 66.96% under irrigated 2014, rainfed 2014 and irrigated 2015, respectively.

Biological yield showed high heritability (84.98%) under irrigated condition during wet season 2014, whereas it showed moderate heritability under TSD (67.63%) and rainfed (58.26%), respectively. During wet season 2015, biological yield exhibited moderate heritability, 68.56% and 65.40% under irrigated and TSD conditions, respectively. This result showed that biological yield can be included in the criteria of selection for grain yield under drought condition. Similar estimates of moderate heritability were reported by Gomez and Kalamani (2003), Bernier *et al.,* (2007) and Verma *et al*. (2013).

Harvest index showed low, high and moderate heritability 35.04% and 84.09% and 67.01% under irrigated, rainfed and TSD conditions, respectively during wet season 2014. Harvest index showed low heritability 38.89% under irrigated condition during wet season 2015 and it also showed high heritability 87.13% under TSD condition during wet season 2015. A similar result of comparatively lower heritability under irrigated condition was reported by Babu *et al.,* (2003).

Broad-sense heritability of grain yield *per se* under different conditions is presented in table 4.1 and it is noted that during both the years heritability under stress conditions were not lower than that of the irrigated control.

Grain yield showed high heritability 77.47% and 98.96% under TSD condition during wet season 2014 and 2015, respectively. Grain yield also exhibit moderate heritability 67.84 and 61.71% under irrigated and rainfed condition, respectively during wet season 2014 and 52.49% under irrigated condition during wet season 2015. Khatun *et al.* (2015) and Shellamal *et al.* (2014) reported high heritability for grain yield. Moderate heritability for grain yield was observed in numerous studies under drought condition (Babu *et al.,* 2003; Yue *et al.,* 2005; Venuprasad *et al*., 2007; 2008; Kumar *et al*., 2008; Bernier *et al*., 2009a and Verulkar *et al*., 2010). High heritability of grain yield under stress conditions were reported by Atlin *et al*. (2004), Bernier *et al*. (2007), Kumar *et al*. (2008) and Venuprasad *et al*. (2007) and thus direct selection for yield under a managed upland drought environment is successful. Moderate to high heritability for grain

yield under drought stress indicated selection for yield under drought stress is repeatable and support direct selection for yield rather than secondary traits related to drought tolerance.

100 seed weight showed high heritability 72.26% and 83.63% under irrigated and rainfed condition, respectively during wet season 2014. It also exhibited moderate heritability under irrigated (59.63%) and TSD (69.90%) conditions, respectively during wet season 2015. Higher heritability for 50 % flowering, plant height and 100 seed weight was also reported by Gomez and Kalamani (2003), Srinivasan *et al.* (2008), Umadevi *et al*. (2009), Nandan *et al.* (2010), Verulkar *et al*. (2010), Seyoum *et al*.,(2012), Verma (2013), and Shellamal *et al.* (2014).

Spikelet fertility showed moderate heritability under irrigated (55.52%) and rainfed (48.63%) during wet season 2014. Spikelet fertility showed low heritability 36.38% and 39.97% under irrigated and TSD (93.97%) conditions, respectively during wet season 2015. Babu *et al.,* (2003) reported moderate to low heritability for spikelet fertility.

Root length showed high heritability 79.13% and 73.44% under irrigated conditions during wet season 2014 and wet season 2015, respectively. Root volume showed moderate heritability 67.31% and 59.50% under irrigated condition during wet season 2014 and 2015, respectively. Average root diameter showed moderate (45.14%) heritability during wet season 2014 and low heritability (26.70%) during wet season 2015. Mohan kumar *et al.* (2011) reported high heritability for root length and root volume. Fukrei *et al.* (2011) reported moderate heritability for root length.

Overall in wet season 2014 and 2015, days to 50%flowering and plant height were the only two traits which exhibited high heritability in all the conditions. Apart from this, grain yield and panicle length were the two traits having high heritability in TSD condition under both the years. As far as root length is concerned, it was the only trait showing high heritability in irrigated condition of both the years.

4.2.3.3 Genetic Advance

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection and depends on three main factors (Allard, 1960). It is influenced by genetic variability, heritability and selection intensity.

Under irrigated condition in 2014, the genetic advance varied from 5.87 for panicle length to 70.06 for root volume, followed by plant height (37.23), biological yield (35.92), grain yield (33.07), root length (32.89) and seedling height (32.42). High estimates of heritability and genetic advance was reported for seedling height, plant height, second leaf length and root length. Tillers Number, biological yield, grain yield and root volume showed moderate heritability and high genetic advance.

Under rainfed condition in 2014, highest genetic advance was recorded for harvest index (85.20) followed by grain yield (58.97), seedling height (32), plant height (30.43), shoot biomass (28.22), biological yield (27.64), 100 Seed Weight (25.60) spikelet fertility (24.42) and second leaf length (23.24). Lowest genetic advance was reported for panicle length (2.73) followed by flag leaf width (3.26). The heritability and genetic advance estimates was reported high for shoot biomass, plant height, second leaf length, harvest index and 100 seed weight. Grain yield, spikelet fertility and biological yield showed moderate heritability but high genetic advance.

Under terminal stage drought (TSD) condition in 2014, highest genetic advance was observed for grain yield (43.92) followed by harvest index (38.85), plant height (31.09), tillers number (30.89), shoot biomass (25.07), seedling height (25.80), second leaf length (23.36) and flag leaf length (21.74). The lowest genetic advance was reported for flag leaf width (5.99) Followed by length of last internode (8.66). High heritability and high genetic advance was exhibited by seedling height, tillers number, plant height, flag leaf length, second leaf length and grain yield. Seedling shoot biomass and biological yield exhibited moderate heritability but high genetic advance.

Under irrigated condition in 2015, maximum genetic advance was recorded for root volume (60.70), followed by plant height (37.96), root length (930.12),

seedling height (29.98), biological yield (29.34), grain yield (24.78), second leaf length (22.44), shoot biomass (21.04) and flag leaf length (20.12). Minimum genetic advance was reported for average root diameter (2.04) followed by spikelet fertility (7.43). High heritability and high genetic advances was reported for seedling height, plant height, flag leaf length, second leaf length and root length. Few characters were exhibiting moderate heritability but high genetic advance, viz., shoot biomass, biological yield, grain yield and root volume.

Under terminal stage drought (TSD) condition in 2015, highest genetic advance was observed for harvest index (70.33) followed by spikelet fertility (43.78), grain yield (36.88), shoot biomass (33.78), plant height (32.14), biological yield (29.46), 100 seed weight (26.97), seedling height (26.320, tillers number (25.77), second leaf length (25.50) and flag leaf length (21.77). Lowest genetic advance was reported for second leaf width (10.23) and length of last internode (15.70). High genetic advance coupled with high heritability was reported for seedling height, shoot biomass, plant height, flag leaf length, second leaf length, grain yield and harvest index. Moderate heritability but high genetic advance was reported for tillers number, biological yield and 100 seed weight.

Overall there were only three traits namely, grain yield, biological yield and plant height to exhibit high genetic advance in all the conditions over the two years. Thus, plant height was the only trait to have high heritability accompanied with high genetic advance; it indicates that most likely the heritability is due additive gene effects and selection will be effective. Similarly, for the trait having low heritability accompanied with high genetic advance reveals that the character is being exhibited due to environmental effects, so selection may be effective in such cases.

This was in consonance with the findings of Chandra *et al.* (2009) and Bekele *et al.* (2013) for plant height, Bekele *et al.* (2013) for biological yield plant^{-1} and panicle length, Subbaiah *et al.* (2011) and Bekele *et al.* (2013) for seed yield plant-1, Bekele *et al.* (2013) and Kiani (2013) for harvest index. High heritability coupled with high genetic advance indicating that most likely the heritability is due to additive gene effects and selection may be effective for these characters based

on phenotypic values in order to obtain maximum genetic gain for yield improvement in rice by simple selection process.

4.3 Correlation studies

Correlation coefficient is a statistical measure which is used to find out the degree and direction of relationship between two or more variables and is represented as 'r' in plant breeding, correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield. The association between any two variables is termed as simple correlation or total correlation or zero order correlation coefficient. It is of three types namely phenotypic, genotypic and environmental correlation. The association between two variables which can be directly observed is termed as phenotypic correlation. This includes genotypic and environmental effects. The inherent or heritable association between two variables is known as genetic correlation. This may be either due to pleiotropic action of gene or due to linkage or both. The main genetic cause of correlation is pleiotropy which refers to manifold effects of a gene (Falconer, 1960). This type of correlation is stable and is of paramount importance for a plant breeder to bring about genetic improvement in one character by selecting the other character.

Genotypic correlation between root traits, yield attributing traits and grain yield under different conditions was evaluated at $P \leq 0.05$ and $P \leq 0.01$. The estimates of correlation are presented in table 4.4.

Root pulling resistance (irrigated 2014 and 2015) followed by panicle length exhibited positive significant association with grain yield under all the five conditions over both the years i.e. 2014 and 2015. Apart from this traits namely, days to 50% flowering, length of last internode, biological yield and harvest index possessed positive and significant association with grain yield. Kamara *et al.*, (2002) reported positive correlation between vertical root pulling resistance and grain yield under non stress condition.

Seedling height was the trait to have positive and significant association in TSD and negative association in irrigated condition (2014 and 2015) with grain

yield. Grain yield under irrigated condition exhibited positive and highly significant correlation with tillers number 0.326 and 0.566 during wet season 2014 and 2015, respectively. Chandra *et al*. (2009) and Arulmozhi and Muthuswamy (2013) reported similar kind of result. Gopikannan and Ganesh (2013) reported significant positive association of grain yield per plant with number of productive tillers per plant, panicle length, and spikelet fertility percentage.

Table 4.4: Correlation between root, grain yield and yield attributing traits under different conditions

Traits	**Genotypic correlation with grain yield**				
	Wet Season 2014			**Wet Season 2015**	
	I	**RF**	**TSD**	**I**	**TSD**
SH	-0.596**	-0.037	0.236*	-0.642**	0.433**
SBM	-0.08	0.256*	-0.011	-0.067	0.095
RPR	0.352**	-	-	0.483**	-
TLN	0.326**	-	-0.074	0.566**	-0.008
DTF	0.710**	-0.188*	-0.443**	0.714**	-0.685**
PH	-0.226*	-0.232**	0.082	-0.370**	0.14
PL	0.193*	0.676**	0.337**	0.195*	0.270**
FLL	0.01	-0.163	-0.078	-0.161	0.089
FLW	0.557**	-0.329**	0.188*	0.429**	-0.03
SLL	-0.07	-0.449**	0.1	-0.107	0.272**
SLW	0.727**	-0.453**	-0.577**	0.321**	-0.071
LLI	-0.219*	0.180*	0.349**	-0.299**	0.220*
BY	0.817**	-0.567**	0.212*	0.804**	0.202*
HI	0.404**	0.960**	0.835**	-0.303**	0.991*
100 SW	0.077	0.481**	-	0.197*	0.237**
SPF	0.144	0.563**	-	0.565**	0.675**
RL	-0.095	-	-	-0.16	-
RV	-0.251*	-	-	-0.205*	-
AD	-0.535**	-	-	0.174	-

* and ** are significant at 5% and 1% probability level, respectively, SH = Seedling height,SBM =Shoot biomass, RPR =Root pulling resistance, TLN =Tiller number, DTF = Days to 50% flowering, PH= Plant height, PL = Panicle length, FLL = Flag leaf length, FLW = Flag leaf width, SLL= second leaf length, SLW= Second leaf width, LLI = Length of last internode, BY =Biological yield, HI= Harvest index, 100sw = 100 seed weight, SPF = Spikelet fertility, RL= Root length, RV= Root volume, AD = Average root Diameter and '-' denoted as data not recorded,

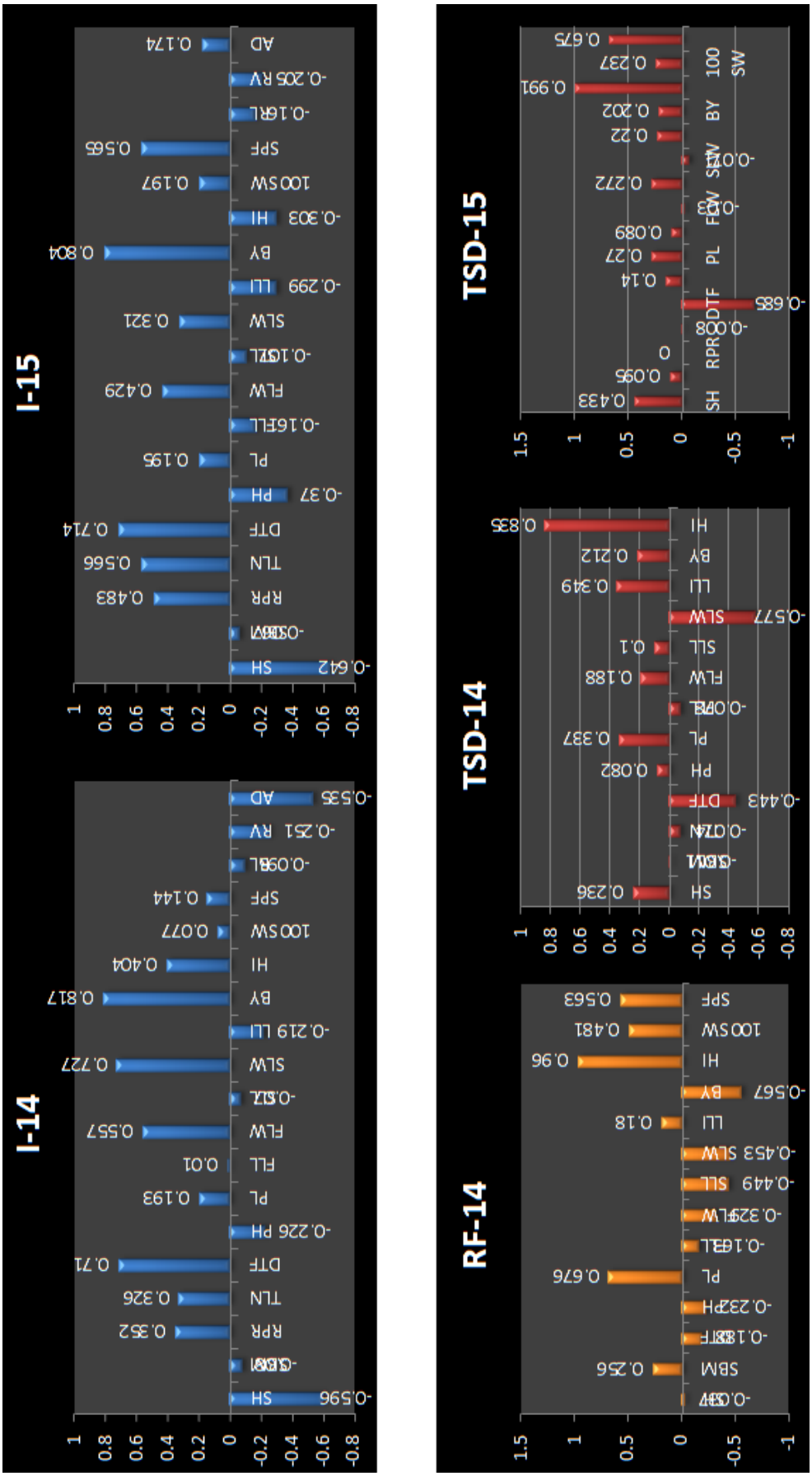

Fig4.1: Graphical representation of correlation between grain yield and other related traits

Flag leaf width exhibited significant positive correlation with grain yield in irrigated condition (2014 and 2015). However, it showed negative relation in rainfed (2014). 100 seed weight and spikelet fertility recorded positive correlation in rainfed (2014) and in irrigated and TSD conditions of 2015. A positive Correlation between grain yield and panicle length, 100 seed weight and number of tillers was reported by Nayak *et al.,* (2001), Madhavilatha (2002), Kuldeep *et al.,* (2004) and Sankar *et al*., (2006) and Chandra *et al*., (2009).

Shellammal *et al.,* (2014) found that yield under stress was significantly and positively correlated with harvest index and 100-grain weight, but negatively associated with days to 50% flowering. Ekka *et al.,* (2011) reported grain yield per plant had positive significant correlation with leaf width, days to 50% flowering, plant height, panicle length, number of filled grains per panicle, 100 seed weight and paddy (grain) length.

Root volume recorded negative association with grain yield in irrigated 2014 and 2015. Average root diameter showed negative and significant correlation with grain yield under irrigated 2014. Whereas, it showed negative correlation with grain yield under irrigated 2015. Such correlation is also reported by Gomez *et al*., 2005 and Haider *et al*., 2012. This probably indicates that greater root length and volume is of no specific advantage under irrigated condition where water is not a limiting factor and probably greater root length and volume require investment of carbon and other food assimilates for these plant parts, which adversely affect the grain yield.

4.3.1 Path analysis

An extensive literature on yield analysis has now developed but the problem that the components of seed yield are to some extent inter-dependent, still exists (Adams, 1967; Hardwick and Andrews, 1980). Phenological and genetic aspects of this interdependence are still debatable (Adams and Grafius, 1971). Under these circumstances, the value of yield analysis in programmes of plant improvement must be called in question (Stucker, 1976). However, a few plant breeders appear to turn to statistical procedures such as factor analysis or path-coefficient analysis. The path of coefficient is free of unit, the concept of path correlation coefficient was originally developed by Wright (1921) but the technique was used by Dewey

and Lu (1959). The path coefficient analysis was carried out by taking grain yield as separate and rest of the characters, as independent variables. The path coefficient analysis was used to partition the correlation coefficients of all the component characters studied with yield per plot, into direct and indirect effects. The results of various causes influencing grain yield (direct effects) are shown in tables 4.5 at the genotypic levels for all five conditions, studied during wet season 2014 and wet season 2015, respectively.

Biological yield and harvest index were the two traits exhibiting high to very high direct effect on grain yield under all the five conditions during wet season 2014 and 2015.

Apart from this, root pulling resistance and second leaf width recorded moderate positive direct effect on grain yield under irrigated condition of 2014. These two traits exhibit negative correlation with grain yield under irrigated condition 2014. Hence, under such situation direct selection for such traits should be practiced to reduce the undesirable indirect effects. On contrary, seedling height and panicle length were the traits to show moderate negative direct effect on grain yield.

In irrigated condition of wet season 2015, root pulling resistance followed by tillers number, flag leaf length, second leaf length, second leaf width and spikelet fertility exhibited high direct effect on grain yield. Root pulling resistance, tiller number, second leaf width and spikelet fertility exhibit positive and significant correlation with grain yield, it reveals true relationship between them and direct selection for these traits will be rewarding for yield improvement. Second leaf length and flag leaf length exhibited negative correlation with grain yield. Hence, under such situation direct selection for such traits should be practiced to reduce the undesirable indirect effects. Whereas, plant height and flag leaf width was the only trait to record high negative direct effect on grain yield.

Path analysis has been widely applied to several crop species like crested wheatgrass (Dewey and Lu, 1959), cereals and legumes (Dixit and Singh, 1975; Singh and Singh, 1976; Mayo 1980). The information obtained by this technique helps in indirect selection for genetic improvement of yield. Selection for a component trait with a view to improve yield is called indirect selection, while selection for yield *per*

se is termed as direct selection. A greater yield response is obtained when the character for which indirect selection is practiced has a high heritability and high correlation with yield.

Table 4.5: Direct effect of traits upon grain yield under different conditions.

Traits	**Direct effect of traits on grain yield**				
	Wet Season 2014			**Wet Season 2015**	
	I	**RF**	**TSD**	**I**	**TSD**
SH	-0.27	0.056	0.147	-0.15	0.056
SBM	0.148	0.066	0.004	0.195	0.034
RPR	0.209	-	-	0.458	-
TLN	-0.121	-	0.031	0.626	0.066
DTF	-0.037	0.007	-0.04	0.014	0.016
PH	-0.009	0.096	-0.069	-0.624	0.013
PL	-0.242	0.058	0.123	0.176	0.008
FLL	0.029	-0.036	-0.076	0.582	0.02
FLW	-0.072	0.086	-0.082	-0.617	-0.034
SLL	0.039	-0.002	-0.085	0.372	-0.035
SLW	0.278	0.013	0.126	0.184	0.109
LLI	0.137	-0.162	0.112	-0.162	-0.027
BY	0.835	0.379	0.538	0.803	0.064
HI	0.434	1.231	1.03	0.206	0.967
100 SW	0.127	0.032	-	-0.079	0.022
SPF	0.086	0.031	-	0.238	0.038
RL	0.079	-	-	0.117	-
RV	0.102	-	-	0.189	-
AD	0.043	-	-	0.052	-
Residual Effect	-0.03086	0.0264	-0.00888	-0.17671	0.00025

SH = Seedling height,SBm =Shoot biomass, RPR =Root pulling resistance, TLN =Tiller number, DTF = Days to 50% flowering, PH= Plant height, PL = Panicle length, FLL = Flag leaf length, FLW = Flag leaf width, SLL= second leaf length, SLW= Second leaf width, LLI = Length of last internode, BY =Biological yield, HI= Harvest index, 100sw = 100 seed weight, SPF = Spikelet fertility, RL= Root length, RV= Root volume, AD = Average root Diameter and '-' denoted as data not recorded,

Biological yield and harvest index also exhibited moderate to high heritability and low to high genetic advance under different condition. Hence, the correlation between grain yield and these traits is due to direct effect of character, it reveals true relationship between them and direct selection for these traits will be rewarding for yield improvement.

Low residual effect was obtained under all the condition at genotypic levels, indicating that factors which have been considered here are sufficient to contribute for yield.

Similarly, the direct effect of component traits on grain yield were reported earlier by Ravindra Babu *et al.,* (2012) and Nagaraju *et al.,* (2013) with number of productive tillers plant, number of grains per panicle and plant height, Satish Chandra *et al.,* (2009) and Nagaraju *et al.,* (2013) with productive tillers per plant, Samonte *et. al.,* (1998) with 100 seed weight and Chitra *et al.,* (2005) with harvest index and Akthar *et al., (*2011) with plant height. Thus, direct selection based on these traits will be rewarding for yield improvement in rice. Patil *et al*., (2016) reported positive direct effect on grain yield by biological yield, harvest index, 100 seed weight, days to 50% flowering and plant height.

Negative direct effect on grain yield was also reported by Naseem *et al.* (2014) for plant height, Zahid *et al*., (2006) for tillers number and Mustafa and Elsheikh (2007) for days to 50% flowering.

The negative direct effect indicates that the direct selection through this trait would not prove to be useful for the improvement of grain yield. The research study of Hairmasis *et al.,* (2010) and Akhtar *et al.,* (2011) was in correspondence with the results obtained.

4.4 Stability analysis

Varietal adaptability to environmental fluctuation is important for the stabilization of crop production both over region and years. Adaptability is the ability of a genotype to produce relatively narrow range of phenotype in different environments. Thus, stability reflects the suitability of a variety for general cultivation over a wide range of environments. In the evolutionary terms, the breeders objectives are to produce population that is better adapted in a given environment. However, adaptation and adaptability is antagonistic terms. The

adaptation refers to those changes in structure or function of an individual or population which lead to better survival or greater fitness in a given environment. Adaptation favors those characters which are advantageous for survival and through which an individual acquires adaptive value or fitness in a given environment. Adaptability on the other hand refers to the capacity of a genotype or population for genetic changes in adaption. The performance of a genotype mainly depends on environmental interaction. Estimation of phenotype stability, which involves regression analysis, has proved to be valuable technique for assessing the response of various genotypes under changing environmental conditions. An evaluation of genotype-environmental interactions given an idea of the buffering capacity of the population under study. The low magnitude of genotype environmental interactions indicates consistence performance of a population over variable environments. In other words, it shows high buffering ability of the population (Gupta *et al*., 1977). Stability analysis is done from the replicated data conducted over several locations or for several years on the same location or both. It includes location or environment wise analysis of variance and pooled analysis of variance for all location or environments. If the G X E interaction is found significant the stability analysis can be worked out. In our study 71 BC_1F_7 (2014) and BC_1F_8 (2015) lines from a cross of Swarna Sub-1 X IR-86918-305-B were grown under two conditions i.e., irrigated and TSD in both the years. Using Eberhart and Russell model the analyzed data (Table 4.6) reveals that the stability analysis partition GXE of each variety into slope of regression line and deviation from regression line

4.4.1 Analysis of variance for stability parameters:

The analysis of variance for stability parameters was done according to the regression model of Eberhart and Russell (1966). The results of this study for grain yield are presented in table 4.5. Variance due to genotype × environment was found significant indicating that the analysis can be further done for estimating the stability parameters. The sum of squares due to environment + genotype × environment were partitioned into environment (linear), genotype × environment (linear) and the remaining part was apportioned to the remainder called pooled deviation. The pooled deviation is further partitioned into component associated

with genotypes. The significance of different effect was tested by variance ratio as per Eberhart and Russell (1966).

Table 4.6: Pooled analysis of variance and stability parameters for grain yield

Sources	d.f.	Mean Sum of Square
Genotypes	70	9794.06**
Environment	3	6258458.7**
Genotype X Environment	210	8665.82**
Environment + (Genotype X Environment)	213	96691.07**
Environment (Linear)	1	1877529.1**
(Genotype X Environment) linear	70	23955.56**
Pooled Deviation	142	1007.14**
Pooled Error	280	3589.41
Total	283	

Under pooled analysis of variance, genotype X environment interaction showed significant at 0.01 probability level. Significant genotype × environment interaction for grain yield was also reported by Panwar *et al*., 2008; Ramya and Senthilkumar, 2008 and Mall *et al*., 2012. The mean sum of square due to environment (linear) was significant when tested against pooled deviation with respect to grain yield, indicated differences between locations and their influence on genotypes for expressing of character. This is in accordance with previous reports on rice by Sawant *et al*., 2005; Panwar *et al*., 2008 and Verma, 2013.

4.4.2 Stability parameters for grain yield

Eberhart and Russell (1966) defined a stable variety as one with b=1 S^2di=0). Using their definition a breeder would usually derive to develop a variety with high mean and satisfying the values of bi and S^2di. The mean performance and stability parameters for grain yield are given in table 4.6. The overall mean grain yield pooled over four environments varied from 222.25 g/m^2 to 414.8 g/m^2

Out of 71 lines, 36 lines showed higher mean grain yield than population mean. The line number 2, 16, and 19 had high value of mean grain yield, unit regression (b_i=1) and deviation was non-significant from zero (S^2di). These three lines gave stable performance under TSD condition in both the years.

Table 4.7: Estimate of different stability parameters for grain yield

Lines	Mean	b_i	S^2di	Lines	Mean	b_i	S^2di
53	414.48	1.47	-598.12	57	332.71	1.07	-759.61
46	412.13	1.45	-279.88	18	330.8	0.76	-1718.94
4	410.52	1.23	-1206.4	49	328.83	1.12	-834.64
5	406.06	1.34	-1583.32	14	328.22	1	-1499.48
52	402.52	1.48	-1387.57	38	323.92	1.05	127.29
11	400.56	1.28	-1087.17	32	323.56	0.95	-1537.65
7	398.08	1.46	-1790.8	59	319.47	0.96	-805.64
6	395.52	1.31	-1757.35	22	316.2	1.08	-1235.81
67	395.22	1.23	-534.49	63	314.75	0.93	-889.07
3	394.34	1.24	-1779.41	58	314.11	0.98	-1507.42
51	391.98	1.43	-1790.24	56	313.22	1.1	-896.36
61	391.79	1.2	-1594.5	33	312.87	0.89	2789.97
54	391.37	1.34	-762.56	30	309.21	0.43	-594.26
8	390.21	1.28	-1787.29	64	308.86	0.89	-1110.59
48	386.43	1.4	-1636.29	44	306.05	0.89	1675.34
50	381.94	1.37	-1286.41	28	300.43	0.49	1675.88
2	378.64	1.05	-1710.04	71	300.34	1.07	-1645.14
68	375.73	1.3	-1155.01	23	298.87	0.91	1502.06
16	374.1	0.96	-542	42	294.01	0.64	98.18
10	371.15	1.25	-1770.17	65	287.54	0.81	-1525.3
47	369.87	1.32	-925.89	26	284.4	0.47	-863.53
70	369.46	1.3	-1420.91	31	282.44	0.49	-1723.93
55	357.9	1.13	650.64	41	282.4	0.88	-1045.44
9	356.34	1.17	-1500.42	43	276.21	0.84	-944.86
62	354.68	1.17	-701.31	17	268.81	0.6	-1559.29
15	352.28	1.03	-1214.21	29	267.5	0.3	-235.72
13	351.33	1.19	-1309.59	40	267.21	0.73	-1292.15
60	348.91	1.01	-1157.72	21	265.63	0.66	-473.07
12	348.46	1.24	-1460.96	25	265.11	0.49	1717.56
69	346.93	1.22	-846.26	27	261.66	0.28	1729.63
1	346.73	1.13	-1108.99	39	260.14	0.69	-1744.73
19	343.98	0.97	-517.21	24	248.92	0.88	-1354.33
34	342.86	1.04	-661.53	35	245.31	0.58	4610.31
66	341.67	1.11	105.64	45	238.63	0.77	-740.21
36	334.25	0.85	-746.01	37	222.25	0.53	-1731.41
20	334.14	0.86	-174.88				

Grain yield in g/m^2 population mean = 333.28 g/m^2, bi = regression, S^2di = Deviation from regression
*** and ** = significant at 0.05 and 0.01probability level, respectively.**

The genotypes had above average mean values for grain yield and bi>1 were line number 55, 46, 52, 7 and 51 indicating their suitability to favorable environment as they were highly sensitive to environmental condition. These lines also showed high DSI value. Higher DSI (>1) values showed that these genotypes are relatively prone to drought stress.

The high mean grain yield and regression (bi<1) were reported for line number 16, 19, 36 and 20. These lines were suitable for cultivation in unfavorable

environment as there were least sensitive to environmental changes. These lines showed low DSI value, indicating that grain yield is relative resistant to stress and use to distinguish suitable variety for drought stress from phenology and yield potential (Naresh Babu *et al*. (2011).

The line number 15 and 16 had high value of mean grain yield, unit regression (bi=1) and deviation is non-significant from zero (S^2di). Hence these lines found stable and suitable for all environments. Kumar *et al*., 2012 and Koli and Chandra, 2012 also studied stability under stress condition and identified stable genotype based on regression coefficient.

4.5 Fine mapping for root traits and grain yield

The mapping population for generating genotypic data in this study was done by crossing two *indica* genotypes Swarna Sub-1 (female) and IR-86918-305-B (male) parents.

The seventy one lines of BC_1F_8 generation was grown in *Kharif* 2015 under two conditions namely irrigated and TSD. Earlier the same line were grown in *Kharif* 2014 under three conditions viz., irrigated, rainfed and TSD. In this study our attempt was to fine map and to validate the earlier reported QTLs for grain yield (qDTY1.1 and qDTY12.1) and for root traits on chromosome # 5, 6 and 9 (Verma, 2013, Vikram *et al.*, 2012 and Mishra *et al.*, 2013). For this study, the phenotypic data was generated on the traits namely grain yield, root length, root volume and average root diameter along with the genotypic data using SSR markers of Chromosome #1, 12 for grain yield and chromosome #5, 6 and 9 for root traits.

For generating genotypic data, a set of 78 primers were taken, Out of 78 SSR primers, 21 primers showed parental polymorphism, were selected (table 4.8). Primers showing polymorphisms were further used for PCR amplification with all of the 71 lines along with parents using standardized PCR protocol. The markers were taken from previously published rice genetic and sequence maps (Singh *et al.*, 2009; IRGSP, 2005; McCouch *et al*., 2002 and Temnykh *et al.*, 2001).

4.5.1 SSR based population analysis (Genotyping)

PCR products were separated on 5 % PAGE gel. The electrophoresis was carried for 1 hour at 180 volts to allow separation of amplified product. The

banding pattern was scored as A, B and H for female, male and heterozygous banding pattern, respectively. The gel pictures for different primers with population are presented in fig 4.2 and 4.3.

The quantitative trait locus, commonly known as QTL, has been defined in various ways by different scientists. Important definitions of QTL are given below. The QTL may be defined as a region of DNA that is associated with the expression of quantitative trait.

A QTL is a chromosomal region (or a locus on the chromosome) containing alleles that differently affect the expression of quantitative trait.

The gene mapping is a technique which is used to identify genes responsible for expression of specific traits.

For identification of QTL, for the above said traits the data were generated from the known marker. For using the technique interval mapping and composite interval mapping large number of marker data were used. But, in our study numbers of markers were limited. So we had to opt Single Marker Analysis (SMA).

Single Marker Analysis (SMA)

It is the earliest and simplest method of QTL analysis. It is based on the idea, that if there is an association between marker genotype and trait value. It is likely that a QTL is close to that marker locus. Thus it finds association between marker genotype and trait value. It involves regression analysis. It is used for quick scanning of the entire genome to find out best possible QTLs. It is also used to identify missing or incorrectly formatted data. There are two main limitation of this method, firstly it underestimates QTL number and secondly it can't determine QTL position precisely.

The concept of detecting QTLs using linked major genes was given by Sax (1923). Recently, progress in DNA markers and development of high density molecular map of rice (Causse *et al.,* 1994) has allowed the localization of QTL and determination of relative magnitudes of their effect on the trait in rice. In selected lines of cross between Swarna Sub-1 and IR-86918-305-B, single marker analysis (SMA) was used to estimate association between marker and trait.

Table 4.8: Primers used for developing genotypic data

Marker	Repeat	Chromosome	Forward sequence	Reverse sequence	Amplicon Size
RM11943	AG	1	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	77
RM3825	AG	1	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC	193
RM6504	CCG	1	ACCGCCTCATTCTTTCCGATCC	AGAGGCGGTAGTATGCTCGTTGC	98
RM12003	AT	1	CAAACCGGGAGTACCAATCC	ACCGGTCCTTAGCTTCTGAGC	758
RM12146	AG	1	AGTATGCCCTGCCCACTACACTAGG	CAGCGAATGGCAAGAGCAACC	100
RM431	AG	1	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	251
RM1361	AG	1	ATTCTCTCCGCCTAAACAAC	TTCTCGTGCACAGTTAATACC	214
RM12091		1			
RM163	AG	5	CGCCTTTATGAGGAGGAGATGG	AAACTCTTCGACACGCCTTGC	179
RM18616	AT	5	TCTAGGCAGTTGGTGTAACTCAGTGG	AACTCAAGTCTCAAGCCATCTACAGG	377
RM18620	AC	5	AATCTGGAAAGTCCCTTGTACGG	AGGCTAATCACCAGCTAGGTTGC	245
RM18615		5			
RM225	AG	6	TATGTGGTTGGCTTGCCTAGTGG	TGCCCATATGGTCTGGATGTGC	196
RM22565	TGTA	8	TCCACGCGTTGTCGTAGAAATTTAGC	AGCCCGAGCACCATGAAACACC	276
RM242	AG	9	AAACACATGCTGCTGACACTTGC	TTACTAGATTTACCACGGCCAACG	265
RM24585	AAG	9	TCCTCCATGATGAACCAGCTACAGG	GCAGATACCGCCTCCTCCATCC	345
RM1233	AG	11	TTCGTTTTCCTTGGTTAGTG	ATTGGCTCCTGAAGAAGG	175
RM28048	CCG	12	TTCAGCCGATCCATTCAATTCC	GCTATTGGCCGGAAAGTAGTTAGC	94
RM28076	AG	12	GGGACTTGGGACCAGTTTATGG	TCAGGTCTGTTGGATTCCATGC	290
RM277	GA	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	124
RM1261	AG	12	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	167

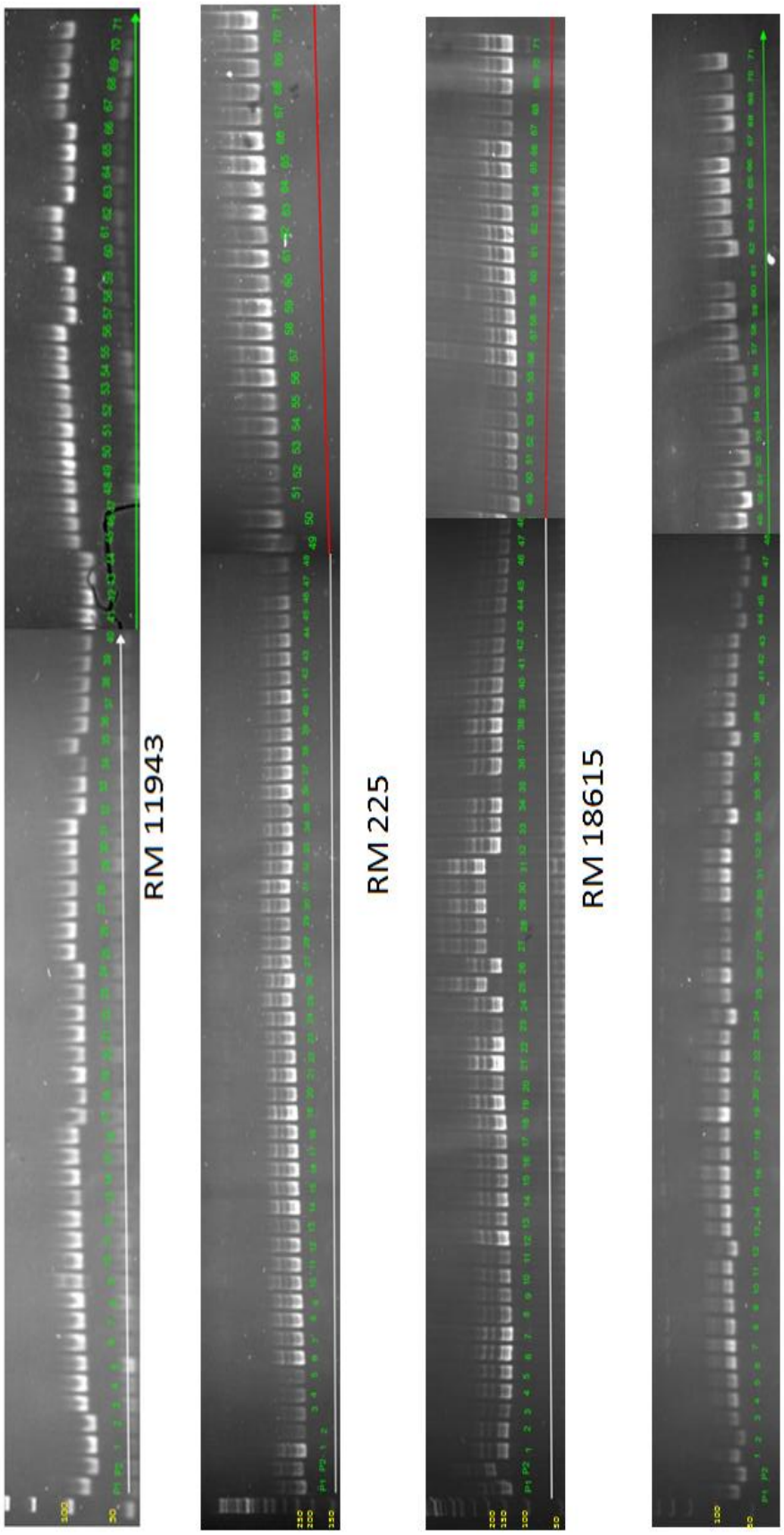

Fig.4.2 Banding pattern of RM 11943, RM 225, RM 18615 and RM 12146

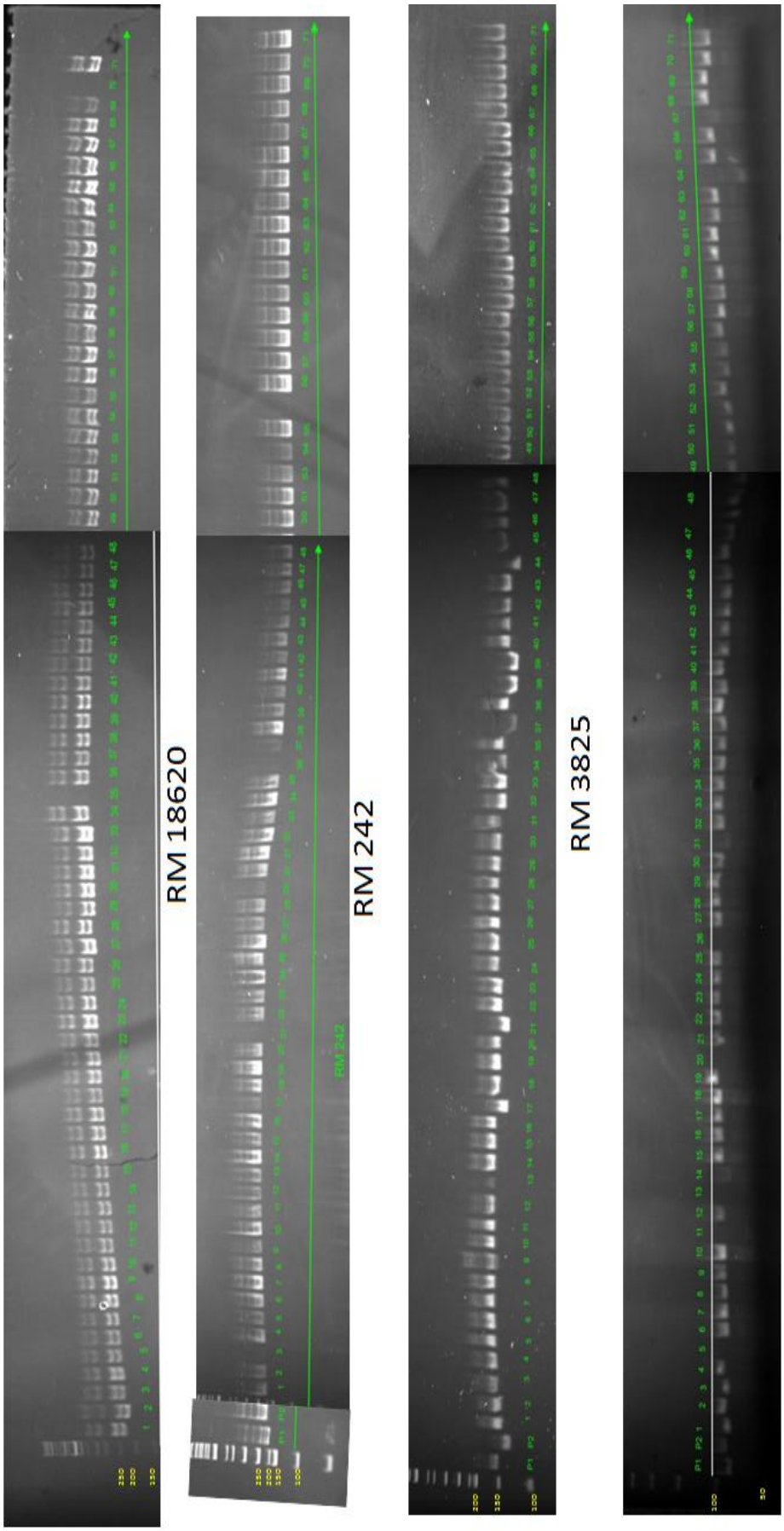

Fig.4.3: Banding pattern of RM 18620, RM 242, RM 3825 and RM 6504

Table 4.9: Scoring pattern of selected lines

Chromosome	Marker	SWARNA SUB 1	IR 305 B	2	21	25	27	28	29	31	38	44	53
1	RM 3825	A	B	A	B	A	A	A	A	A	B	B	A
	RM 11943	A	B	B	B	A	A	A	A	A	B	B	A
	RM 431	A	B	B	B	A	A	A	A	A	A	A	B
	RM 6504	A	B	B	A	A	A	A	A	A	B	A	B
	RM 1361	A	B	B	B	A	B	B	B	A	A	B	B
	RM 12003	A	B	A	B	A	A	A	A	A	B	A	B
	RM 12146	A	B	B	A	A	A	A	A	A	B	B	B
	RM12091	A	B	B	A	A	A	A	A	A	B	B	B
5	RM 163	A	B	A	A	A	A	A	A	B	A	A	A
	RM 18615	A	B	A	A	B	-	B	B	A	A	A	A
	RM 18616	A	B	A	A	A	B	A	A	A	A	A	A
	RM 18620	A	B	A	A	B	B	B	B	B	A	A	A
6	RM 225	A	B	A	A	B	B	B	B	B	A	A	A
8	RM 22565	A	B	A	A	A	-	A	A	B	A	B	B
9	RM 242	A	B	A	A	B	B	B	B	B	A	A	A
	RM 24585	A	B	B	B	A	A	A	A	A	B	A	A
11	RM 1233	A	B	A	A	B	B	B	B	B	A	A	A
12	RM 1261	A	B	B	A	B	B	B	B	B	B	B	A
	RM 28048	A	B	A	A	A	A	A	A	A	A	A	A
	RM 277	A	B	A	A	B	B	B	B	B	A	A	A
	RM 28076	A	B	A	A	B	B	B	B	B	A	A	B

Single marker analysis (SMA) t-test is done to find out significant association between the trait and marker. SMA sometimes gives better results compared to interval mapping (Coffman *et al.*, 2003). The significant association was identified between markers and grain yield and root traits.

SMA was performed and the result from the table 4.10, revealed that 14 QTLs have been identified for trait grain yield, of which 3 each minor gene were found on chromosome # 1 and #5, one each on chromosome # 6 and # 11, two on chromosome #9 and four on chromosome # 12.

In rainfed condition marker RM6504 was significantly linked with the grain yield on chromosome #1. Marker RM1361 and RM12146 had highly significant association with grain yield on chromosome #1under TSD condition.

Table 4.10: The t-test analysis of primers for grain yield and root traits

Trait	Chromosome	Marker	t-value				
			I-14	I-15	RF-14	TSD-14	TSD-15
GY	1	RM6504			0.036*		
	1	RM1361				0.003*	0.002**
	1	RM12146					0.018*
	5	RM163	0.005*		0.003*		
	5	RM18620				0.00***	
	5	RM18615					0.001**
	6	RM225	0.001**	0.00***		0.002**	0.00****
	9	RM242	0.013*		0.07**	0.000***	0.00***
	9	RM24585	0.000***	0.00**	0.017*		
	11	RM1233				0.00***	0.00****
	12	RM1261	0.11*	0.00***		0.001**	0.00****
	12	RM28048	0.000****	0.000*****	0.005**	0.17*	0.00****
	12	RM28076				0.003**	0.00****
	12	RM277		0.007**			
RL	5	RM18615	0.07*				
	5	RM18620		0.02*			
	8	RM22565		0.033**			
RV	1	RM12003	0.041*	0.039*			
	1	RM12091	0.049*				
	5	RM18616	0.0008***				
	5	RM18615		0.01*			
	8	RM22565	0.003**	0.003**			
	9	RM24585	0.028*	0.027*			
	12	RM28048	0.009**	0.008**			
ARD	1	RM12091	0.018*	0.017*			
	5	RM163	0.014*				
	5	RM18615	0.001**				

GY= grain yield, RL, Root length, RV= Root Volume and ARD= Average Root Diameter

On chromosome #5, RM163 showed significant association with grain yield in irrigated well as rainfed condition. However, RM18620 and RM18615 showed significant association with grain yield under TSD condition.

RM225 exhibited significant association with grain yield under both the TSD condition in both the year. Similarly, RM242 (chromosome #9) exhibited significant association with grain yield under irrigated, rainfed and TSD conditions. Another marker on chromosome # 9 (RM24585) exhibited significant association with grain yield under irrigated and rainfed condition. RM1233 (chromosome #11) showed significant association with grain yield under TSD condition. RM28048 (chromosome #12) exhibited significant association with grain yield under all condition. RM1261 exhibited significant association with

grain yield under irrigated and TSD conditions. RM28076 and RN277 exhibited significant association with grain yield under TSD and rainfed conditions, respectively.

Three QTL have been identified for the trait root length. Of which, two QTLs were found on chromosome #5 and one QTL on chromosome #8. RM18615 (chromosome #5) was significantly linked with root length under irrigated condition 2014 whereas, RM18620 (chromosome#5) and RM22565 (chromosome #8) had significant association with root length under irrigated condition during wet season 2015.

Likewise, seven QTLs for root volume have been identified of which, two QTL each on chromosome# 1and chromosome #5; one each on chromosome #8, #9 and #12.

On chromosome #1, RM12003 showed significant association with root volume under irrigated 2014 and irrigated 2015, whereas, RM12091 exhibited same relationship under irrigated 2014.

RM18616 and RM18615 of chromosome #5 exhibited significant association with root volume under irrigated condition during 2014 and 2015, respectively. RM22565 (chromosome #8), RM24585 (Chromosome #9) and RM28048 (chromosome #12) exhibited significant association with root volume under irrigated condition 2014 and 2015.

Three QTL for average root diameter have been identified on chromosome #1 and chromosome #5. On chromosome #1, RM12091 exhibited significant association with average root diameter under irrigated condition 2014 and 2015. RM163 and RM18615 on chromosome #5, exhibited significant association with average root diameter.

Eight markers of chromosome # 1; adjoining to QTL DTY 1.1, were taken for study. Of these eight markers RM 11943 (140.2 cM) and RM 12091 (155.49 cM) were the upstream and downstream markers, respectively. The peak markers of this QTL (qDTY 1.1), was RM 3825, RM 431, RM 6504, and RM 1361. RM 12146, RM 6504 and RM1361 exhibited highly significant association with grain yield. Hence, we conclude that QTL qDTY1.1 is present between these regions. Earlier reported region for this QTL was 135.8 cM to 194 cM, but now using these

8 markers we narrowed down from 135.1 to 155.49 cM. With due consideration of marker RM11943 and RM6504, twenty eight and thirty eight, BC_1F_8 lines of cross between Swarna Sub-1 and IR-86918-305-B carries qDTY 1.1, respectively. Apart from this, there were five BC_1F_8 lines, (line # 2, 21, 38, 44 and 53) of the same cross carries qDTY 1.1. These lines can be used for further selection for grain yield.

Four markers of chromosome # 12, for qDTY 12.1 were taken for study. Of these four markers, RM 28048 (52.24 cM) and RM 1261 (61.60 cM) were the two upstream and downstream markers respectively. The peak markers for this QTL was RM 28048 and (52.24 cM) and RM28076 (56.06 cM). All the four markers exhibited highly significant association with grain yield. This association represents the presence of QTL qDTY 12.1 as well as narrow down the region earlier reported by Mishra *et al.* (2013). There were five BC_1F_8 lines (25, 27, 28, 29, 30 and 31) carries qDTY 12.1 on the basis of RM 28048, RM 277, RM 1261 and RM 28076.

To study the root traits four markers of chromosome 5, one marker of Chromosome #6 and two Markers of chromosome #9 were taken for study.

Out of four markers of chromosome # 5, RM18615 (71.1 (cM), RM18616 (71.2 cM) and RM 18620 (71.5 cM) were reported as peak markers. RM18615 and RM18620 associated with root length, whereas, RM18615 and RM18616 exhibited significant association with root volume. RM163 andRM18615 exhibited significant association with average root diameter. Hence, we can conclude that the region and markers taken for study belongs to different root traits. QTL region was earlier reported by Verma (2013) for average root diameter was 27cM to 74 cM. The markers we have taken for study not only falls in this region but also co-segregate with root traits. Hence this fine mapped region of chromosome #5 and # 9 were useful. The result indicates that, five lines of BC_1F_8 (viz., line #25, 27, 28, 29 and 31) were significantly carries root genes. RM22565 (chromosome #8) exhibited significant association with root length and root volume. RM242 (chromosome #9) and RM28048 (chromosome #12) exhibited significant association with root volume. Similarly, RM12091 (chromosome #1) associated

with root volume and average root diameter. RM12003 (chromosome #1) significantly associated with average root diameter.

Above all the five lines viz., 25, 27, 28, 29, and line # 31, carry the genes for qDTY 12.1 as well as for root traits. Selection of these lines will be extremely beneficial from farmer's point of view, because qDTY 12.1 known to enhance yield under stress and genes related to root traits will help to develop root under stress which ultimately enhance the yield.

However most of the other populations used in QTLs analysis were derived from the *indica* × *japonica* crosses because high level of polymorphism attributable to wide variation facilitates in the construction of linkage maps and QTLs mapping. Many authors however emphasized the necessity of QTLs identification based on variation from the crosses between two related varieties belonging to same subspecies so as to make rice breeding fruitful (Yano and Sasaki, 1997; Redona and Mackill, 1996).

Number of RIL population developed by different cross combination have been used by different scientist to analysis QTL's. RIL population are considered to be more useful than the other mapping population. RIL populations are genetically true-breeding or homozygous, stable and permanent and well suited to QTL analysis. Further RILs undergoes multiple round of meiosis before homozygosity is reached, there is a greater chances for linked gene to recombine, providing an opportunity for accurate detection of QTLs (Burr and Burr, 1991; McCouch and Doerge, 1995). Considering the above facts the genotypic data based on SSR markers has been developed.

4.6 Gene Expression study of root traits

As an important organ of rice plant, root performs vital functions, acquisition of resources and anchorage of rice plant. In addition, root systems serve the secondary functions, such as propagation, synthesis of growth regulators, storage (Fitter, 2002). The release of the organic substances from the root can modify, physical, chemical and biochemical characterization of the soil in the rhizosphere (Grayston *et. al.,* 1996). Roots sense and response to abiotic and biotic stresses and communicate with the shoot via signaling pathways. Roots can regulate not only stomatal conductance, and also affect the posture of leaf blade

and photosynthesis rate under soil impedance, nutrients, drought and salt stresses. Since Weaver (1919) performed a pioneering investigation on rice root, great progress has been made in rice root biology. It is well known that the function of absorption and support of root system are an important guarantee for biological yield and grain yield of rice (Carvallais *et al.,* 2011). Therefore, root traits have been claimed to be critical for increasing yield under soil related stresses (Lynch, 2007). Root morphology and physiology are closely associated with the growth and development of above ground part of plants (Yang, 2011).

Root researchers have been paid more and more attention in recent years with the aid of novel molecular biology methods, continuing progress on root genetic research has been achieved in rice. A number of genes related to root morphology characteristics and physiological functions have been identified or cloned, which opened an opportunity for further improvement of rice productivity (Rebouillat *et al.,* 2009 and Uga *et al.,* 2013).

Rice bears a shallow root system that is comprised of one seminal root (radical), numerous adventitious roots (crown roots) arising from successive nodes and large and small lateral roots emerging from primary roots. Dissecting genetic and molecular mechanism controlling rice root development is critical for the development of new rice ideotypes that are better adapted to adverse condition and for the production of sustainably achieved rice yield potential (Rebouillat *et al.,* 2009). Early in 1980s Chang *et al.,* 1982 and Ekanayake *et al.,* 1985 have made some genetic researches on rice root. Fustuhara and Kitano (1985) reported a crown root inhibiting gene *RT1*. To date, advances have been made in root genetics. Through the utilization of several mapping population (including DH, RI and F2 population) more than 100 QTLs related to rice root architecture (root length, root number and root thickness etc.) have been mapped (Horii *et al.,* 2006; Kamoshita *et al.,* 2002; Price *et al.,* 2002; Nguyen *et al.,* 2004).

The experiment was designed to test genome-wide gene expressions from the roots of rice seedlings during the period of stress. Rice seedling at 21-days-old stage is very fragile and sensitive to abiotic stresses and has been used in many studies (Ferdose *et al.,* 2009; Hua *et al.,* 2009; Gao *et al.,* 2011; Zhou *et al.,* 2011; Hue *et al.,* 2013); hence this stage was selected for the gene expression analysis.

In the experiment, the response of plants to soil water deficit condition was observed. Water stress condition can be divided into three stages. In stage 1, when water is freely available to plant, i.e. irrigated condition (1000 ml water to wooden tray), hence, plants are under normal development. Stage 2: 500 ml water is supplied to wooden tray; hence, only susceptible plants exhibit drought symptoms. Stage 3: No water is applied to wooden tray, severe stress, susceptible lines showed mortality symptoms. This kind of irrigation schedule was repeated for seven days. Then root of resistant and susceptible lines were separated carefully, washed it and dried it. RNA was then extracted and subsequently, cDNA was prepared from RNA. The cDNA was used for semi quantitative RT-PCR and qPCR to assess the expression level of particular genes.

For gene expression study, three resistant and three susceptible lines from the cross of Swarna Sub-1 X IR-86918-305-B were selected. The seven genes under consideration were *DRO1, GLR3.1, OsGLU3, OsAGAP, OsIAA23, OsPIN3t* and *OsRR2* along with a house keeping gene α-tubuline.

Table: 4.11 Reported root development related genes

Phenotype	Gene	Locus ID	Reference
Root angle	DRO1	LOC_Os09g26840	Yi *et al.* (2002)
Root elongation	GLR3.1	LOC_Os04g49570	Li *et al.* (2006b)
Root elongation	Os GLU3	LOC_Os04g41970	Zhang *et al.* (2012a)
Root elongation	OsAGAP	LOC_Os02g10480	Zhuang *et al.* (2005)
Root elongation	OsPIN3t	LOC_Os01g45550	Zhang *et al.* (2012)
Root elongation	OsRR2	LOC_Os02g35180	Zhao *et al.* (2009)
Lateral root development	OsIAA23	LOC_Os06g39590	Ni *et al.* (2011)

4.6.1 Semi-quantitative RT-PCR

Expression analysis of genes by semi-quantitative RT-PCR was undertaken to understand and identify the network of genes involve in drought tolerance in plant. Seven genes taken for study were expressed in all conditions (Control, Moderate and Stress). Some genes showed higher expression in resistance lines and lower expression in susceptible lines and vice-versa.

4.6.1.1 Semi quantitative expression of genes responsible for root elongation

Following genes involved in root elongation and respective primers were used for semi quantitative expression analysis in 6 rice genotypes.

4.6.1.1.1 Semi quantitative expression of GLR3.1 gene in rice genotypes/ lines with N2 primer under water stress in comparison to control condition:

Glu receptors are known to function as Glu-activated ion channels that mediate mostly excilatory neurotransmission in animals. Glu receptor like genes have also been reported in higher plants, although their function is largely unknown. GLR3.1 is a Glu receptor like gene in rice, the root meristematic activity of mutant is distorted and accompanied by enhanced programmed cell death. GLR3.1 is essential for the maintenance of cell divison and individual cell survival in the root apical meristem at the early seedling stage (Li et. al., 2006b)

GLR3.1 seems to be involved in root development in rice and to act as a regulator of cell death and cell proliferation. The function of glutamate receptors remains obscure in plants; however, several recent publications suggest that L-glutamate acts as a specific exogenous signal to modulate root growth and branching.

Under moderate condition decreased expression of *GLR3.1* gene in lines 55, 33, 39 and 59; increased expression in lines 50 and 53 was observed. In stress condition decreased in 55, 33, 39 and 59; increased in, lines 50 and 53 were found. Significant weak expression was observed for *GLR3.1* gene in susceptible lines 33, 39 and 59 under stress condition in comparison to control condition. Similarly significant strong expression was observed for *GLR3.1* gene in resistant line 50 under stress condition. Present investigation supports the role of *GLR3.1* gene for enhanced root elongation (one of the factor responsible for drought tolerance in rice). Singh *et al.* (2016) reported similar kind of result and they concluded that loss of function of *AtGLR3.6* (atglr3.6-1) leads to reduced primary root growth and fewer lateral roots, whereas, *AtGLR3.6* over expression induced both primary and lateral root growth.

4.6.1.1.2 Semi quantitative expression of OsGLU3 gene in rice genotypes/ lines with N3 primer under water stress in comparison to control condition:

OsGLU3, encodes a putative membrane bound endo-1, 4-b-glucanase. *OsGLU3* can affect root cell wall cellulose synthesis to modulate root elongation (Zhang *et al.*, 2012a).

In moderate condition, decreased expression of *OsGLU3* gene in susceptible lines 33, 39 and 59; increased expression in tolerant lines 50, 53 and 55 was achieved. Stress condition: decreased in lines 50, 53, 39 and 59; increased in lines 55 and 33. There is no apparent association of expression of *OsGLU3* gene in the present investigation. Significant strong expression was observed for lines 55 and 33 under stress condition in comparison to control condition. Significant weak expression was observed for lines 50 and 59 under stress condition in comparison to control condition. This gene is responsible for root elongation and the locus ID is LOC_Os04g41970

4.6.1.1.3 Semi quantitative expression of OsPIN3t gene in rice genotypes/ lines with N18 primer under water stress in comparison to control condition:

The rice genome contains 12 putative *PIN* genes encoding auxin efflux transporters, including four *PIN1* and one *PIN2* genes. *OsPIN1* plays an important role in auxin-dependent adventitious root emergence and tillering, crown root development along with root gravitropism and drought tolerance. *OsPIN1* expresses in the vascular tissues and root primordial in a manner similar to *AtPIN1* (Xu *et al.,* 2005). *OsPIN3t* acts in auxin polar transport but is also involved in the drought stress response. Over-expression of *OsPIN3t* led to improved drought tolerance, while knockdown of *OsPIN3t* caused crown root abnormalities in the seedling stage (Zhang *et al.,* 2012c).

In moderate condition, decreased expression of *OsPIN3t* gene in line number 55 and 59 and increased expression in line 39. Under stress condition, decreased expression was observed in 50, 53, 55 and 33 and increased in 39 and 59. Significant increase in expression was observed for line 39 and 59 under stress condition in comparison to control condition. In contrast to the property of *OsPIN3t* (where resistant line should have enhanced expression), we observed strong expression of this gene in two susceptible lines 39 & 59 under severe water stress / drought condition. The rice *OsPIN3t* was responsive to drought stress, and over-expression of this gene in rice led to improved drought tolerance (Zhang *et al.,* 2012). Lee *et al.,* 2012 and Zhang *et al.,* 2012 reported over expression for *OsPIN3t* gene.

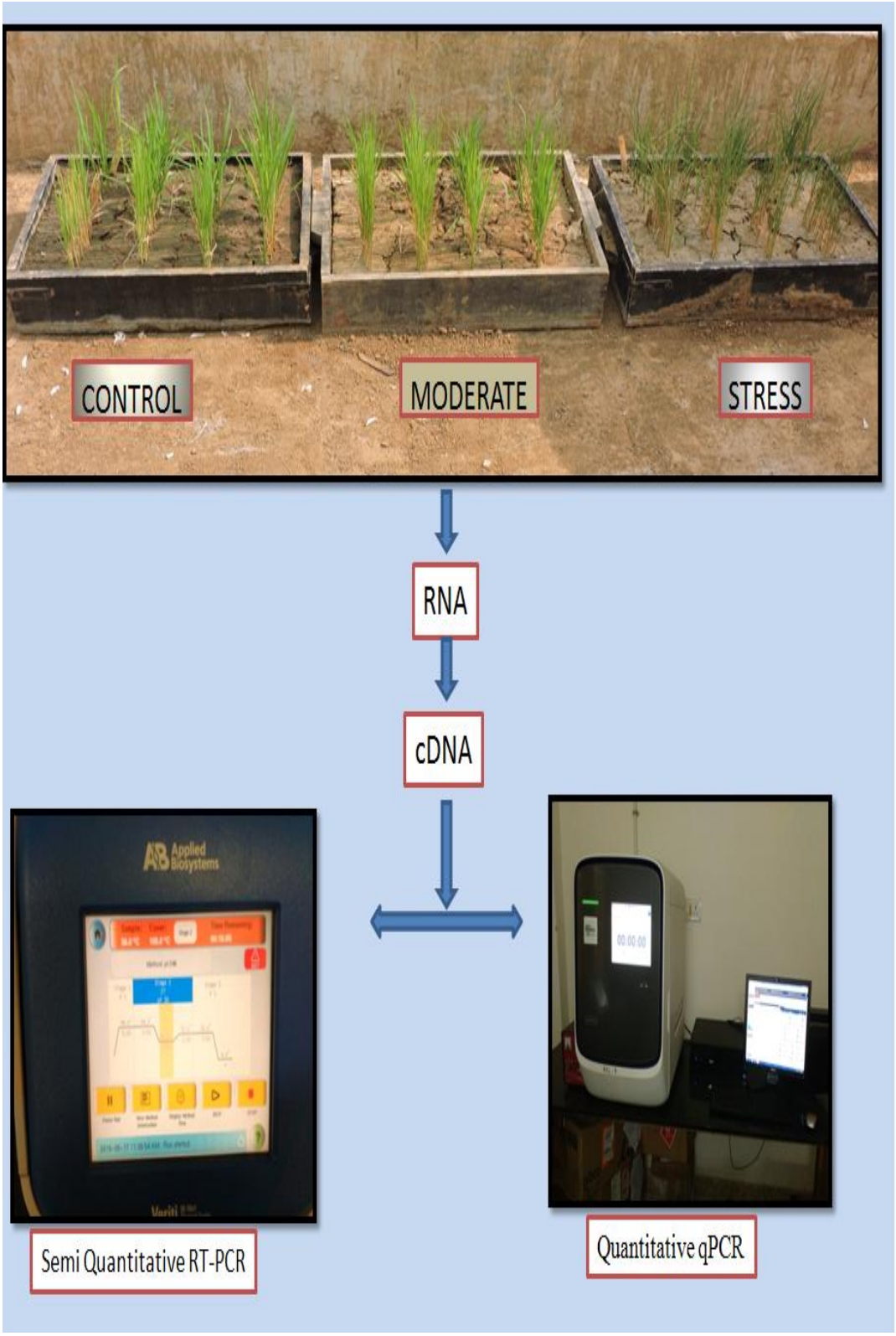

Fig. 4.4 Procedure for expression analysis Study

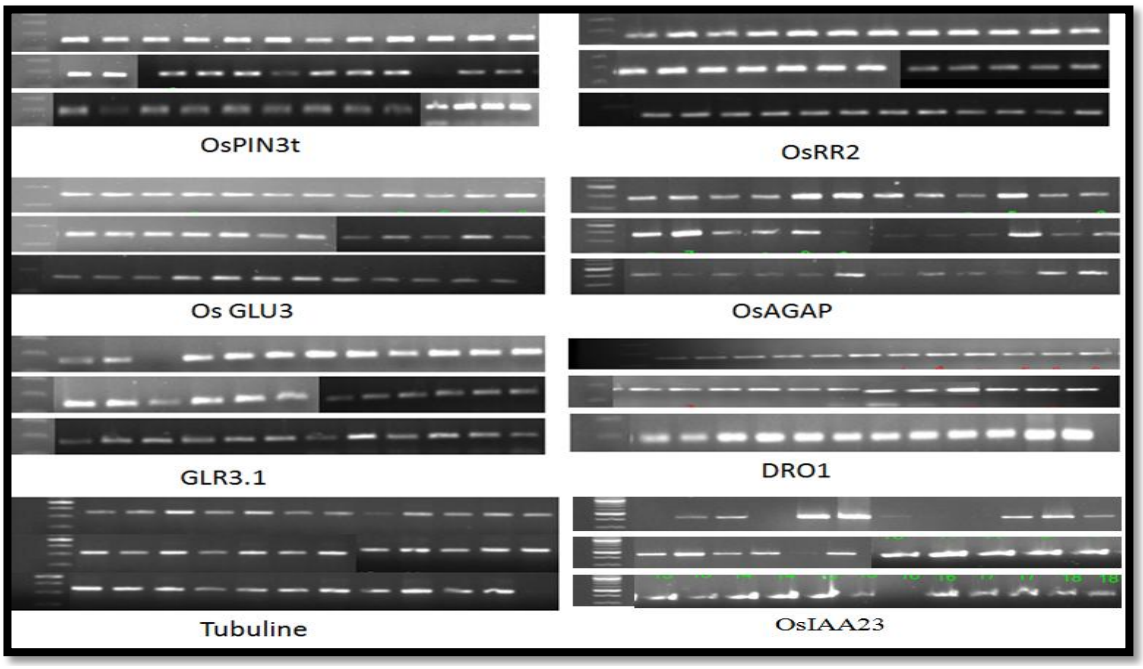

Fig. 4.5 Expression patterns of Root Specific Genes in rice seedlings under different water stress condition.

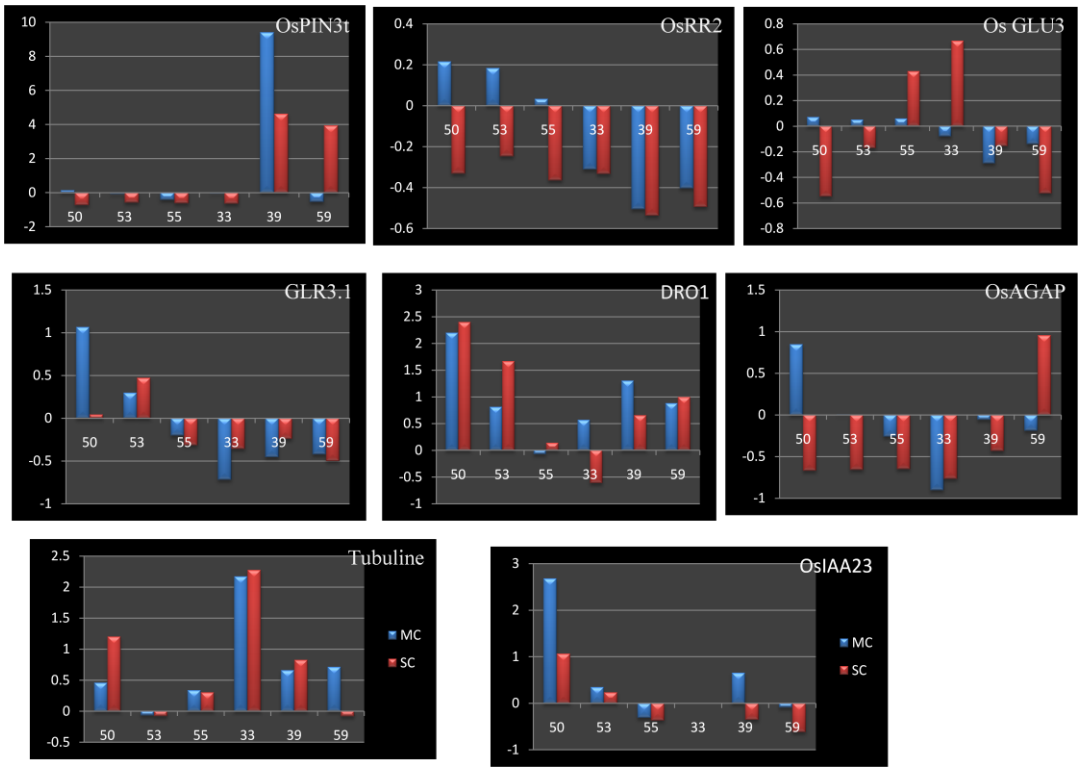

Fig: 4.6 Confirmation of expression profiles by semi-quantitative (RT-PCR) experiment with 7 selected root specific gene markers compared to microarray data in roots of rice under 3 drought stress treatments.

4.6.1.1.4 Semi quantitative expression of *OsRR2* gene in rice genotypes/ lines with N20 primer under water stress in comparison to control condition:

OsRR2, a cytokinin-responsive regulator gene, was reported to be involved in crown root formation (Zhao *et al.,* 2009). In *WOX11* over expression lines, *OsRR2* is switched off, resulting in enhanced crown root development (Gao *et al.,* 2014 and Zhao *et al.,* 2009).

Under moderate condition, decreased expression of *OsRR2* gene in lines 33, 39 and 59 and increased expression in line 50, 53 and 55 was observed. in stress condition, decreased in all the lines; i.e., line 50, 53, 55, 33, 39 and 59. Significant increase in expression was observed for line 50 and 53 moderate condition in comparison to control condition. Similarly, significant weak expression was observed for susceptible line 39 and 59 under both moderate and stress condition with respect to control. Under stress condition overall decreased expression of *OsRR2* gene was observed in all the lines (irrespective of being drought resistant or susceptible). This gene is responsible for root elongation trait with the locus ID LOC_Os02g35180 (Zhao *et. al.,* 2009)

4.6.1.1.5 Semi quantitative expression of *OsAGAP* gene in rice genotypes/ lines with N5 primer under water stress in comparison to control condition:

OsAGAP involved in the mediation of plant root development by regulating auxin level. Constitutively *OsAGAP* expression showed reduced apical dominance, shorter primary roots, increasing number of longer adventitious roots. This gene is also governing the root elongation trait with the locus ID LOC_Os02g10480 (Zhuang *et. al.,* 2005)

Under moderate condition, decreased expression of *OsAGAP* gene in lines 55, 33, 39 and 59 and increased expression in line 50 was found. In stress condition, decreased in the lines; i.e., line 50, 53, 55, 33 and 39. Increased expression in line 59. Significant increase in expression was observed for line 50 under moderate condition in comparison to control condition. Under stress condition overall decreased expression of *OsGAP* gene was observed in all the lines (irrespective of being drought resistant or susceptible) except line 59. Hence, down regulation of this gene associate with tolerance response.

4.6.1.1.6 Semi quantitative expression of *DRO1* gene in rice genotypes/ lines with N1 primer under water stress in comparison to control condition:

A large number of QTLs related to root development in rice have been detected, but just few major QTLs have been cloned. Root system architecture improves drought avoidance through the cloning and characterization of *DEEPER ROOTING 1* (*DRO1*), a rice quantitative trait locus controlling root growth angle was mapped and sequenced (Uga *et al.,* 2013). *DRO1* is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and downward bending of the root in response to gravity. Higher expression of *DRO1* increases the root growth angle, whereby roots grow in a more downward direction. Introducing *DRO1* into a shallow-rooting rice cultivar by backcrossing enabled the resulting line to avoid drought by increasing deep rooting, which maintained high yield performance under drought conditions relative to the recipient cultivar.

Under moderate condition, decreased expression of *DRO1* gene was observed in line 55; increased expression in line number 50, 53, 33, 39 and 59 and in stress condition, decreased expression was observed in line 33 and increased in line 50, 53, 55, 39 and 59. Significant strong expression was observed for *DRO1* gene in line 50 under both moderate and stress condition in comparison to control condition. Similarly, significant weak expression was observed for line 33 under stress condition. Higher significant expression of *DRO1* gene under stress condition in resistant lines 50 & 53 might be responsible for better performance than other lines. However, other factors may add on for better drought tolerance character in resistant lines which may be poorly expressed in susceptible lines. Uga *et al.* (2010) also reported similar kind of result for *DRO1*.

4.6.1.2 Semi quantitative expression of genes responsible for lateral root development:

4.6.1.2.1 Semi quantitative expression of *OsIAA23* gene in rice genotypes/ lines with N5 primer under water stress in comparison to control condition:

The quiescent centre (QC) is critical to root development. OsIAA23 is semi dominant mutant related QC development. It inhibits pleiotropic defects in root tissues, which includes the root cap, lateral and crown roots. Expression of

OsIAA23 is specific to the QC of the root tip during the development of primary, lateral and crown roots. The maintenance of the QC is dependent on OsIAA23 mediated auxin signaling (Ni et al., 2011)

OsIAA23 (Os06g39590) belongs to *Aux/IAA* family. *The* Expression of *OsIAA23* is specific to the quiescent center (QC) of the root tip during the development of primary, lateral and crown roots. A stabilizing mutation of OsIAA23 exhibited defects in postembryonic maintenance of the QC that caused the disintegration of the root cap and termination of root growth. The initiation of lateral root and crown root primordia was also blocked. OsIAA23-mediated auxin signaling has been shown to be crucial for the maintenance of the quiescent center in root tips (Jun *et al.,* 2011).

Under moderate conditions, decreased expression of *OsIAA23* gene in lines 55 and 59 and increased expression in lines 50, 53 and 39. In stress condition, decreased expression in lines 55, 39 and 59 and increased expression in line 50 and 53 was observed. Significant increase in expression was observed for line 50 under both moderate and stress condition in comparison to control condition. Similarly, significant weak expression was observed for susceptible line 59 under stress condition with respect to control. Under stress condition two resistant lines 50 & 53 expressed enhanced expression of *OsIAA23* gene in comparison to control condition and vice versa *i.e.* decreased expression was observed under stress condition for two susceptible lines 39 & 59. The results indicate some correlation of expression of *OsIAA23* gene with resistant and susceptible character and possible role of the gene for drought tolerance in terms of enhanced lateral root development.

Up regulation was reported for tubuline for all the lines under both moderate stress and severe stressed conditions, except for line 53 which showed down regulation under both the stress conditions.

Susceptible lines showing decreased expression under stressed condition for *OsRR2, OsGLU3, GLR3.1* and *AsAGAP,* whereas; tolerant lines showing over expression under moderate stress but also exhibit decreased expression under severe stress condition. Tolerant line 50 and 53 showed over expression under both stress condition for *GLR3.1* and *DRO1* genes. Susceptible line 59 showed over

expression under severe stress condition for gene *OsPIN3t, DRO1* and *OsAGAP* genes.

4.6.2 Quantitative qPCR assay

In quantitative analysis we tried to eliminate experimental inconsistencies by adopting normalization strategies at different experimental steps. Normalization is essentially the process of neutralizing the effects of experimental variability such as the nature and amount of starting sample, the RNA isolation process, reverse transcription, and, of course, real-time PCR amplification. Normalizing to sample quantity was performed by initiating the RNA isolation with a similar amount of sample (100 mg root tissue). Normalizing to RNA quantity: precise quantification and quality assessment of the RNA samples are necessary and therefore almost equal concentration (~700 ng) of RNA samples were taken for cDNA preparation. Later on, for qPCR set up an equal quantity of cDNA (1 to 2 µl) was taken for preparing reaction mixture (assuming equal concentration of cDNA for all the samples). Pipetting error was tried to minimize by careful handling and avoiding bubbles. Normalizing to a reference gene: The use of a normalizer gene (also called a reference gene or endogenous control) is the most thorough method of addressing almost every source of variability in real-time PCR. Relative expression levels of the different genes (root development and drought related) in each cDNA sample were obtained by normalization to either of the two endogenous reference genes ("housekeeping" genes) actin or tubulin genes. In the present work α-tubulin was taken as housekeeping reference gene. Comparative quantification was applied to the present gene expression study where, the expression level of a gene of interest is assayed for up- or down-regulation in a calibrator (normal) sample and one or more experimental samples.

4.6.2.1 Tubulin Vs DRO1 gene

Root system architecture improves drought avoidance through the cloning and characterization of *DEEPER ROOTING 1* (*DRO1*), a rice quantitative trait locus controlling root growth angle. *DRO1* is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and downward bending of the root in response to gravity. Higher expression of *DRO1* increases the root growth angle, whereby roots grow in a more downward

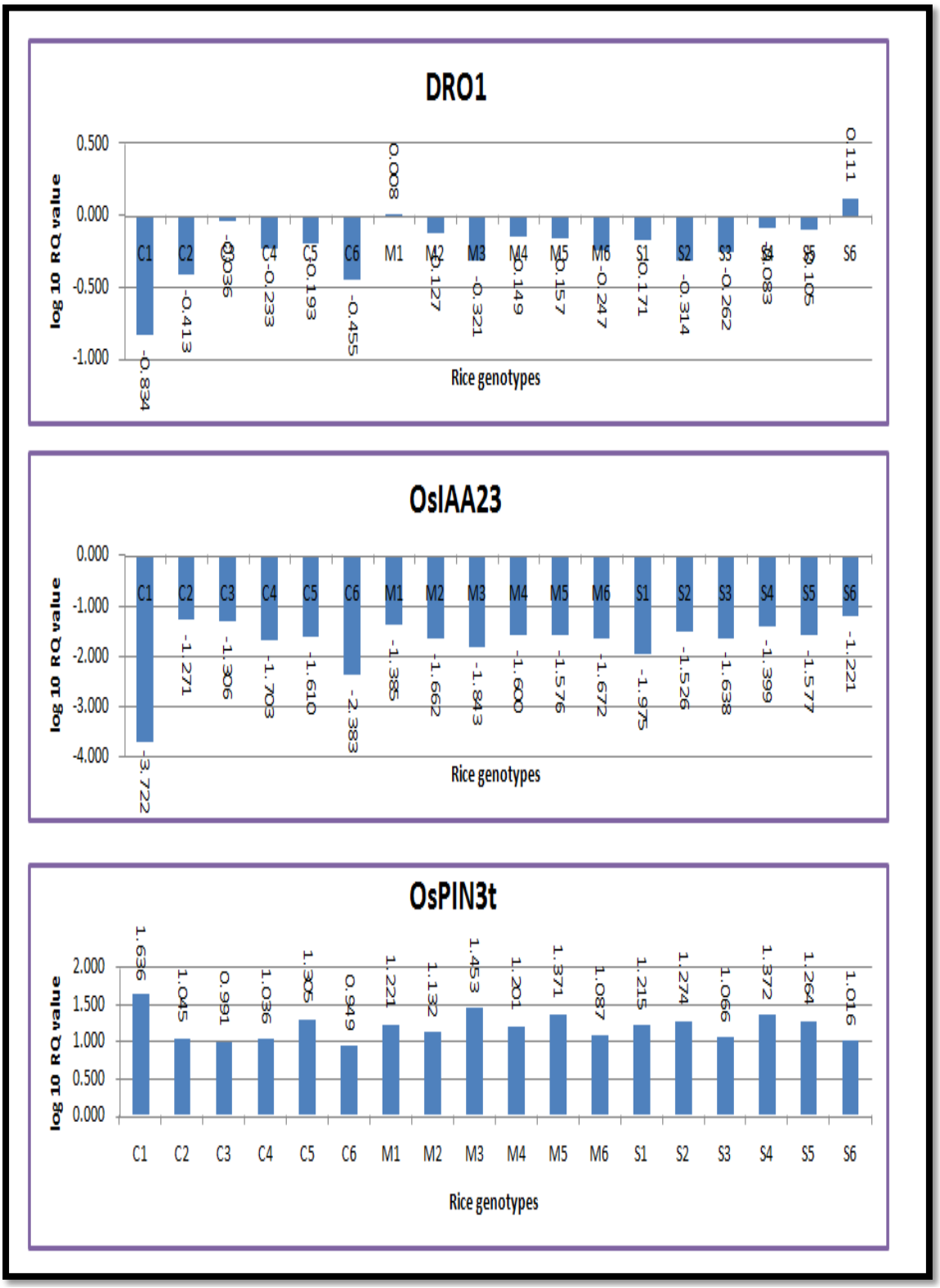

Fig: 4.7 Confirmation of expression profiles by qRT-PCR experiment with 3 selected root specific gene markers compared to microarray data in roots of rice under 3 drought stress treatments.

direction. Introducing *DRO1* into a shallow-rooting rice cultivar by backcrossing enabled the resulting line to avoid drought by increasing deep rooting, which maintained high yield performance under drought conditions relative to the recipient cultivar.

In the present q PCR experiment, there was weak expression of DRO1 gene in all the six selected lines under control, moderate and stress conditions (fig.4.7). However, line 59 which was identified as one of the highly susceptible line for drought had strong expression for DRO1 gene under stress condition. The expression of *DRO1* has been shown to be negatively regulated by auxin signaling downstream of Aux/IAA and ARF, and is involved in cell elongation in the root tip that causes gravitropic bending (Uga *et al.* 2013). These results suggest that polar auxin transport and auxin signal transduction during the gravitropic response is an important determinant of the root growth angle (RGA) in rice plants, as it is in *Arabidopsis*.

4.6.2.2 Tubulin Vs *OsIAA23*

OsIAA23 (Os06g39590) belongs to Aux/IAA family. *The* Expression of *OsIAA23* is specific to the quiescent center (QC) of the root tip during the development of primary, lateral and crown roots. A stabilizing mutation of OsIAA23 exhibited defects in postembryonic maintenance of the QC that caused the disintegration of the root cap and termination of root growth. The initiation of lateral root and crown root primordia was also blocked. OsIAA23-mediated auxin signaling has been shown to be crucial for the maintenance of the quiescent center in root tips (Jun *et al.*, 2011).

OsIAA23 gene for lateral root development shows weak expression with respect to housekeeping gene tubulin under all the three conditions (control, moderate and stress). Significant weak expression was observed for resistant line 50 under control condition. There is no direct correlation between selected resistant and susceptible lines under different water stress condition with respect to OsIAA23 gene expression.

4.6.2.3Tubulin Vs *OsPIN3t*

The rice genome contains 12 putative PIN genes encoding auxin efflux transporters, including four PIN1 and one PIN2 genes. OsPIN1 plays an important

role in auxin-dependent adventitious root emergence and tillering, crown root development along with root gravitropism and drought tolerance. OsPIN1 expresses in the vascular tissues and root primordial in a manner similar to AtPIN1 (Xu *et al.,* 2005). OsPIN3t acts in auxin polar transport but is also involved in the drought stress response. Over-expression of OsPIN3t led to improved drought tolerance, while knockdown of OsPIN3t caused crown root abnormalities in the seedling stage (Zhang *et al*., 2012c).

In the present q PCR experiment, there was strong expression of OsPIN3t gene in all the six selected lines under control, moderate and stress conditions with respect to house keeping gene tubulin. The quantitative expression result obtained with primer for OsPIN3t gene is opposite to that obtained with primers for genes DRO1 and OsIAA23.

We could not establish any association between expression of genes with resistant and susceptible lines under different water stress and control conditions.

It is the roots that absorb most nutrients and water (Russell, 1977). Among essential nutrient elements required for rice growth, inorganic carbon is absorbed mainly by leaves in the form of carbon dioxide, the other essential mineral elements are all absorbed mainly through root surface from the soil.

Root is the foundation of rice development. As described in the report by Zhang *et al.* (2009), the high grain yield was mainly due to a larger sink size (total number of spikelets) as a result of a larger panicle. The low percentage of filled grains was closely associated with a quick decreased root activity during grain filling. Further research is needed to understand the mechanism involved in the low percentage of filled grains and yield fluctuation and to improve the yield performance in elite hybrid lines. The yield of elite varieties can be further increased by an increase in filled grains through enhancing root activity during grain filling (Yang, 2011; Cheng *et al.*, 2007c).

Drought is one of the most severe abiotic stresses limiting rice productivity in the world, and poses a serious threat to the sustainability of rice yields in rainfed agriculture. Development of drought resistant rice is one of the objectives in the water-saving agriculture programs (Dat, 1986). Acquisition of more water from soil is a mechanism for drought tolerance in rice. Improving the understanding of

the interaction between root function and drought in rice could have a significant impact on global food security (Gowda *et al.*, 2011). Therefore, improving root system with deep root and high water uptake ability would be the key to developing elite rice varieties suitable for water-saving farming system.

Although increasing knowledge on rice root has given an insight into mechanisms of root development, it remains unclear what root traits should be taken into account in rice breeding programs. There are difficulties that hampered progress of root genetics: research efforts devoted to the root system have been much less than to the above ground part. Lacking stable and credible morphological data makes it difficult to perform genetic research on rice root traits (De Dorlodot *et al.*, 2007).

4.7 Pyramiding of QTL for root traits in rice

For quantitative traits such as root traits and yield, a single QTL rarely accounts for a significantly large portion of the trait variation. Several QTLs with additive effects need to be stacked to achieve significant improvement. Gene pyramiding is a method aimed at assembling multiple desirable genes from multiple parents into a single genotype. The end product of a gene pyramiding program is a genotype with all of the target genes. Marker assisted gene pyramiding has gained popularity in the last decades (Ye and Smith, 2010). In principle, pyramiding multiple genes is achieved by crossing parental lines with complementary desirable genes and then selecting the desired recombinants among the progeny populations (Allard, 1999). Given that breeding is time-consuming, breeders aim to combine as many desirable alleles as possible in a single breeding cycle (from crossing to the generation of near-homozygous breeding lines). Once the number of genes to be assembled is known, gene pyramiding then aims to obtain near-homozygous breeding lines that are fully homozygous for the desirable alleles of the target genes by using the minimum number of generations, as well as the lowest genotyping and phenotyping costs.

Drought being a major abiotic factor affecting rice production especially in the critical reproductive growth phase. Novel approach, such as QTL pyramiding through marker assisted selection for root morphological traits and evaluation of

Table: 4.12 Primers used for developing genotypic data for gene pyramiding

Marker	Chromosome	Position	Forward sequence (5' --------->3')	Reverse sequence (5' --------->3')
HvSSR 1-80	1	34.9	TTTGAGCAAATAAACTTGAGG	GCTTCTACTTCCACAAGGC
Hvssr 1-87	1	38.9	TTGGTACACGACCATGATTA	ATGGATCTGTGTGTGCGT
HvSSR 1-89	1	40.6	TGCGACGGATAGAGTACATA	GGATGCAAAGAAAGAACAAG
RM449	1	81.9	TTGGGAGGTGTTGATAAGGC	ACCACCAGCGTCTCTCTCTC
RM475	2	92.5	CCTCACGATTTTCCTCCAAC	ACGGTGGGATTAGACTGTGC
HvSSR3-6	3	2.8	AGATGAGCTTCAGTGCTAGG	TTCACCACAAAGTTCACAAA
HvSSR 3-9	3	3.8	TGTATTCAAGGAGGGCTAGA	CAACTGTTTCCTGGAATGAT
HvSSR 3-35	3	13.5	TTGATTACGTGAATAGCTCG	CATAGCTAACTTGTGCGTTG
HvSSR 3-40	3	15.4	CGAGAGGTTCAGAGAGAATG	GCATTCACCCTAAGGATACA
RM135	3	157.3	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC
HvSSR 4-35	4	22.9	ACCAACCTAATACCGATGTG	CGCGAGTGTTGTAACTTTAAC
HvSSR 4-38	4	24.1	CCAAGCACCTCTTAACTTGA	CCGTTCTTATTAGGTTGTGG
HvSSR 4-39	4	26.8	CAAATAAGATCGCTGAAACC	TTCGGAGTAAATTGGACATC
RM348	4	113.2	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
RM163	5	74.5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
RM440	5	92.7	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
HvSSR 8-29	8	15.3	AACTGAGAGGCTGCTTGTAT	TAAAGGGTTCACTCATGGAC
RM310	8	57	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC
HvSSR 9-19	9	10.5	TCGAATTTAGTCCAGGGTAA	GGTGAGAGATCTTGAGTTCG
HvSSR9-27	9	15.5	TGGGCATCTGGTACTATCTT	AGCTCATTCCACAGGTTAGA
HvSSR9-37	9	16.8	AATCTCAACTGCTCGGATTA	TTGATTGATTGATTGAACGA
HvSSR 9-57	9	22.8	GGAGGTTGTTGTTACGTTGT	GGGAGGGTAATTCAGGTAAG
RM410	9	64.4	GCTCAACGTTTCGTTCCTG	GAAGATGCGTAAAGTGAACGG
RM242	9	73.3	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
HvSSR 11-02	11	0.4	TAGATTGGGTGATGGATAGC	CTACTTGATCCAGGGAAATG
RM254	11	110.1	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
RM20	12	3.2	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
HvSSR 12-51	12	27	AATCATCATATTGCCGAAAG	ATCACCATCTATCATTGCAC
RM17	12	109.1	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA

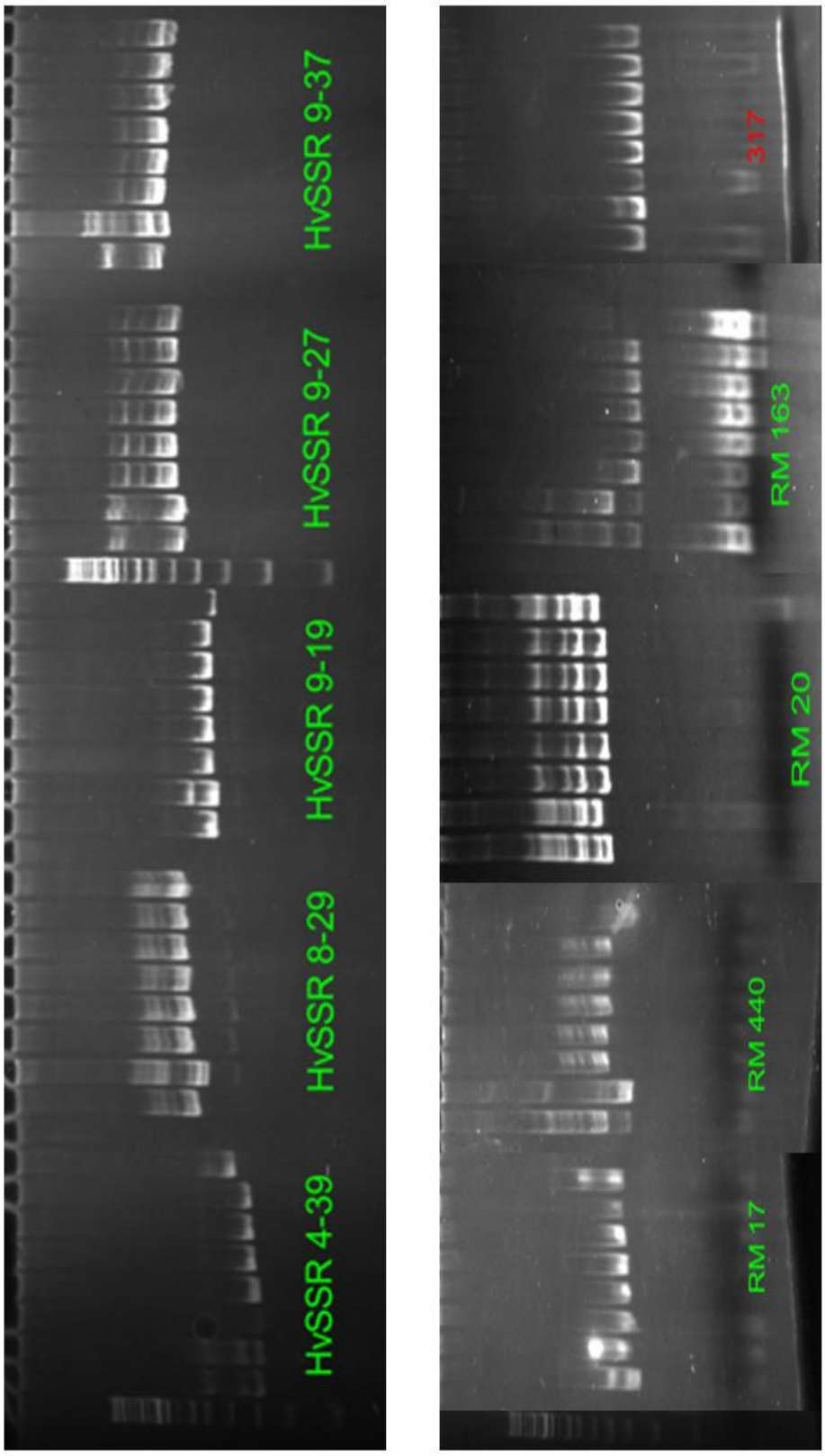

Fig 4.8: Banding pattern of parents and progenies for different markers

the effects of root QTLs in combination is attempted to develop new drought tolerant rice genotypes (Selvi *et al.*, 2015).

Initially, a RIL population was developed using Danteshwari (released variety) and a local land race, Dagad Deshi (deep rooted drought tolerant) during 2003. A set of 122 RILs were selected. For pyramiding of QTLs for root traits, out of 122 RILs a set of 38 RILs were selected on the basis of leaf rolling which clearly classify into stress tolerant and stress susceptible. Therefore, out of 38 RILs, 2 tolerant lines were taken for crossing program to develop R X R cross. From this R X R cross, F_2 and F_3 segregating generation was developed. Six lines from above said cross was taken to generate the genotypic data (table 4.13), using 29 polymorhic markers (table 4.12). Further, graphical genotyping was prepared by the software (GGT 2.0) (fig. 4.9).

The regions for root traits and grain yield were identified by Verma *et al.*, (2013). Our objective was to bring together the QTLs, responsible for root traits as well as grain yield. A set of 29 polymorphic markers were used to generate genotypic data. Result of GGT clearly indicate the variation present among the parent (line number 31 and line #24) as well as this variation is well distributed in selected F_3 progeny (of cross between line # 31 and 24). GGT graph clearly indicate the flow of genomic regions between parents and progenies.

The GGT results (fig. 4.9) represent the regions of different chromosomes that come from their original parents (i.e., Danteshwari and Dagad Deshi).

The region for root volume, root length and average root diameter has been reported in chromosome # 5 between 74 cM to 92 cM (Verma, 2013) and this region is present in second parent, taken for crossing (line #24) as well as progeny

6. Another region governing the root volume is present in first parent in chromosome # 8 (between 15 to 57 cM). This region showed variation among all selected progeny and present in progeny # 6 (Fig 4.8a). Hence, we can conclude that progeny # 6 is pyramided line for root traits. This pyramiding include region of chromosome #5 and chromosome #8.

Table 4.13: Scoring pattern of parents and progenies

Marker	Parent 1 (Line #31)	Parent 2(Line #24)	1	2	3	4	5	6
HvSSR 1-80	B	A	B	B	B	B	B	B
Hvssr 1-87	A	B	B	B	A	A	A	U
HvSSR 1-89	A	B	A	A	A	A	A	A
RM449	B	H	B	B	B	B	B	H
RM475	A	B	A	A	A	A	A	A
HvSSR3-6	B	A	A	A	B	B	B	B
HvSSR 3-9	B	H	B	B	B	B	B	H
HvSSR 3-35	A	B	A	A	A	U	A	B
HvSSR 3-40	A	B	A	A	A	A	U	B
RM135	B	A	A	A	A	A	A	B
HvSSR 4-35	A	B	A	A	A	A	U	B
HvSSR 4-38	B	H	B	B	B	B	B	H
HvSSR 4-39	B	A	A	A	B	B	B	U
RM348	B	A	B	A	B	B	B	B
RM163	U	A	B	B	B	B	B	A
RM440	A	B	A	A	A	A	A	B
HvSSR 8-29	A	H	A	A	A	A	A	A
RM310	B	A	B	B	B	B	B	B
HvSSR 9-19	A	H	A	A	A	A	A	H
HvSSR9-27	B	H	B	B	B	B	B	B
HvSSR9-37	A	B	A	A	A	A	A	B
HvSSR 9-57	A	B	A	A	A	A	A	B
RM410	B	A	B	B	A	A	A	A
RM242	B	A	B	B	B	A	A	A
HvSSR 11-02	B	A	B	B	B	B	B	B
RM254	B	A	A	A	A	A	A	U
RM20	B	A	B	B	B	B	B	A
HvSSR 12-51	B	A	B	B	B	B	B	A
RM17	B	A	A	A	A	A	A	B

*A denotes Danteshwari type, * B denotes Dagaddeshi type, H denotes Hybrid type

First parent of cross carries the grain yield QTL in chromosome # 9 (10 to 15 cM) and second parents carries the grain yield QTLs in chromosome # 3 (127 to 157 cM) and # 11(85 to 95 cM). Progeny # 1, 2, 3, 4 and 5 contain the grain yield QTLs from both the parents (Fig. 4.8c) Hence, these progeny lines are the pyramided lines for grain yield.

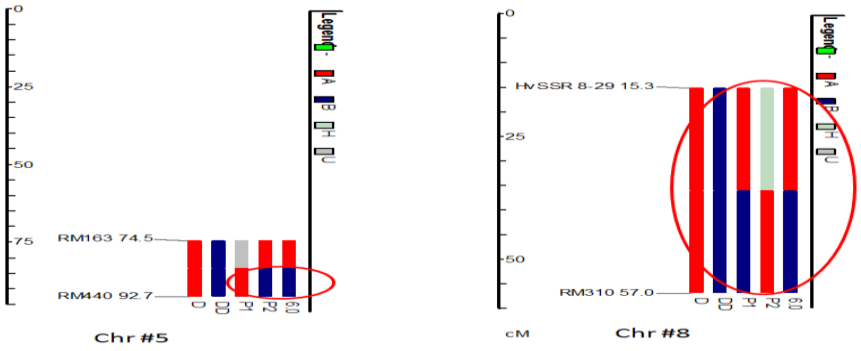

Fig:4.9a. Graphical genotype of chromosome 5 and 8 of Danteshwari × Dagad Deshi, P1(31), P2 (24) and progeny #6 population showing expected proportion of introgression

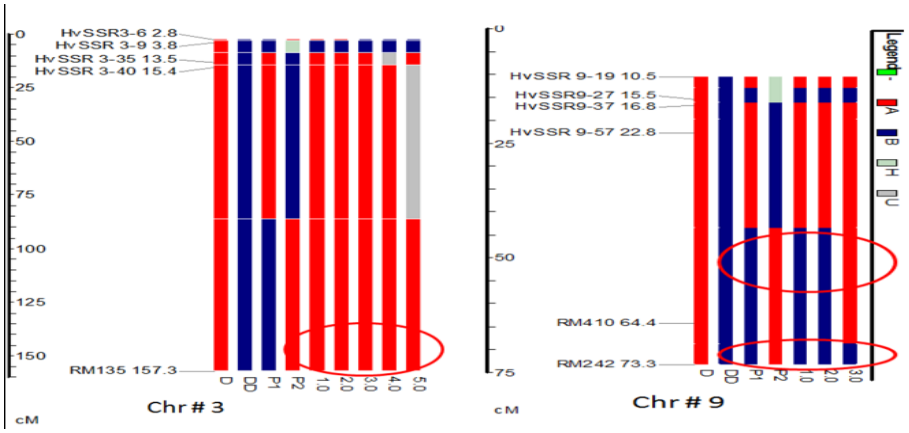

Fig:4.9b. Graphical genotype of chromosome 3 and 9 of Danteshwari × Dagad Deshi, P1(31), P2 (24) and progeny #1, 2, 3 showing expected proportion of introgression

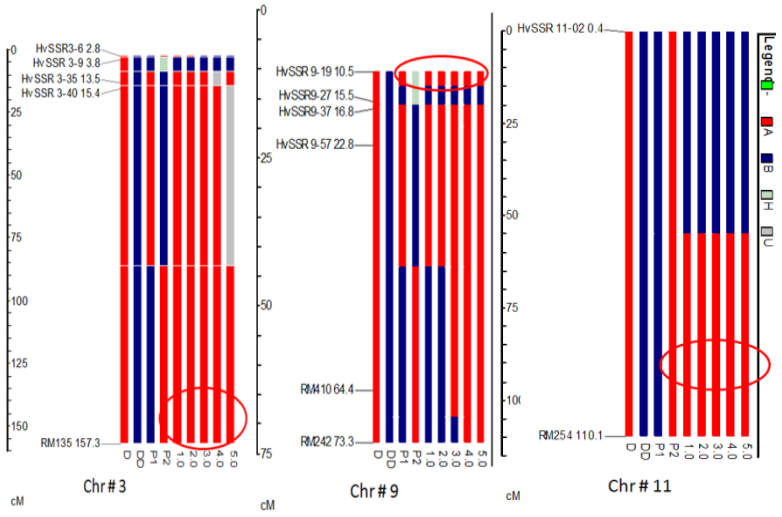

Fig:4.9c. Graphical genotype of chromosome 3, 9 and 11 of Danteshwari × Dagad Deshi, P1(31), P2 (24) and progeny #1, 2, 3, 4 and 5 showing expected proportion of introgression

Apart from these, we found that, progeny # 1, 2 and 3 contain the QTLs region for root length and root volume in chromosome # 9 (between 46 cM to 57 cM and 70 cM to 73 cM, respectively) from parent one of cross. The same progeny lines also contain the grain yield QTLs in chromosome #3 (between127-157 cM) from second parent of cross (Fig 4.8b). Hence, we can conclude from above combinations that, progeny # 1, 2 and 3 are the pyramided lines for grain yield and root trait.

n the basis of gene pyramiding study, we conclude that progeny number 6 was pyramided for various root traits. Progeny number 1, 2 and 3 were pyramided for root traits and grain yield, whereas progeny number 1, 2, 3, 4 and 5 pyramided for grain yield only.

Steele *et al.* (2006) pyramided six QTLs in the background of an Indian upland rice variety Kalinga III. Five segments on different chromosome were targeted for introgression. Four segments carried QTLs for improved root morphological traits and fifth carried a recessive QTL for aroma.

Three drought yield QTLs, *qDTY* $_{2.2}$, *qDTY* $_{3.1,}$ and *qDTY* $_{12.1}$ with consistent effect on grain yield under reproductive stage drought stress were pyramided by Shamsudin *et al.* (2015).

IRRI's drought breeding team has successfully developed pyramided lines containing different combinations of the four major drought QTLs ($qDTY_{2.2}$, $qDTY_{4.1}$, $qDTY_{9.1}$, and $qDTY_{10.1}$) (Swamy and Kumar, 2011). Introgressed lines with three and two QTLs in an IR64 background show yield advantages under drought.

Drought is a major challenge in achieving sustainable world rice production in the rainfed ecosystems of Asia. Breeding drought-tolerant rice cultivars can increase rice production yields especially in rainfed ecosystems under drought stress. The identification and introgression of QTL regions with a large and consistent effect on grain yield and root traits, presents an opportunity to improve high-yielding drought-susceptible mega-varieties through MAB.

In the present study, it was demonstrated that it is possible to combine QTLs in a common genetic background using co-dominant markers that reside in

the QTL region. The combinations of more than one QTL present in a single genotype that performed superior in both stress and well watered condition is being tested on large scale and in natural water deficit situation.

CHAPTER IV
SUMMARY AND CONCLUSION

Rice (Oryza sativa L.), a semi-aquatic cereal is adapted to a variety of climates ranged from flooded to aerobic condition. Increased productivity in irrigated rice has out-paced because 40 per cent irrigation water will get reduced by 2020. Abiotic stresses, especially water stress is a major constrain to instability in production and yield under rainfed rice ecosystem. The development of cultivars with improved drought tolerance is thus an important element in increasing productivity and alleviating poverty in communities depended on rainfed ecosystem. The complex nature of drought tolerance, genotype × environment interaction, lack of understanding of inheritance of drought tolerance, poor understanding of physiological basis of yield under water limited condition and difficulties of effective drought tolerance screening complicate the development of drought tolerance varieties. A number of shoot and root morphological traits have been proposed to improve performance of rice under drought condition. Among the root characters maximum root length, root volume, root number and root dry weight are found to be associated with the yield under water scarcity in aerobic condition. Therefore, the present study was undertaken to understand the association of root traits with grain yield; fine mapped the region of root traits and study of expression of genes, specific to root under different stress condition.

Most of the mapping populations used in QTLs analysis was derived from the cross between *indica* × *japonica* crosses, the mapping population used in this study was the result of crossing of two *indica* subspecies adapted to local environment. The BC_1F_7 and BC_1F_8 generation of the mapping population was developed using modified single seed descent method. Seventy one RILs were evaluated under irrigated, rainfed and TSD conditions during wet season 2014 and wet season 2015 along with and rhizotrons for root studies to generate phenotypic data. For generating genotypic data, DNA from each line along with parents was extracted using CTAB method and subjected to DNA quantification. Finally, DNA was diluted to approximate concentration of 50 ng/µl. The diluted DNA was then

subjected to PCR base amplification using SSR and HvSSR primers. The PCR products were then analyzed on 5 % PAGE gel. A set of 78 primers were screen to detect parental polymorphism, out of which 21 primers exhibited parental polymorphism and selected to generate genotypic data. The phenotypic data was statistically analyzed to calculate heritability, correlation and stability of grain yield with yield contributing traits. The phenotypic and genotypic data thus obtained was used for Single marker analysis in QTL Cartographer2.5 and QTL IciMapping3.2 to test the significant association between marker and grain yield and root traits. Gene pyramiding was our one of the objective of study. Our objective was to combine the genes/QTLs (grain yield and root traits). Based on the GGT analysis we have obtained lines carrying these genes/QTLs in combination.

Conclusions

- The analysis of variance indicated substantial variation among the RILs for grain yield and yield contributing traits.
- The drought intensity index of population was 81.42 and 83.43 under rainfed direct seeded and TSD transplanted condition, respectively during wet season 2014 and 85.59 under TSD transplanted conditions during wet season 2015. This indicated very high level of stress.
- During both the years line number 53, 52, 46 and 7 had highest grain yield under irrigated condition, line number 8, 9, 13 and 18 had highest grain yield under rainfed condition, while line number 30 and 29 had highest grain yield under terminal stage drought (TSD) condition.
- Line number 18, 30 and 31 had highest yield under stress conditions (rainfed and TSD) in both the years.
- High genetic advance coupled with high heritability was reported for seedling height, shoot biomass, plant height, flag leaf length, second leaf length, grain yield and harvest index. Medium heritability but high genetic advance was reported for tillers number, biological yield and 100 seed weight.

- Root length was ranged from 12 to 58.02 cm under rhizotron. Root volume was ranged from 0.10 to 1.12 mm. Average root diameter ranged from 0.17 to 0.35 mm with population mean of 0.25 mm.
- Root pulling strength showed positive and significant correlation with grain yield under both the irrigated conditions; viz., 2014 and 2015, respectively.
- Root length and root volume exhibited negative correlation with grain yield under irrigated condition.
- Shoot biomass, root pulling resistance, second leaf width, biological yield, harvest index, spikelet fertility, root length, root volume and average root diameter showed positive direct effect on grain yield under all three conditions during wet season 2014 and two conditions during wet season 2015.
- Line number 2, 16, 17, 18, 20, 21, 25, 26, 27, 28, 29, 30, 31, 32, 36, and 42 showed low value of DSI under all sets of stress conditions. Line number 1, 3, 5, 6, 7, 12, 22, 24, 34, 41, 46, 47, 48, 49, 50, 51, 52, 53, 54, 56, 57, 61, 68, 69, 70 and 71 showed high value of DSI under all sets of stress conditions.
- Stability analysis revealed line number 15 and 16 to be most stable; line no 16, 19, 20 and 36 suitable under unfavorable condition and line number 7, 46, 51, 52 and 55suitable under favorable condition.
- Screening of parental polymorphism using SSR primer reveal low level (26.92%) of polymorphism between two parents of the population.
- Number of markers, RM 3825, RM 1361, RM 12146, RM 6504, RM431 RM 1261, RM 28048 and RM 28076 exhibited highly significant co-segregation with grain yield.
- Number of markers, RM 18615, RM 18620, RM 18615, RM 163, RM 277, RM 24585 significant association with root traits.
- Line number 25, 27, 28, 29 and 31 were significantly carrying the gene related to root traits.
- Under semi-quantitaive RT-PCR, susceptible lines showing down regulation under stressed condition for OsRR2, OsGLU3, GLR3.1 and

AsGAP, whereas; tolerant lines showing up-regulation under moderate stress but also exhibit down regulation under severe stress condition. Tolerant line 50 and 53 showed up-regulation under both stress condition for GLR3.1 and DRO1 genes. Susceptible line 59 showed up-regulation under severe stress condition for gene OsPIN3t, DRO1 and OsAGAP genes.

- q PCR experiment there was strong expression of OsPIN3t gene in all the six selected lines under control, moderate and stress conditions with respect to housekeeping gene tubuline. The quantitative expression result obtained with primer for OsPIN3t gene is opposite to that obtained with primers for genes DRO1 and OsIAA23.
- In gene pyramiding study we reported that progeny number six was pyramided for various root traits. Progeny number 1, 2 and 3 was pyramided for root traits and grain yield, whereas progeny number 1, 2, 3, 4 and 5 pyramided for grain yield only.

Suggestions for future work:

- Under stability analysis line number 15 and 16 to be most stable; can be evaluated under multi location trials for development of new variety.
- Line number 16, 19, 20 and 36 suitable under unfavorable condition (stress) can be used as donor for drought.
- The identified markers to be cross validated on other mapping populations.
- Use these markers for subsequent marker assisted selection, QTL pyramiding etc.
- Fine mapping and candidate gene approach to identify gene controlling drought related traits on other mapping populations.
- Study the expression pattern of tolerant and susceptible lines with other drought related gene and also use these to validate other mapping populations.

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APPENDICES

Appendix (A): Standard meteorological week's average weather data during the crop growth period (June 18, 2014 – Oct. 25, 2014)

Wk No.	Date	Max. Temp. (°C)	Min. Temp. (°C)	Rain-fall (mm)	Rainy days	Relative Humidity (%)		Vapour Pressure (mm of Hg)		Wind Velocity (Kmph)	Evapo-ration (mm)	Sun Shine (hours)
						I	II	I	II			
23	**Jun 04-10**	44.1	30.9	0.0	0	54	23	20.6	14.8	7.8	13.0	8.0
24	**11-17**	40.4	28.4	14.0	2	56	36	18.2	16.7	9.7	10.6	5.0
25	**Jun 18-24**	33.6	26.0	30.0	3	79	60	21.5	21.1	9.5	5.7	3.0
26	**25-01**	35.7	26.0	27.6	2	78	50	21.7	20.2	8.1	6.4	3.3
27	**Jul 02-08**	37.7	27.0	9.0	1	72	44	21.6	19.7	9.0	8.5	5.3
28	**09-15**	34.3	23.8	152.8	7	92	72	23.6	24.8	8.4	6.6	4.1
29	**16-22**	28.5	24.6	260.2	6	95	88	22.8	22.6	12.1	2.8	0.5
30	**23-29**	28.7	23.8	37.2	4	95	82	22.1	23.0	9.4	2.7	1.6
31	**30-05**	29.8	24.8	136.0	7	95	86	23.2	24.3	9.7	4.0	1.9
32	**Aug 06-12**	30.2	24.8	42.1	3	91	71	22.7	22.5	9.1	3.6	2.8
33	**13-19**	31.8	25.3	45.0	3	91	70	23.7	22.6	7.0	4.7	5.5
34	**20-26**	32.3	25.1	25.8	2	92	73	24.0	23.7	4.0	3.7	3.4
35	**27-02**	31.8	25.0	84.8	4	91	76	23.1	23.6	5.8	4.1	3.6
36	**Sep 03-09**	28.3	24.2	79.5	4	94	83	22.5	22.6	6.2	1.7	0.5
37	**10-16**	30.5	24.3	41.0	3	95	79	23.0	24.0	5.8	3.3	3.4
38	**17-23**	32.1	24.6	57.6	3	94	68	23.6	23.3	3.6	3.7	4.4
39	**24-30**	33.4	24.0	0.0	0	93	57	22.5	20.6	2.1	4.1	8.3
40	**Oct 01-07**	33.2	24.0	0.0	0	91	57	22.0	20.6	2.5	3.9	8.3
41	**08-14**	30.4	23.6	52.2	2	89	66	20.8	20.4	6.9	3.6	4.9
42	**15-21**	31.5	22.5	1.2	0	91	56	20.4	18.8	2.6	3.4	8.4
43	**22-28**	29.1	19.4	5.4	1	92	52	17.0	14.9	2.0	2.8	5.9
44	**29-04**	30.1	16.9	0.0	0	94	37	15.1	11.5	1.9	3.0	8.0

Appendix (B): Standard meteorological week's average weather data during the crop growth period (June 18, 2015 – Nov. 11, 2015)

Wk No.	Date	Max. Temp. (°C)	Min. Temp. (°C)	Rain-fall (mm)	Rainy days	Relative Humidity (%)		Vapour Pressure (mm of Hg)		Wind Velocity (Kmph)	Evapo-ration (mm)	Sun Shine (hours)
						I	II	I	II			
25	**Jun 18-24**	33.8	25.8	201.8	3	85	72	23.3	22.4	7.9	5.3	4.9
26	**25-01**	33.5	25.0	25.8	4	87	59	22.8	21.6	9.3	6.4	4.3
27	**Jul 02-08**	33.6	25.2	41.8	2	79	64	21.7	22.0	9.1	6.4	5.9
28	**09-15**	31.2	25.2	72.8	5	89	80	23.2	24.1	7.9	3.3	1.7
29	**16-22**	31.8	25.6	7.8	1	91	71	23.4	23.6	8.0	4.7	2.4
30	**23-29**	30.7	25.1	43.6	1	90	70	22.3	21.6	7.9	4.1	3.4
31	**30-05**	31.2	25.2	48.7	3	86	69	21.3	21.2	10.4	4.6	4.6
32	**Aug 06-12**	30.8	24.7	36.6	1	94	73	23.2	23.7	4.8	3.1	2.5
33	**13-19**	31.7	25.3	126.4	3	94	73	24.0	24.2	7.5	4.7	4.1
34	**20-26**	32.3	25.9	23.6	1	87	65	22.6	22.0	8.1	5.0	6.5
35	**27-02**	30.8	25.0	37.9	6	94	80	23.5	24.7	4.9	2.5	1.2
36	**Sep 03-09**	33.0	25.5	10.0	1	93	64	23.7	21.5	4.7	3.9	6.9
37	**10-16**	33.5	25.4	68.4	2	93	62	23.9	22.0	3.8	4.7	6.8
38	**17-23**	30.1	25.1	135.4	2	94	78	23.6	24.0	5.8	2.6	3.1
39	**24-30**	32.5	24.6	0.0	0	92	57	22.3	20.2	3.0	3.8	7.2
40	**Oct 01-07**	33.7	24.4	0.0	0	92	51	22.7	19.3	2.4	4.4	7.7
41	**08-14**	33.9	22.2	0.0	0	89	47	19.5	17.9	3.0	4.3	8.7
42	**15-21**	33.4	22.8	0.0	0	91	45	20.2	16.7	2.4	3.8	8.7
43	**22-28**	33.7	21.3	0.0	0	90	37	18.5	13.8	2.1	3.6	8.2
44	**29-04**	30.0	19.4	0.0	0	90	55	16.6	16.4	4.1	3.2	6.7
45	**Nov 05-11**	31.7	18.8	0.0	0	91	37	15.8	12.4	2.6	3.5	7.8

Appendix (C): Scoring of population

MARKERS	Swarna Sub-1	IR 305 P1B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
RM163	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM225	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B
RM242	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B
RM277	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B
RM431	A	B	A	B	A	A	A	A	A	A	-	A	A	B	A	A	A	A	A	B	B	B	B	B	B	B	A	A	A
RM1233	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B
RM1261	A	B	A	B	A	A	A	A	A	A	B	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A	B	A	B
RM1361	A	B	A	B	B	B	B	A	B	B	A	B	B	B	-	A	B	B	B	B	A	B	B	A	A	-	A	A	B
RM3825	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A
RM6504	A	B	A	B	A	B	-	A	A	A	A	A	-	A	A	A	A	A	A	A	B	-	A	A	A	A	A	-	A
RM11943	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A
RM12003	A	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	A	A	A	B	A	B	A	A	A	A
RM12091	A	B	A	B	A	A	A	A	A	A	A	-	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM12146	A	B	A	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A
RM18615	A	B	A	A	-	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	-
RM18616	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
RM18620	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B
RM22565	A	B	-	A	A	A	A	-	A	A	B	-	B	B	A	A	B	-	A	A	A	A	A	-	A	A	A	A	-
RM24585	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A
RM28048	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
RM28076	A	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	A	A	A	A	A	A	B	A	B	B	B

Appendix (C): Scoring of population

MARKERS	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
RM163	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM225	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM242	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM277	B	B	B	B	-	A	A	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM431	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	A	A	B	A	B	B	A	A	A	A	B	A	A	B
RM1233	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM1261	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A
RM1361	B	B	A	A	A	A	A	B	A	A	A	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	A
RM3825	A	A	A	A	A	A	A	A	B	A	B	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A
RM6504	A	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	A	A	B	A	A	A	A	A	A	B	A	A	B
RM11943	A	A	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A
RM12003	A	A	A	A	B	A	A	B	B	A	B	A	A	B	B	B	A	-	A	A	-	A	A	A	B	B	A	B	A
RM12091	A	A	A	A	A	A	A	A	A	A	B	A	-	A	A	A	B	A	A	-	-	A	B	A	B	B	B	B	B
RM12146	A	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	B	A	B	B	A	A	B	A	B	B	A	B	B
RM18615	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
RM18616	A	A	A	A	A	B	A	B	A	B	A	A	A	A	A	A	A	-	A	A	-	A	A	A	A	A	A	A	A
RM18620	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM22565	A	A	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	B	-	-	B	B	B	B	B	B	B	A
RM24585	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A
RM28048	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	-
RM28076	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A

Appendix (C): Scoring of population

MARKERS	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
RM163	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM225	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
RM242	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM277	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM431	A	A	A	B	B	A	A	A	A	A	A	B	B	B	A
RM1233	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM1261	B	B	B	A	A	A	B	B	B	B	A	A	A	A	A
RM1361	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM3825	B	B	B	A	A	A	B	B	B	B	A	A	A	A	A
RM6504	A	A	A	B	B	A	A	-	A	A	A	B	B	A	A
RM11943	B	B	B	A	A	A	B	B	B	B	A	A	A	A	A
RM12003	A	B	A	B	B	B	B	B	B	A	A	A	B	A	A
RM12091	A	A	A	B	-	-	A	A	A	A	B	B	B	B	A
RM12146	A	A	A	A	-	B	A	A	A	A	B	B	B	B	A
RM18615	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM18616	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM18620	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
RM22565	B	A	A	A	B	-	B	B	A	A	B	A	A	-	-
RM24585	A	A	B	A	A	B	B	B	B	A	A	A	A	A	A
RM28048	A	A	A	A	-		-	-	A	A	A	A	A	A	A
RM28076	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

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2. ICAR-Junior Research Fellowship (ICAR-JRF) for pursuing Post Graduation
3. ICAR-Senior Research Fellowship (ICAR-SRF (PGS)) for pursuing Ph.D.
4. Qualified ICAR-NET 2013 conducted by Agriculture Scientist Recruitment Board, New Delhi

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